[APPLICATION NOTEBOOK]

IMPURITIES

Developing Safe and Effective Drugs

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INTRODUCTION

The pharmaceutical industry is integral in maintaining public health by providing therapeutic and preventative medicines. The cornerstones of bringing new medicines to market are quality, safety, and efficacy. These attributes are controlled using a variety of analytical procedures aimed at characterizing drug substance and drug product purity. A key step in delivering on these aspects is a detailed understanding of the impurity profile of a drug substance and final product. This knowledge provides insight to improve synthetic processes, formulation selection, and shelf life while ensuring safety and efficacy throughout the product lifecycle.

Targeted analytical procedures provide the framework guiding the drug development process for the pharmaceutical industry, but also establish standardization for regulatory agencies aiming to measure drug product integrity prior to use in the marketplace. Since 1993, the regulatory focus on impurities has intensified. In recent years, guidance has been issued relating to stability methods (ICH Q1A-F; 1996-2003), drug substance/drug product impurities (ICH Q3A-Q3B; 2006), genotoxic impurities (ICH S2(R1); 2011 & M7; 2014), extractables and leachables (ICH Q8-Q9; 2005-2009), and life cycle management (ICH Q12; 2014). In addition, therapeutic molecules are becoming more complex, giving rise to greater analytical challenges. At Waters, we strive to understand the requirements of these guidances and provide innovative solutions to address the changing pharmaceutical landscape.

The role of impurity analysis is dynamic and conducted throughout the pharmaceutical drug development process. Impurity based activities throughout the development of therapeutics are uniquely focused in terms of their application needs as the drug product progresses downstream to commercialization. Central to the discovery and ongoing monitoring of impurities is liquid chromatography and mass spectrometry coupled with appropriate informatics solutions. Waters' portfolio provides the most comprehensive of liquid chromatographic techniques, mass spectrometry, data management, and column chemistries to address your challenges. Our ongoing innovation, guided by the pharmaceutical industry, has led to novel introductions such as Empower® Chromatography Data Software, MS^{E}, ACQUITY UPLC,® Bridged-ethylene hybrid stationary phases, and ACQUITY® QDa® mass detection. These solutions are shaping the approaches and landscape of the pharmaceutical laboratory focusing on implementing streamlined pathways to understanding and controlling impurities.

In this compilation, we revisit the last decade of Waters developments in impurity analysis. As needs vary from one analyst to another, the design of this application compilation was structured to navigate primarily from two perspectives:

1. the analysts' responsibilities within the pharmaceutical pipeline
2. the types of applications that are most challenging

Additionally, we recognize the changing regulatory requirements and future challenges regarding impurities. Therefore, a separate table of contents is provided to facilitate navigation to topics such as genotoxic impurities (GTI), extractables and leachables (E&L), method modernization, and stability testing.

NAVIGATION

This application notebook is broken into three distinct sections to help you easily find information that's relevant to your needs. **Pharmaceutical Pipeline** identifies those app notes relevant to the discovery, development, and manufacturing of new drugs. If you're more interested in which app notes detail the process and workflow in a variety of application areas, then **Application Workflow** is the section you want to spend more time on. Finally, **Trending Topics** highlights scientific experiments that leverage the latest technologies and therapeutic focus. Finally, each table of contents highlights the technologies, which are emphasized in the respective app note.

Hover and click on any one of the navigation aids below to quickly link to the appropriate set of application notes.

Pharmaceutical Pipeline

Application Workflow

Analytical Separations · Purification · Mass Spectrometry · Informatics

Method Development

Isolations

Elucidation

Monitoring

Trending Topics

	Analytical Separations	Purification	Mass Spectrometry	Informatics	
GTI					
Analysis of Benzenesulfonic Acid and P-Toluenesulfonic Acid Esters in Genotox Monitoring Using UPLC/UV-MS	■		■		149
Quantitative Determination of Genotoxic Impurities Using Xevo TQD	■		■		159
Rapid Identification of Genotoxic Impurities in Tablets Using the ASAP Probe			■		237
E&L					
Streamlining Current Approaches for Extractable Analysis Utilizing Waters MV-10 ASFE and ACQUITY UPC² Systems	■		■		83
Application of UPC² in Extractables Analysis	■		■		89
Routine Trace-Level Contamination Testing in High-Quality Manufactured Parts and Assemblies	■		■	■	170
Identifying Leachables and Extractables from Packaging Materials	■		■	■	163
Detection and Identification of Extractable Compounds from Polymers			■		241
Stability					
Utilizing UPLC-MS for Conducting Forced Degradation Studies	■		■		27
A Stress Testing Study of Glimepiride Utilizing UPLC-MS Methodology			■		37
Impurity Isolation and Scale-up from UPLC Methodology: Analysis of an Unknown Degradant Found in Quetiapine Fumarate	■	■	■	■	42
UPC² Method Development for Achiral Impurity Analysis	■		■		101
Method Modernization					
A Quality by Design (QbD) Based Method Development for the Determination of Impurities in a Peroxide Degraded Sample of Ziprasidone	■			■	72
Forced Degradation Analysis of Omeprazole Using CORTECS 2.7 µm Columns	■		■		119
Chromatographic Purity of Estradiol Using the ACQUITY UPC² System	■				178
Improving Resolution Using e***X***tended ***P***erformance (***XP***) Columns	■				180
Transfer of USP Methods for Impurities Analysis of Ziprasidone HCl between HPLC Systems and to UPLC	■			■	183
Transfer of Two USP Compendial Methods for Impurities of Ziprasidone HCl to a Single UPLC Method	■			■	193
USP Method Transfer of Donepezil Tablets from HPLC to UPLC	■			■	201
USP Method Transfer of Levonorgestrel and Ethinyl Estradiol Tablets from HPLC to UPLC	■			■	207
Transfer of an Isocratic USP Assay from an Agilent 1100 Series LC System to a ACQUITY UPLC H-Class System: Analysis of Tioconazole and Related Impurities	■			■	221
Improving Performance and Throughput of the USP Organic Impurities Analysis of Tioconazole on an Alliance HPLC System	■				243
Using e***X***tended ***P***erformance (***XP***) Columns to Modernize the USP Organic Impurities Analysis of Tioconazole	■				248

[APPLICATION NOTE]

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Mass-Directed Isolation of a Pharmaceutical Compound Using AutoPurify with an ACQUITY QDa Detector

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APPLICATION BENEFITS

- The ACQUITY® QDa® Detector can be seamlessly integrated into the Waters® AutoPurification™ System, making mass-directed purification more readily accessible to chromatographers and is ideal for upgrading existing UV-directed systems.
- Pre-optimization and automatic calibration routines make the ACQUITY QDa Detector easy to use and reduce the time required for operator training.
- The Waters AutoPurification System with ACQUITY QDa Detector provides flexible automated compound isolation options that simplify the purification workflow and increase process efficiency by empowering scientists to perform other tasks.
- Mass-directed purification of compounds improves productivity by collecting only target compounds, thereby reducing fraction handling and analysis that would be necessary for UV-directed systems.

WATERS SOLUTIONS

Waters AutoPurification System

FractionLynx® Application Manager with AutoPurify™

MassLynx® Software

ACQUITY QDa Detector

XSelect® Columns

KEY WORDS

Isolation, purification, mass-directed purification, AutoPurification, AutoPurify, ACQUITY QDa, pharmaceutical, MassLynx, FractionLynx, XSelect, Charged Surface Hybrid, CSH

INTRODUCTION

Pharmaceutical compounds synthesized in chemistry laboratories almost always require purification before they can be used in experimental studies. Preparative chromatography is often the technique used to clean up synthetic mixtures, but the multi-step purification process can be cumbersome and time-consuming. AutoPurify, a feature provided within the FractionLynx Application Manager of MassLynx Software, streamlines the purification process with flexible, automated strategies that reduce or eliminate user intervention between isolation steps. While the benefits of AutoPurify have been discussed in detail previously,[1] the integration of the ACQUITY QDa Detector into the AutoPurification System makes mass-directed purification more readily accessible and provides the added assurance that mass spectral data brings to chromatographic separations. With its pre-optimized hardware and automated calibration routine, the ACQUITY QDa Detector can be easily added to purification systems. In this application note, we demonstrate the feasibility of successfully isolating a synthetic pharmaceutical compound from a crude mixture using AutoPurify on an AutoPurification System configured with an ACQUITY QDa Detector.

EXPERIMENTAL

LC conditions

LC system:	Waters AutoPurification System
Detectors:	ACQUITY QDa (mass); 2998 Photodiode Array
Analytical column:	XSelect CSH C_{18}, 4.6 x 50 mm, 5 µm (p/n 186005287)
Preparative column:	XSelect CSH C_{18} OBD Prep, 19 x 50 mm, 5 µm (p/n 186005420)
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Methanol, neat
Column temperature:	Room
Sample temperature:	Room
Injection volume:	Reported in figures
Flow rate:	Reported in figures
Gradient:	Reported in figures

MS conditions

Ionization mode:	Electrospray +
Data:	Centroid
Mass range:	100-850 amu
Scan time:	10 min
Cone voltage:	15
Sampling frequency:	5 Hz
Capillary voltage:	0.8
Probe temperature:	600
Detector gain:	1
Makeup solution:	90:10 water:acetonitrile with 0.01% formic acid

Note: Makeup solution is only used for preparative separations

Data management

MassLynx v4.1

FractionLynx Application Manager

Sample description

Dry pharmaceutical intermediate was dissolved in dimethylsulfoxide to a concentration of 50 mg/mL.

RESULTS AND DISCUSSION

Before beginning the isolation process, the AutoPurify method was configured in FractionLynx. The AutoPurify method defines the set of parameters that will be used for analyzing the crude sample, selecting the purification method, running the isolation protocol, and evaluating the collected fractions. In high throughput laboratories where there is broad sample diversity, the system administrator may choose to define multiple AutoPurify methods to adequately address the different types of molecules that require purification. For example, there may be a specific method for acids, bases, or hydrophobic molecules. While AutoPurify can be configured to run automatically from analytical crude sample screening and prep isolation to fraction analysis, the three stages may also be executed in a semi-automated fashion. The software can be programmed to run the analytical screening and generate the prep sample list. The user can then review the FractionLynx browser report, make changes to the purification strategy if desired, and then manually start the system to perform the compound isolation. In the same manner, the user may review the newly generated purification results in the browser report and edit the sample list before fraction analysis occurs. Thus, the chemist can interact with AutoPurify as much or as little as he deems necessary for a given set of samples. Detailed descriptions of the AutoPurify process have been communicated previously.[2,3]

Once the AutoPurify method was defined to perform the completely automatic purification process from analytical screening through fraction analysis, the synthetic crude mixture of the pharmaceutical intermediate was placed in the sample manager and the system was started. After completing the analysis of the crude material, the FractionLynx browser report showed that the sample was approximately 67% pure. According to the parameters defined in the AutoPurify method, the sample required purification. The software selected an appropriate gradient for purification (the focused gradient named Narrow B in Figures 1 and 2), and immediately started the isolation.

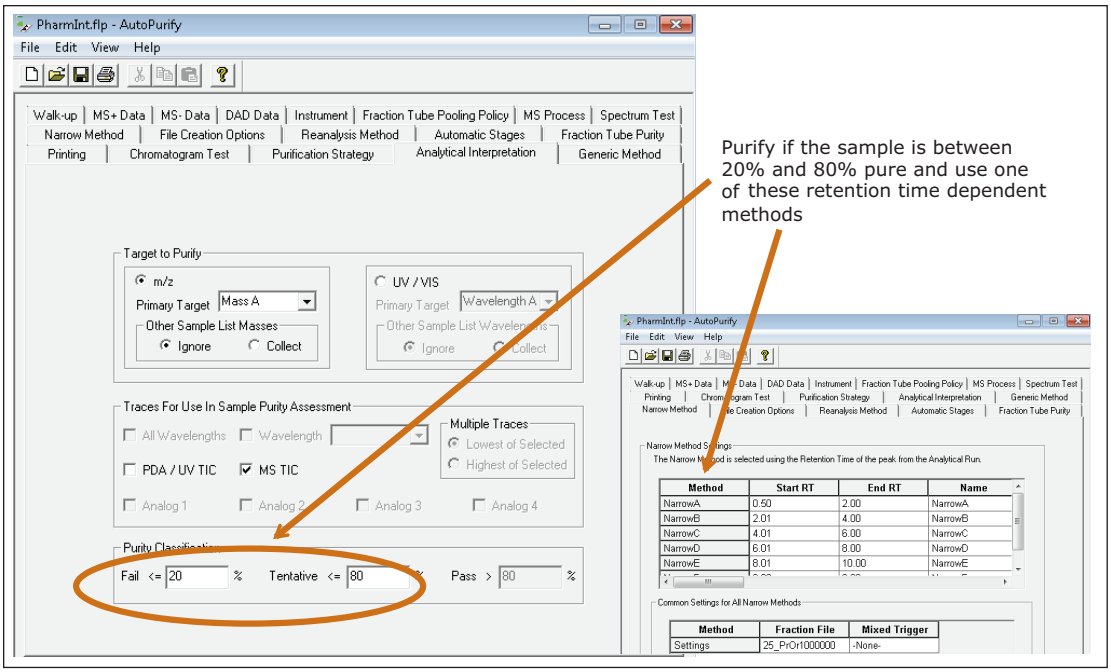

Figure 1. The Analytical Interpretation tab in the AutoPurify method specifies the primary target and its required purity. The Narrow Method tab defines the retention time ranges for each focused gradient method.

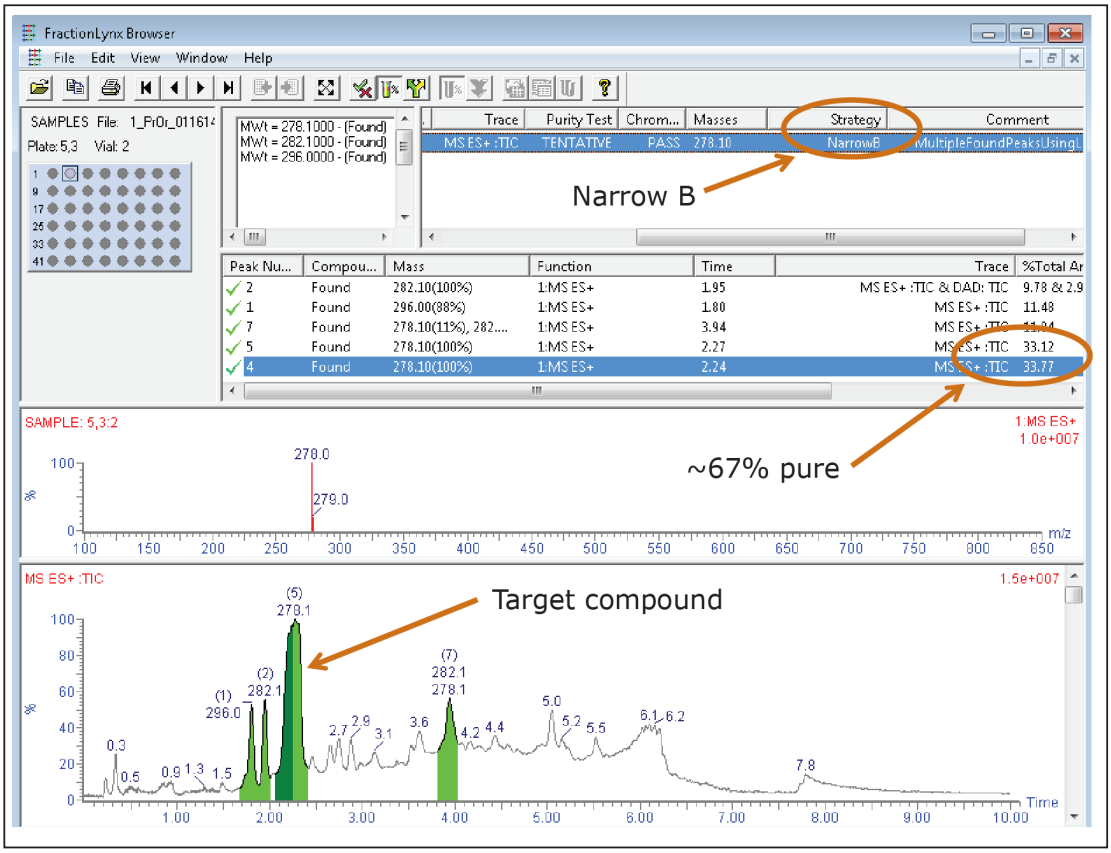

Figure 2. The analytical browser report shows the chromatogram of the crude sample mixture, the purity of the target compound, and the purification method that will be run. The analytical gradient ran from 5–95%B in 6 minutes. The injection volume was 5 µL.

AutoPurify uses the retention time of the target peak to select one of the predefined narrow gradients. Narrow gradients, also known as focused gradients,[4] are useful in preparative chromatography because they effectively increase the resolution between the target peak and its closely eluting neighbors without increasing run time. Increased resolution ultimately leads to higher loading and greater product purity. Figure 3 shows the preparative chromatography of the isolation using the Narrow B gradient method described in Table 1.

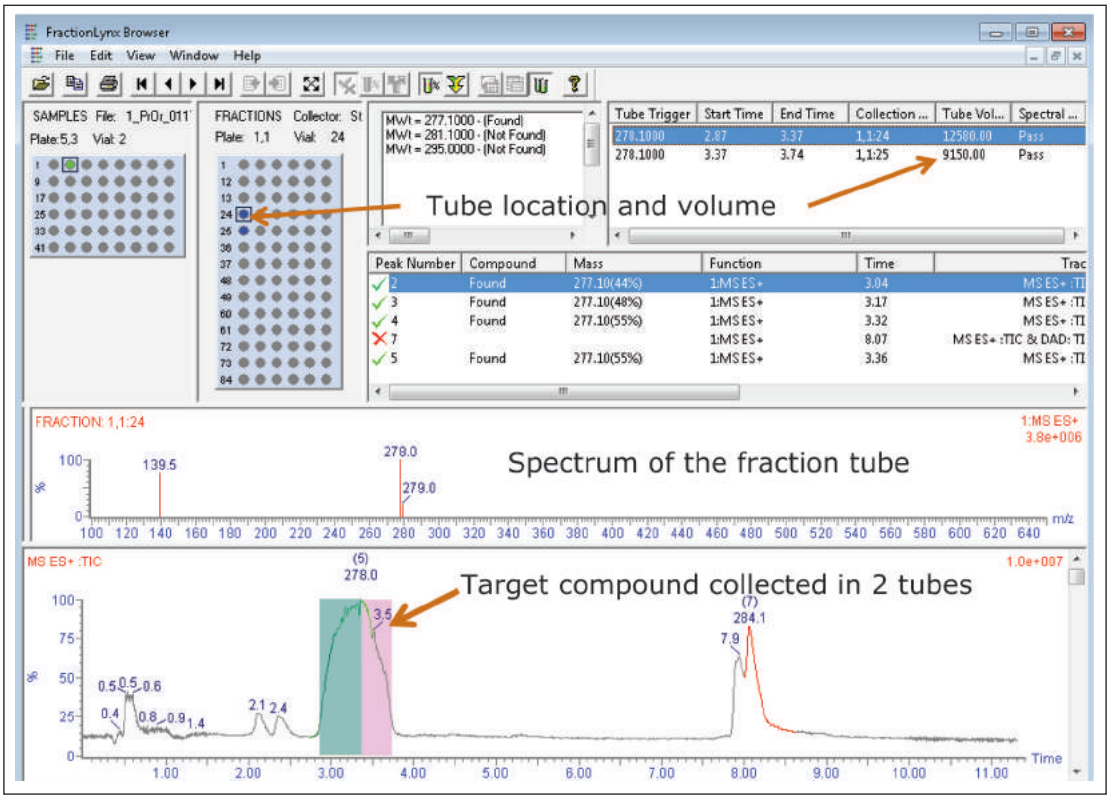

Figure 3. Browser report for the preparative chromatography with Narrow Method B. The focused gradient increased the resolution between the contaminant peak eluting at 2.4 minutes and the target peak. The spectrum of the fraction tube showed excellent purity. The mass at 139.5 was most likely due to in-source fragmentation. The injection volume was 85 µL, geometrically scaled from the 5-µL injection on the analytical column.

Without user intervention, analysis of the collected fractions was performed immediately following the completion of the preparative isolation. The analysis method used was the same one as the original screening gradient, running from 5–95%B in 6 minutes. The estimated purity of the fraction shown is 97%, as the peak in red is actually part of the column washout. The results of the fraction analysis are shown in Figure 4.

Time	Flow	%A	%B
0.00	25	95.0	5.0
0.35	25	95.0	5.0
1.31	25	86.3	13.7
7.31	25	75.0	25.0
7.41	25	5.0	95.0
8.41	25	5.0	95.0
8.51	25	95.0	5.0
11.31	25	95.0	5.0

Table 1. Narrow Method B. The 0.35 minute hold at the beginning of the gradient is to account for the difference in system volume between the analytical and preparative system flow paths.

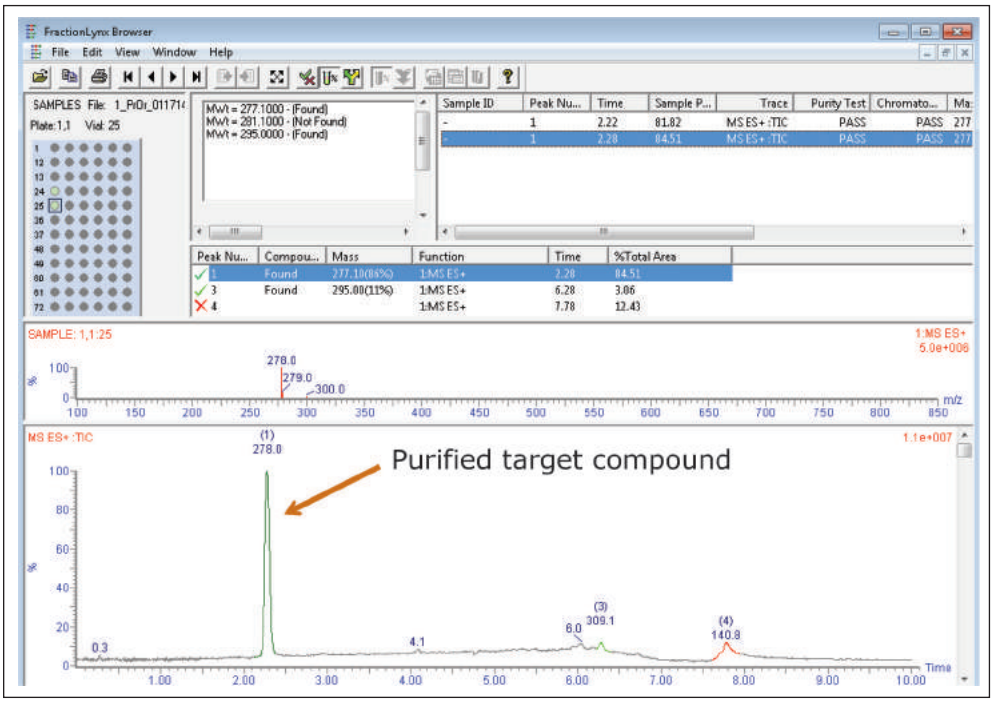

Figure 4. Browser report for the analysis of a fraction. The injection volume was 20 µL, taken directly from the collection tube immediately after purification.

CONCLUSIONS

- The ACQUITY QDa Detector is suitable for integration into purification systems, making mass-directed purification more accessible to all isolation chemists.
- Mass-directed purification of compounds reduces cost and saves time by collecting only specific target compounds, thereby reducing the number of fractions requiring analysis and handling.
- The ACQUITY QDa Detector is compatible with AutoPurify, the software feature in FractionLynx that executes all phases of the purification process from crude sample analysis to final fraction evaluation.
- AutoPurify effectively improves productivity by increasing sample throughput with minimal user intervention at each step of the isolation process.

References

1. Lefebvre PM, Brailsford A, Brindle D, North C, Cleary R, Potts III WB, Smith BW. Compound Purification Workflow Management and Optimization. Waters Poster Reprint 720000696EN. 2003 March.
2. Lefebvre PM, Cleary R, PottsIII WB, Granger J, Plumb RS. Automated Characterization, Purification and Fraction Analysis. Waters Poster 720001014EN. 2004.
3. Cleary R, Lefebvre P. Purification Workflow Management. Waters Application Note 720001466EN. 2007 June.
4. Jablonski JM, Wheat TE, Diehl DM. Developing Focused Gradients for Isolation and Purification. Waters Application Note 720002955EN. 2009 September.

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Supporting Pharmaceutical Synthetic Process R&D Using LC/MS

GOAL

To demonstrate enhancements to UPLC®/MS technology and its use with open access software to improve synthetic process monitoring for process R&D chemists.

BACKGROUND

Once a chemical hit is found through a library screening process and is verified, investigation of the compounds' synthetic route takes place. This step involves an iterative process of different synthetic approaches to generate the identified compound efficiently and safely.

Because these reactions may take a long time, chemists need to know as soon as possible if their syntheses are proceeding as desired. This means utilizing measurement capabilities that require minimal sample preparation and provide a fast response giving low detection limits.

High throughput approaches can provide important time savings in the optimization of process parameters and help identify false positives. Open access LC/MS is replacing TLC as a reaction monitoring tool. Sample preparation of reaction mixtures can be as minimal as filtering and dilution before injecting into the LC/MS system. This allows fast turnaround of results to allow the chemist to advance to the next step or to eliminate the process.

Thus, sample throughput is a critical issue for process R&D chemists. UltraPerformance LC® (UPLC) leverages sub-2-µm LC particle technology to generate high efficiency and faster separations.

Enhance productivity through confirmed identification of synthesized compounds and impurities using UPLC with the SQ Detector 2.

Peak I.D.	Description	RT (min)	Mass (*m/z*)
1	Impurity 1	1.51	267.2
2	Impurity 2	1.63	313.2
3	Impurity 3	1.88	418.4
API	API	1.68	419.4
By-product	By-product	1.54	437.4

Table 1. Retention time and mass of impurities and identified peaks.

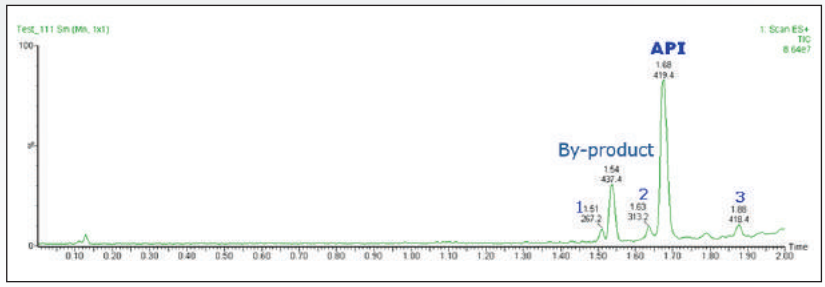

Figure 1. Chromatographic separation of three impurities, API, and by-product.

Open access software offers the power of chromatography and mass spectrometry to chemists who are not analytical instrumentation specialists. It allows them to quickly and easily know what they've made and allows the experts to work on the difficult analytical problems.

An open access UPLC/MS system was investigated for process R&D support. In this technology brief, we describe some of the enhancements to LC and LC/MS technologies that have generated useful tools that improve the throughput and accuracy of these assays.

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LC conditions

LC system:	ACQUITY UPLC with SQ Detector 2
Column:	ACQUITY UPLC BEH, 2.1 x 30 mm, 1.7 µm
Mobile phase A:	0.1% Formic acid in water
Mobile phase B:	0.1% Formic acid in acetonitrile
Gradient:	5% to 95% B over 2 minutes
Flow rate:	800 µL/min
Column temp.:	50 °C
Sample temp.:	8 °C
Injection volume:	2.0 µL

MS conditions

MS system:	SQ Detector 2
Ionization mode:	ESI+/ESI-
Acquisition range:	150 to 600 *m/z*
Capillary voltage:	3.0 KV
Cone voltage:	20 V
Desolvation temp.:	450 °C
Desolvation gas:	900 L/Hr
Source temp.:	150 °C

THE SOLUTION

A representative sample from a synthetic chemistry laboratory was analyzed a using gradient of 2 minutes. Chromatographic separations were carried out using an ACQUITY UPLC® System coupled to an SQ Detector 2 single quadrupole mass spectrometer, using OpenLynx Application Manager for MassLynx™ Software.

By using a walk-up UPLC/MS system, chemists were able to quickly and easily determine the successful completion of their reactions with mass confirmation, noting the API synthesis as well as seeing the formation of any side products or other impurities. As a consequence chemists can spend less time setting up and analyzing the samples, get mass confirmation quicker than previously, and therefore increase their reaction throughput.

SUMMARY

Synthetic chemists frequently need to confirm the identity of synthesized compounds and confirm the presence of any impurities quickly and easily using very small quantities of material in the quickest possible time. The ability to do this using an open access LC/MS system utilizing UPLC with the SQ Detector 2 allows them to make rapid, informed, high quality, data rich decisions on their synthetic processes thereby enhancing their productivity and improving their support of the drug discovery and development process.

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[TECHNOLOGY BRIEF]

Characterization of Impurities in Synthesized Fine Chemical Products

GOAL

To successfully characterize impurities in synthesized fine chemicals by exact mass MS/MS using *Fast*DDA on the Xevo® G2 QTof.

BACKGROUND

Analytical laboratories studying the products of organic synthesis have to consider many things from confirmation of the final product to identification of impurities. Impurity identification, whether expected or not, is an essential part of the manufacture of fine chemicals, as any impurities could adversely affect the properties of the final product. This applies equally to the raw starting materials and the final synthesized product.

Obtaining MS/MS product ion spectra is one useful way of elucidating the structure of impurities, while exact mass provides additional confidence to structural assignments. However, the manual setup of each product ion experiment can be time consuming and prone to human error, so a Data Directed Analysis (DDA) is beneficial with regard to laboratory resources. DDA automatically selects ions for MS/MS acquisitions in real time, as components elute from the column. Recent improvements in the spectral acquisition rate (30 Hz) now enable DDA to be compatible with UPLC® peaks.

_Fast_DDA automatically produces MS/MS product ion spectra that can be used to successfully elucidate the structure of trace level impurities.

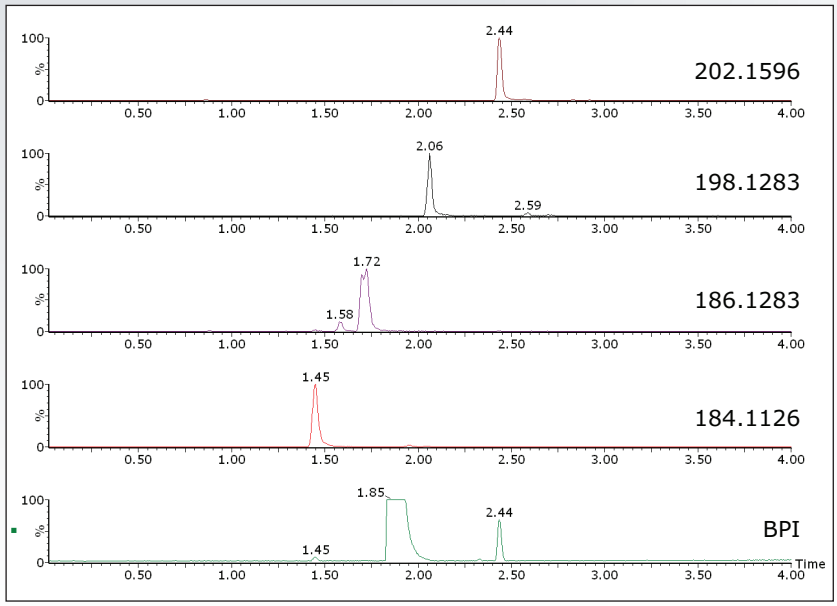

Figure 1. BPI chromatogram for octahydroacridine (Rt = 1.85 min) with extracted mass chromatograms for four of the impurities found automatically by FastDDA.

Octahydroacridine (>97% purity) was used here for illustration purposes. Base peak intensity (BPI) chromatogram and extracted ion chromatograms of some of the impurities found automatically by *Fast*DDA are shown in Figure 1. Octahydroacridine is interesting as it plays an important role in the preparation of alkaloids, dyes, drugs, and other biologically active compounds.

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THE SOLUTION

Waters® Xevo G2 QTof, coupled with the ACQUITY UPLC® System and operated in *Fast*DDA acquisition mode, rapidly and automatically generated MS/MS product ion spectra of all the impurities, enabling structural elucidation to be performed with MassFragment™ Software.

*Fast*DDA intelligently selects ions for MS/MS acquisitions in real time, as components elute from the chromatographic system. Embedded algorithms rapidly interrogate MS survey spectra and co-eluting precursor ions are selected for MS/MS analysis based on threshold intensity and pre-defined exact mass include/exclude lists.

In this example, octahydroacridine (*m/z* 188.1439) was excluded from the *Fast*DDA setup as the expected compound. This setup allowed seven discrete impurities to be characterized with the automatic generation of high resolution MS/MS product ion spectra, four of which are shown in Figure 2.

MassFragment Software, the automated structural elucidation tool which uses a systematic bond cleavage and ranking algorithm, was employed to rationalize and identify fragment ion structures from potential impurities. By submitting a postulated precursor structure and a MS/MS product ion spectrum, the MassFragment Software tool generates a report of possible fragmentation with exact mass confirmation. An extract from the MassFragment results summary for the impurity, *m/z* 202.1596, is shown in Figure 3.

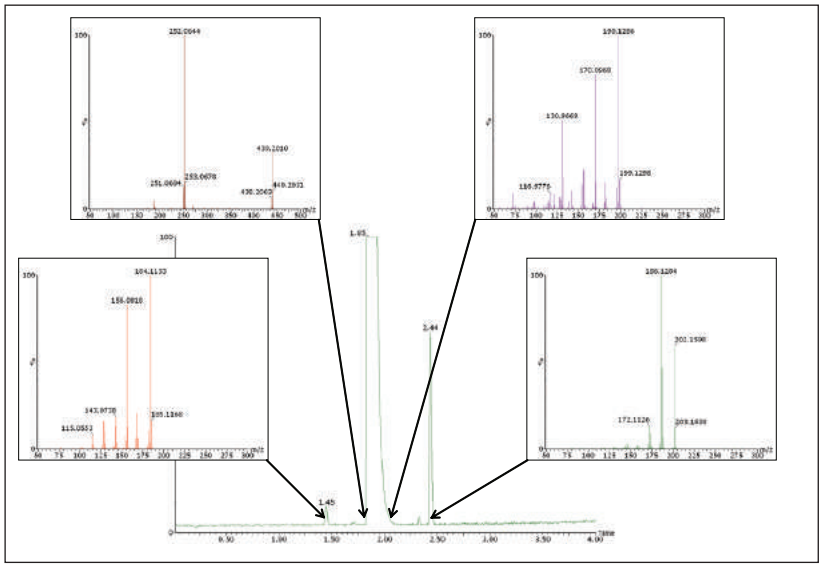

Figure 2. FastDDA spectra for some of the impurities of octahydroacridine.

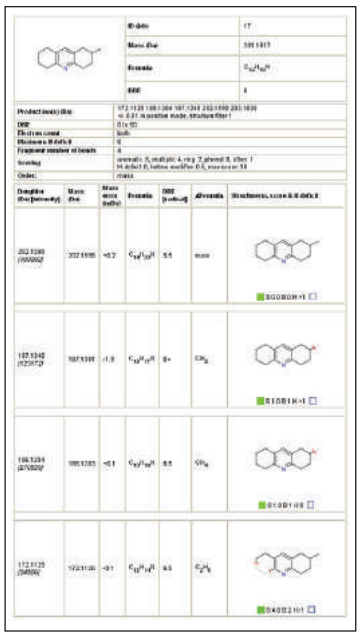

Figure 3. MassFragment report summary for m/z 202.1596.

SUMMARY

The synthesized product and trace-level impurities were successfully characterized using the:

- High resolution capabilities of UPLC
- Rapid, automated, and intelligent acquisition rates of *Fast*DDA
- Sensitivity, resolution, mass accuracy, and dynamic range of Xevo G2 QTof
- Automation of MassFragment Software

The rapid, automated nature of this solution minimizes the need for manual intervention, which reduces the drain on laboratory resources and maximizes the information obtained from a single analytical experiment.

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[TECHNOLOGY BRIEF]

Low Level Enantiomeric Impurity Analysis Using the ACQUITY UPC² System

GOAL

To demonstrate the fast chiral separation of benzyl mandelate and the enantiomeric excess determination at 0.02% impurity level using the Waters® ACQUITY UPC²™ System.

BACKGROUND

According to the September 2005 issue of *Chemical & Engineering News*, 9 out of the top 10 drugs (based on sales figures) have chiral active ingredients, and five of those nine drugs have single enantiomeric active ingredients. The single enantiomeric form of a chiral drug is considered an improved chemical entity, which may offer a higher efficacy, a better pharmacological profile, and a more favorable adverse reaction profile. For the manufacturers of single enantiomeric drugs, the undesired stereoisomers should be considered in the same manner as other organic impurities. Regulatory requirements for the identification, quantification, and control of impurities in drug substances and their formulated products have been explicitly defined by the International Conference of Harmonization (ICH). The threshold for identification and quantification of organic impurities is 0.1% for the majority of compounds, according to the ICH.

The high detection sensitivity of the ACQUITY UPC² System enables the identification and quantification of enantiomeric impurities in drug substances.

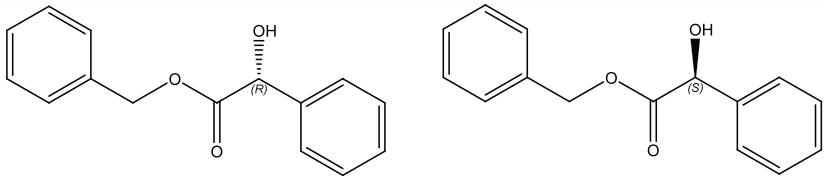

Figure 1. Chemical structures of R- and S-benzyl mandelate.

THE SOLUTION

Benzyl mandelate, shown in Figure 1, is an important synthetic intermediate for pharmaceutical synthesis. A racemic mixture of R- and S-benzyl mandelate (0.20 mg/mL in methanol for each enantiomer) was separated using UltraPerformance Convergence Chromatography™ (UPC²™), and the chromatogram is shown in Figure 2. Key experimental parameters are listed in Table 1.

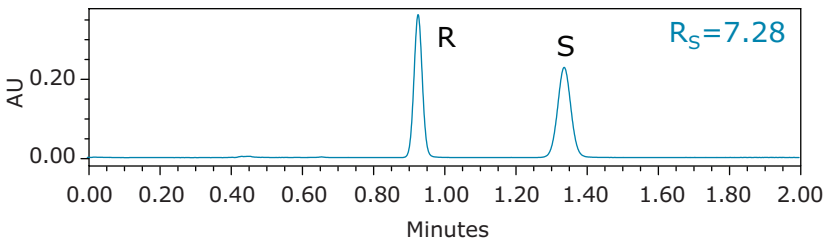

Figure 2. UPC² chromatogram of R- and S- benzyl mandelate at 0.20 mg/mL of each enantiomer.

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The overall analysis time was less than 1.5 min. Average base peak widths were less than 6 s. Based on the peak area, the ratio of the R- and S-benzyl mandelate was 0.997. Retention time and peak area repeatability measurements were based on five replicate injections, as summarized in Table 2. At 0.20 mg/mL concentration, repeatability for retention time was better than 0.23% RSD and better than 0.5% RSD for peak area.

Analyte		Benzyl mandelate	
Isomer		R-	S-
Retention time	^{t}R (min)	0.933	1.344
	Std. dev.	0.00179	0.00283
	% RSD	0.23	0.21
Peak area	Peak area (µV*s)	593374	594972
	Std. dev.	2815.4700	2986.2300
	% RSD	0.47	0.50

Table 2. Retention time and peak area repeatability at 0.20 mg/mL of each enantiomer.

Figure 3 shows the UPC^2 chromatogram of R-benzyl mandelate at 2 mg/mL. The minor peak at 1.30 min corresponds to S-benzyl mandelate as confirmed by the UV spectrum (results not shown). This S-benzyl mandelate impurity peak has an S/N of ~3 (LOD) and represents 0.02% of the major peak based on the peak area. This increased detection sensitivity can be attributed to the holistically designed ACQUITY UPC^2 System, which includes an improved pumping system and an optimized detector design. The enantiomeric excess (e.e.) in this case was 99.96%.

Flow rate	4 mL/min
Mobile phase	CO_2:methanol=70:30
Back pressure	120 bar
Temperature	40 °C
Column	CHIRALPAK AD-H (4.6 x 150 mm, 5 µm)
Injection volume	5 µL

Table 1. Key experimental parameters.

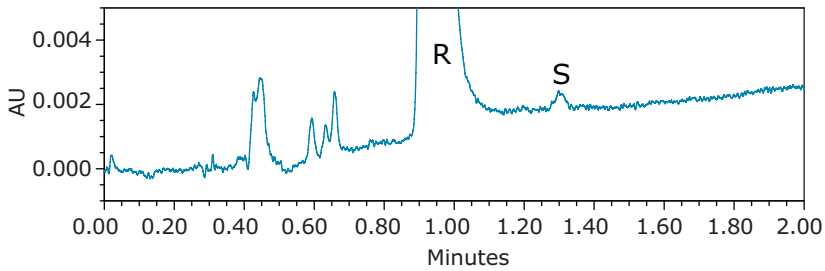

Figure 3. UPC^2 chromatogram of R- and S-benzyl mandelate showing the LOD of the S-benzyl mandelate at 0.02% of the main peak (2 mg/mL).

SUMMARY

UPC^2 chiral separations of R- and S-benzyl mandelate in less than 1.5 min were successfully demonstrated using the ACQUITY UPC^2 System. At 0.20 mg/mL concentration of each enantiomer, excellent repeatability (better than 0.23% RSD for retention time and better than 0.5% RSD for peak area) was obtained. Improved detection sensitivity, resulting from a new pumping system and optimized detector design, made detection of a 0.02% enantiomeric impurity and e.e. determination possible. The AQUITY UPC^2 System is suitable for the analysis of low level enantiomeric impurities, enantiomeric excess determinations, and QA/QC analyses.

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Chiral Purification with Stacked Injections and Collections Using the Prep 100 SFC MS Directed System

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APPLICATION BENEFITS

Implementing the stacked injection mode for chiral purification demonstrates the versatile and flexible collection scheme offered by the Prep 100 SFC MS Directed System. This open-bed platform for collection at atmospheric pressure conditions provides higher efficiency and greater success when multiple detectors are used including a mass detector trigger.

WATERS SOLUTIONS

Prep 100 SFC MS Directed System

2998 Photodiode Array (PDA) Detector

3100 Mass Detector

2767 Sample Manager

MassLynx™ Software

FractionLynx™ Application Manager

Stacked Injection Module

KEY WORDS

Chiral

Prep 100 SFC

Stacked injections

Mass directed

Open-bed collection

INTRODUCTION

Chiral chromatography has become the preferred tool for enantiomer separations in the early stages of pharmaceutical development for the purpose of accurately identifying single pure enantiomers with pharmacologic, toxicological, and clinical information, as stipulated by the FDA.[1]

Supercritical fluid chromatography (SFC) has proven to be a mainstream technology for chiral separations based on its higher efficiency, throughput, and wide applicability. Chiral SFC has seen increased interest and applicability, in some cases, becoming the method of choice.

Frequently, enantiomeric mixtures contain some significant impurities, which decrease the efficiency of the actual purification process where stacked injection and signal-threshold-based collection strategies (such as UV/PDA detection) are commonly used. In most cases, a pre-cleanup step is necessary but impractical because of resource and labor restrictions. This requires a versatile detection scheme capable of distinguishing the enantiomers from other impurities. The 3100 Mass Detector is an ideal choice in addition to UV/PDA detectors that are widely used in chiral separations.

In this application note, the Prep 100 SFC MS Directed System, with stacked injections and collections on the open-bed platform is demonstrated as a tool in chiral compound purification. The system configuration and methodology for the chiral separation cases are reviewed and presented.

EXPERIMENTAL

Chemicals

CO_2 from Airgas (Salem, NH, USA) is supplied in pressurized liquid form at approximately 1100 to 1300 psi through house lines to the Prep 100 SFC MS Directed System.

Methanol and trans-stilbene oxide (TSO, MW196) were provided by Sigma-Aldrich (St. Louis, MO, USA).

SFC columns

ChiralPak AD-H and ChiralCel OD-H (both 21 mm x 250 mm, 5 µm) were supplied by Chiral Technologies (West Chester, PA, USA).

SFC system

Prep 100 SFC MS Directed System was implemented with an additional stacking injector. The 2767 Sample Manager was configured as a simplified, repetitive fraction collector.

METHOD CONDITIONS

SFC gradient and flow program

For all data presented, the maximum total flow of 100 g/min was used with various isocratic modifier programs.

Mass detector conditions

The standard ESI mode for all experiments with the 3100 Mass Detector used the following key parameters:

Capillary voltage:	3.5 KV
Cone voltage:	40.0 V
Extractor voltage:	3.0 V
RF Lens voltage:	0.1 V
Source temp.:	150 °C
Desolvation temp.:	350 °C
Desolvation gas flow:	400 L/hr
Cone gas flow:	60 L/hr

0.1% formic acid in methanol is used as make-up/conditioning flow into MS for improved ionization efficiency.

Data management

MassLynx/FractionLynx, version 4.1

RESULTS AND DISCUSSION

Scale-up purification with stacking injection mode

A commonly accepted best practice in chiral analyses is to utilize the stacking mode for sample injection and fraction collection, maximizing efficiency and reducing production costs.

The mass-directed-based system has advantages in difficult situations where significant impurities are present, by selectively identifying targets of interest and collecting them while correctly ignoring unwanted impurities; thus, maintaining the high efficiency of chiral SFC for purifications, realizing wider applicability, and becoming the routine mainstream tool for chiral drug development.

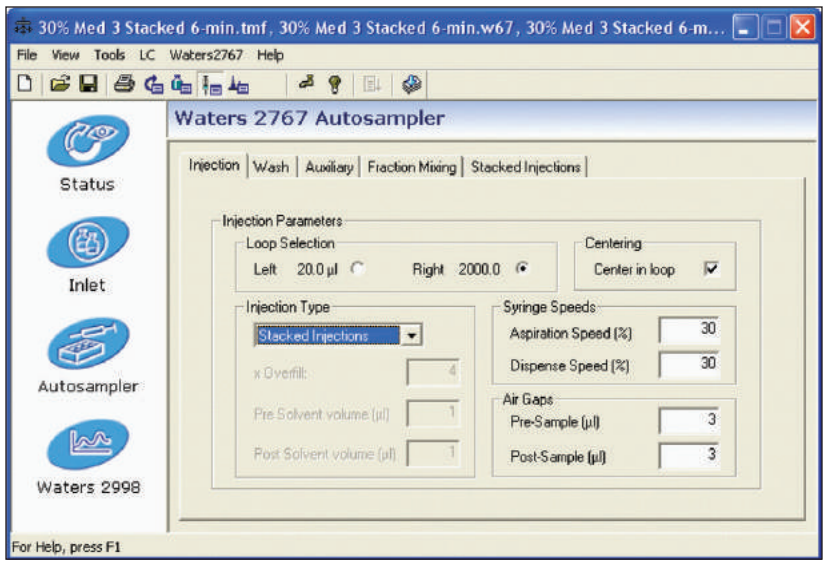

Figure 1. Stacked injection mode is integrated into MassLynx Software.

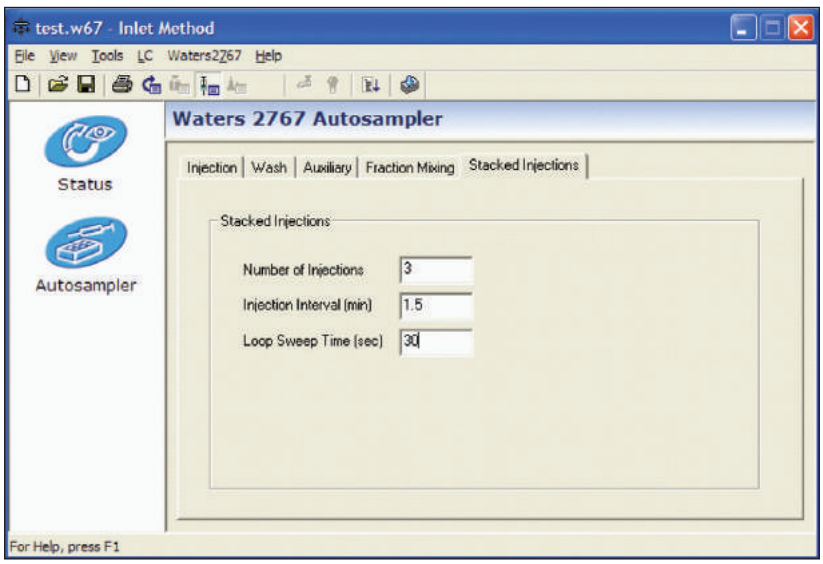

Figure 2. Stacking injection parameters as part of method settings.

To use the mass-directed system for chiral purification to its maximum benefit, certain modifications had to be made on the Prep100 SFC System. These included the addition of a dedicated injector and collection bed layout modifications to accommodate larger containers for repetitive fraction collections of the enantiomers.

Stacking of injection /injector implementation

A Waters® Stacked Injection Module was integrated into the Prep 100 SFC System. Users select "Injection Type," and enter the total number of stacked injections and other related parameters in the software program, as shown in Figures 1 and 2. A customized injector sequence is implemented to run the injector in stacking mode. The injector can draw sample aliquots from a single, bulk sample vessel.

When not in stacked injection mode, the 2767 Sample Manager maintains its capabilities of performing single injections from the sample racks on the bed, as defined in the Sample List.

Figure 3 shows representative chromatograms from stacked injections of a two-peak mixture. Both UV and MS trace of desired mass are identified correctly, ensuring the reliability and successful fraction collections by either UV or MS trigger. In this case, UV signal was used as the collection trigger; if needed, MS signal can also be used.

Customization of collection bed layout for single bottles

The Prep 100 SFC MS Directed System utilizes the 2767 Sample Manager as the dedicated fraction collector. In chiral purifications, since the number of the collected fractions are two (or may be up to four in some cases), larger containers with repetitive back-and-forth collection mode are desired in place of the routine tube rack format in one-to-one fashion.

The 2767 Sample Manager was, therefore, customized by defining the locations of the desired larger containers on the bed, to allow repetitive back-and-forth stacking collection sequences for the same enantiomer from all the stacked injections collected into the same bottle.

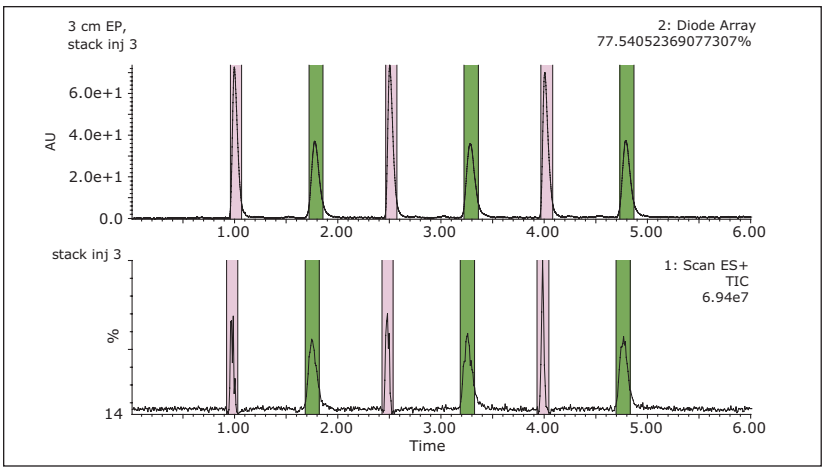

Figure 3. Stacked injections and demonstrated correspondent collection bands.

As shown in Figure 3, the two enantiomer fractions were collected into bottle 1 (pink band) and bottle 2 (green band), respectively. This was done in a back-and-forth fashion on the 2767 Sample Manager bed from a single injection line in the sequence. This demonstrated that the process was successful, meeting the key criterion using MassLynx Software and FractionLynx Application Manager to correctly identify and collect the enantiomer pair by its signal intensity level.

Figure 4 shows a selective separation and collection that represents a case where an unwanted peak co-existed with the desired enantiomer pair. Only two separated, targeted compounds were collected via targeted mass. This is depicted by colored bands. However, the extra third peak (unwanted impurity) was not collected.

The MassLynx/FractionLynx AutoPurify™ platform has many advanced detection and collection algorithms that can be adopted for sophisticated workflow, such as Boolean logic of multiple detector signals as triggers. If the sample is sufficiently clean, a user may opt to use UV/PDA for detection. If the sample contains significant portions of impurities, the operator may opt to use combined signal and slope algorithms and specific targeted masses to ensure purer collections.

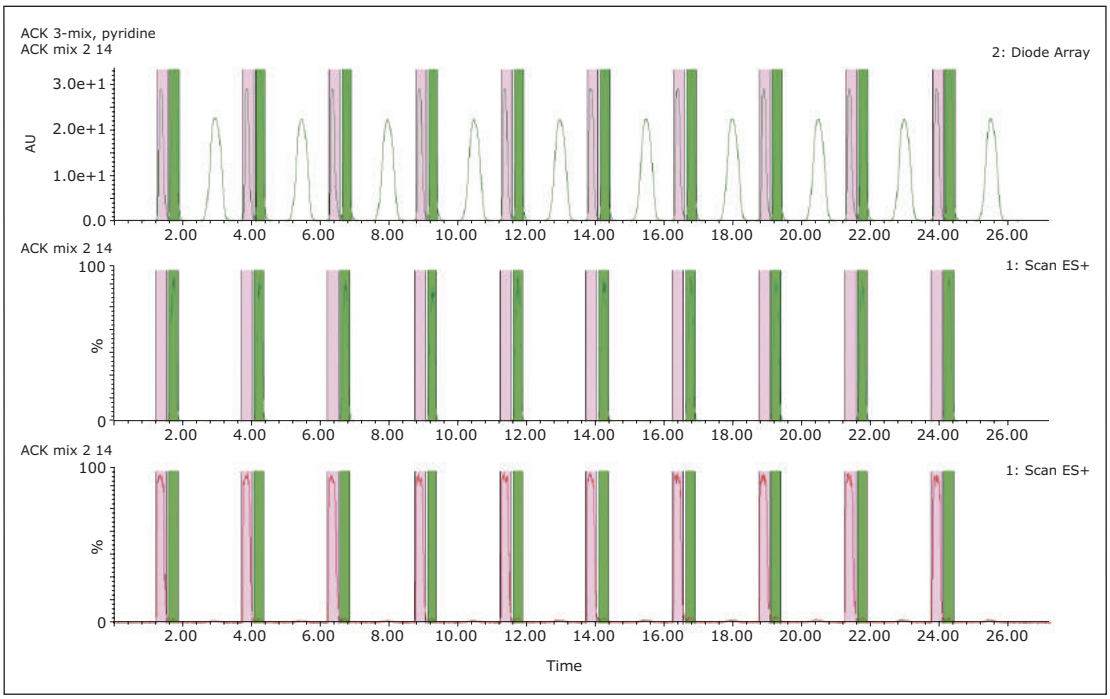

Figure 4. Stacked injection and collection of two peaks from a three-peak mixture.

CONCLUSIONS

The Prep 100 SFC MS Directed System has demonstrated its high efficiency, applicability, and versatility in various pharmaceutical developments. The added feature of stacked injections and collections of the Prep 100 SFC MS Directed System demonstrated here, enables more customized capabilities for chiral separation applications that benefit chromatographers in purification labs such as:

- Multiple and versatile detection modes for a higher success rate.
- The same stacked injection and collection mode on an open-bed platform facilitating ease-of-use.
- A safer lab environment in compliance with industrial and governmental regulations.

The Waters Prep 100 SFC MS Directed System is a powerful tool for chiral purification in drug discovery as well as other preparative chromatography, meeting the increased demand for productivity and greater success.

Reference

1. http://www.fda.gov/cder/guidance/stereo.htm

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[TECHNOLOGY BRIEF]

Importance of Selectivity for Reaction Monitoring

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James McKearin, Prime Organics Inc., 25 Olympia Ave, Woburn, MA, USA

GOAL

To demonstrate the benefit of different selectivity of UPC^2 compared to LC when used for the analysis of synthetic reaction mixtures.

BACKGROUND

During the development of new chemical entities (NCE), there is a need to analyze reaction mixtures, synthetic intermediates, and isolated compounds. Understanding the purity of the materials used in a reaction provides information that is used to aid reaction rates, inhibit catalyst quenching mechanisms, and provide insight to scalability.

Due to the complexity of reaction mixtures, a single chromatographic method or technique is not sufficient for providing the information scientists require. For example, during the synthesis of a molecule, lack of chromatographic selectivity can lead to poorly separated impurities that often affect the target molecule yield throughout the synthetic pathway.

In terms of high-throughput screening (HTS) using LC, screening strategies including high /low pH have recently been implemented to alter chromatographic selectivity. However, this reaction monitoring process may still be inefficient in separating structurally similar components generated during the synthetic process. It is critical when submitting an NCE to a compound library that identity and purity are assessed, thus, making orthogonal separations strategies important.

Alternative chromatographic selectivity with UPC^2 allows for robust and accurate characterization of synthetic reaction mixtures.

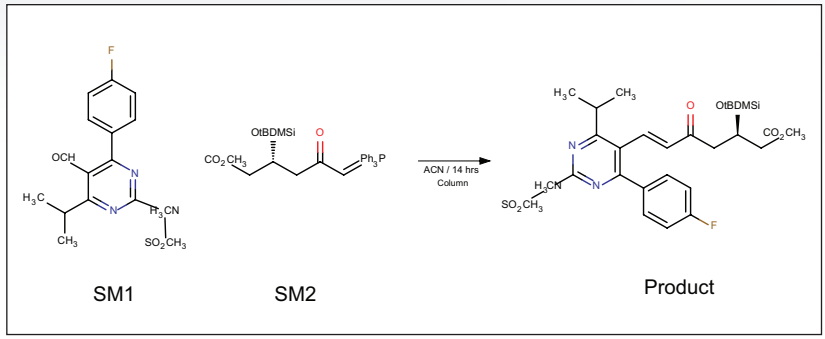

Figure 1. A single reaction step from the rosuvastatin synthesis reaction scheme.

THE SOLUTION

The primary aspects of this investigation evaluate Convergence Chromatography for monitoring the total synthesis of the pharmaceutical drug substance rosuvastatin, an HMG-CoA inhibitor. We used an ACQUITY UPC^2™ System configured with an ACQUITY UPC^2 PDA for UV detection and an ACQUITY® QDa™ Detector for mass detection. For simplicity, we compared the ACQUITY UPC^2 results from one of the synthetic stages to the results acquired with reversed-phase UPLC® using high/low pH screening. The details of the synthetic step are shown in Figure 1.

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Differences in chromatographic selectivity were clearly apparent as shown in Figures 2 and 3. At T= 0 the ACQUITY UPC^2 (A), UPLC Low pH (B) and UPLC High pH (C) separations were able to resolve each of starting materials in the reaction mixture (Figure 2). The reaction was monitored using both techniques after several hours at reflux. The UPC$^{2®}$ separation resolved both the starting materials and the final product in the mixture (Figure 3). In contrast, neither of the UPLC separations provided enough specificity between the product (PDT) and starting material (SM1). Additionally, the starting material labeled "SM2" appeared quenched in both UPLC separations, but not in the UPC^2 results.

The monitoring wavelength of 270 nm was suitable for the starting materials; however, 270 nm was not suitable for monitoring the product material during the UPLC analysis. The systems were configured to acquire a 3D PDA MaxPlot data channel ranging from 210 nm to 410 nm. This capability allows for UV spectral analysis and selected wavelength extracted chromatograms. The PDA spectra of the product indicated a lambda max of 295 nm. Therefore, chromatograms were extracted from the MaxPlot data channel to best monitor the reactions without having to change methods prior to next experiments.

Interestingly, the SM2 material is not as easily detected by UPLC at 295 nm. The UV absorbance of the mobile phase interferes with the detection of the SM2, hence appearing as a quenched reaction. Although changing the wavelength was needed to monitor the production of the target molecule, the PDA 3D data collection (MaxPlot) provided the ability to view the results at 270 nm. Deriving a chromatogram at 270 nm combined with the MS data enabled us to determine the presence of SM2 in the clean-up stages of the final material.

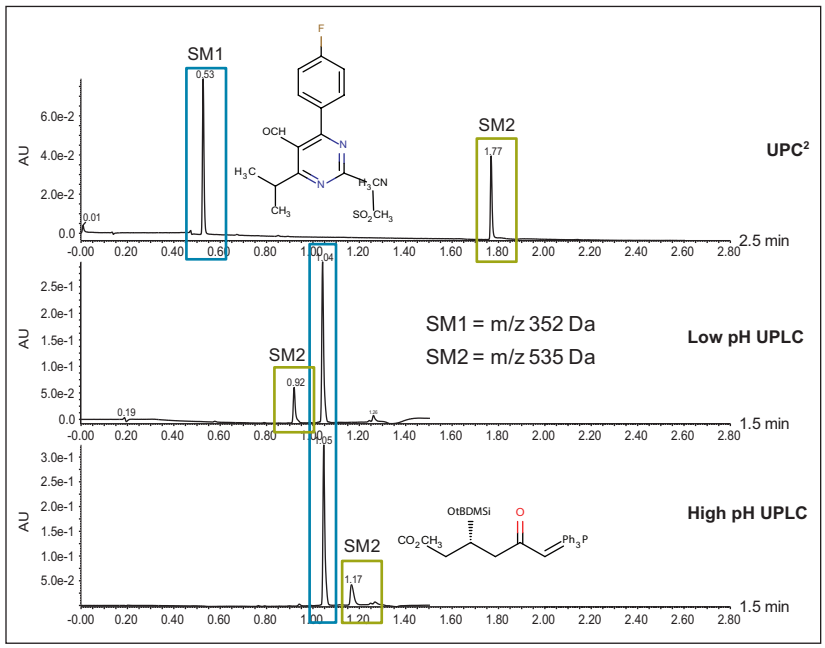

Figure 2. Chromatographic overlays at 270 nm of (A) ACQUITY UPC^2, (B) UPLC low pH, and (C) UPLC high pH results for the initial time point (T=0 min). See figure 1 for SM1 and SM2 structures.

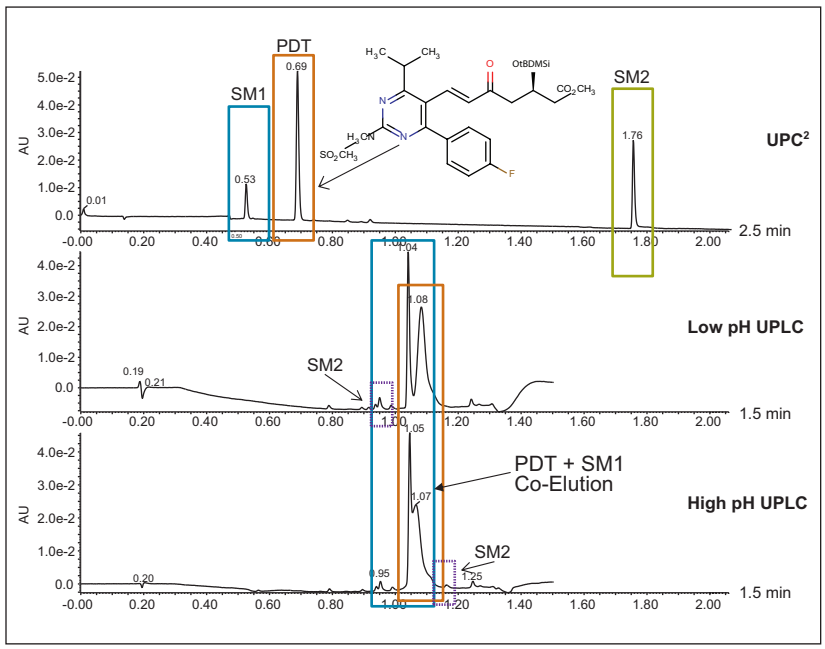

Figure 3. Chromatographic overlays at 298 nm of (A) ACQUITY UPC^2, (B) UPLC low pH, and (C) UPLC high pH results for time point 5 (T5 = 6hrs).

SUMMARY

ACQUITY UPC^2 for the analysis of reaction mixtures provides added selectivity and orthogonality when compared to reversed-phase LC high/low pH screening separations. Extracted wavelengths from the PDA Maxplot provided the ability to monitor reactions at various wavelengths and verify UV spectral profiles without having numerous data files to investigate. In addition, using both MS and PDA detection for either technique provides an accurate and quantitative assessment of the reaction progress and purity.

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Improving Productivity in Purifying Antroquinonol Using UltraPerformance Convergence Chromatography (UPC²) and Preparative Supercritical Fluid Chromatography (Prep SFC)

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APPLICATION BENEFITS

- For antroquinonol and its derivative, UPC² results in improved resolution, compared to RPLC, allowing for increased mass loading in the ensuing prep SFC method.
- UPC² and prep SFC methods yielded a more favorable elution order compared to RPLC, further facilitating the purification step due to increased mass loading.
- Purification via prep SFC offered a nine-fold improvement in overall productivity and reduced the organic solvent use by 77% compared to the prep HPLC approach.

WATERS SOLUTIONS

ACQUITY UPC²™ System with ACQUITY TQD

ACQUITY UPLC® H-Class System with SQ Detector 2

AutoPurification™ LC System with 3100 Mass Spectrometer

Prep 100q SFC System with 3100 Mass Spectrometer

ACQUITY UPC² 2-EP and BEH 2-EP Columns

Viridis® Silica 2-EP Column

HSS T# Columns

KEY WORDS

Natural products, purification, prep, UPC², SFC, convergence chromatography, CC, antroquinonol

INTRODUCTION

Natural products are a productive source of leads for new drugs due to their high chemical diversity, biochemical specificity, and many "drug-likeness" molecular properties.[1-4] A large portion of today's existing drugs on the market are either directly derived from naturally occurring compounds or inspired by a natural product. In addition, natural products are used directly in the forms of food supplements, nutraceuticals, and alternative medicines.[5] Isolation and purification of bioactive compounds play an important role in natural product research. The most commonly used process involves extraction of target compounds from the cellular matrix and pre-purification by various low to medium pressure liquid chromatographic techniques, predominantly reversed-phase liquid chromatography (RPLC).[6] While being a generally applicable chromatographic technique for a variety of compound classes, RPLC does not guarantee adequate resolutions for all analytes, especially for those structural analogs and isomers of similar polarities often found in natural products. As a result, the purification step is perceived by many as a rate-limiting step and a major bottleneck for natural product drug discovery.[7] To that end, supercritical fluid based chromatographic techniques, including UltraPerformance Convergence Chromatography™ (UPC²®), a novel analytical chromatographic technique that applies the performance advantages of UPLC to supercritical fluid chromatography (SFC), and preparative supercritical fluid chromatography (prep SFC) have brought viable new additions to the natural product research toolbox by offering a wide range of selectivity complementary to RPLC.

Antroquinonol, with its structure shown in Figure 1, is a ubiquinone derivative recently isolated from the mycelium of Antrodia camphorata, a parasitic fungus

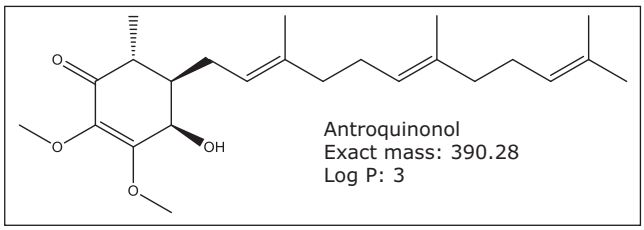


Figure 1. The chemical structure, molecular mass, and Log P of antroquinonol.

EXPERIMENTAL

Materials and Reagents

HPLC grade methanol and isopropanol (IPA) were purchased from Thermo Fisher Scientific (Fair Lawn, NJ, USA). Denatured ethanol (reagent grade) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The antroquinonol raw product (98% purity) was from a commercial source and dissolved in methanol at 1 mg/mL for analytical experiments and 20 mg/mL for preparative experiments.

Chromatography

The UPLC®/MS experiments were performed on a Waters® ACQUITY UPLC H-Class with SQ Detector 2. The UPC^2/MS experiments were performed on a Waters ACQUITY UPC^2 with ACQUITY® TQD Mass Spectrometer. Both systems were controlled by MassLynx® Software. The LC/MS experiments were performed on a Waters AutoPurification LC System with 3100 Mass Detector. The preparative SFC experiments were performed on a Waters Prep 100q SFC System with 3100 Mass Detector. Both systems were controlled by MassLynx Software and FractionLynx™ Application Manager. Detailed experimental parameters are summarized in Tables 1 and 2.

unique to Taiwan.[8] Antroquinonol has proven cytotoxic activities against multiple tumor cell lines.[9-11] Pre-purification of antroquinonol from the mycelium extract involves two RPLC steps of using silica gel and size exclusion gel, respectively, resulting in a raw product of 98% purity.[9] In order to support medicinal research where a higher purity (>99%) product is generally required, it is imperative to develop an efficient and cost-effective purification strategy to further purify the raw product. Described herein is a comparative study on using prep LC and prep SFC to purify the antroquinonol raw product to achieve >99% purity. Chromatographic behavior of the analytes, including resolution and elution order, using each technique and their implications on downstream preparative chromatography is discussed. The productivity and solvent consumption for each purification technique are also compared.

	Figure 2A		Figure 2B		Figure 4A	
Instrument	ACQUITY UPLC H-Class/ SQD 2 System		AutoPurification LC MS System		ACQUITY UPLC H-Class/ SQD 2 System	
Flow rate (mL/min)	0.60		1.46		0.75	
Mobile phase A	Water		Water		Water	
Mobile phase B	Methanol		Methanol		Methanol	
Backpressure (psi)	N/A		N/A		N/A	
MS detection	ESI+		ESI+		ESI+	
Column	ACQUITY UPLC HSS T3 (3.0 x 150 mm, 1.8 µm)		Atlantis T3 (4.6 x 150 mm, 5 µm)		ACQUITY UPLC BEH C_{18} (2.1 x 50 mm, 1.7 µm)	
Temperature (°C)	60		Ambient		60	
Injection volume (µL)	1		Varying		0.5	
Gradient	Time (min)	%B	Time (min)	%B	Time (min)	%B
	0	92	0	88	0	80
	5	96	3.08	88	4	80
	5.25	92	8.21	94		
	6	92	8.61	100		
			9.22	88		
			20.90	88		

Table 1. Key experimental parameters for LC.

	Figure 3A		Figure 3B	
Instrument	ACQUITY UPC^2 /ACQUITY TQD		Prep 100q SFC System with 3100 Mass Detector	
Flow rate (mL/min)	1.5		80	
Mobile phase A	CO_2		CO_2	
Mobile phase B	Isopropanol		Isopropanol	
Backpressure (psi)	1740		1740	
MS detection	APCI+		ESI+	
Temperature (°C)	45		40	
Injection volume (µL)	1		600	
Column	ACQUITY UPC^2 2-EP (3.0 x 100 mm, 1.7 µm)		Viridis Silica 2-EP (19 x 150 mm, 5 µm)	
Gradient	Time (min)	%B	Time (min)	%B
	0	5	0	5
	2.50	25	1	5
	2.75	40	6.5	9
	3.25	40	7	9
	3.50	5	7.25	5
	4	5	8	5

Table 2. Key experimental parameters for UPC^2 and Prep SFC.

RESULTS AND DISCUSSION

Figure 2A shows the UPLC/MS chromatogram of the antroquinonol raw product. The peak at *m/z* 391 is the sodium adduct of antroquinonol and the impurity peak at *m/z* 383 is the sodium adduct of the demethoxylated antroquinonol. Although baseline resolved, the structural similarity between antroquinonol and its derivative resulted in a rather limited resolution, which severely hampered the sample loadability in the prep LC. Figure 2B summarizes a loading study of the raw product on an analytical column (4.6 x 150 mm, 5 µm). The baseline resolution was only preserved with a 10-µL injection. The resolution deteriorated as the injection volume increased, and completely diminished with an 80-µL injection. If geometrically scaled up to a 19 x 150 mm semi-prep column, the maximum loading is projected to be 170 µL. At 20 mg/mL, this translates into a maximum loading of 3.4 mg/injection.

UPC^2 offers an attractive alternative. Figure 3A shows the UPC^2/MS chromatogram of the antroquinonol raw product. Compared to UPLC (Figure 2A), the UPC^2 method provided a better resolution between antroquinonol and its derivative, allowing for an increased mass loading in the ensuing prep chromatography. It is also noted that the elution order of antroquinonol and its derivative is the opposite of that in RPLC. When a polar stationary phase, such as 2-EP, is used, UPC^2 resembles normal phase chromatography (NPLC) and offers orthogonal selectivity to RPLC. As a result, the elution order of the analytes is often the reverse of that in RPLC. Elution order could play an important role in the overall productivity of prep chromatography, especially for those closely eluting target/impurity pairs. Since the peak front generally accounts for a higher weight percentage of the total peak than the peak tail of the same time interval, it is highly desirable to have the target compound elute before the impurity, so that when the target and impurity are less than baseline resolved, only a small portion of the target peak is excluded during collection. In the current study using RPLC, the impurity elutes before the target. With a 40-µL injection (Figure 2B), high purity antroquinonol can only be obtained at the expense of target recovery and total productivity. In contrast, the prep SFC chromatograms depict a much more favorable scenario for prep chromatography (Figure 3B). With impurity eluting after the target, high purity antroquinonol can be collected with negligible loss in productivity, even at the loading level where antroquinonol and the impurity slightly overlap, as shown in Figure 3B.

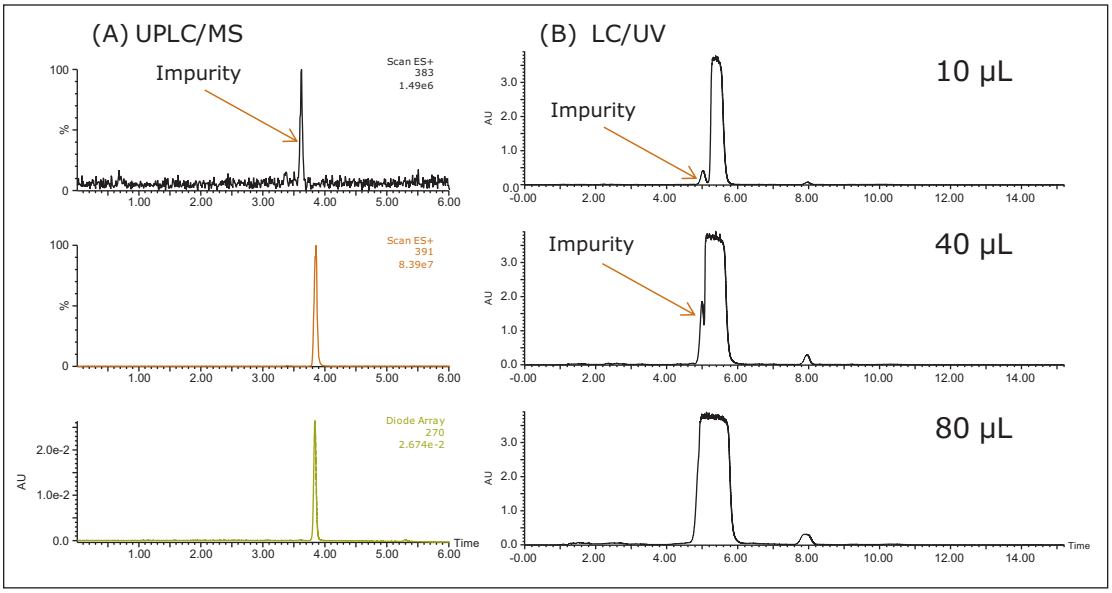
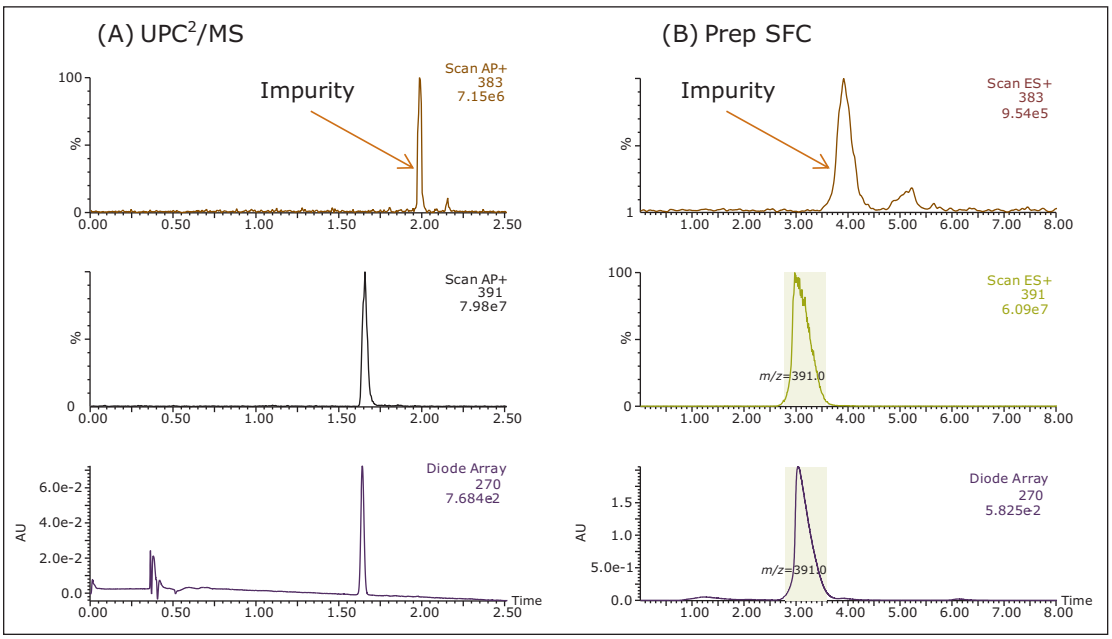


Figure 2. (A) UPLC/MS of the raw antroquinonol product at 1 mg/mL and (B) LC/UV chromatograms of the raw antroquinonol product at 20 mg/mL.

The UPC^2 method was scaled up to a 19 x 100 mm semi-prep column. Based on the chromatographic behavior shown in Figure 3A, a focused gradient ranging from 5 to 9 B% was used. The resulting chromatogram is shown in Figure 3B. The total run time was 8 min, compared to the 20-min run time using RPLC. The maximum loading was empirically determined to be 600 µL. At 20 mg/mL, this represents a 12 mg/injection.



Aliquots of the purified antroquinonol product were analyzed by UPC^2/PDA/MS and the results are shown in Figure 4. The main impurity at *m/z* 361 was successfully removed, as shown in the corresponding mass spectrum. The final antroquinonol product has a >99% purity by UV at 270 nm.

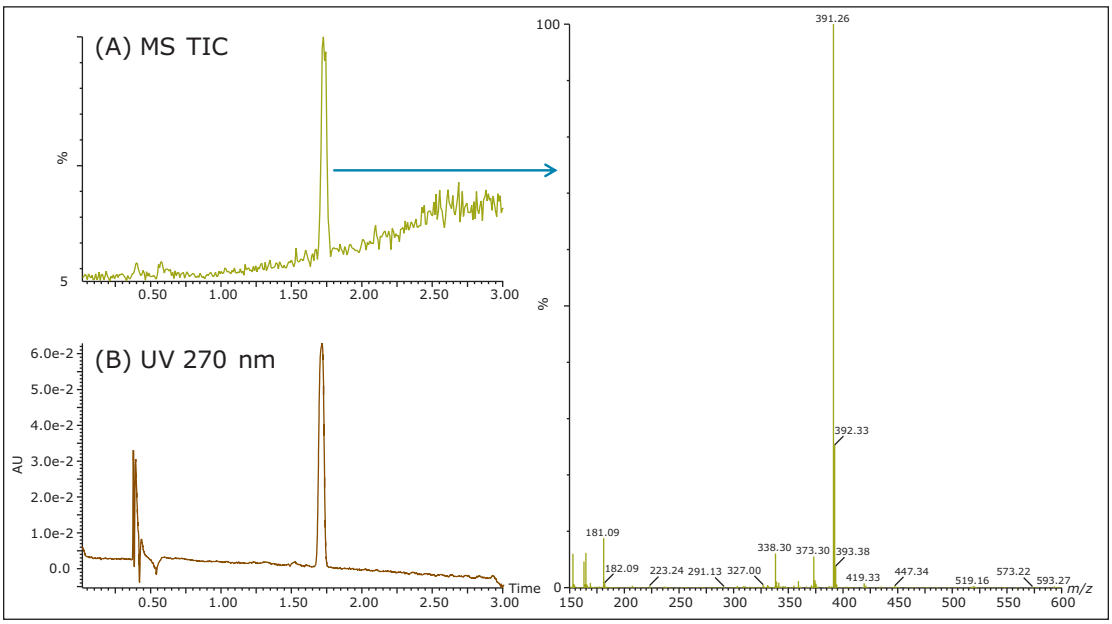


Figure 4. Purity analysis of the final antroquinonol product by UPC^2 with UV and MS detection.

A comparison on the productivity and solvent consumption was summarized in Table 3. Overall, by using prep SFC to replace prep RPLC, the purification productivity was increased by nine-fold with the following breakdown: 2.5-fold from the reduced run time and 3.5-fold from the increased sample loading. The organic solvent use was also reduced by 77%.

Prep chromatographic technique	Productivity (g/24 hr)	Solvent	Organic solvent consumption (L/24 hr)	CO_2 use (kg/24 hr)
HPLC	0.25	MeOH	33.52	N/A
SFC	2.25	MeOH/IPA	7.70	105

Table 3. Comparison on productivity and solvent consumption of two purification approaches.

CONCLUSIONS

Two different chromatographic approaches to purify a raw antroquinonol product to the desired 99% purity have been demonstrated. In the HPLC approach, the critical pair of antroquinonol and its demethoxylated derivative resulted in a limited resolution; hence, limited mass loading in prep chromatography and limited purification productivity. The same critical pair was better separated, and had a more favorable elution order versus RPLC, using Waters UPC^2 and Prep 100q SFC technologies, allowing for an increased mass loading in prep SFC when the analytical UPC^2 method was scaled up. Overall, the prep SFC approach offered a nine-fold improvement in productivity and reduced the organic solvent use by 77% compared to the prep HPLC approach. The supercritical fluid-based techniques, UPC^2 and prep SFC, augment the conventional toolbox for natural product research by offering complementary selectivity to RPLC, and enable laboratories and manufacturers in pharmaceutical, traditional medicine, nutraceutical, and dietary supplement industries with more efficient and more cost-effective natural product purification.

References

1. Harvey, A. Strategies for discovering drugs from previously unexplored natural products. *Drug Discovery Today* 2000; 5 (7):294-300.
2. Harvey, A. Natural products in drug discovery. *Drug Discovery Today* 2008; 13 (19/20):894-901.
3. Li J, Venderas J. Drug Discovery and natural products: end of an era or endless frontier? Science 2009; 325 (10):161-165.
4. Harvey A. Natural Products as a screening source, *Curr. Opin. Chem. Biol.* 2007, 11: 480-484.
5. Sarker S, Latif Z, Gary A. Natural product isolation: an overview, Natural Product Isolation, 2nd ed. Eds. Sarker SD, Latif Z, Gary AI, *Humana Press Inc.* Totowa, NJ. 2006, P1-25.
6. Sticher, O. Natural product isolation, *Nat. Prod. Rep.*, 2008, 25, 517–554.
7. Koehn F, Carter G. The evolving role of natural products in drug discovery, *Nat. Rev. Drug Discov.* 2005, 4: 206-220.
8. Geethangili M, Tzeng Y. Review of Pharmacological effects of Antrodia camphorata and its bioactive compounds, *Evid. Base Compl. Alternative Med.* 2009, 2011: 212641-58.
9. Lee T, Lee C, Tsou W, Liu S, Kuo M, Wen W. A new cytotoxic agent from solid-state fermented mycelium of *Antrodia camphorata, Planta Med.* 2007, 73: 1412-1415.
10. Chiang P, Lin S, Pan S, Kuo C, Tsai I, Kuo M, Wen W, Chen P, Guh J. Antroquinonol displays anticancer potential against human hepatocellular carcinoma cells: a crucial role of AMPK and mTOR pathways, *Biochemical Pharmacology* 2010, 79: 162-171.
11. Yu C, Chiang P, Lu P, Kuo M, Wen W, Chen P, Guh J. Antroquinonol, a natural ubiquinone derivative, induces a cross talk between apotosis and senescence in human pancreatic carcinoma cells, *J. Nutr. Biochem.* 2012, 23: 900-907.

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Utilizing UPLC/MS for Conducting Forced Degradation Studies

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INTRODUCTION

Chemical stability is one of the most important issues that impacts the quality and safety of a pharmaceutical product. The FDA and ICH require stability testing data to understand how the quality of an API or a drug product changes with time under the influence of environmental factors such as heat, light, and humidity.[1,2] Knowing the stability characteristics of a pharmaceutical allows for the establishment of storage conditions and shelf life, the selection of proper formulations and protective packaging, and is required for regulatory documentation.

Forced degradation, or stress testing, is similar to stability testing but carried out under harsher conditions than those used for accelerated testing. Forced degradation is generally performed early in the drug development process and is the main tool used to predict stability related properties, to understand degradation products and pathways and to develop stability indicating methods.[3]

Figure 1. The ACQUITY UPLC® System with the SQ Mass Spectrometer.

The most common analytical technique for monitoring forced degradation experiments is HPLC with UV and/or MS detection, allowing for peak purity, mass balance, and identification of degradation products. These methodologies are often time consuming and of medium resolution, requiring analysis times of 30 minutes or more[4] to ensure that all of the degradation products are accurately detected. The use of UltraPerformance LC® (UPLC®)/UV/MS allows for faster and higher peak capacity separations, which can aid in the analysis and identification of degradation products and shorten the time required to develop stability indicating methods. The purpose of this application note is to demonstrate the advantages of resolution and sensitivity that UPLC brings to forced degradation studies.

RESULTS AND DISCUSSION

Simvastatin standard

A standard solution of simvastatin was injected at a concentration of 0.1 mg/mL as a control (Figure 2). The major peaks were identified and confirmed by orthogonal acceleration time-of-flight (oa-TOF) MS to be the commonly observed impurities often found in simvastatin. This chromatographic data is the baseline that will be used to evaluate results of stress testing studies.

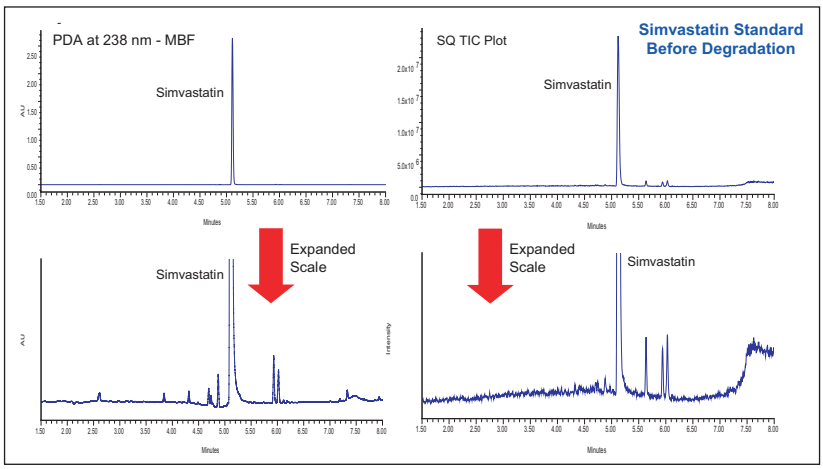

Figure 2. UPLC/UV/MS analysis shows evidence of impurities present in simvastatin standard.

EXPERIMENTAL

Forced degradation studies were carried out on simvastatin under varied conditions of acid/base hydrolysis, thermal degradation, peroxide oxidation, and photo degradation, with the ultimate goal of achieving 10 to 20% degradation (loss of API). Additional degradation products to those normally observed in real time or accelerated stability testing were generated.

Acid and base hydrolysis and peroxide degradation were carried out on simvastatin in solution (10 mM ammonium acetate, pH 4.5) while thermal degradation was performed on simvastatin solid. Photostability measurements were performed on both simvastatin solid and in solution. Solution degradation experiments were carried out at a simvastatin concentration of 1 mg/mL. The degraded samples were diluted to a concentration of ~0.1 mg/mL (1:10 dilution) prior to injection on the UPLC/UV/MS system. The data generated was used to monitor the effects of the stress conditions on the simvastatin.

LC conditions

LC system:	ACQUITY UPLC
LC data software:	Empower® 2 CDS
Column:	ACQUITY UPLC BEH C_{18} Column, 2.1 x 50 mm, 1.7 µm
Column temp.:	45 °C
Flow rate:	600 µL/min
Mobile phase A:	10 mM ammonium acetate, pH 4.5
Mobile phase B:	Acetonitrile
Gradient:	Linear gradient 25 to 90% B over 7 min

MS conditions

MS system:	SQ Mass Spectrometer
Ionization mode:	ESI positive
Capillary voltage:	3200 V
Cone voltage:	20 V
Desolvation temp.:	350 °C
Desolvation gas:	900 L/Hr
Cone gas:	50 L/Hr
Source temp.:	130 °C
Acquisition range:	100 to 900 *m/z* (5000 Da/sec)

Acid/base hydrolysis

UPLC/UV/MS analysis of the acid and base hydrolysis of simvastatin showed it to be extremely sensitive to pH (Figure 3). Above pH 8, simvastatin rapidly undergoes hydrolysis to be completely converted to simvastatin acid, which agrees with previously published work.[5] These studies demonstrated that hydrolysis of simvastatin in 100 mM hydrochloric acid for 1 hour and 15 mM sodium hydroxide for 45 minutes both resulted in the desired 10 to 20% loss of the initial API.

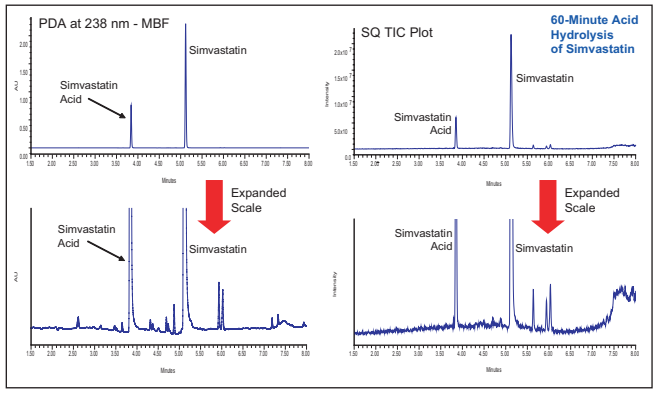

Figure 3. The example of acid hydrolysis of simvastatin demonstrates the only major degradation product – simvastatin acid.

Photo degradation

Photostability studies were performed on simvastatin both as a dry powder and in solution (1 mg/mL in 10 mM ammonium acetate, pH 4.5). Samples were exposed for 8 hours and 24 hours at the maximum Suntest CPS lamp intensity (583 and 1750 Watt-Hrs/m2, 320 to 400 nm). In the solid state, simvastatin was observed to be very stable with little evidence of degradation (Figure 4).

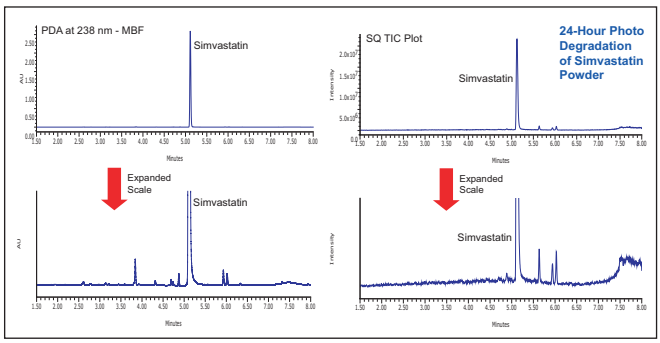

Figure 4. In the solid state, simvastatin exhibited no degradation under the photostability conditions evaluated.

In solution, the simvastatin exhibited significant degradation after 24 hours, and a unique profile of degradation products was observed (Figure 5).

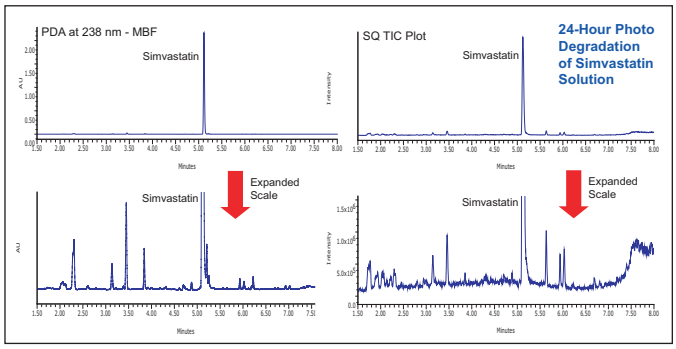

Figure 5. Simvastatin solution demonstrated significant photodegradation after 24 hours.

Peroxide oxidation

Oxidative degradation is most commonly achieved using peroxides, metal ions (metal salts), or radical initiators such as AIBN (autoxidation). This study found than a 7.5% hydrogen peroxide solution at 55 °C for 45 minutes was sufficient to degrade the simvastatin by ~15% of intitial concentration (Figure 6), resulting in many more degradation products than observed by simple acid or base hydrolysis.

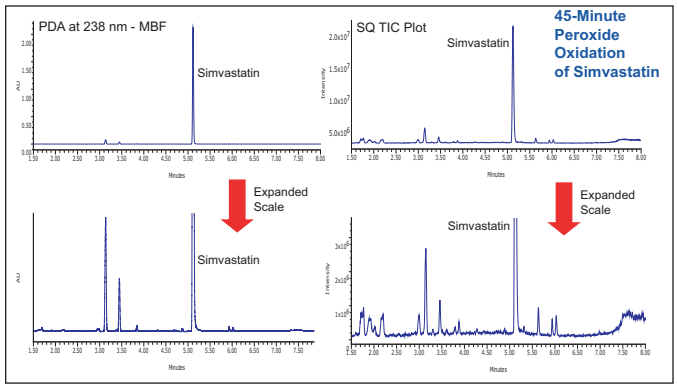

Figure 6. Peroxide oxidation is harsher than acid/base hydrolysis, resulting in a more complex mixture of degradation products.

Thermal degradation

Simvastatin was thermally stressed as a dry powder at 115 °C. After 60 minutes the starting material was degraded sufficiently to detect a number of degradation products. Many of the same degradation products observed from the peroxide oxidation study were also present in the temperature degradation sample (Figure 7).

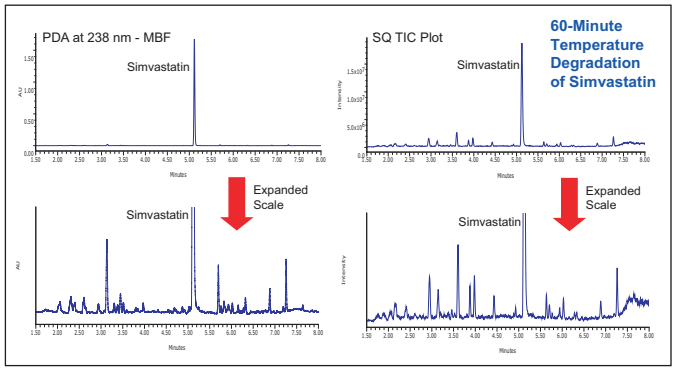

Figure 7. Degradation products obtained from thermal degradation at 115 °C for 60 minutes.

SUMMARY

The comparison of chromatograms obtained from analysis of the forced degradation of simvastatin by acid and base hydrolysis, thermal degradation, peroxide oxidation, and photo degradation demonstrate the varied degradation product profiles that result from these various procedures (Figure 8). Although acid or base hydrolysis yields only simvastatin acid as a degradation product, other procedures such as photo and thermal degradation produce much more complicated and unique profiles of degradation products. The high efficiency separations obtained with the ACQUITY UPLC Systems allow for the easy analysis of these complex mixtures.

Figure 8 also demonstrates the utility of the combined detection of photodiode array and MS detection. Many of the degradation products were observed in the MS data channel only as the components do not contain chromophores thus are not detected by UV absorption. For other degradation products, UV detection was determined to be more sensitive than MS detection, this is mainly due to the lack of ionizable groups on the molecules. Table 1 lists the major degradation peaks observed for each degradation method including which type of detection was able to detect the degradation products. A multi-detector approach is clearly desirable in order to detect the maximum number of degradation products.

Peak Rt (min)	Acid Hydrolysis		Base Hydrolysis		Temperature Degradation		Peroxide Oxidation		Photo Degradation	
	UV	MS	UV	MS	UV	MS	UV	MS	UV	MS
1.699							★			
1.760								★		★
1.886								★		
1.926										★
2.062					★				★	★
2.116									★	
2.161						★				
2.191								★		
2.308					★				★	★
2.397					★	★				
2.938						★				
2.986							★	★		
3.107					★			★		
3.137						★	★		★	★
3.449					★		★	★	★	★
3.594						★				
3.841	★	★	★	★			★	★	★	★
3.862						★				
3.970						★				
4.423						★				
5.209									★	
5.254									★	
5.699					★					
6.222									★	
6.322					★					
6.881					★	★				
7.257					★	★				

Table 1. Major degradation product peaks are highlighted (★) in table with detector and degradation method with which they were observed.

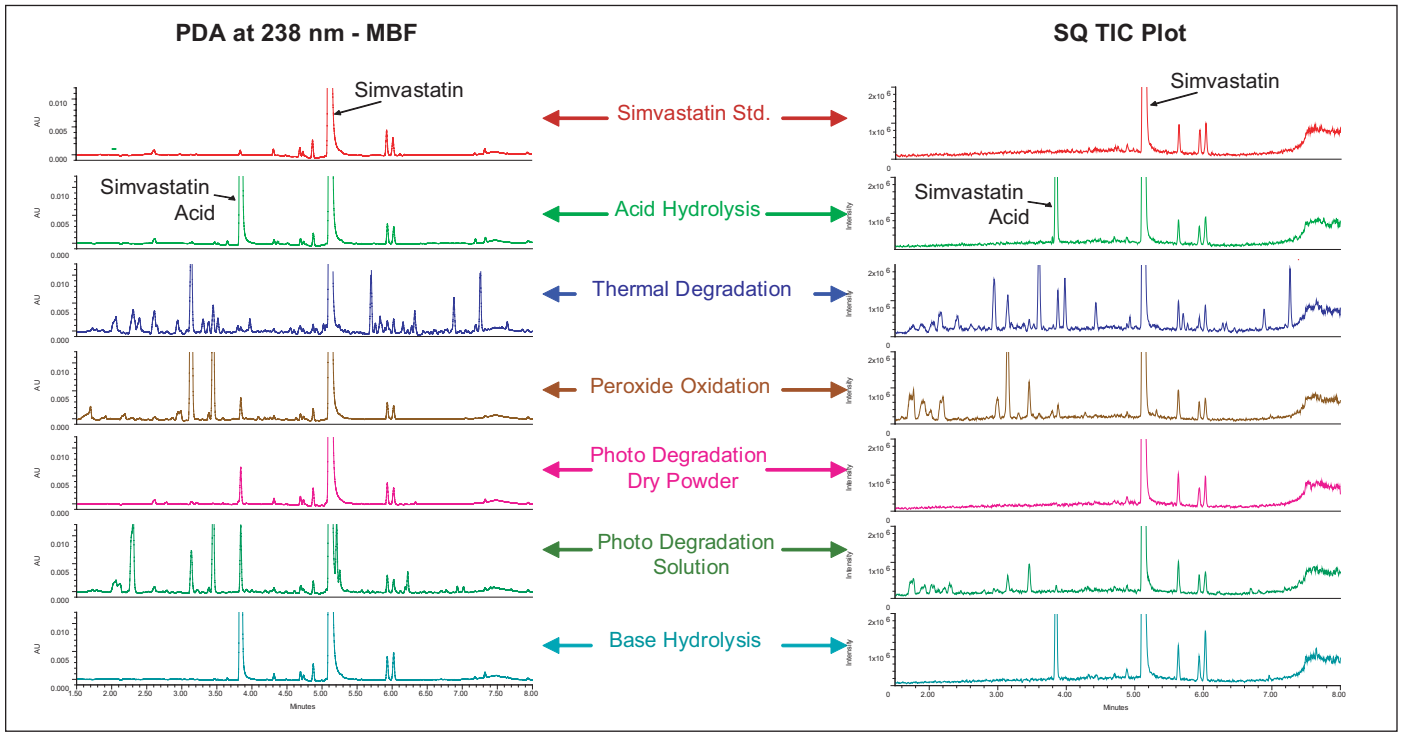

Figure 8. Overlay of chromatograms from degradation samples visually demonstrates the different degradation product profiles produced by the different methods of degradation.

CONCLUSIONS

In this application note, we have demonstrated the ability of an ACQUITY UPLC System combined with an ACQUITY UPLC PDA and SQ Mass Spectrometer to separate degradation products in a pharmaceutical product, such as simvastatin. These high peak capacity separations for complex mixtures of degradation products result in faster analyses, improve identification of impurity products and shorten the time required to develop stability indicating methods, improving the quality and throughput of forced degradation studies.

References

1. Guidance for Industry, Q1B, *Photostability Testing of New Drug Substances and Products*. 1996; Nov.
2. Guidance for Industry, Q1A (R2), *Stability Testing of New Drug Substances and Products*. 2003; Nov.
3. Upadrashta S. 4th Annual Forced Degradation Conference, Las Vagas, NV.
4. Wu Y. *Biomed. Chromatogr.* 2000; 14: 384–396.
5. Alvarez-Lueje A. et al, *J. of AOAC International*, 2005; 88 (6).
6. Wang H et al. J. *Mass Spectrom.* 2001, 36: 58–70.

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Rapid, Simple Impurity Characterization with the Xevo TQ Mass Spectrometer

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Waters Corporation, Milford, MA, USA

INTRODUCTION

The detection and characterization of impurities and degradation products of an active pharmaceutical ingredient (API) are regulatory filing requirements. The detection and identification of impurities not only ensures medicine safety but can also be used as a fingerprint for patent protection and counterfeit drug analysis.

Impurity characterization and identification is normally carried out using information-rich analytical techniques such as NMR and LC/MS. Analysis by LC/MS provides parent ion mass from full-scan MS and structural information from the fragments generated in MS/MS experiments. With traditional tandem quadrupole instrumentation, the generation of this data requires multiple experiments to obtain MS and MS/MS information.

Modern Linear Ion Trap (LIT) mass spectrometers allow the collection of MS, multiple reaction monitoring (MRM), and MS/MS data in the same analytical run, allowing quantitative and qualitative data to be obtained simultaneously. However, the duty cycle of these instruments when switching between MS and MS/MS modes is typically 2 to 3 seconds. With modern high-resolution, sub-2-µm column particle chromatography such as UPLC,® peak widths of 2 to 3 seconds are now commonplace. With these LIT MS systems, this would result in just 1 to 2 points across the peak, with the peaks either poorly defined or missed completely; thus slower, lower-resolution LC systems must be used, resulting in reduced throughput and lower data quality.

The Waters® Xevo® TQ Mass Spectrometer is equipped with a novel collision cell design that is continuously filled with collision gas, allowing rapid switching between MS and MS/MS modes. The Xevo TQ MS is capable of operating at up to 10,000 Da/sec and can correctly define the very sharp peaks produced by UPLC, with more than 10 points across a 2-second-wide peak, even on a multi-scan experiment.

This collision cell is capable of enhanced high-sensitivity operation in MS/MS mode. In this mode of operation, ions are constrained in the final third section of the collision cell using both DC and RF barriers. These ions are then ejected from the collision cell, in a controlled manner, from high to low *m/z* in synchronization with the scanning of the final resolving quadrupole. This increases the duty cycle of the instrument, resulting in enhanced sensitivity that is ideal for the detection and characterization of low-concentration impurities that may result in toxic effects.

Figure 1. Xevo TQ Mass Spectrometer with the ACQUITY UPLC® System.

EXPERIMENTAL

To evaluate the performance of this system, the impurities of the common pharmaceutical drug quetiapine, used to treat biopolar disorder, was investigated using UPLC/MS/MS.

LC /MS conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp.:	65 °C
Flow rate:	800 µL/min
Mobile phase A:	20 mM ammonium bicarbonate pH 10
Mobile phase B:	Acetonitrile
Gradient:	15% to 95% B/18 min
MS system:	Xevo TQ MS
Ionization mode:	ESI positive ion mode
Capillary voltage:	30 V
Collision energy:	15 eV

RESULTS

The unique collision cell design allows the Xevo TQ MS to be operated in several different modes of operation: full scan MS, MRM, as well as MS/MS mode. As the collision cell is continuously filled with collision gas, the instrument can rapidly switch between MS and MS/MS in the same analytical run. This allows MRM and MS scans to be performed in the same run. Combined with the high scan rate, this allows for rapid survey scans to be performed, such as MS neutral loss or parent ion, before switching to MS/MS.

This high data-capture rate allows for the accurate definition of the peak, even with the very narrow peaks produced by UPLC. Figure 2 shows the UPLC/MS chromatogram produced in the analysis of an API batch of quetiapine at a concentration of 1 µg/mL. Here we can see that impurity peaks are 2 to 4 seconds wide at the base. The data shown in Figure 3 illustrates the number of scans achieved in MS and MS/MS modes.

Maximizing LC peak definition

In this example, the Xevo TQ MS was operated in ScanWave™ MS mode, switching to ScanWave DS (daughter ion scan) mode when a peak was detected above a user-defined threshold. In this mode of operation, the instrument selects the most intense peak in the MS spectrum and acquires MS/MS data on this peak before returning to MS mode. Since the collision cell is continuous filled with collision gas, there is a no delay in switching between MS and MS/MS modes.

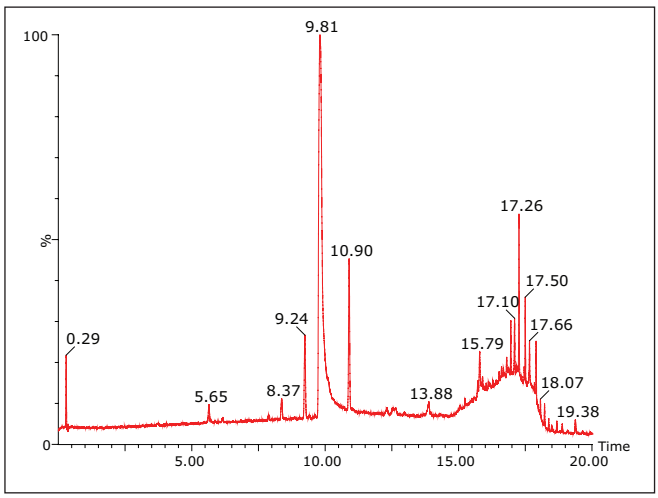

Figure 2. UPLC/MS analysis of quetiapine at 1 µg/mL.

We can see from this data that the instrument has acquired 9 points across the peak in MS mode, and 15 points across the peak in MS/MS mode – despite the fact that the peak is only 2 seconds wide at the base. This high data-capture rate enables the Xevo TQ MS to perform high quality, data-dependent MS-to-MS/MS experiments in a UPLC timeframe with sufficient data points to accurately define the peak. This dual mode of operation can also be used to acquire full-scan MS data simultaneously with MRM data, or to detect a peak with precursor ion scanning before switching to MS/MS mode.

Precursor ion scanning

The detection of new impurities, degradation products, or, in a DMPK study, drug metabolites, is often confounded by the signal from the matrix. To detect and visualize these analytes, the analytical chemist can use the specificity of the mass spectrometer.

Since compounds can undergo fragmentation as a result of the degradation or metabolism process, the use of simple, predicted MRM transitions for common degradation/metabolism pathways may result in the non-detection of a potentially toxic impurity, degradation product, or metabolite. A more comprehensive way to detect these compounds is to monitor for the common fragment ions of the molecule of interest. The Xevo TQ MS's Survey Scan functionality utilizes the fast data-capture rate of the instrument to facilitate the collection of precursor ion data as well an MS/MS spectrum of the peaks detected.

This functionality was used to evaluate a commercially-purchased API sample of quetiapine. The MS/MS spectra of quetiapine revealed that it gave rise to three major product ions having *m/z* values 221, 253, and 279. This data was used to detect drug-related impurities in the API batch by performing a Survey Scan analysis on each of these ions.

The data collected for the parent ion chromatogram of *m/z* 279 is displayed in Figure 4. Here, we can see the presence of seven major peaks, six impurities and the quetiapine active peak, eluting with a retention time of 9.86 minutes. A similar analysis using the common fragment ion *m/z* 221 to trigger data collection produced the chromatogram shown in Figure 5. With this fragment ion, a total of 12 peaks were detected and MS/MS data acquired.

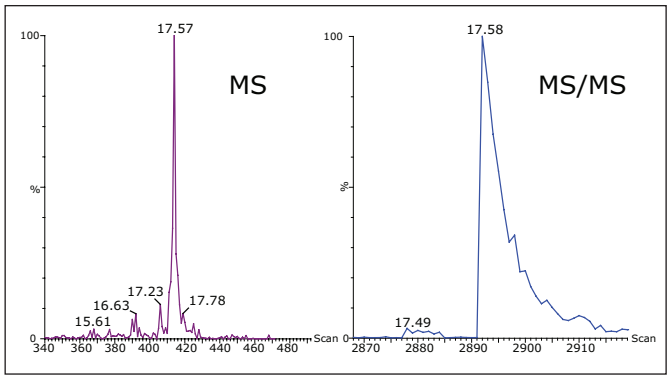

Figure 3. Rapid data collection is performed simultaneously in both MS and MS/MS modes.

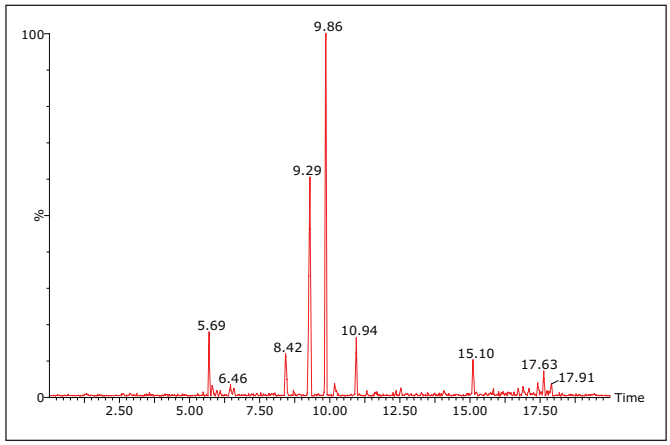

Figure 4. Survey Scan UPLC/MS analysis of quetiapine for ion m/z 279 in positive ion mode.

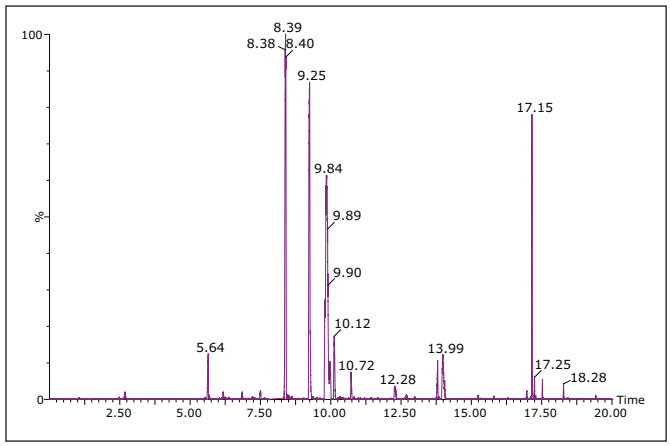

Figure 5. Survey Scan UPLC/MS analysis of quetiapine for ion m/z 221 in positive ion mode.

The MS/MS spectra obtained from the peak eluting with a retention time of 5.6 minutes is displayed in Figure 6. This impurity has a *m/z* value of 400 amu and has been identified as the S-Oxide impurity of quetiapine.

ScanWave Technology

The detection of low-level impurities is becoming increasingly important, especially when monitoring potential genotoxins. Collection of the MS/MS spectrum from Survey Scan experiments, either precursor ion or common neutral loss, can be performed in two modes of operation: standard MS/MS or ScanWave MS/MS.

As described previously, ScanWave Technology allows for increased sensitivity in the collection of MS/MS data. This increase in sensitivity is illustrated by the MS spectra obtained for the desthanol impurity of quetiapine (Figure 7). The top spectrum is obtained in standard MS/MS mode, while the lower spectrum is obtained in ScanWave MS/MS mode. In this example, we can see that the ScanWave MS/MS data is 13 times more sensitive than that in standard MS/MS mode. This increase is essential for the correct confirmation or identification of low-level impurities.

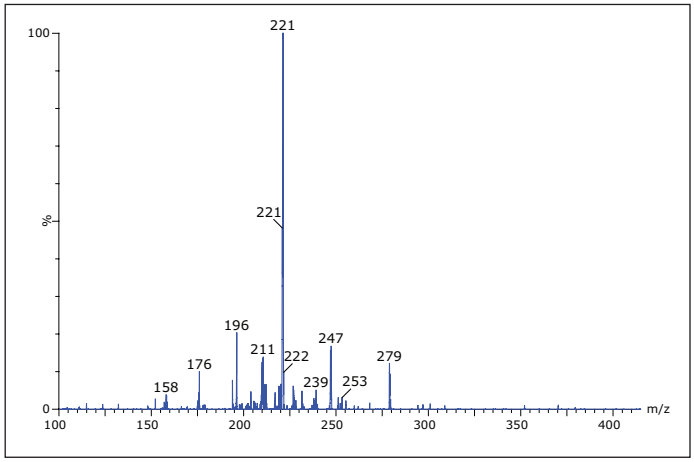

Figure 6. AutoScanWave MS/MS spectrum of S-Oxide of quetiapine eluting at 5.6 minutes.

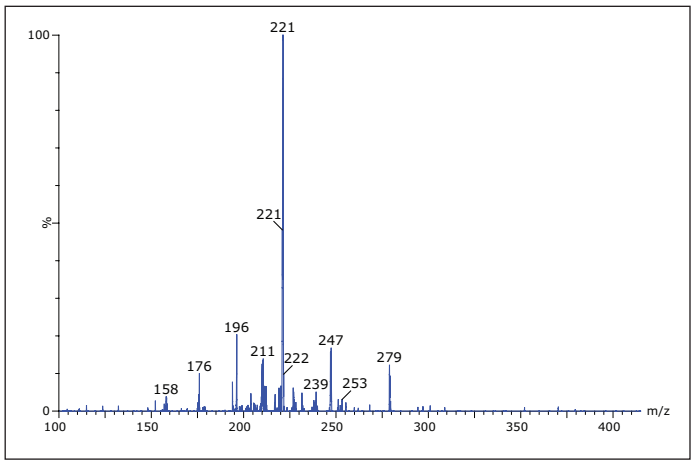

Figure 7. Comparison of standard and ScanWave MS/MS sensitivity.

CONCLUSIONS

- The Xevo TQ MS provides unrivaled levels of sensitivity and functionality.
- The high data-capture rates of the instrument, and its unique collision cell design and ScanWave Technology, allows the maximum amount of data to be collected in one analytical run.
- This reduces the number of experiments needed to make a decision, allowing impurities to be detected and identified quicker, and making maximum use of instrumentation.
- The rapid switching between MS and MS/MS possible with the Xevo TQ MS allows the collection of qualitative data and quantitative data in the same analytical run.
- The instrument's high data-capture rate ensures that, even with the narrow peaks of 2 to 3 seconds produced by today's modern sub-2-µm particle LC systems, sufficient points can be collected across for accurate quantification.
- The use of ScanWave Technology ensures that even the lowest-level peaks are detected and MS/MS spectra acquired, ensuring comprehensive impurity detection.

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A Stress Testing Study of Glimepiride Drug Substance Utilizing UPLC/MS Methodology

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INTRODUCTION

Forced degradation or stress testing studies are done to identify likely degradation products and to help establish the degradation pathways and intrinsic stability of a drug molecule. They are also performed to demonstrate specificity for development and validation of stability-indicating analytical methods.

Forced degradation studies may be used later in development to distinguish between degradation products in formulations that are related to a drug substance, and those that are related to non-drug substances or excipients.

The ability to improve and streamline the analytical procedures used to identify potential impurities is important to goals of providing safe medicines to the marketplace faster.

One of the challenges with forced degradation studies is that there are no definitive procedures describing how the testing should be performed. In addition, the analytical methods used to monitor forced degradation studies must be sufficiently specific and sensitive to ensure detection of all of the potential impurities.

In this application, a forced degradation study will be performed for the drug substance glimepiride. Waters UltraPerformance LC® (UPLC®) Technology and the Empower® 2 Software chromatography data system facilitate the analysis of the degradation behavior of drug substances during stress testing.

The sensitivity and chromatographic efficiency of UPLC enables the detection of even very low levels of degradants. The enhanced resolution of UPLC and specificity of MS detection ensures that all impurities are detected, achieving a comprehensive evaluation of the forced degradation of glimepiride.

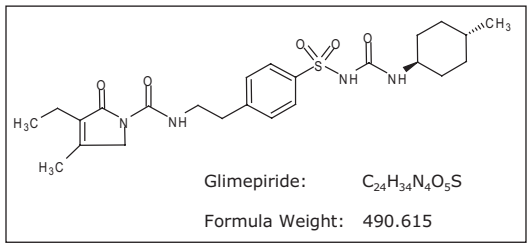

Figure 1. Structure and formula weight of glimepiride.

RESULTS AND DISCUSSION

System suitability was determined by calculating the percent relative standard deviation (RSD) for area and retention time for five replicate injections of the 125 µg/mL glimepiride standard. The area %RSD was calculated to be 0.9% and the retention time %RSD was calculated to be 0.0%.

Determination of the linearity of the UPLC method was required to accurately quantitate the rate of decomposition of glimepiride. Five standards of separate concentrations 25, 50, 100, 125, and 250 µg/mL glimepiride in methanol were prepared. A calibration curve was created using the five standard solutions injected in triplicate. The resulting correlation coefficient was calculated to be $R^2 = 0.99982$ (Figure 2).

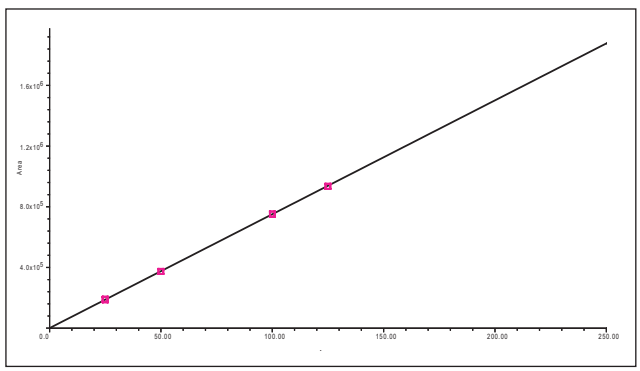

Figure 2. Calibration curve of glimepiride for the range of concentration 25 µg/mL to 250 µg/mL.

EXPERIMENTAL

Accelerated degradation of glimepiride was carried out under acid, alkaline, oxidative, and elevated temperature conditions. An accurate weighing of glimepiride reference standard was dissolved to yield a stock solution of 500 µg/mL glimepiride in methanol. Preparations of 0.5 N HCl, 0.5 N NaOH, and 4% hydrogen peroxide were used as the separate reagent mediums for the degradation procedure.

Two milliliters of glimepiride stock solution were pipetted to each of four 5-mL reaction vessels. Two milliliters of each reagent medium was pipetted separately to each respectively-labeled reaction vessel – 0.5N HCl + glimepiride stock, 0.5N NaOH + glimepiride stock, and 4% H_2O_2 + glimepride stock – to yield final concentrations of glimepiride in solution to 250 µg/mL. Two milliliters of methanol was added to the fourth reaction vessel to yield a concentration of 250 µg/mL glimepiride in methanol. The fourth reaction vessel was used for thermal degradation at 90 °C.

LC conditions	
LC system:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 50 mm, 1.7 µm
Column temp.:	30 °C
Flow rate:	800 µL/min
Mobile phase A:	20 mM ammon. formate, pH 3.0
Mobile phase B:	Acetonitrile
Gradient:	5 to 95% B/5 min

MS conditions	
MS system:	ACQUITY® SQD with the SQ Detector
Ionization mode:	ESI Positive
Capillary voltage:	3200 V
Cone voltage:	20 V
Desolvation temp.:	500 °C
Desolvation gas:	1200 L/Hr
Source temp.:	150 °C
Acquisition range:	100 to 600 *m/z*

Separate blanks were also prepared for each degradation medium by adding 2 mL of reagent medium with 2 mL of methanol (diluent) resulting in a total of eight separate reaction vials. The eight reaction vessels were tightly closed with Teflon-lined caps and placed in a heating oven monitored with a thermacouple to maintain a temperature of 90 ± 30 °C. Aliquots of 200 µL were taken at time points 0, 30, 60, 90, 120, and 180 minutes and diluted in 200 µL of methanol to yield a resulting working glimepiride solution of 125 µg/mL for analysis.

Kinetic behavior

Empower 2 was used to facilitate the quantitation of glimepiride decomposition. The rate of decomposition was determined by measuring the decrease in the amount of glimepiride over the sampling period. The kinetic slopes are shown in Figure 3 whereas the log% remaining was plotted versus time.

Each of the degradation mediums – acidic, alkaline, oxidative, and thermal – exhibited a linear kinetic behavior. The R^2 values were in the range of 0.93 to 0.99. It was expected that some experimental error was to occur due to evaporation, given the volatile nature of the methanol diluent used to dissolve the glimepiride at the elevated temperature of 90 °C.

The average rate of change was calculated and determined from the slope of the kinetic curves in Figure 3. The values were: 1.1 x 10^{-3} min^{-1} for acidic medium, 1.2 x 10^{-3} min-1 for oxidative medium, 2.9 x 10^{-3} min^{-1} for thermal effects in methanol diluent, and 4.3 x 10^{-3} min^{-1} for alkaline medium.

To best describe the effects of each degradation medium, the effects can be categorized in increasing susceptibility as acidic < oxidative < thermal < alkaline in the review window of Empower 2.

Chromatographic characteristics

Low level impurities were easily detected and identified using the ACQUITY UPLC System and the SQ Detector, for single quadrupole MS detection, with Empower 2. Chromatograms generated from sampling the different degradation conditions were easily compared using Empower 2 (Figure 4). The major peaks were labeled A through F in the four chromatograms generated from the different degradation conditions.

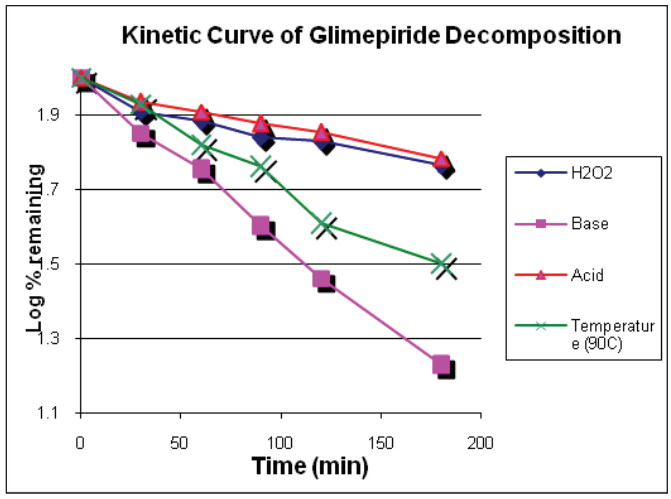

Figure 3. Kinetic curves of the decomposition of glimepiride.

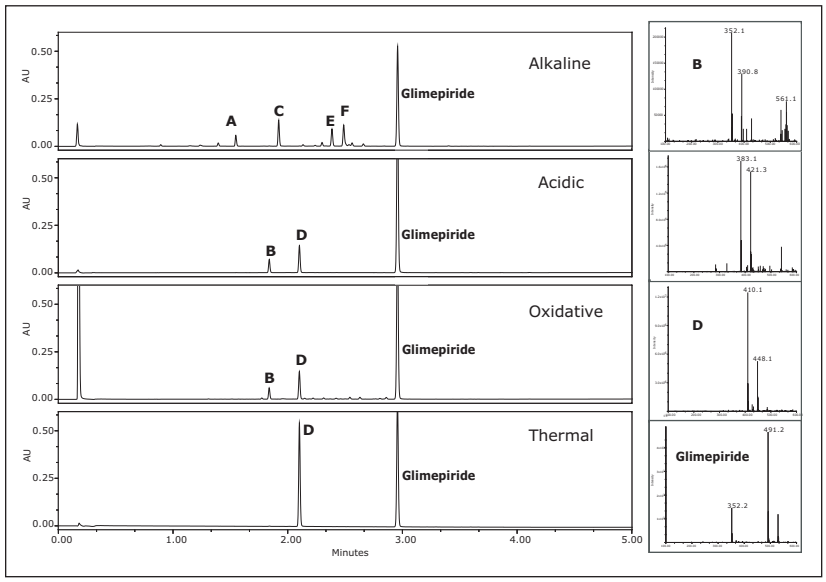

Figure 4. UPLC chromatograms showing the decomposition of glimepiride. The MS spectra (right) were used to identify and confirm recurring degradants between degradation conditions. Each of the representative chromatograms were taken from time point of 90 minutes.

The alkaline degradation produced the largest number of peaks with the major peaks labeled A, C, E, and F at retention times 1.55 min, 1.92 min, 2.31 min, and 2.40 min, respectively. The oxidative degradation yielded a large number of peaks, however the peaks were quite small and indicative of the slower rate of decomposition of glimepiride in the oxidizing environment.

Two peaks, B and D at 1.84 min and 2.10 min, were at significant levels. The acidic conditions also yielded peaks B and D as confirmed by the mass spectra. The observed *m/z* for peak D in the chromatograms representing the acidic, oxidative, and thermal degradation conditions was determined to be 410.1 Daltons. Peak B exhibited different *m/z* values at various scans across the peak. The major observed masses were 353, 390, and 561. Overlaid extracted MS scans of the masses indicated that a co-elution may be present within peak B.

Extracted chromatograms of the observed spectral masses of 352.1 Da and 390.1 Da eluted at a retention time of 2.082 min and mass 561.1 Da eluted at 2.088 min. Integration of the peak at various cross-sections resulted in mass spectral data in which it was apparent that there was a co-elution. The mass spectra for the first integrated segment gave a base peak of 352.1 Da with the second largest intensity peak at 390.1 Da. The integrated tail end of the peak gave a base peak mass of 390.1 Da with a second largest intensity *m/z* at 561.1 Da.

The initial assumption of an adduct was negated due to the decrease of intensity of the 352.1 spectral trace and increased mass spectral intensity of the 390.1 spectral trace. The spectra exhibiting 561.1 Da is unknown at this time (Figure 5).

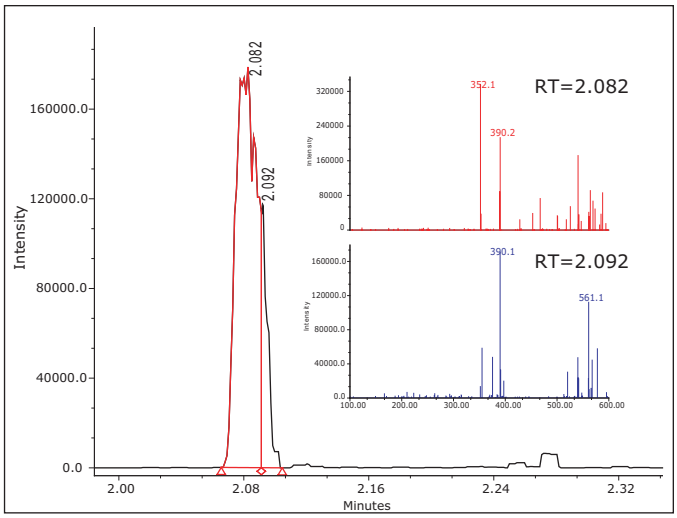

Figure 5. MS-integrated trace of Peak B. The MS spectra identifies the likelihood of co-eluting peaks rather than the presence of adduct formation.

DISCUSSION

The use of a generic LC method for forced degradation screening normally has a benefit of adding speed to the analysis. Differently, in compound-specific assays, more resolution can be obtained and confidence increases as separation of all the expected and unexpected peaks can be achieved.

In this example, UPLC/MS provided the necessary speed for the analysis with little compromise in resolution of many of the peaks, with the exception of a possible co-elution under the peak labeled B. Obviously by utilizing the MS data, much information was obtained about the masses and purity of any peak in the chromatogram. We determined that the peak was most likely related to the active ingredient because Peak B (*m/z* 352.1) was an observed product ion fragment of the active pharmaceutical glimepiride (see the glimepiride spectra in Figure 4).

CONCLUSIONS

In this application, a generic UPLC method was used to assess degradant peaks during a stress testing analysis of glimepiride drug substance.

The generic method demonstrated linearity over a large range of concentration as automatically calculated by Empower 2 CDS. The calibration curve was used to establish the kinetic behavior of glimepiride when subjected to the various stress conditions. Using single quadrupole MS spectral data assisted in the evaluation of peak purity of each major peak of interest.

This stability information will help to determine the appropriate diluents, method conditions, matrix options, standard and/or sample preparation for future experiments and will prove useful in the final method development of the drug product. The use of ACQUITY UPLC coupled to the SQ Detector and data analysis within Empower 2 decreased analysis time and enabled a workflow that proved to be more efficient than the traditional manual – and sometimes less information-rich – approaches that are used today.

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Impurity Isolation and Scale-up from UPLC Methodology: Analysis of an Unknown Degradant Found in Quetiapine Fumarate

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INTRODUCTION

A company that manufactures drug product or drug substance has a vested interest in determining the impurities associated with their compounds. The analysis of impurities can be a very labor-intensive task involving method development, impurity synthesis, isolation techniques, and various analytical approaches to determine the unambiguous identification of the impurity of interest. The lack of a pure impurity can delay a drug development program. Techniques concerning procurement of a targeted impurity are generally based on the project timelines. Impurity synthesis can be a time-consuming process requiring skilled synthetic chemists.

However, purification provides a rapid alternative to chemical synthesis that is appropriately suited to the skill set of an analytical chemist. The purification of an impurity can help with the structural elucidation by providing sufficient material for experiments such as 2D NMR. Also, collection of impurities via purification leads to reference standards of high purity.

UltraPerformance LC® provides a rapid, high-resolution approach to impurity identification and profiling. The process of scaling a UPLC® analytical method to a preparatory method can be a difficult task. Traditionally, scaling from analytical UPLC to preparatory HPLC involves calculations that transfer the flow rate and gradients associated with the original column/particle dimensions to that of the new column/particle dimensions.[1]

In this application note, a strategic approach utilizing high-resolution chromatographic theory and a forced degradation study was applied to maximize the yield of a targeted impurity of the drug substance quetiapine fumarate, an antipsychotic drug. A degradant with *m/z* 402 was found under acid stress conditions (0.1 N HCl) and chosen as the primary target for isolation. Mass-directed purification facilitated this isolation by fractionation collection of the targeted unknown impurity.

EXPERIMENTAL

In transferring the UPLC method to preparative HPLC, three key factors for the new working conditions for scale-up must be considered:

- **Separation efficiency** – L/d_p (column length/particle size) is an indication of the resolving power of the particular column. For example, a 50-mm column with 1.7-µm particles has an L/d_p of 29,411, which is equivalent to a 150-mm preparative column with 5-µm particles and an L/d_p of 30,000.
- **Productivity** – Can a shorter preparative column be utilized? In the separation of impurity *m/z* 402, a 100-mm prep column could still provide enough column efficiency to adequately isolate the impurity.
- **Column volumes** – If each of the gradient segments is scaled appropriately to maintain the equivalent number of column volumes between UPLC and preparative HPLC, the separation profile will be preserved *considering there is no change in stationary phase composition.*

Analytical conditions

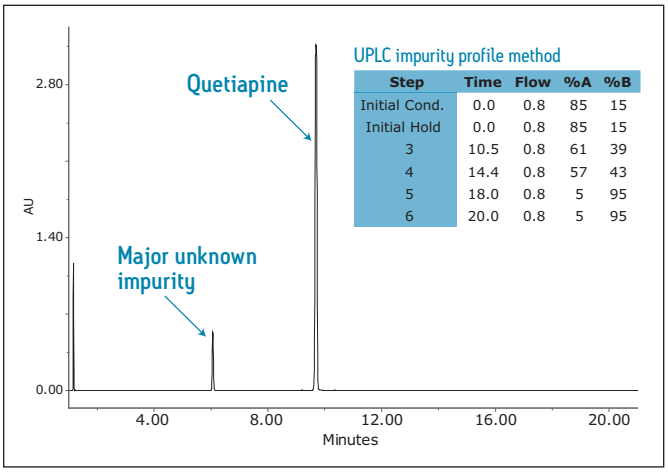
LC system:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18} 2.1 x 50 mm, 1.7 µm (optimized)
Column temp.:	Ambient
Mobile phase A:	10 mm Ammonium bicarbonate, pH 9.0
Mobile phase B:	Acetonitrile
Flow rate:	800 µL/min
Gradient (Starting):	See Figure 1
MS system:	ACQUITY® SQD
Ionization mode:	ESI positive
Capillary voltage:	1500 V
Cone voltage:	35 V
Desolvation temp.:	450 °C
Desolvation gas:	900 L/Hr
Source temp.:	150 °C
Acquisition range:	50 to 600 *m/z*

Preparative conditions

LC/MS system:	AutoPurification™ MS
Pump:	2545 Binary Gradient Module
Injector/Collector:	2767 Sample Manager
UV Detector:	2998 Photodiode Array Detector
MS Detector:	3100 Mass Detector
Column:	19 x 100 mm XBridge,™ 5 µm
Solvent A:	10 mm Ammonium bicarbonate, pH 9.0
Solvent B:	Acetonitrile
Flow rate:	25 mL/min
Gradient:	5% to 60% B over 10.5 min, flushed for approx 5 min 95% organic
Data management:	FractionLynx™ Application Manager for MassLynx™ Software

RESULTS AND DISCUSSION

A forced degradation was performed on the drug substance quetiapine. During the study, major degradants were formed for each of the various stress conditions. An impurity profile utilizing UPLC, optimized to produce maximum resolution with a 2.1 x 100 mm, 1.7 µm ACQUITY UPLC BEH C_{18} Column, was used to search for the presence of any degradants (Figure 1). Each of the major degradants was assessed by its *m/z* ratio as reported by the ACQUITY SQD single quadrupole mass detector. Peaks with masses of particular interest needed to be isolated and characterized.



Step	Time	Flow	%A	%B
Initial Cond.	0.0	0.8	85	15
Initial Hold	0.0	0.8	85	15
3	10.5	0.8	61	39
4	14.4	0.8	57	43
5	18.0	0.8	5	95
6	20.0	0.8	5	95

Figure 1. Acid hydrolysis results utilizing a highly-specific UPLC impurity profile that is optimized for maximized resolution, using a 2.1 x 100 mm, 1.7 µm ACQUITY UPLC BEH C_{18} Column.

Importance of an efficient impurity isolation process

The method was developed on an ACQUITY UPLC BEH C_{18} Column and allowed for seamless transfer to an XBridge C_{18} preparatory HPLC Column. Both XBridge HPLC particles and 1.7-µm ACQUITY UPLC BEH particles feature the same BEH Technology.

The mathematical calculations for scaling the impurity profile's UPLC methodology to preparatory conditions did not allow for an efficient isolation procedure. The L/d_p geometric scaling of the column used in the original UPLC impurity profile method resulted in a 300-mm preparatory column, as represented by the calculation:

For original UPLC quetiapine impurity profile:

$$L/d_p = 100 \text{ mm}/1.7 \text{ µm} = 58{,}823$$

Transfer to prep to maintain resolving power:

$$L/d_p = (X) \text{ mm}/5 \text{ µm} = 58, 823$$

$$X = 294 \text{ mm} \approx 300 \text{ mm column}$$

Based on this L/d_p calculation, applying the geometrically-scaled run time would result in a 60-minute preparative method – which would be excessive when considering the number of injections required to isolate milligrams of the target compound from the enriched sample preparation, thus wasting considerable time and solvent. In addition, the geometrically-scaled flow rate of 65.5 mL/min for a 19 x 300 mm column would overpressure the instrument (Figure 2). Therefore, it was necessary to make adjustments to the original UPLC impurity profile to enable a seamless scale-up.

UPLC impurity profile method

Step	Time	Flow	%A	%B
Initial Cond.	0.0	0.8	85	15
Initial Hold	0.0	0.8	85	15
3	10.5	0.8	61	39
4	14.4	0.8	57	43
5	18.0	0.8	5	95
6	20.0	0.8	5	95

Geometrically-scaled method

Step	Time	Flow	%A	%B
Initial Cond.	0.0	65.488	85	15
Initial Hold	0.3	65.488	85	15
2	31.8	65.488	61	39
3	43.5	65.488	57	43
4	54.3	65.488	5	95
5	60.3	65.488	5	95

Figure 2. Geometrically-scaled results for the preparative gradient based on quetiapine's impurity profile under UPLC gradient methodology. Calculations were performed using the Waters OBD™ Prep Calculator.

Re-optimizing for efficiency

The impurity method used to monitor the presence of degradants generated from the forced degradation study was highly specific. The forced degradation resulted in the production of only one major impurity peak with ample resolution from the active pharmaceutical ingredient (API). The decision was made to modify the impurity profile method itself to optimize for speed, while maintaining baseline resolution of the impurity peak of interest.

As stated earlier, impurity procurement is dictated by project timelines. Re-optimizing the impurity profile method to a more generic gradient satisfied analytical needs such as faster run time, lower temperature, and shorter column length (50 mm), while maintaining adequate resolution of the major impurity from quetiapine. By reducing analysis times associated with isolation, we also benefit from decreasing solvent consumption, reducing the amount of waste, and increasing sample production per unit of time.

The resulting re-optimized UPLC method utilized the same method parameters: ammonium bicarbonate at pH 9.0, ACQUITY UPLC BEH C_{18} chemistry, and acetonitrile. The peak purity of the resulting chromatogram (Figure 3) was verified by examining the mass spectral information provided by the ACQUITY SQD.

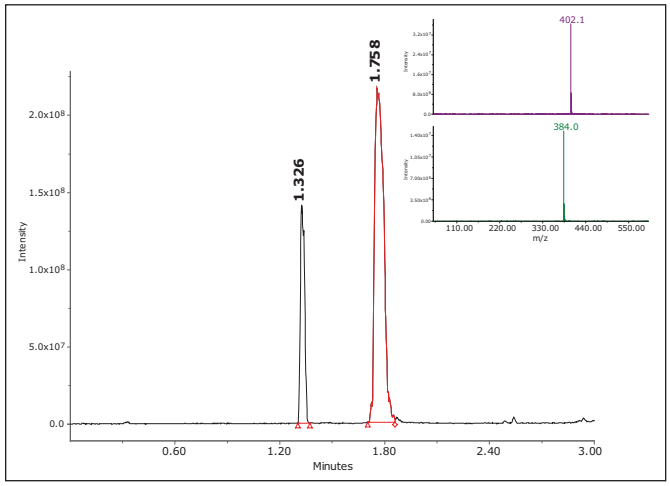


Figure 3. Re-optimized UPLC method for the peaks of interest. The resulting conditions provide an easier and more efficient solution for scaling to preparatory HPLC.

The new UPLC method with a 50-mm column provided rapid determination of the ability to use a shorter preparative column to maintain resolution. The new method resulted in an L/d_p value of 29,411, which would require a preparative column of 150 mm and a 5-µm particle size. However, after reviewing the data the observed resolution is quite large between the peaks of interest. Based on this observation, a shorter 19 x 100 mm preparative column was chosen to further decrease the preparative cycle time.

Optimizing for efficient preparative isolation

The gradient conditions for the preparative isolation analysis were optimized to maintain the same number of column volumes per gradient segment as used with the UPLC methodology. It was observed that the last peak eluted in less than two minutes, resulting in a 60% acetonitrile composition to elute all components in the re-optimized UPLC method. This observation allowed us an approach that would decrease the run time of the preparative method by applying one focused gradient segment. This practice is similar to an approach using multiple focused gradients to facilitate impurity isolation of many closely-resolved peaks.[2,3]

Step-by-step calculations were followed to determine the gradient segment durations needed for use with a 19 x 100 mm column flowing at 25 mL/min. The resulting preparative gradient is represented in Figure 4.

UPLC Column 2.1 x 50 mm, 1.7 µm	Time (min)	Flow Rate (mL/min)	%A	%B	Segment Duration Time (min)	Segment Duration (c.v)
	0.0	0.8	95	5	0	0
	2.0	0.8	40	60	2	9.25
	3.0	0.8	5	95	1	4.62

Calculated migration from UPLC to prep

Prep Column 19 x 100 mm, 5 µm	Time (min)	Flow Rate (mL/min)	%A	%B	Segment Duration Time (min)	Segment Duration (c.v)
	0.0	25	95	5	0	0
	10.5	25	40	60	10.5	9.25
	15.7	25	5	95	5.2	4.62

Figure 4. Final gradient table used to transition from UPLC to preparative analysis.

Mass-directed autopurification

The isolation of the major impurity *m/z* 402 was facilitated both analytically and chemically. In order to maximize production yield of the degradant present during the hydrolysis-forced degradation study, a stock solution of 8 g/mL quetiapine was refluxed in 0.1 N HCl for eight hours to increase the abundance of the impurity. A preparative load study allowed for 5-mL injections on-column. Together, the sample preparation and load study reduced the number of injections needed to isolate a sufficient amount of the impurity substance required for NMR analysis, while still maintaining resolution of the impurity without interferences (Figure 5).

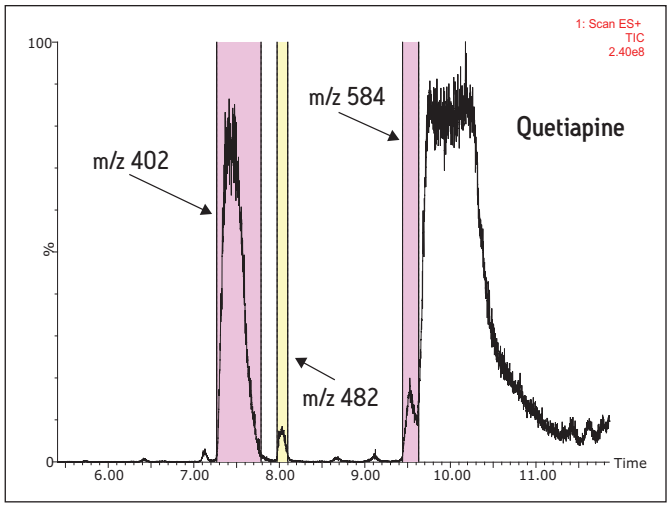


Figure 5. Preparative chromatogram of the forced degradation sample.

Using this instrumentation, automatic isolation was performed by mass triggering using the FractionLynx Application Manager for MassLynx Software. The specificity and purity of the mass triggering process was verified by UPLC/MS using the ACQUITY SQD System for the fraction analysis of the target impurity peak (Figure 6).

Re-optimizing for the impurity of interest via UPLC provided rapid methods for further analysis, such as for confirmation by UPLC/oa-Tof MS and UPLC/MS/MS.[4]

Confirmation of isolation

The isolated fractions collected for *m/z* 402 by the mass-directed purification system were pooled and evaporated to dryness. It was determined that the isolation process yielded 28.6 mg of impurity *m/z* 402. A stock solution was prepared at a concentration of 286 µg/mL, diluted to 2.86 µg/mL in methanol, and injected using the 3-minute UPLC/MS method to determine quality of the resulting isolation (Figure 6).

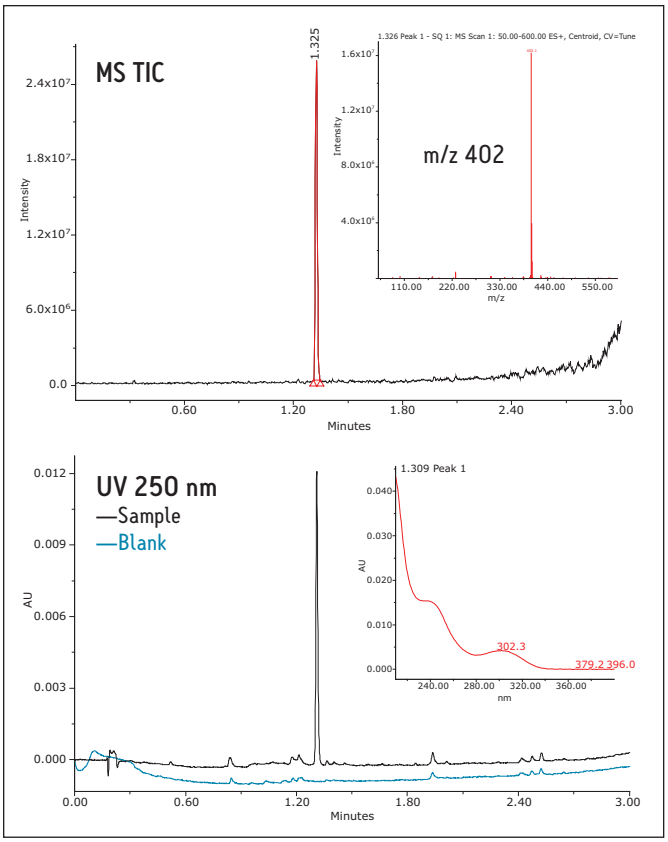


Figure 6. UPLC/MS confirmation of isolated impurity m/z 402. The resulting pooled fraction was determined to be pure, based on the MS and UV spectra that indicated zero presence of foreign substances.

CONCLUSIONS

Analytical benefits

- Maintaining L/d_p ratio was a driving factor to best manage scale-up possibilities.
- The use of a common chemistry platform for analytical-scale UPLC and preparative-scale HPLC is essential for maintaining selectivity.
- Utilizing forced degradation techniques increases production that maximizes yield.
- Mass-directed collection of the fractions assures high purity during impurity collection.
- ACQUITY SQD provides rapid confirmation of the purity composition of the pooled fractions.

Time and fiscal savings

Waters software solutions streamlined data processing and calculations:

- The dedicated FractionLynx browser presents sample and fraction information in a single, interactive location that reduced time needed to decipher data and fraction location.
- The Waters OBD Prep Calculator, a free download, facilitates scaling calculations (**www.waters.com/prepcalculator**).

Re-optimizing the UPLC method prior to preparative-scale fraction collection provided:

- A rapid and highly-specific method that utilizes less solvent and increases confidence in fraction purity.
- A scaled preparative method that requires less analysis time, considerably less solvent consumption, and less waste.
- Significant savings: 30% less time, 60% less solvent.

References

1. Diehl, DM, et al. Method Migration from UPLC Technology to Preparative HPLC. Waters Corporation. 2007; 720002375en.
2. Jablonski, JM, et al. Developing Focused Gradients for Isolation and Purification, Waters Corporation. 2009; 720002955en.
3. Cleary, R, et al. The Impact of Focused Gradients on the Purification Process. Waters Corporation. 2007; 720002284en.
4. Jones, MD, et al. Identification and Characterization of an Isolated Impurity Fraction: Analysis of an Unknown Degradant Found in Quetiapine Fumarate. Waters Corporation. 2009; 720003079en.

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A Workflow Approach for the Identification and Structural Elucidation of Impurities of Quetiapine Hemifumarate Drug Substance

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INTRODUCTION

The ability to understand the levels of pharmaceutical impurities is not only a regulatory necessity, but a business imperative. Analytical determination of impurities is often time-constraining and resource-consuming. Analysts require a range of mass spectrometry capabilities as well as sophisticated software to facilitate data processing of these complex impurity data sets.

Here, we explore a multidisciplinary approach to impurity analysis using a systematic workflow that is capable of highly specific and highly sensitive detection and determination of impurities that are present in quetiapine hemifumarate active pharmaceutical ingredient (API) drug substance. The designed approach incorporates superior chromatographic resolution, confident impurity identification, and rapid structural elucidation facilitated by intelligent and user-friendly software. This workflow-based methodology improves the ability to evaluate known and unknown impurities in a pharmaceutical drug substance.

Using a variety of software solutions within a central chromatographic data system, results are reported in the MetaboLynx™ XS data browser. The software intelligently processes chromatographic and exact mass data to report retention times, peak area, mass accuracy, and isotope distribution values for *m/z* found. Elemental compositions are confirmed for known impurities and proposed for unknown impurities. The software also performs a fragment analysis, correlating the precursor ion information of the low-energy-collision MS scan to that of the product ion information of the high-energy MS scan. The high-collision-energy MS scan data is imported into the MassFragment™ Software, where structural fragmentation pathways of the impurity compounds are proposed based on the likelihood of breaking certain bonds.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18}, 100 x 2.1 mm, 1.7 µm
Temperature:	65 °C
Injection vol.:	3 µL
Mobile phase A:	20 mM Ammonium Bicarbonate, pH 10
Mobile phase B:	Acetonitrile

Gradient:

	Time (min)	Flow (mL/min)	%A	%B	Curve
1	Initial	0.800	85.0	15.0	Initial
2	1.31	0.800	85.0	15.0	6
3	10.49	0.800	61.0	39.0	6
4	14.40	0.800	57.0	43.0	6
5	18.03	0.800	5.0	95.0	6
6	20.00	0.800	5.0	95.0	6

Detection: ACQUITY UPLC PDA at 250 nm

MS conditions

MS system:	SYNAPT® MS
Source:	ES positive
Capillary:	1.5 kV
Sample cone (V):	40 V for reference 35 V for analyte
Extraction cone:	4.0 V
Desolvation temp.:	450.0 °C
Source temp.:	120.0 °C
Desolvation flow:	900.0 L/Hr
Acquisition range:	100 to 1000 *m/z*
Scan time:	0.095 sec
Interscan delay:	0.02 sec
Lock mass:	300 pg/µL Leucine/Enkephalin at 50 µL/min
MS^E settings:	4 eV low collision energy 20 eV high collision energy

Software

MetaboLynx XS and MassFragment application managers for MassLynx® 4.1 Software

Workflow

The workflow approach shown in Figure 1 may require several iterations to determine the accurate result for the unknown peak of interest. Evaluation of the data can be more involved depending on the complexity of the compound; however, the general workflow remains constant. The benefit of this approach is that it provides a systematic data-driven association to correlate the variety of data acquired by the two scan functions generated by MS^E experiments.

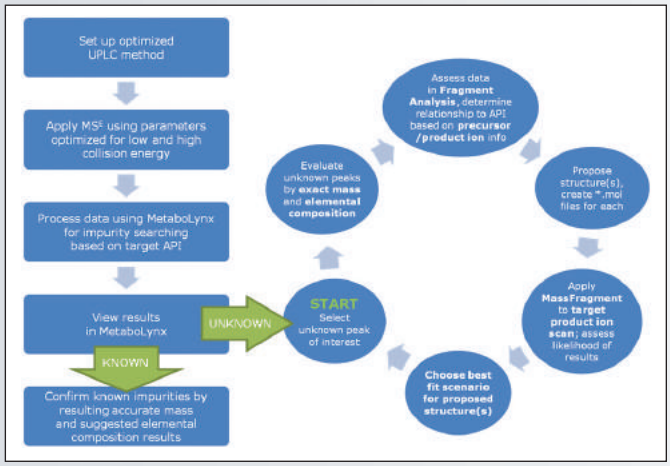

Figure 1. Workflow for impurity identification in an API impurity profile.

RESULTS AND DISCUSSION

The MetaboLynx XS Application Manager provides the flexibility to apply user-defined filters to configure how the reported data is viewed in the browser window. Some useful techniques to apply meaningful data filters were identified by investigating proper integration parameters. Mass defect filters, the dealkylation tool, spectrum intensity thresholding, and selection of components relative to the compound in the elemental composition tab all proved highly useful in displaying more confident data.

For example, to get elemental composition for every peak found in a chromatogram, the analyst would typically have to combine MS scans and perform background subtraction for each peak of interest and then generate individual elemental composition reports. To streamline this process, the MetaboLynx XS browser populates all impurity peaks integrated in the Tof-MS ES+ chromatographic trace with associated elemental compositions, mass accuracy, and isotope pattern scoring using i-FIT,™ and displays the results in a single window (Figure 2).

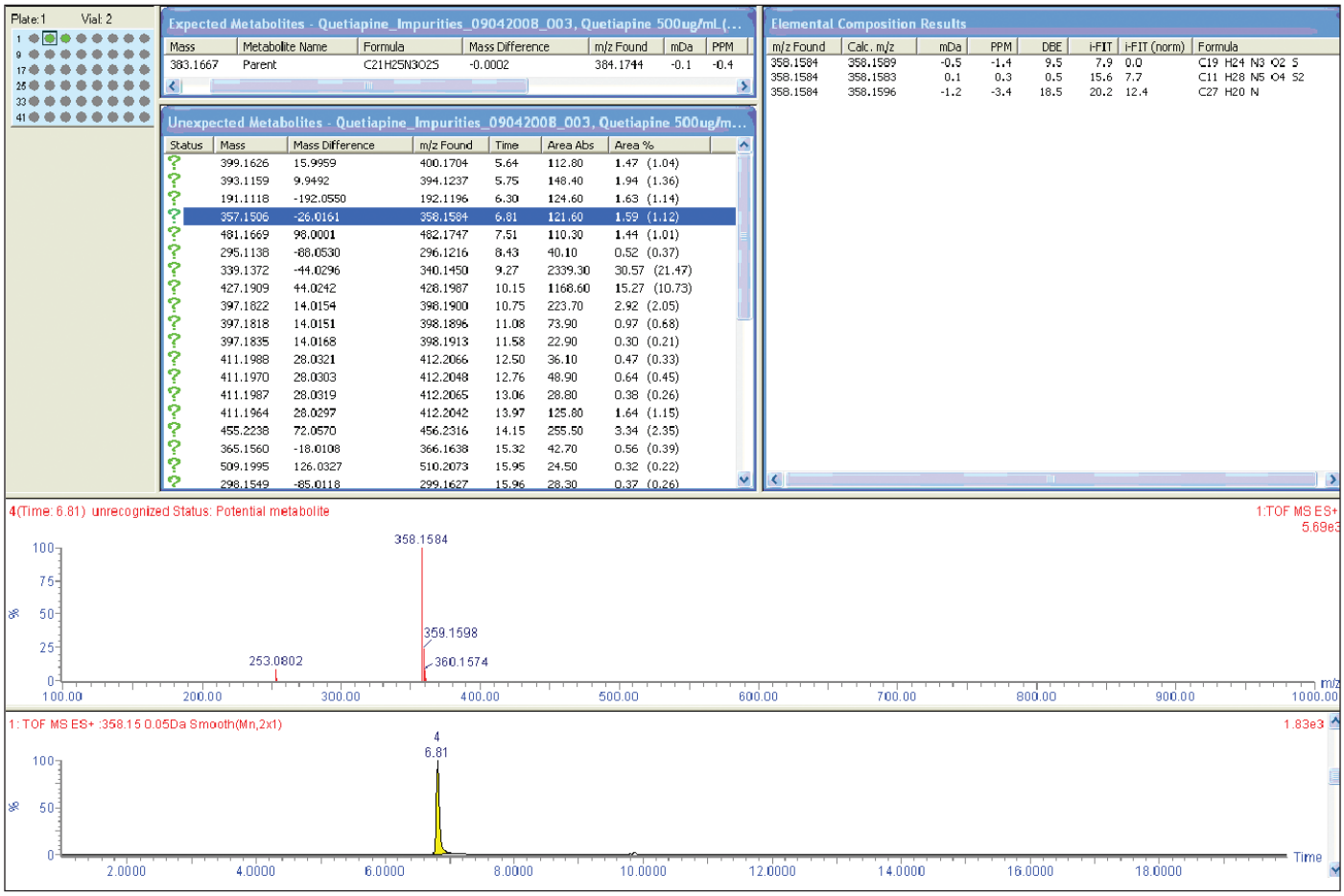

Figure 2. MetaboLynx XS browser window displaying the various chromatographic and MS spectral information generated by the MS^E experiments.

Evaluating known and unknown impurities

Evaluation of the unknown impurity peaks by exact mass and elemental composition of quetiapine hemifumarate using MetaboLynx XS indicated that the mass accuracy of the API quetiapine was reported to be 0.4 ppm. A total of 80 impurity peaks were listed. Upon adjustments to integration and data filtering, 44 peaks were found to be relevant. Non-relevant peaks were observed to be anomalies of initial integration of noise and peaks with extremely low-level response in UV and MS detection.

Ten known impurities were observed with an average mass accuracy of 1.3 ppm. Two known masses, 398.19xx and 412.20xx, had three and four separate retention times listed, respectively. The masses with multiple chromatographic retention times, which indicated possible structural isomers, were:

[M+H] = 398.19xx observed four peaks, three of which met the reporting threshold. The observed [M+H] = 398.1900, 398.1896, 398.1913 at retention times (RT) of 10.75 min., 11.08 min., and 11.58 min., with measured mass accuracies of 0.5 ppm, 1.5 ppm, and 2.8 ppm, respectively, resulted in an identified elemental composition of $C_{22}H_{28}N_3O_2S$

[M+H] = 412.20xx observed five peaks, four of which met the reporting threshold. The observed [M+H] = 412.2066, 412.2048, 412.2065, and 412.2059 at retention times (RT) of 12.50 min, 12.76 min, 13.06 min, and 13.97 min, with measured mass accuracies of 1.7 ppm, 2.7 ppm, 1.5 ppm, and 4.1 ppm, respectively, resulted in an identified elemental composition of $C_{22}H_{29}N_3O_2S$.

In terms of the unknowns that were identified, of 21 entries for 15 chromatographic peaks:

Peaks identified as doubly charged species:

- $[M+2H]2^+$ = 353.1512, [M+H]+ 705.3013 at RT = 17.20 min
- $[M+2H]2^+$ = 309.1256, [M+H]+ 617.2514 at RT = 17.36 min
- $[M+2H]2^+$ = 684.2089 with a large fragment at [M+H] = 382.3485

Peaks with multiple *m/z* ions; which could be possible coelutions, included:

- Peak RT = 15.96 min observed [M+H] = 510.2073, 299.1627, 399.2523 (three intense *m/z* values)
- Peak RT = 17.42 min observed [M+H] = 653.3301, 592.1955 (two intense *m/z* values)

From these data, we can generate and assess the data in the Fragment Analysis function of MetaboLynx XS by determining the relationship to the API based on the MS^E precursor/product ion information.

Fragment analysis

The Fragment Analysis tool aligned the high and low collision energy data that were simultaneously collected during the MS^E acquisition. The resulting information was displayed in a collective window where the precursor and the collision-induced product ions were evaluated spectrally and presented chromatographically. The Fragment Analysis window allowed for numerous iterations by the analyst to assess common fragment ions between peaks of interest (Figure 3). Commonalities were observed between known impurity structures and fragmentation patterns that aided in proposing the structures of other unknown impurity entities.

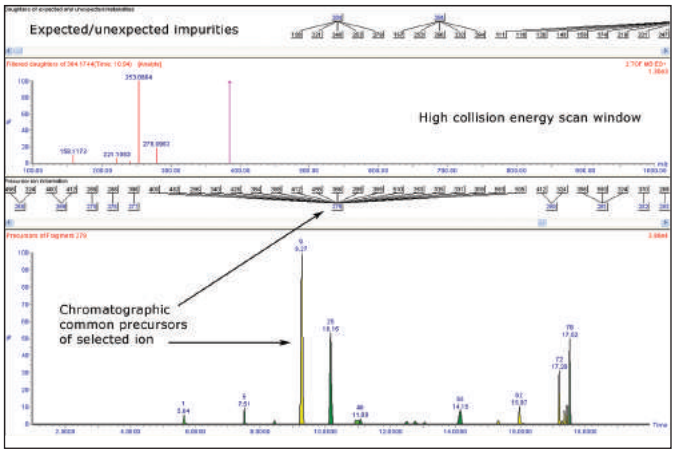

Figure 3. Fragment Analysis window of MetaboLynx XS correlating the low-collision-energy MS scan data with the high-collision-energy MS scan data.

The assessment of the common fragment ions of quetiapine identified the major fragment ions to be *m/z* 279, 253, 221, and 158:

- XIC of precursor 279 was identified in 22 impurity peaks
- XIC of precursor 253 was identified in 25 impurity peaks
- XIC of precursor 221 was identified in 23 impurity peaks
- 14 impurity peaks were deemed not to be directly related to the parent

Structural elucidation

MassFragment is a chemically-intelligent software tool that combines the aligned high and low collision energy data in the MetaboLynx XS Fragment Analysis window with the user's input about a hypothesized structure to facilitate structural elucidation. Prior to performing the elucidation procedure, a proposed parent structure (or structures) is saved as a "*.mol" file.

Upon opening MassFragment, a dialog window prompts the selection of the *.mol file. The fragment ion information from the Fragment Analysis product ion's high-collision-energy scan window of the selected observed impurity mass automatically exports to MassFragment along with the *.mol file. Potential structures are assigned and scored for the precursor ions in the isotopically-filtered spectrum.

Figure 4 shows an example of the report generated by MassFragment for the unknown impurity [M+H] 456.2305. Other conclusions determined by the MassFragment data included:

- Many of the impurities have the common fragment ions *m/z* 279, 253, 221, and 158, as observed in the API quetiapine
- MassFragment confirmed similar fragmentation patterns of the imported structures with excellent mass accuracy generally less than 2.0 mDa
- It was also hypothesized that the structure undergoes a structural rearrangement after the cleavage of the piperazine ring,[1] however this did not seem to affect the mass accuracy of many of the proposed fragmentation pathways of the assumed parent structure of the unknown impurity

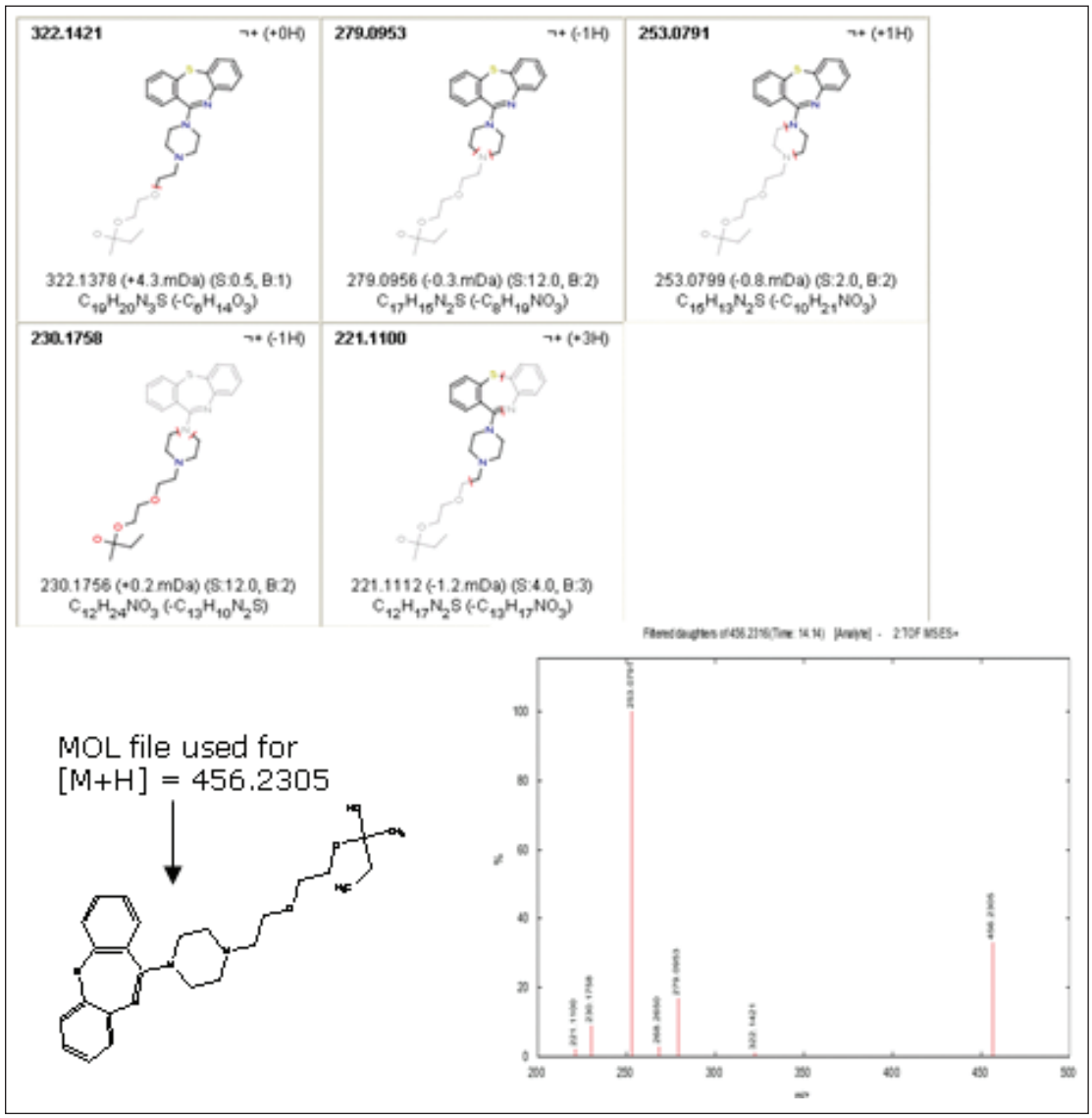

Figure 4. Snapshot of a MassFragment summary report of possible structures for each of the fragment ions in the isotopically-filtered spectrum.

CONCLUSIONS

Data collection using ACQUITY UPLC with the SYNAPT MS provided high chromatographic resolution, ample sensitivity, and superior mass accuracy to identify many of the impurities in the quetiapine hemifumarate drug substance. MS^E provided simultaneous acquisition of both high and low collision energy, maximizing the information gathered from a single injection. This analytical workflow was followed by a deliberate data processing workflow that streamlined the fragment analysis and structural elucidation process and provided greater confidence in the end results.

The MetaboLynx browser provided:

- A comprehensive list of elemental compositions for the known and unknown peaks
- 10 known impurities were rapidly identified with an average mass accuracy <3.0 ppm
- [M+H] = 398 and 412 were observed to have a series of structural isomers

Using MetaboLynx's Fragment Analysis:

- A minimum of 25 impurity peaks were identified as being related to quetiapine utilizing the common fragment ions *m/z* 279, 253, 221, and 158
- 14 integrated impurity peaks were identified with no common fragment ions

Using MassFragment:

- The structures of the 10 known impurities were rapidly confirmed
- Information of the possible structural isomers for [M+H] = 398 and 412 were easily compared to various proposed structural isomers for best-fit correlation to the high collision energy data.

In some cases where the peak identification was more challenging, MetaboLynx was able to help formulate decisions about compound determination. The combination of these three software tools, along with the optimized instrument configurations for impurity analysis and efficient MS^E acquisition, provided a systematic workflow approach that can readily be applied to identify and confirm known and unknown peaks in an impurity profile.

This workflow-based approach delivers the rapid and systematic set of comprehensive results that are needed to identify and confirm impurities in an API impurity profile.

Reference

1. Xu H, et al. J. Pharma. *Biomed. Anal.* 2007; 44: 414–20.

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Identification and Characterization of an Isolated Impurity Fraction: Analysis of an Unknown Degradant Found in Quetiapine Fumarate

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Waters Corporation, Milford, MA, USA

INTRODUCTION

Impurity profiling is a critical part of the drug development process. Structural elucidation of unknown substances such as synthetic impurities is a key factor to refining pharmaceutical drug potency and safety attributes.[1] Success in solving these very complicated and challenging structural puzzles has been facilitated by evolutions in LC and MS instrumentation; however it still requires a high level of experience to solve these analytical problems.

Typically MS/MS data from a triple quadrupole mass spectrometer is interrogated for structural elucidation in impurity profiling, in order to provide fragmentation spectra. However, using MS/MS analysis alone – particularly nominal mass spectra – may not provide sufficient specificity to determine the analyte structure. Complementing MS/MS with time-of-flight (Tof) data acquisition provides elemental composition information. For small molecules, this significantly improves the elucidation process since it significantly reduces the number of possible elemental compositions.

Quetiapine (Figure 1) is an atypical antipsychotic that has been shown to form more than 20 impurities/degradation products.[2] In this application note, we demonstrate the application of high-resolution mass spectrometry to generate exact mass MS/MS fragment analysis data in conjunction with the use of software to automatically identify product ion fragments. This software applies a series of novel, chemically-intelligent algorithms to elucidate an unknown impurity peak of quetiapine that was previously isolated by mass-directed autopurification.[3]

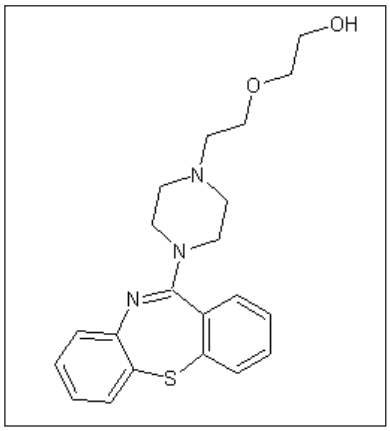

Figure 1. Structure of quetiapine, [M+H] = 384.1746.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC®
Column:	ACQUITY UPLC BEH C_{18} Column, 2.1 x 50 mm, 1.7 µm
Column temp.:	65 °C
Flow rate:	800 µL/min
Mobile phase A:	20 mM ammonium bicarbonate, pH 9.0
Mobile phase B:	Acetonitrile
Gradient:	5 to 95% B for 3.0 min

MS conditions

MS system:	Waters SYNAPT® MS System
Ionization mode:	ESI positive
Capillary voltage:	1.5 kV
Cone voltage:	15 V
Desolvation temp.:	350 °C
Desolvation gas:	650 L/Hr
Source temp:	120 °C
Acquisition ranges:	100 to 1000 *m/z* for MS 50 to 600 *m/z* for MS/MS
Collision energies:	Ramp from 15 to 30
Lock mass:	300 pg/µL leucine/enkephalin flow at 50 µL/min

Data management

MassLynx® 4.1 Software with MassFragment™

RESULTS

In a previous application note, forced degradation was employed to maximize the amount of an impurity formed during hydrolytic conditions to generate a sufficient mass of impurity for subsequent NMR analysis.[2] The peak giving rise to the *m/z* 402 ion was isolated using preparative chromatography and mass-directed fraction collection. Although the desired substance was collected, the compound's structure remained unknown.

Importance of mass accuracy for determination of elemental composition

Instrumentation capable of producing high mass accuracy information is an essential tool in the area of impurity determination. Modern Q-Tof™ technology makes the acquisition of sub-5-ppm mass accuracy data readily achievable. This, coupled with fast data acquisition speeds that allow the detection of the narrows peaks produced by UPLC® chromatography, make it an ideal tool for impurity identification.

The sample was analyzed on the SYNAPT MS System to obtain accurate mass MS data and hence elemental composition determination. The impurity had an observed accurate mass of *m/z* 402.1838. This is an addition of 18.0092 amu to that of quetiapine (*m/z* 384.1746).

The high mass accuracy of the SYNAPT MS System produced a short list of possible elemental compositions. The potential elemental composition list was further reduced by a series of filters. These filters included the ability to limit molecular formulas based on:

- Elemental symbols and limits (e.g., Nitrogen, 0 to 5)
- Mass accuracy tolerance (ppm or mDa)
- *i*-FIT™ isotopic pattern
- Electron state (odd, even, or both)

The Elemental Composition browser returned three possible molecular formulas (Figure 2).

The Elemental Composition calculator was set to order the list of proposed elemental compositions according to the fit of the experimental data to the theoretical isotope distribution using i-FIT. The i-FIT criterion calculated a molecular formula of $C_{21}H_{28}N_3O_3S$ as the best fit. The double bond equivalency (DBE) reported a value of 9.5 and a mass error of -1.3 mDa.

Possible structures that fit the elemental compositions based on the mass accuracy data for 402.1838 include:

- Oxidation of the sulfur on quetiapine structure in conjunction with reduction of the C=N double bond in the seven member ring (Figure 3a)
- Cleavage of the C=N bond producing a nitroso-based structure (Figure 3b)
- Cleavage of the C=N bond producing a ketone- and amine-based structure (Figure 3c)

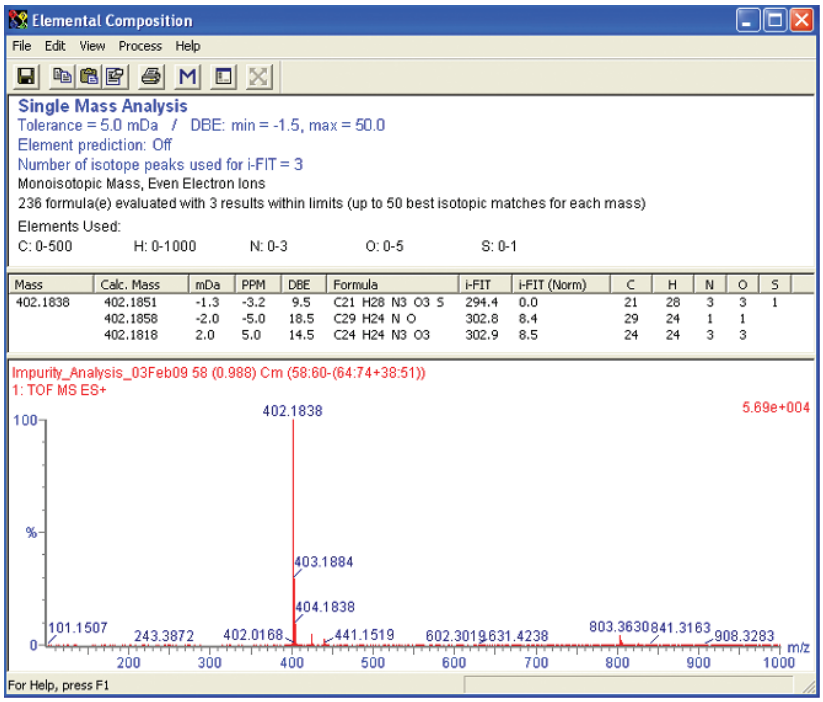

Figure 2. Elemental composition for impurity m/z 402.1838. The double bond equivalence (DBE) value, low i-FIT value, and low mDa results support the "first hit" formula.

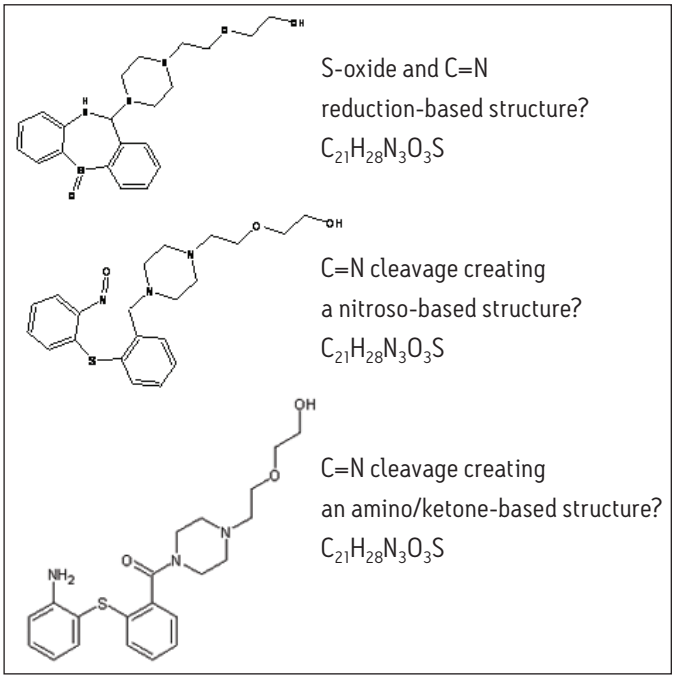

Figure 3. Structures that fit the m/z 402.1838 mass accuracy results reported in the elemental composition.

Examining the forced degradation reaction that enriched the production of the impurity suggests the most probable structure is as shown in Figure 4. Other chemical structures could be proposed based on the accurate mass information of the base *m/z* alone. Knowing the reaction that produced the addition of 18 amu aids the elucidation decision process.

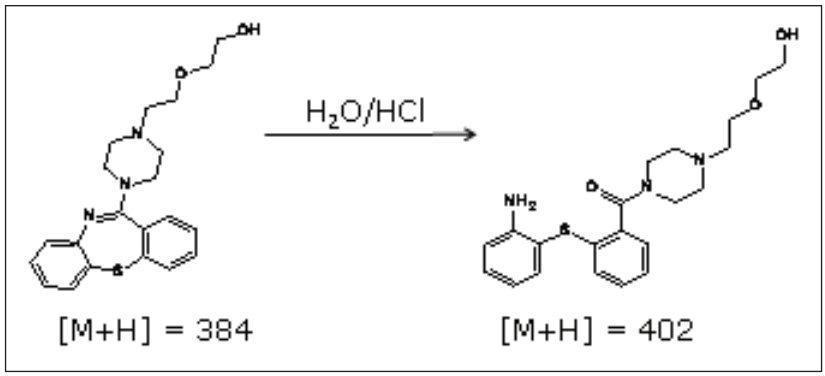

Figure 4. Proposed hydrolysis reaction of quetiapine.

Accurate mass MS/MS analysis

A careful examination of the fragmentation patterns obtained during related impurity analysis allows the impurities' structural information to be associated with that of the active pharmaceutical ingredient (API). The key to informative MS/MS data is the quality of the fragmentation spectra. The quality of MS/MS spectra assessed in small-molecule analysis is based on the number of fragments and spatial location of the fragments, thus providing added structural informative value for elucidation determination.

The SYNAPT MS Systems performs both MS^E and traditional MS/MS experiments. MS^E experiments allow for simultaneous high and low collision energy data to be collected with one injection. Traditional MS/MS experiments provide additional information in the form of product ion scans, precursor ion scans, and common neutral loss scans. An accurate mass MS/MS product ion scan of 402.1838 was performed to evaluate the fragmentation pattern of the isolated impurity to further support the proposed structure shown in Figure 3c (Figure 5). The fragments produced are most likely associated to cleavage of the seven-member ring that differentiates the three unknown impurities' proposed structures.

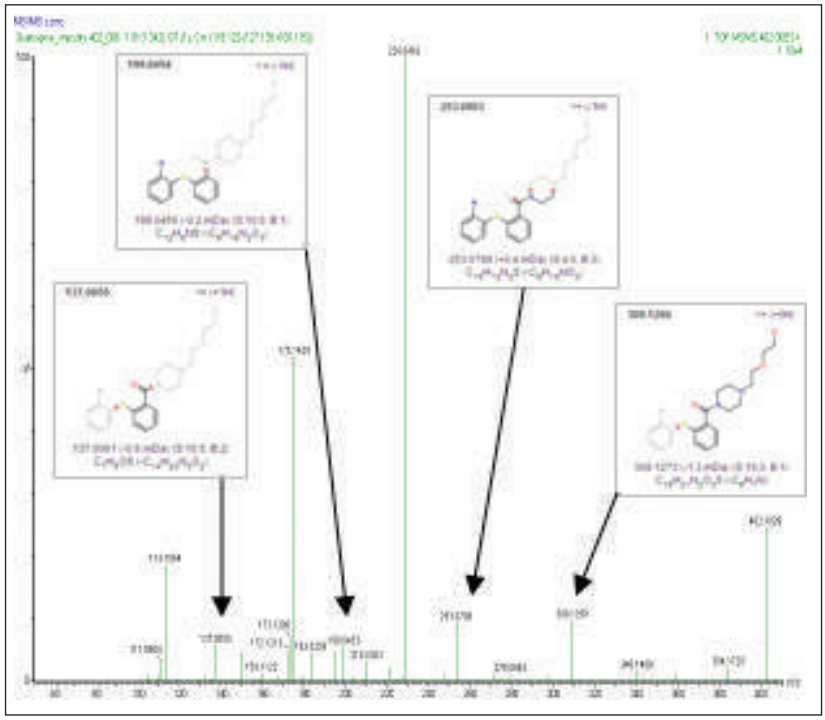

Figure 5. Fragment analysis following the hypothesis of the amino/ketone-based structure as the unknown impurity highlighted with MassFragment results.

The key structural fragments that added greater confidence in differentiating amine/ketone-based structures to that of the alternatively-proposed structures were the *m/z* 137.0063, *m/z* 199.0454, *m/z* 228.0464, *m/z* 253.0803, and *m/z* 309.1260 ions.

MassFragment for structural elucidation

Structural elucidation requires an expert knowledge of chemistry and bond reactions. MassFragment is a software solution that is capable of automatically identifying product ion fragments using a series of chemically-intelligent algorithms. This approach is based on systematic bond disconnection of the precursor structure instead of the traditional rule-based approach. MassFragment was used to help visualize the correlation of the product ion data with possible structural assignments for the observed fragment ions (Figure 6).

MassFragment increases our confidence in the MS/MS fragment analysis of the proposed structure in Figure 3c. The proposed structures were drawn and saved as *.mol files. Each *.mol file was loaded separately into MassFragment to be structurally correlated with the spectra in Figure 5. The MassFragment results ruled out the proposed nitroso-based and S-oxide based structures.

The fragment ion *m/z* 137.0063 was observed in the MS/MS spectra in Figure 5. The MassFragment reports for the S-oxide based structure and the amino/ketone-based structure observed the ion fragment *m/z* 137.0063 and displayed possible structures. MassFragment was not able to report a structure for this particular fragment ion when the nitroso-based precursor structure was loaded into MassFragment (Table 1).

The fragment ion *m/z* 199.0454 was also observed in the MS/MS spectra. MassFragment reported a candidate structure for fragment ion *m/z* 199.0454 based on the amino/ketone-based structure (Figure 6). There were no reported structures for this fragment ion when the S-oxide based structure was loaded into MassFragment (Table 2).

NMR supporting data

The collected fraction was dried down and prepared in deuterated methanol for analysis by NMR. It was determined from the NMR data (not shown) that the impurity *m/z* 402 was consistent with the proposed structure.

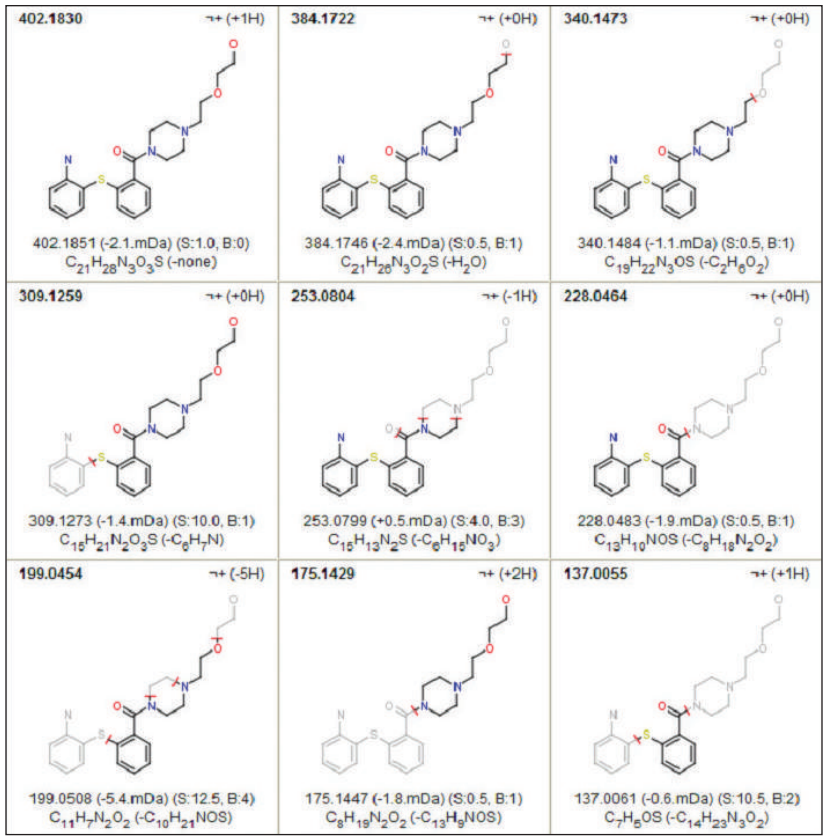

Figure 6. MassFragment results for the structure proposed in Figure 3c (amino/ketone-based).

Daughter (Da)	Mass (Da)	Mass error (mDa)	Formula	DBE	ΔFormula	Structure(s), score & H-deficit
137.0055	137.0061	-0.6	C_7H_5OS	5.5	$C_{14}H_{22}N_3O_2$	none
	137.0048	+0.7	$C_5H_3N_3S$	6	$C_{16}H_{24}O_3$	none
	137.0027	+2.8	$C_{10}HO$	10.5	$C_{11}H_{26}N_3O_2S$	none
	137.0021	+3.4	$C_2H_5N_2O_3S$	1.5	$C_{19}H_{22}N$	none
	137.0113	-5.8	$C_6H_3NO_3$	6	$C_{15}H_{24}N_2S$	none
	136.9987	+6.8	$C_5HN_2O_3$	6.5	$C_{16}H_{26}NS$	none
	137.0140	-8.5	C_9HN_2	10.5	$C_{12}H_{26}NO_3S$	none
	137.0147	-9.2	$C_3H_7NO_3S$	1	$C_{18}H_{20}N_2$	none

*Table 1. MassFragment results for m/z 137.0055 based on spectra from Figure 6 and nitro-based *.mol file.*

Daughter (Da)	Mass (Da)	Mass error (mDa)	Formula	DBE	ΔFormula	Structure(s), score & H-deficit
199.0454	199.0456	-0.2	$C_{12}H_9NS$	9	$C_9H_{18}N_2O_3$	none
	199.0429	+2.5	$C_9H_{11}O_3S$	4.5	$C_{12}H_{16}N_3$	none
	199.0422	+3.2	$C_{15}H_5N$	14	$C_6H_{22}N_2O_3S$	none
	199.0415	+3.9	$C_7H_9N_3O_2S$	5	$C_{14}H_{18}O$	none
	199.0508	-5.4	$C_{11}H_7N_2O_2$	9.5	$C_{10}H_{20}NOS$	none
	199.0395	+5.9	$C_{12}H_7O_3$	9.5	$C_9H_{20}N_3S$	none
	199.0382	+7.2	$C_{10}H_5N_3O_2$	10	$C_{11}H_{22}OS$	none
	199.0541	-8.7	$C_8H_{11}N_2O_2S$	4.5	$C_{13}H_{16}NO$	none
	199.0548	-9.4	$C_{16}H_7$	13.5	$C_5H_{20}N_3O_3S$	none

CONCLUSIONS

For small molecule analysis, the advances in MS technologies with greater mass accuracy readily complement the advances in LC technology and sub-2-µm column particles capable of peak widths of 1 to 3 seconds. Laboratories performing impurity analysis can realize significant benefits by using chemically-intelligent software that improves how this LC/MS^E data is analyzed.

Analytical benefits

- The high resolution possible with the SYNAPT MS System provided excellent mass accuracy for elemental composition determination of precursor and product ions.
- MassFragment facilitated the confirmation of a proposed structure for an unknown impurity isolated from quetiapine in a forced degradation experiment.

Realized benefits

- Increasing confidence in the structural determination due to the high mass accuracy data.
- Minimizing the number of possible elements in the elemental compositions for the compound(s) and fragments.
- Using criteria filters such as isotopic pattern values, elemental filters, and mass accuracy values saves time during data evaluation.
- Correlating spectral MS information to possible structural fragmentation and assigning a rank allows for more confidence in elucidation decisions.

Proper characterization of a compound provides greater insight to the reactive behavior of the compound that will lead to more informative formulation and packaging decisions.

References

1. Ahuja S and Alsante K. Handbook of Isolation and Characterization of Impurities in Pharmaceutical Compounds. 2003; Elsevier.
2. Jones MD et al. Method Development of an Impurity Profile for Quetiapine Fumarate Drug Substance. Waters. 2009; 720003080en.
3. Jones MD et al. Impurity Isolation and Scale-up from UPLC Methodology: Analysis of an Unknown Degradant Found in Quetiapine Fumarate. Waters. 2009; 720003078en.

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A QbD with Design-of-Experiments Approach to the Development of a Chromatographic Method for the Separation of Impurities in Vancomycin

Peter G. Alden, Warren Potts, and Dana Yurach
Waters Corporation, Milford, MA, U.S.

APPLICATION BENEFITS

Using specialized software in conjunction with UPLC® Technology, an optimized QbD method for the impurities in vancomycin can be developed that will be robust for method validation and transfer.

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ACQUITY UPLC®
Fusion Method Development
Empower™ 2 CDS

KEY WORDS

Quality by design (QbD), method development, design of experiment (DOE)

INTRODUCTION

Analytical methods are developed at various stages of the drug develoment process for samples of varying complexity. Due to the inherent nature of the method development process, redundant efforts take place across an organization, resulting in a very costly and time-consuming activities. If we can streamline the process by which we develop methods, products can be brought to market faster and in a more cost effective manner.

Many different approaches are typically used to develop chromatographic methods today including trial and error, method/column scouting, and software approaches such as first principles approaches and simplex optimization procedures. All these approaches suffer from the inability to determine complex interactions effects between method variables or measurably consider method robustness during the method development process.

Vancomycin is a tricyclic glycopeptide antibiotic derived from *Amycolatopasis orientalis* (formerly *Nocardia orientalis*) and is indicated for the treatment of serious or severe infections caused by susceptible strains of methicillin-resistant (beta-lactam-resistant) staphylococci. Vancomycin is a large molecule (MW 1485.71 daltons) and contains many impurities that are difficult if not impossible to separate. Traditional HPLC gradient methods have shown the ability to separate out as many as 13 of these impurities, while the use of sub-2-µm ACQUITY UPLC Column chromatography has demonstrated the separation of as many as 26 impurities.

This paper describes a novel method development approach using Quality by Design (QbD) with Design of Experiments to develop a UPLC method for separating 39 impurities in vancomycin resulting in an optimally performing analytical method while simultaneously applying robustness limits to ensure success in final method validation and ultimately in method transfer.

EXPERIMENTAL

Analytical instrumentation

The vancomycin studies described here were carried out using an automated integrated system consisting of Fusion Method Development Software, Empower 2 Chromatography Data Software (CDS), and an ACQUITY UPLC System with PDA, Column Manager, and Solvent Select Valve allowing for the screening of up to four different column chemistries, six different aqueous buffers/pHs, and two different organic mobile phases in one run.

Data management

Fusion Method Development Software (S-Matrix Corporation, Eureka, CA) is a Quality by Design based LC Method Development software package with built-in robustness metrics. Fusion includes a built-in interface with the Empower 2 CDS Software that controls the ACQUITY UPLC System. Using the chromatographic results collected from Empower 2 CDS, Fusion manages complex statistics and models for method optimization. Fusion builds experiments, analyzes data, and presents results as visual and numerical method predictions.

RESULTS AND DISCUSSION

Phase 1: Rapid screening

Experiment design

The first phase of the method development involves the screening of the major effectors of selectivity, primarily the column chemistry, buffer pH, and organic mobile phase. The variables and ranges screened along with the constant conditions are listed in Figure 1's tables.

Column Assignments

Column Valve Position	Column Level
ValvePosition_1	BEH C18, 2.1x100mm, 1.7um
ValvePosition_2	BEH RP18, 2.1x100mm, 1.7um
ValvePosition_3	BEH Phenyl, 2.1x100mm, 1.7um
ValvePosition_4	BEH C8, 2.1x100mm, 1.7um

Reservoir Assignments

Reservoir A1-1	Level
pH	3.0
Reservoir A1-2	**Level**
pH	5.0
Reservoir A1-3	**Level**
pH	6.5
Reservoir A2	**Level**
Aqueous Solution	---
Reservoir B1	**Level**
Acetonitrile	---
Reservoir B2	**Level**
Methanol	---

Experiment Constants

Constant Name	Constant Value
Sample Concentration	0.25
Pump Flow Rate	0.450
Injection Volume	2.5
Oven Temperature	45.0
Wavelength	254
Equilibration Time	10.0
Equilibration % Organic	2.0
Initial Hold Time	1.0
Initial Hold % Organic	2.0
Final Hold Time	2.0
Final Hold % Organic	40.0
Ramp Up to Wash Time	0.1
Column Wash Time	2.0
Column Wash % Organic	95.0
Ramp Down from Wash Time	0.1
Re-equilibration Time	1.0
Re-equilibration % Organic	95.0

Figure 1. Screened variables and ranges.

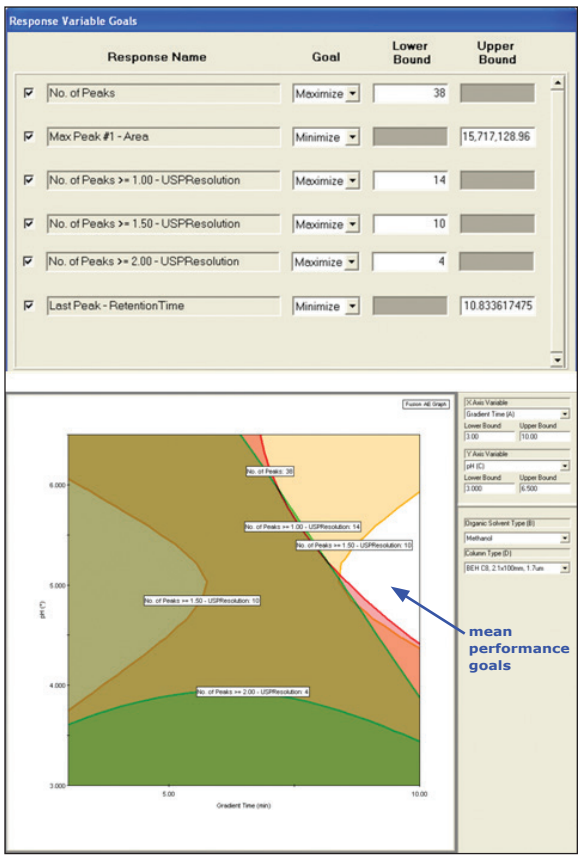


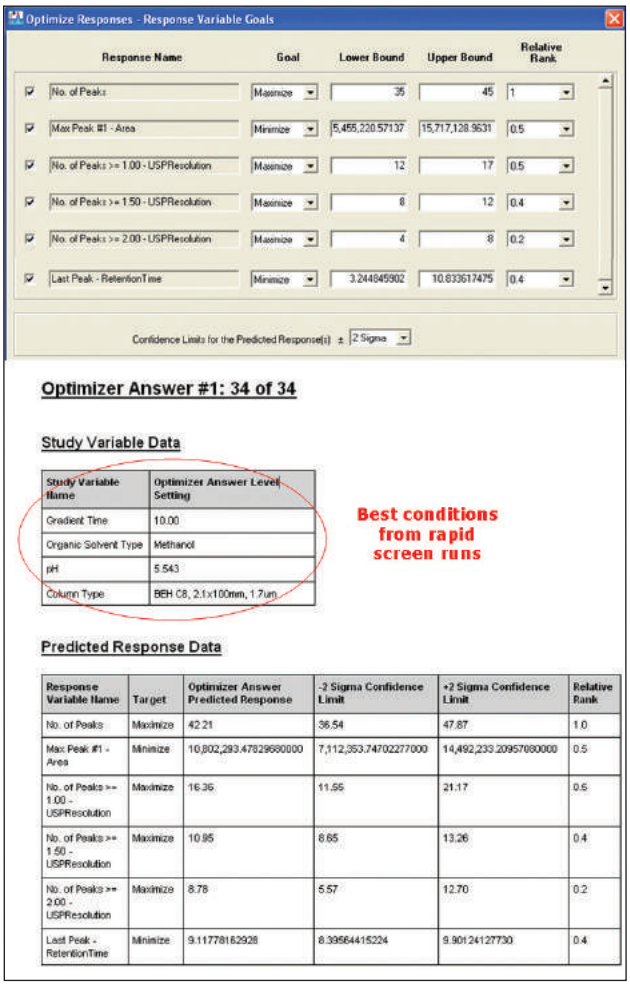
Figure 2. Processed data are imported to Fusion, where an overlay graph illustrates in white the region where the mean performance goals are achieved.

Overlay graphics

The experimental design is run and data processed on the chromatographic system and the results are imported back into Fusion. The software predicts the optimum LC method after modeling all significant effects – linear, interaction, and complex – on each critical method performance characteristic. The unshaded (white) area of the overlay graph shown for the BEH C_8 column with methanol as the organic mobile phase (Figure 2) highlights the experimental region where the mean performance goals are obtained.

Optimization

The Automated Optimizer wizard defines the LC method performance goals and ranks them in order of importance. The software searches for the LC method that meets all the performance goals simultaneously. The best result(s) are reported along with predicted results for an experimental run (Figure 3). These conditions are used for the next stage, Method Optimization.



Optimize Responses - Response Variable Goals

Response Name	Goal	Lower Bound	Upper Bound	Relative Rank
No. of Peaks	Maximize	35	45	1
Max Peak #1 - Area	Minimize	5,455,220.57137	15,717,128.9631	0.5
No. of Peaks >= 1.00 - USPResolution	Maximize	12	17	0.5
No. of Peaks >= 1.50 - USPResolution	Maximize	8	12	0.4
No. of Peaks >= 2.00 - USPResolution	Maximize	4	8	0.2
Last Peak - RetentionTime	Minimize	3.244845902	10.833617475	0.4

Confidence Limits for the Predicted Response(s) ± 2 Sigma

Optimizer Answer #1: 34 of 34

Study Variable Data

Study Variable Name	Optimizer Answer Level Setting
Gradient Time	10.00
Organic Solvent Type	Methanol
pH	5.543
Column Type	BEH C8, 2.1x100mm, 1.7um

Best conditions from rapid screen runs

Predicted Response Data

Response Variable Name	Target	Optimizer Answer Predicted Response	-2 Sigma Confidence Limit	+2 Sigma Confidence Limit	Relative Rank
No. of Peaks	Maximize	42.21	36.54	47.87	1.0
Max Peak #1 - Area	Minimize	10,802,293.47829680000	7,112,353.74702277000	14,492,233.20957080000	0.5
No. of Peaks >= 1.00 - USPResolution	Maximize	16.36	11.55	21.17	0.5
No. of Peaks >= 1.50 - USPResolution	Maximize	10.95	8.65	13.26	0.4
No. of Peaks >= 2.00 - USPResolution	Maximize	8.78	5.57	12.70	0.2
Last Peak - RetentionTime	Minimize	9.11778162928	8.39564415224	9.90124127730	0.4

Figure 3. Fusion's Automated Optimizer facilitates determination of the LC method that meets all performance goals.

Design Variables

Variable	Range
Pump Flow Rate	0.25 - 0.45 mL/min
Gradient Time	6.0 - 10.0 min
Final % Organic	25% - 40% B
Column Temperature	35 – 60 °C

Reservoir Assignments

Reservoir A1-1	Level
pH	5
Reservoir A2	**Level**
Aqueous Solution	---
Reservoir B2	**Level**
Methanol	---

Experiment Constants

Constant Name	Constant Value
Column Type	BEH C8 100mm
Injection Volume	2.5
Wavelength	254
pH	5.0
Initial % Aqueous	95
Initial % Organic	5
Equilibration Time	10.0
Equilibration % Organic	5.0
Initial Hold Time	1.0
Final Hold Time	2.0
Ramp Up to Wash Time	0.1
Column Wash Time	2.0
Column Wash % Organic	95.0
Ramp Down from Wash Time	0.1
Re-equilibration Time	1.0
Re-equilibration % Organic	5.0

Figure 4. Fusion determines optimal method conditions and exports this information back to Empower 2 to be run and processed.

Phase 2: Method optimization

Experiment design

Phase 2 experiments use the column (ACQUITY UPLC BEH C8, 2.1 x 100 mm, 1.7 µm) and mobile phase (pH 5.0 buffer, methanol B solvent) results from Phase 1 plus additional variables with tighter ranges to determine the optimum LC method. The experimental design is created using pump flow rate, gradient time, final percent organic, and column temperature as final optimization variables in the ranges shown (Figure 4).

Fusion Software creates the experimental design and exports it to Empower 2, automatically creating all the necessary instrument methods, method sets, and sample sets. The experimental design is run and data processed on the chromatographic system and the results are imported back into Fusion.

In addition to the data analysis for method optimization, Fusion applies a combination of Monte Carlo Simulation and Process Capability statistics to evaluate method robustness without running additional experiments.

Multiple response surface plots

Visualizing the results with Fusion's 3D Response Surface Plots demonstrates the combined effects of variables on key chromatographic responses such as resolution, peak tailing, and retention time. Colors represent the magnitude of interaction and the curvature indicates the type of interaction (Figure 5).

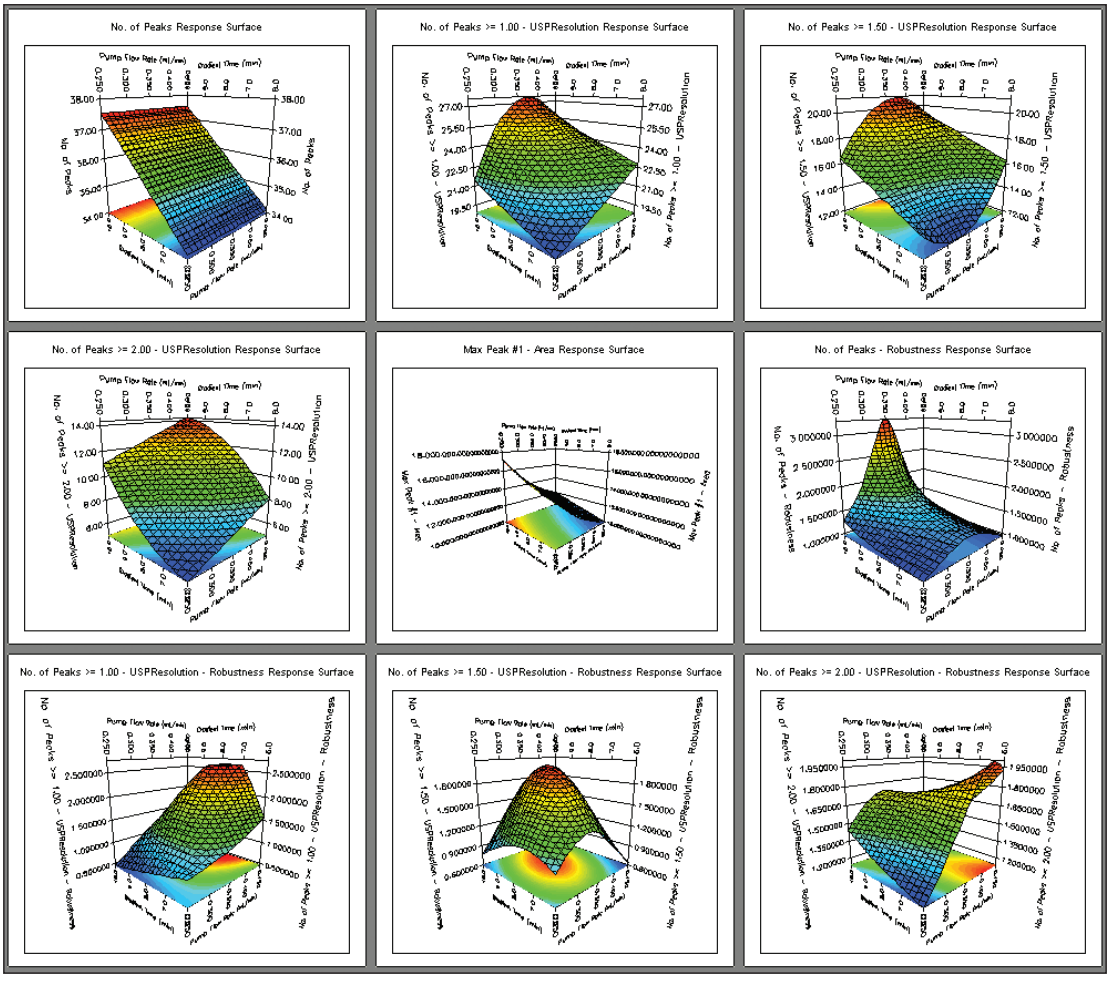


Figure 5. Surface plot responses.

Multiple response effects plots

Another option for visualizing the interactions between variables is through Multiple Response Effects Plots. These plots clearly identify simple linear additive effects (1), complex interactions of variables (2), and other types of response effects (Figure 6).

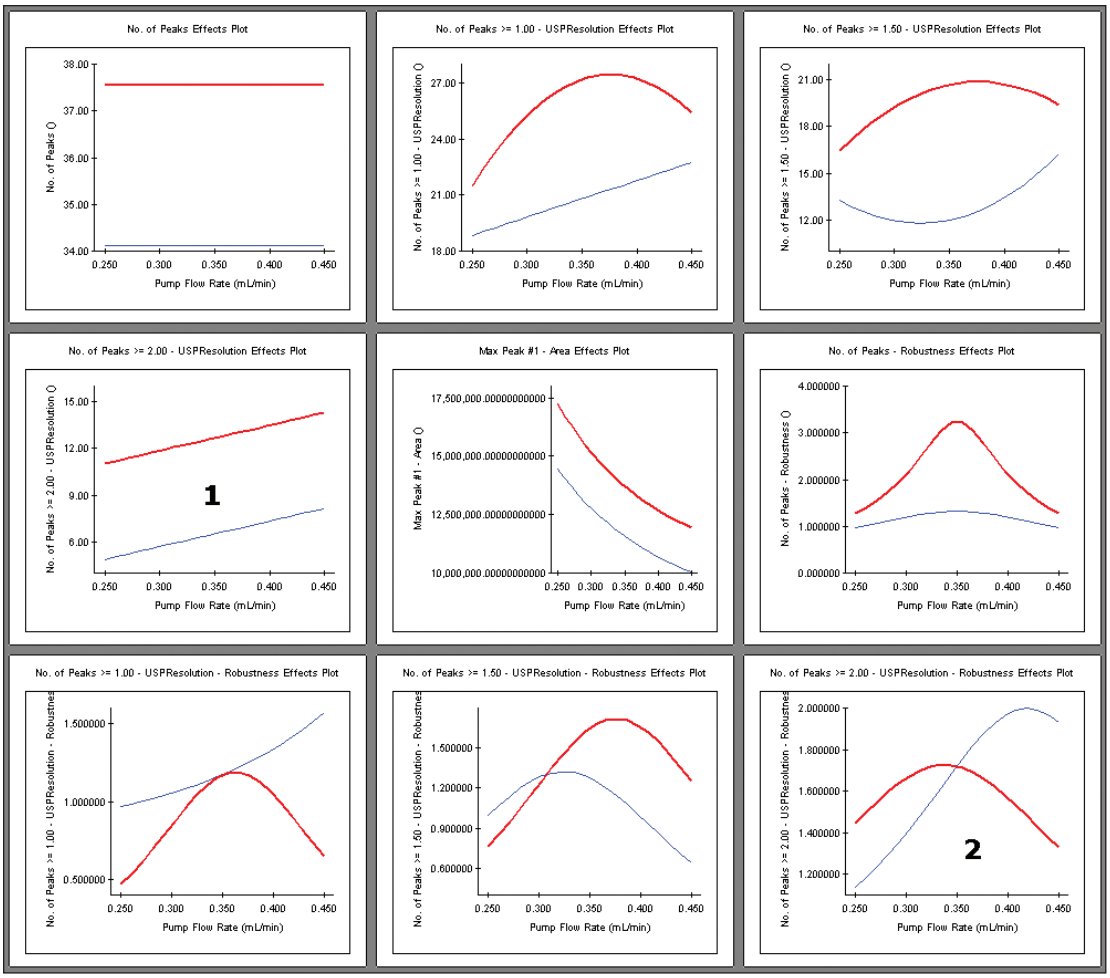


Figure 6. Effects plot responses.

Final results of method optimization

Next, an overlay graph is created using a number of critical chromatographic responses. Of primary importance for the vancomycin separation is maximizing the number of peaks observed and the number of peaks exceeding different levels of resolution while minimizing the area of the vancomycin peak, which equates to separating out the most impurities. The overlay graph shows the QbD Design Space (unshaded region) where the method meets the mean performance goals and robustness criteria (Figure 7).

Using ranked response variables, the Method Optimizer determines the optimum method to best meet the performance and robustness goals specified. The final method conditions are listed along with predicted response results with confidence limits for this method (Figure 8).

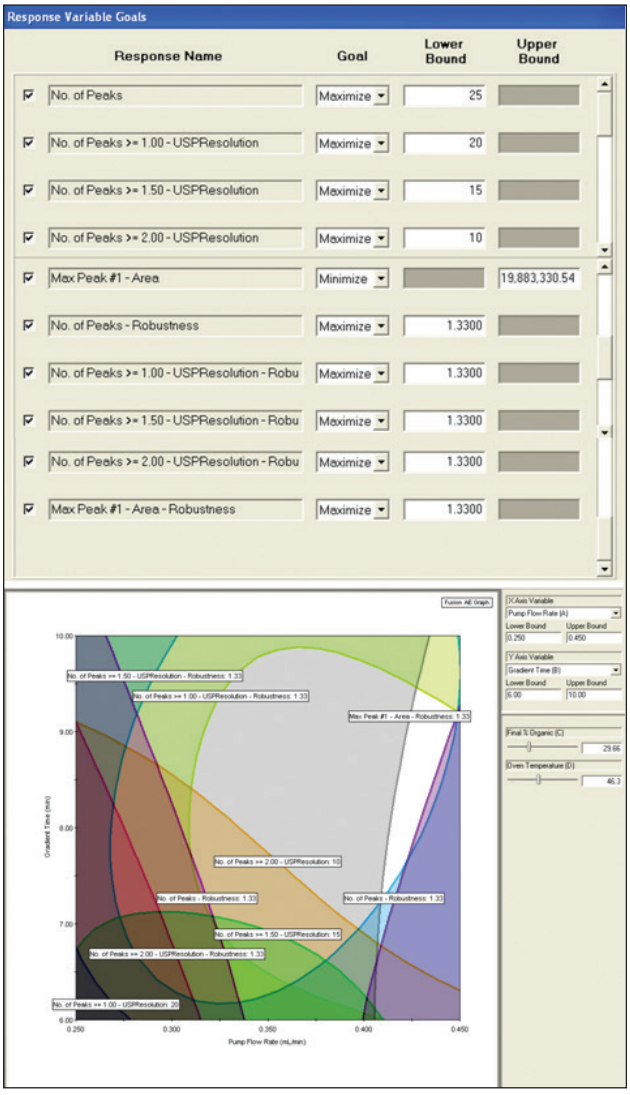


Figure 7. In the final overlay graph, the optimal QbD Design Space is the white or unshaded area.

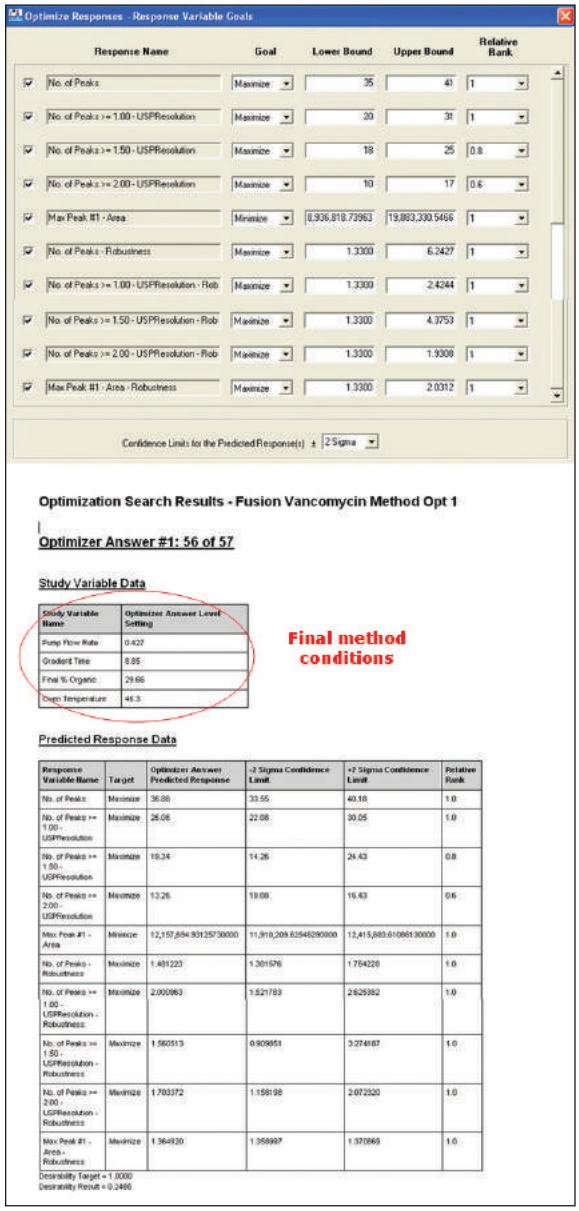


Figure 8. The Method Optimizer determines the optimum method to meet performance and robustness goals, and lists the final method and predicted responses.

Phase 3: Confirmation

Optimization results

The optimum method determined by the Fusion Method Optimizer was:

Column:	ACQUITY UPLC BEH C_8 Column, 2.1 x 100 mm, 1.7 µm
Mobile phase A:	10 mM Ammonium Acetate, pH 5.0
Mobile phase B:	Methanol
Flow rate:	0.427 mL/min
Gradient:	5% to 29.66% Methanol in 8.85 min
Column temperature:	46.3 °C

This method was exported to Empower 2 and the vancomycin sample was run to evaluate the prediction accuracy. The chromatogram in Figure 9 shows the separation of vancomycin impurities obtained with the optimized method.

The experimental results compare favorably with Fusion Software's predictions.

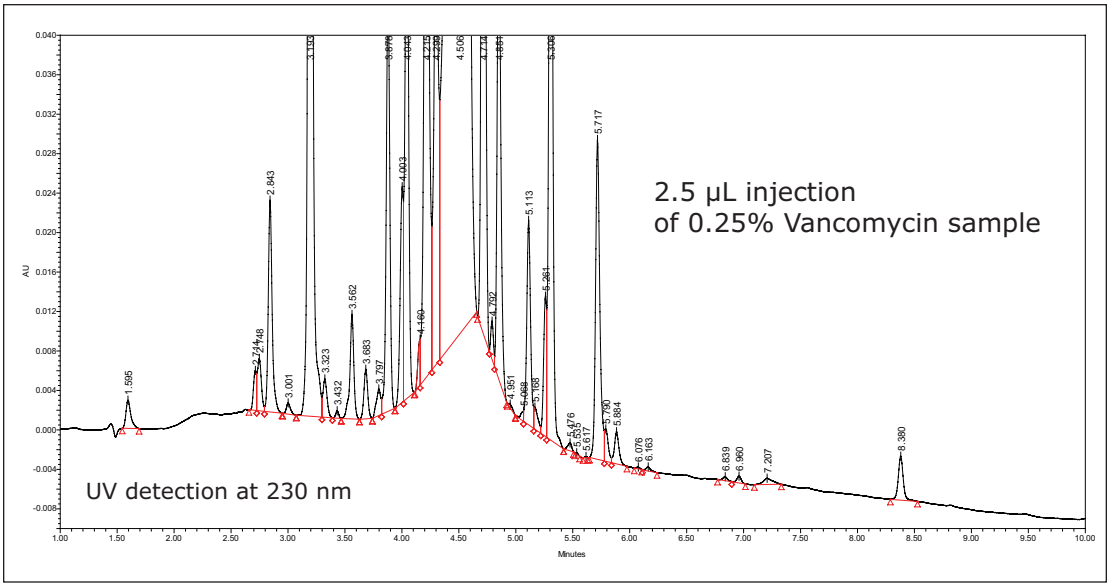


Figure 9. Confirmation run of the final UPLC method recommended by Fusion Software, where the number of impurities observed increased from 26 to 39.

Response variable	Predicted response	Experimental response
# of Peaks	36.9 Peaks	39 Peaks
# of Peaks ≥ 1.0 Rs	26.1 Peaks	27 Peaks
# of Peaks ≥ 1.5 Rs	19.3 Peaks	18 Peaks
# of Peaks ≥ 2.0 Rs	13.3 Peaks	12 Peaks

The QbD-based Fusion Software method improved the separation of impurities in vancomycin from 26, obtained previously with UPLC methods developed manually, to 39 impurities observed with the method shown.

CONCLUSIONS

- Fusion Method Development Software, used with the ACQUITY UPLC System, generated an optimized method for the analysis of vancomycin and its impurities in two business days.
- The use of UPLC data managed and processed by Fusion and Empower 2 software established a valid design space with both mean performance (set point optimization) and robustness (operating space).
- The QbD method's resolution improved from 26 peaks in previous method to 39 peaks.
- Integrated robustness calculations ensure a reproducible method, which increases confidence in the ability to validate and transfer that method.

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Enhancement of UV Detection Sensitivity in SFC Using Reference Wavelength Compensation

Lakshmi Subbarao, Jacquelyn Cole, and Rui Chen, Ph.D.
Waters Corporation, U.S.A.

APPLICATION BENEFITS

Using the built-in feature of Waters® 2998 PDA Detector, reference wavelength compensation, an average 3- to 5-fold increase in S/N was achieved for all tested compounds. The reported LOD and LOQ results indicate that SFC is an enabling analytical technique and suitable for use in the analysis of impurities, enantiomeric excess (EE) determinations, and QA/QC.

WATERS SOLUTIONS

Resolution™ SFC MS System

2998 Photodiode Array (PDA) Detector

3100 Mass Detector

MassLynx Software

KEYWORDS

SFC

Sensitivity

LOD

LOQ

Photodiode Array Detector (PDA)

Reference wavelength compensation

INTRODUCTION

Regulatory requirements for the identification, quantification, and control of impurities in drug substances and their formulated products are increasingly being explicitly defined, particularly through the International Conference of Harmonization (ICH). According to ICH, the threshold for identification and qualification of organic impurities is 0.10% for the majority of compounds, which implies a limit of quantification (LOQ) of 0.05% will be required for the involved analytical technology. With an increasing number of single enantiomers and stereoisomers being developed as drug candidates, detection and quantitation of chiral impurities to the 0.10% level are of great importance. Supercritical fluid chromatography (SFC) is a superior chromatographic technique for chiral separation; however, traditionally SFC UV has not been considered a highly sensitive technique.

While much effort has been applied to hardware improvement, using appropriate reference wavelength(s) compensation in data acquisition, a common built-in feature of photodiode array (PDA) detectors and the like, offers a facile means to effectively reduce most non-wavelength-dependent noise; thereby, increasing the overall signal/noise ratio (S/N). Reference wavelength compensation collects wide-band absorbance data in a region where the analytes have minimal or no absorption. The detector calculates the compensation value by averaging the absorbance values within the selected range of wavelengths. The averaged value is then subtracted from the absorbance value. Since the main absorbance includes the reference bands, noises from common sources including pump and back pressure regulator can be effectively reduced. The closer the reference bands are to the λ_{max} of the analyte of interest, the more effective the noise reduction.

In this application note, we demonstrate the enhancement of UV detection sensitivity in SFC by using reference wavelength compensation, a built-in feature of the 2998 PDA Detector under both MassLynx™ and Empower™ software.

EXPERIMENTAL

All experiments were carried out using a Waters Resolution SFC MS System. The system consists of a Fluid Delivery Module (FDM), Alias® Autosampler, Column Oven, Automated Back Pressure Regulator (ABPR), 3100 Mass Detector, and 2998 PDA Detector. MassLynx Software was used for data acquisition and analysis. In all experiments, the sampling rate for the 2998 PDA Detector was five point/s and the resolution was 3.6 nm.

For the experiments of hydrocortisone and caffeine, a 4.6 x 50 mm silica column was used. Key experimental parameters were as follows:

- Flow rate: 3 mL/min
- System pressure: 150 bar
- Temp.: 40 °C
- Injection volume: 5 µL (full loop)
- Isocratic method: 25% methanol
- Compensated wavelengths: 290 to 330 nm for hydrocortisone and 310 to 350 nm for caffeine

For the warfarin experiments, a 4.6 x 250 mm OD-H column was used. Key experimental parameters were as follows:

- Flow rate: 3 mL/min
- System pressure: 150 bar
- Temp.: 40 °C
- Injection volume: 5 µL (full loop)
- Isocratic method: 30.0% methanol with 0.4% N,N-dimethylethylamine (DMEA)
- Compensated wavelengths: 330 to 370 nm

All samples were dissolved in methanol. The concentrations for each compound were as follows: warfarin (5.0 mg/mL, or 2.5 mg/mL for each enantiomer); hydrocortisone (2.5 mg/mL); caffeine (2.0 mg/mL).

RESULTS AND DISCUSSION

Figure 1 shows a comparison of two hydrocortisone chromatograms at 0.125 µg/mL. Figure 1A represents a standard chromatogram at λ_{max}, whereas figure 1B shows a chromatogram acquired using reference wavelength compensation. While the peaks at 0.6 min have similar heights, the S/N of 1B is almost four times higher than 1A, suggesting that reference wavelength compensation provides a four-fold reduction in noise. On average, a minimum 3- to 5-fold increase in S/N was obtained in all compounds tested. Another example of reference wavelength compensation is shown in Figure 2 with caffeine at a concentration close to its LOD (0.025 µg/mL).

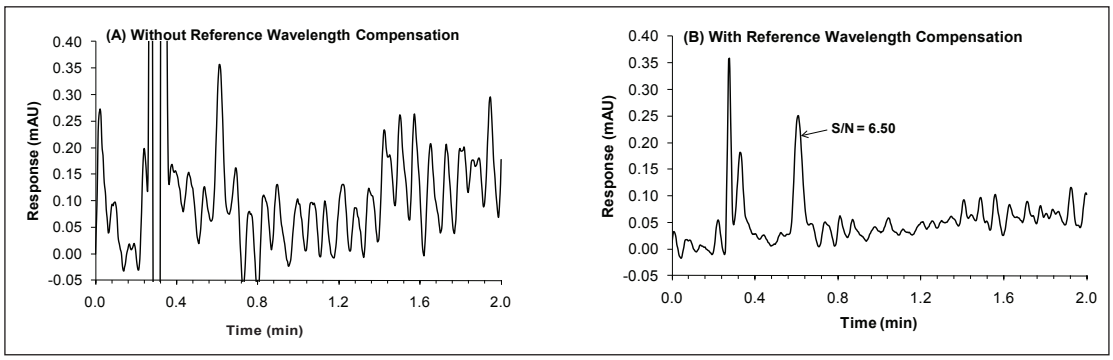

Figure 1. SFC UV chromatograms of hydrocortisone at 0.000125 mg/mL: (A) without reference wavelength compensation and (B) with reference wavelength compensation.

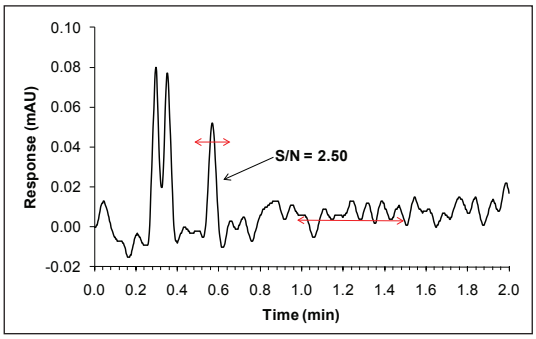

Figure 2. SFC UV chromatogram of caffeine at 0.00125% of the nominal concentration.

Next, we demonstrate the quantitative analysis of warfarin by SFC UV. Figure 3 shows the overlay of SFC UV chromatograms from five replicate injections of warfarin at 0.1% of the nominal concentration. Excellent reproducibility was achieved on both retention time and peak area as shown in Table I. At this concentration, the average S/N is above 100. Figure 4 shows the SFC UV chromatogram of warfarin at 0.005% of the nominal concentration. This concentration represents 0.000625 µg (0.625 ng) of each enantiomer on the column. Even at this low concentration, the S/N is still above 10 for peak 1, and slightly lower than 10 for peak 2.

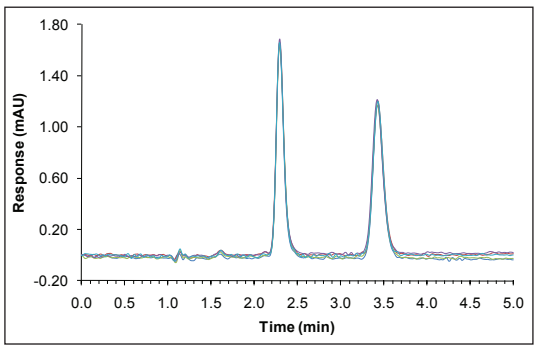

Figure 3. Overlay of the SFC UV chromatograms from five replicate injections of warfarin at 0.100% of the nominal concentration.

Nominal Concentration	Retention time (min)	RSD%	Peak area	RSD%	Avg. S/N
100%	2.288	0.080	184626	0.860	58070
10%	2.290	0.070	18170	0.670	5354
1%	2.293	0.100	1816	0.830	569
0.1%	2.300	0.080	175	0.770	135
0.01%	2.300	0.110	16	3.440	28
0.005%	2.300	0.070	8	2.680	12

Table 1. Statistics for the analyses of warfarin by SFC UV.

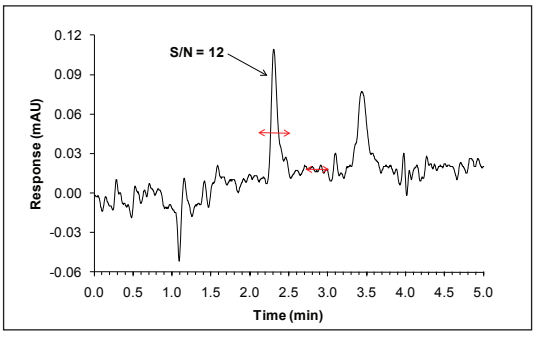

Figure 4. SFC UV chromatograms of warfarin at 0.005% of the nominal concentration.

Figure 5 shows the calibration curves for both peak 1 and peak 2, with a correlation coefficient of > 0.99999 for both curves. The linearity range expands from 0.005% to 100%, over four orders of magnitude of the nominal concentration. The curves for the two enantiomers also displayed excellent agreement between each other. It is noted, however, that in order to truly gauge the linearity encompassing such a wide concentration range, two calibration curves (one for low concentrations and one for high concentrations) are typically required.

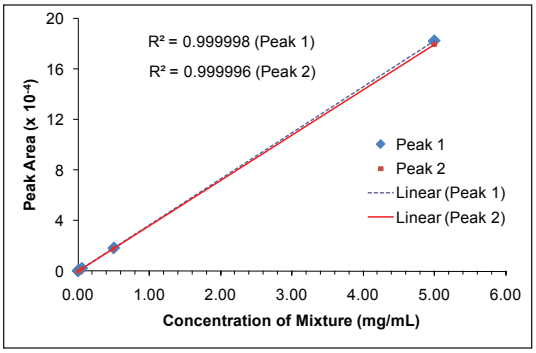

Figure 5. Calibration curve for the analyses of warfarin by SFC UV.

CONCLUSIONS

Using reference wavelength compensation featured in the 2998 PDA Detector, an average 3- to 5-fold increase in S/N was achieved for all tested compounds, indicating a 3- to 5-fold reduction in noise. Reference wavelength compensation produced an LOQ of 0.125 µg/mL (0.625 ng on the column) of each enantiomer of warfarin and over four orders of magnitude of linearity, the best sensitivity and widest dynamic range ever reported in SFC. These results indicate SFC is ready for prime time and suitable for use in the analysis of impurities, enantiomeric excess (EE) determinations, and QA/QC.

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A Quality by Design (QbD) Based Method Development for the Determination of Impurities in a Peroxide Degraded Sample of Ziprasidone

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Waters Corporation, 34 Maple St., Milford, MA, USA

APPLICATION BENEFITS

- Faster, efficient separations and data management using an ACQUITY UPLC® H-Class with Column Manager and Solvent Select Valve in conjunction with Empower™ 2 software.
- Built-in method robustness using a Quality by Design (QbD) approach to generate a method that is amenable to continuous improvement without re-validation in the future.
- Streamlined method transfer from UPLC® to HPLC using the ACQUITY UPLC Columns Calculator and Method Transfer Kits facilitates transfer of methods to labs that may not equipped with UPLC.
- Significant time savings using a statistical design of experiments approach to method development to generate efficient sample sets that cover a wide experimental space.

WATERS SOLUTIONS

ACQUITY UPLC H-Class system
ACQUITY UPLC CSH C_{18} Columns
XSelect™ CSH™ C_{18} Columns
Empower 2 CDs
Fusion AE™ Method Development Software (S-Matrix)

KEY WORDS

Method development, UPLC, method transfer, Quality by Design, ziprasidone, degradation, CSH, ACQUITY UPLC Columns Calculator

INTRODUCTION

Method development can be a time-consuming process that can be repeated many times thoughout a drug development pipeline. Methods are commonly developed using a one-factor-at-a-time (OFAT) approach where one variable is changed sequentially until a suitable method is produced. This type of development may create an adequate method but provides a limited understanding of method capabilities and method robustness. Rather, a systematic screening approach that evaluates a number of stationary phases, pH ranges and organic modifiers provides a more thorough approach to method development. A Quality by Design (QbD) approach to method development uses statistical design of experiments (DoE) to develop a robust method 'design space'. The design space defines the experimental region in which changes to method parameters will not significantly affect the results. This approach builds-in robustness to the method as the method is being developed[1].

A better understanding of the overall method capabilities and limitations in development ensures a greater chance of successful downstream method validation, transfer and routine use. Software-driven method development affords considerable time savings for the scientist and the use of QbD can produce a significantly more robust and quality submission to regulatory authorities.

In this application note, a QbD approach to method development and subsequent method transfer to HPLC is presented on a forced degradation sample of ziprasidone, an anti-psychotic drug. Method development was performed using an ACQUITY UPLC H-Class system equipped with a column manager and solvent select valve to allow for automated exploration of a wide range of conditions, while obtaining efficient separations with shorter chromatographic run times. Fusion AE Method Development software was used in conjuction with Empower 2 to facilitate a more comprehensive QbD approach to method development.

EXPERIMENTAL

ACQUITY UPLC H-Class Conditions

Mobile phase:

A: Acetonitrile

B: Methanol

D1: Water with 0.1% Formic Acid (pH 2.5)

D2: Water with 0.1% Ammonium Hydroxide (pH 10.5)

Columns (All 2.1 x 50 mm, 1.7 µm)

1. ACQUITY UPLC CSH C_{18}
2. ACQUITY UPLC CSH Fluoro-Phenyl
3. ACQUITY UPLC BEH Shield RP18
4. ACQUITY UPLC HSS C_{18} SB
5. ACQUITY UPLC HSS T3
6. ACQUITY UPLC HSS Cyano

Needle Wash:	10:90 Water:Methanol
Sample Purge:	90:10 Water:Methanol
Seal Wash:	90:10 Water:Methanol
Detection:	UV at 254 nm

SCREENING (PHASE 1)

Flow Rate:	0.6 mL/min
Injection Volume:	2 µL
Column Temp.:	30°C
Gradient Time:	5 min
Variables:	stationary phase, mobile phase, gradient endpoint % organic, mobile phase pH 2.5 to 10.5

OPTIMIZATION (PHASE 2)

Column:	ACQUITY UPLC CSH C_{18}, 2.1 x 50 mm, 1.7 µm
Mobile Phase:	A: Acetonitrile
	D1: Water with 0.1% Formic Acid (pH 2.5)
Gradient endpoint:	87.5% Acetonitrile
Variables:	gradient time, column temperature, injection volume, flow rate
Data Management:	Empower 2 CDS Fusion AE Method Development Software (S-Matrix)

SAMPLE PREPARATION

Ziprasidone peroxide degradation sample:

To 0.4 mg/ml ziprasidone in 50:50 water:methanol, add one equal volume of 3% hydrogen peroxide solution in water, heat at 80°C for 30 min. Dilute to 0.1 mg/mL final concentration with water.

RESULTS AND DISCUSSION:

Phase 1: Screening

Method development was performed using an ACQUITY UPLC H-Class system, Empower 2 and Fusion AE Method Development software. The H-Class was equipped with a 6-position column manager and a solvent select valve to enable full method development capability in one system. The initial screening varied column chemistries having CSH, BEH and HSS base particles for maximum selectivity. Organic modifier (acetonitrile or methanol) was screened varying the gradient endpoint from 50% to 100% organic, over a mobile phase pH range from 2.5 to 10.5.

Using these parameters, an experimental design was generated within Fusion AE, including randomization and replicate injections. The design generated encompassed the entire knowledge space defined by the constants and variables entered during the experimental setup. A partial factorial statistical design was selected by the software to obtain the maximum amount of information with the least number of experimental runs. The experimental design was transmitted to Empower2 software where all methods, method sets and sample sets were automatically generated and ready to run.

After initial integration and processing, results from the screening analysis for ziprasidone were imported back into Fusion AE and processed to generate an initial method for subsequent optimization. For the ziprasidone peroxide degradation sample, a water/acetonitrile gradient at pH 2.5 with an 87.5% acetonitrile gradient endpoint on a CSH C_{18} column was found to be optimal. The method developed is compatible with mass spectrometric detection and was directly transferred to LCMS to rapidly identify the ziprasidone forced-degradation products (Figure 1).

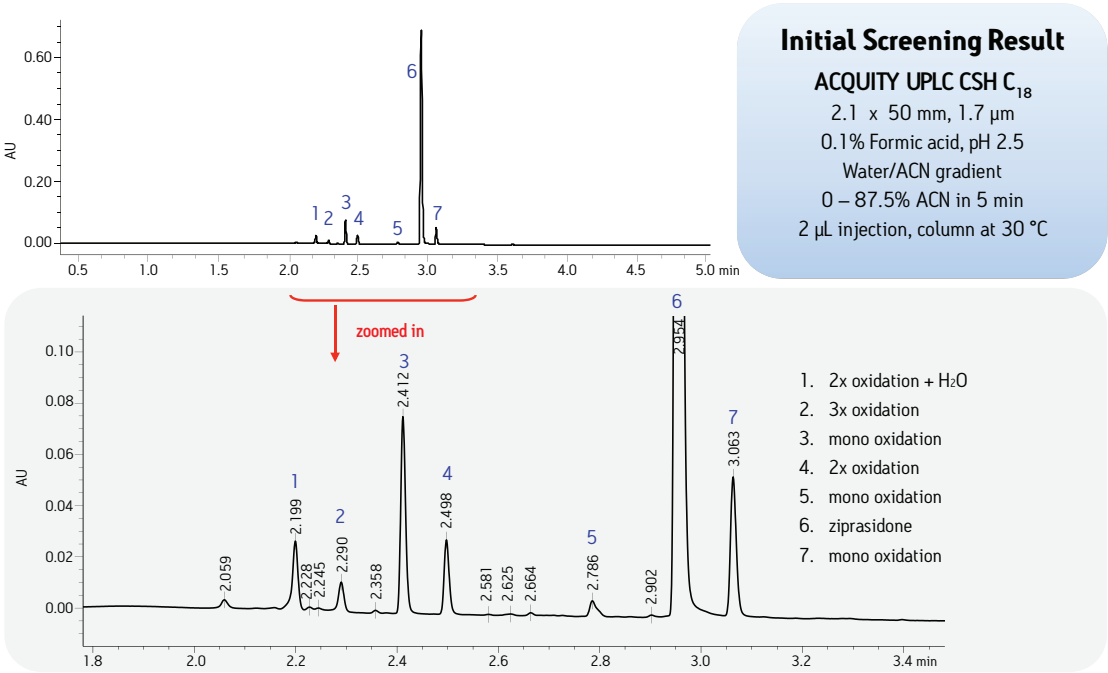

Figure 1. Initial method from screening experiments for ziprasidone peroxide degradation.

Phase 2: Method Optimization

The initial method was further optimized in a second experiment where secondary effectors such as column temperature, injection volume, gradient slope (modified using gradient time) and flow rate were varied. A new experimental design was generated by Fusion AE and new methods and sample sets were automatically created within Empower 2.

After processing data in Fusion AE, the final optimized method was generated, demonstrating the method that best meets the success criteria defined by the user. In the case of the ziprasidone peroxide degradation separation (Figure 2), an improvement in the tailing of peak 2 is seen along with better resolution of baseline impurity peaks and a newly resolved impurity is observed at 2.075 min.

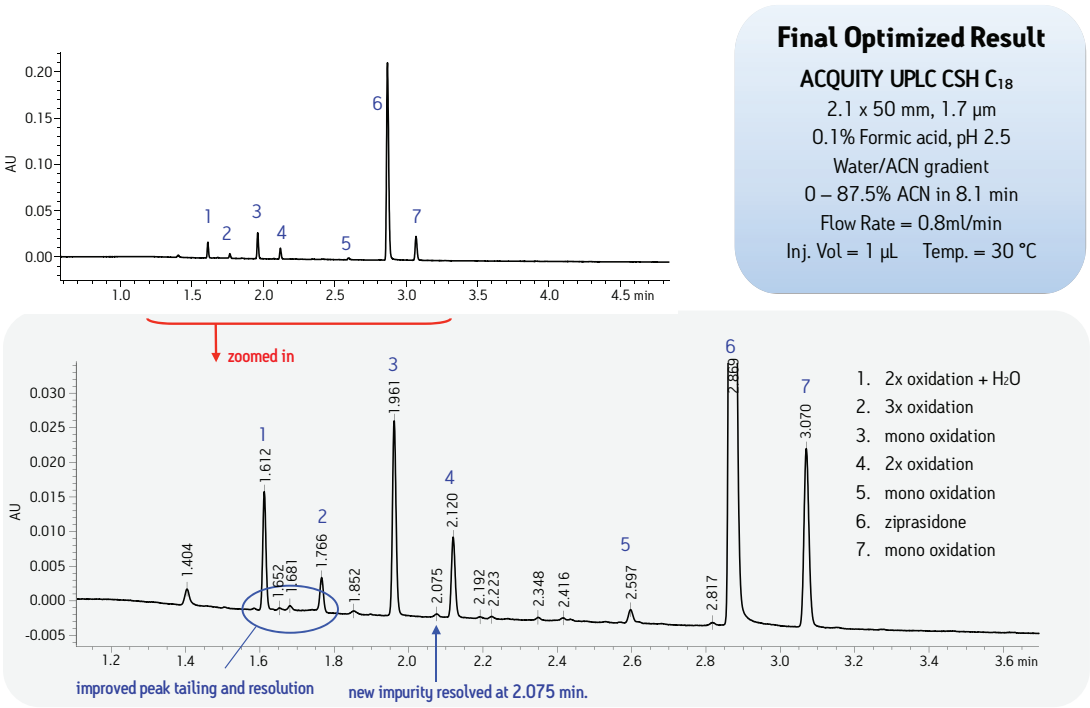

Figure 2. Final optimized method for ziprasidone peroxide degradation showing improved peak tailing and resolution.

Multi-dimensional plots in Fusion AE facilitates visualization of the effect of each factor on the separation (Figure 3). The white region of the 2D contour plot depicts the design space, which defines the robust region of the method where results are within designated criteria. By changing the factors on each axis, the design space can be explored in detail and method robustness can be fully understood.

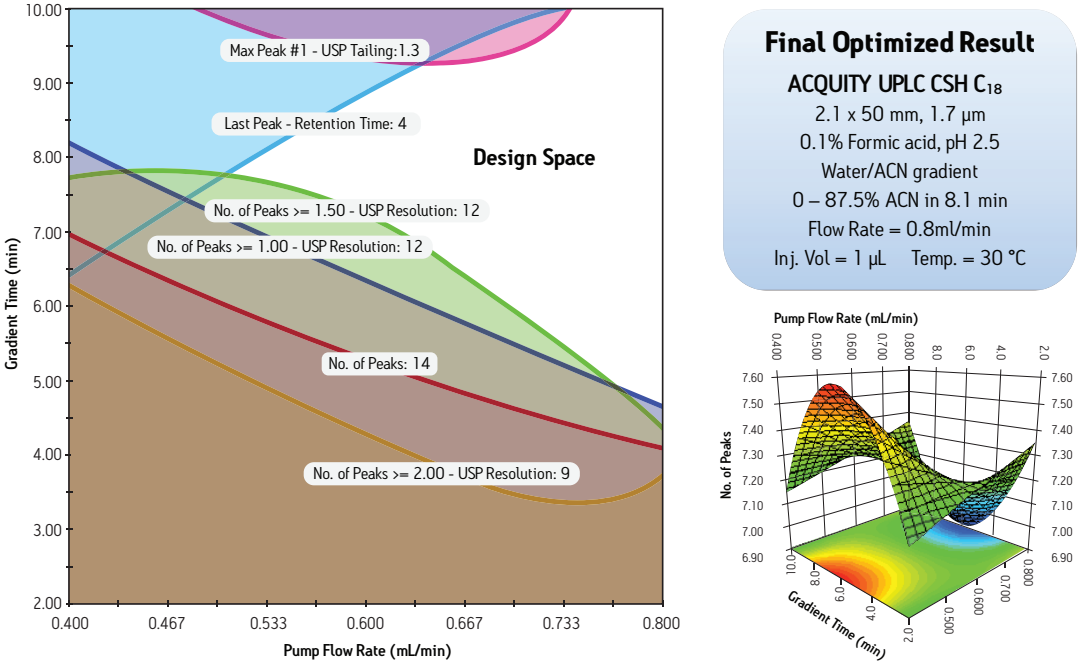

Figure 3. The design space region showing the independent effects of gradient time and pump flow rate on method success. Data can also be visualized in 3D plots as shown.

Method Transfer

The UPLC method developed using Fusion AE software was transferred to HPLC to demonstrate transferability from a method development laboratory to a quality control (QC) laboratory that might not be equipped with UPLC. Method transfer was performed using the ACQUITY UPLC Columns Calculator and Method Transfer Kit, scaling for particle size[2]. The method was scaled from the ACQUITY UPLC CSH C_{18} 2.1 x 50 mm 1.7 µm particle column to the corresponding XSelect CSH C_{18} 4.6 x 150 mm 5 µm HPLC column. A comparison of the UPLC and HPLC separation demonstrates that the peak profile and resolution is maintained when scaling to HPLC conditions from method development on UPLC.

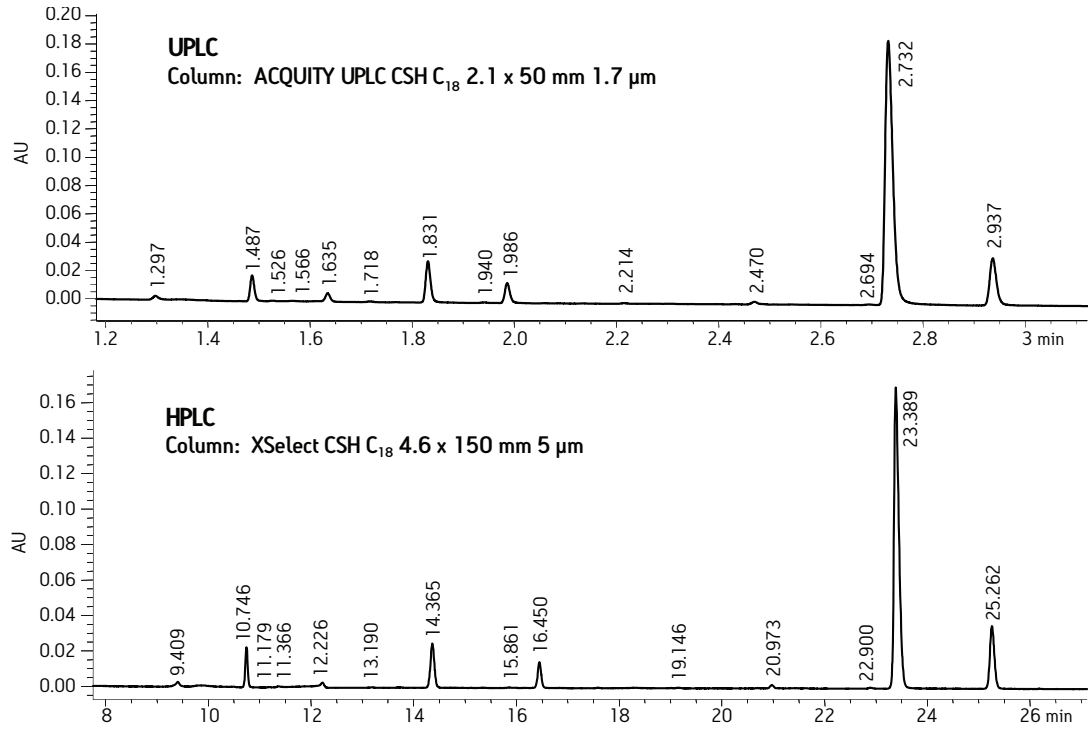

Figure 4. Method transfer from UPLC to HPLC for ziprasidone peroxide degradation.

CONCLUSIONS:

- A robust method for ziprasidone peroxide degradation was developed in two days using a Quality by Design approch on an ACQUITY UPLC H-Class system running Empower 2 and Fusion AE Method Development software.
- QbD method development software in conjunction with ACQUITY UPLC H-Class system automation allows for rapid screening and optimization across a wide range of column chemistries, mobile phases and pH ranges, while evaluating the effects of secondary factors such as column temperature, flow rate, injection volume and gradient slope on the separation.
- A comprehensive method development experiment can be rapidly performed by combining fast separations using UPLC with efficient experimental designs by Fusion AE Method Development software.
- The UPLC method developed for ziprasidone peroxide degradation was transferred to HPLC in one step using a Method Transfer Kit and ACQUITY UPLC Columns Calculator, demonstrating ease of transfer of developed methods to labs that may not be equipped with UPLC.

REFERENCES:

1. **Alden PG, Potts W, Yurach D. A QbD with Design-of-Experiments Approach to the Development of a Chromatographic Method for the Separation of Impurities in Vancomycin. Waters Application Note 70003719EN. 2010 Sept.**
2. **Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721EN. 2010 Sept.**

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Simplified Approaches to Impurity Identification using Accurate Mass UPLC/MS

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Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

This work demonstrates the use of Xevo G2 Tof and novel MS^E technology, and its ability to collect exact mass precursor and fragment ion information from every detectable component in a sample, in the analysis and identification of sildenafil impurities in counterfeit tablet samples.

WATERS PRODUCTS

Xevo® G2 Tof

ACQUITY UPLC ® System

ACQUITY UPLC BEH Column

MassLynx™ Software

MassFragment™ Software

KEY WORDS

Sildenafil, Impurity Analysis, Mass Defect Filter

INTRODUCTION

The profiling, identification, and quantification of impurities plays a critical role at all stages of the drug development and manufacturing process. The levels of impurities of pharmaceutical products are strictly controlled and regulated in order to ensure the safety of the administered drug product. The structure of the impurities must be identified and their pharmacology understood in order for the overall safety of the drug to be characterized.[1] In addition, pharmaceutical manufacturers monitor potential counterfeit drugs through the use of impurity profiling.

During the development process, many different routes of synthesis may be evaluated, each producing its own distinct impurity profile. It is critical that these routes of synthesis are thoroughly evaluated and that the impurities produced are identified. This activity is often performed using a combination of NMR and LC/MS/MS. Although modern nominal mass LC/MS instrumentation, such as a tandem quadrupole or ion trap mass spectrometer, can quickly produce high-sensitivity, fast scanning data, the interpretation of its data can be very time consuming and of limited utility due to low mass spectral resolving power. These techniques often require further confirmatory experiments to identify the structure of the impurities.

Modern high-sensitivity exact mass instruments with time-of-flight (Tof) technology capable of fast acquisition rates offer the opportunity to dramatically simplify the process of impurity data capture and analysis.

The use of simultaneous low and high collision energy data collection (MS^E) allows the interpretation of precursor and fragment ion data from a single analytical run. As this data is collected with a high degree of mass accuracy, elemental compositions can be obtained for both intact molecular ions and structurally significant fragments.

Sildenafil citrate is one of the approved synthetic phosphodiesterase Type-5 (PDE-5) inhibitors used in the treatment of erectile dysfunction (ED) and is widely counterfeited. In this application note, we present the use of the Xevo G2 Tof for the applicability of novel MS^E technology in the analysis and identification of sildenafil impurities present in counterfeit tablet samples.

EXPERIMENTAL

Sildenafil tablets were dissolved in a mixture of methanol/water (1:1), filtered, and transferred to an autosampler vial for analysis. The chromatography was performed on a 2.1 x 100 mm, 1.7-µm ACQUITY UPLC BEH C_{18} Column. The column was maintained at 45 °C. Reversed phase gradient elution was performed at 600 µL/min, using 10 mM NH_4OAC in water as the aqueous mobile phase, and acetonitrile as the organic modifier.

Positive ion electrospray, with a cone voltage of 30 V and a capillary voltage of 3k V were used. Data was collected over the mass range of 50 to 1000 *m/z*. The data was acquired in MS^E mode with the low energy data acquired using a collision energy of 4 eV. Elevated collision energy data was acquired using a collision energy ramp of 25 to 45 eV.

MassLynx 4.1 Software was used to acquire the data.

RESULTS

The total ion chromatogram (TIC) obtained from the analysis of a counterfeit tablet sample of sildenafil is displayed in Figure 1A. Here we can see that the parent drug elutes with a retention time of 8.49 min and there are at least eleven other peaks that elute between 2.75 and 9.79 min. Any of these peaks could represent drug-related impurities or excipient components from the tablet.

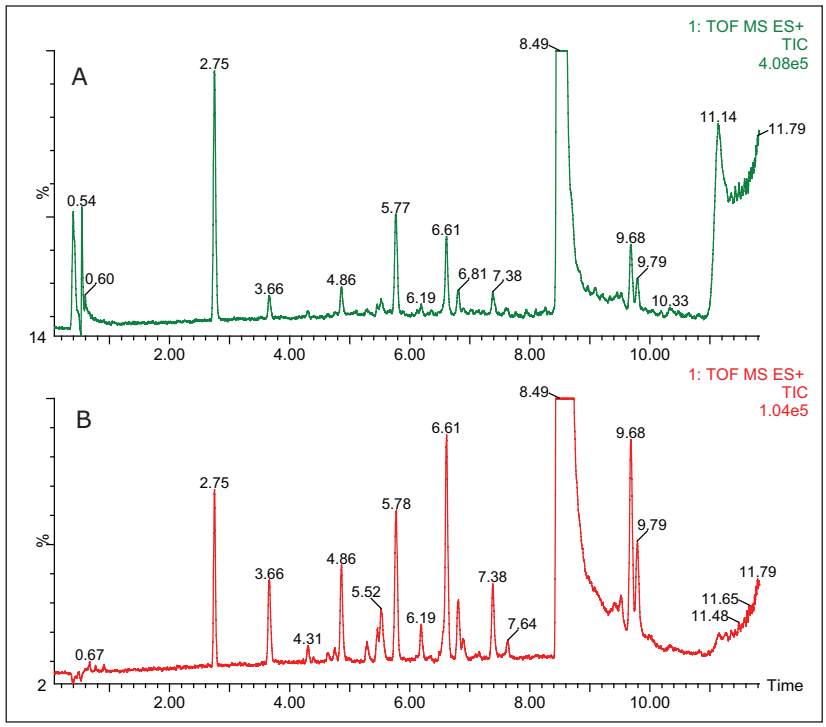

Figure 1. A) Total ion chromatogram obtained from the analysis of a counterfeit tablet of sildenafil. B) LC/MS data after filtering with the MDF.

The mass spectra displayed in Figure 2 show the elevated and low collision energy of the sildenafil peak. The low collision energy spectrum includes a peak with a measured mass of 475.2127, allowing an elemental composition of $C_{22}H_{31}N_6O_4S$ with a mass error of -0.1 mDa to be assigned. The high collision energy data gave rise to two main fragment ions, *m/z* = 100.0997 and 283.1191, which had mass errors of -0.3 mDa and -0.4 mDa, respectively.

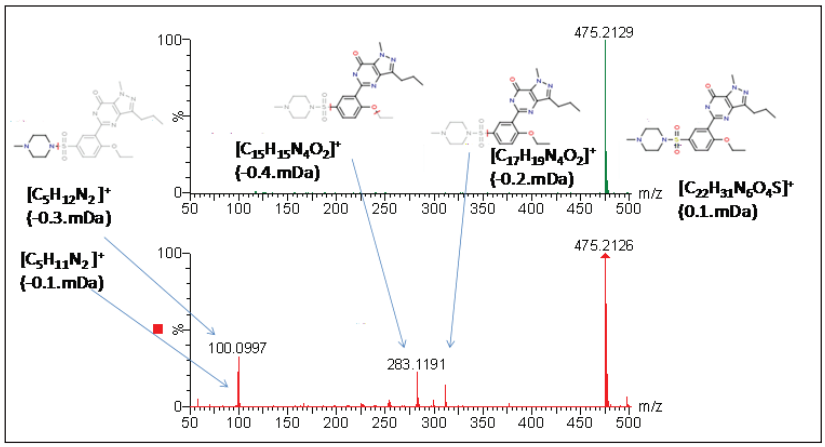

Figure 2. Mass spectra showing the elevated and low collision energy of the sildenafil peak.

The elemental composition and fragment ion structures generated using MassFragment are shown in Figure 2. MassFragment is software that automatically identifies product ion fragments using a series of chemically intelligent algorithms.[2]

The accurate mass capability of the Xevo G2 Tof mass spectrometer in conjunction with the software capabilities in MassLynx can be used to simplify data analysis. The fractional part of the exact mass of the parent drug *m/z* can be used to visualize only the drug related material by applying a mass deficiency filter (MDF) to the data. In this case, the exact mass of the sidenafil ion is 475.2127. A 20 mDa range was applied to the mass measurement after the decimal point to limit display.

The data displayed in Figure 1B shows UPLC/MS data after filtering with the MDF. Here we can see that there are at least 17 drug-related impurities. The use of MDF has greatly improved the signal-to-noise of the related components, most notably at RTs 5.52 min, 6.81 min and 7.64 min. This is due to the reduction in background noise, which allowed improved peak detection for the low concentration drug-related components.

The common fragment ions may also be used as an approach to highlight drug-related peaks. The data shown in Figure 3 shows the use of narrow window extracted ion chromatograms for the fragment ions *m/z* 283.1195 and 100.1001 using a 5 mDa window. From this data, we can see six possible drug-related impurities in each trace.

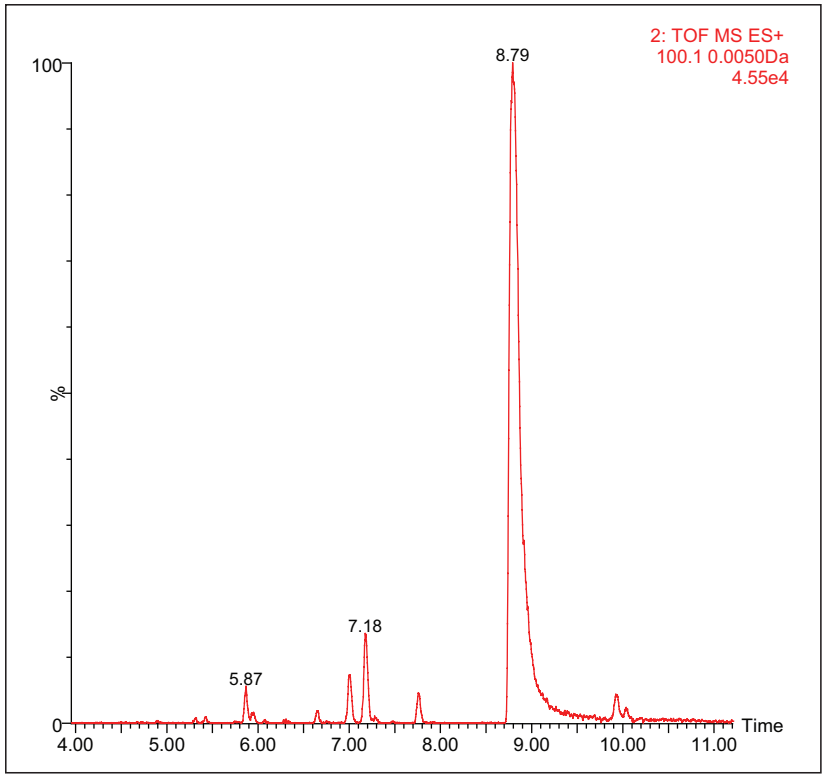

Figure 3. The use of narrow window extracted ion chromatograms for the fragment ions m/z = 283.1195 and m/z = 100.1001 using a 5 mDa window.

All of this data were available from one simple UPLC/MS experiment taking just 13 minutes.

Data was processed using MetaboLynx, an Application Manager within MassLynx Software. MetaboLynx MS^E fragmentation analysis was used to interrogate both low and high collision energy data simultaneously. This enables the visual alignment of precursor with collision induced dissociation fragment ions for sildenafil and its impurities. The automated structure elucidation tool, MassFragment, was employed to rationalize and identify fragment ion structures.

The elucidation of the impurity structures is facilitated by the elemental composition, which was obtained for both the fragment ions and parent compound molecular mass after data processing with MetaboLynx. The data in Figure 4 shows the elemental composition of the sildenafil-related impurities found in the sample.

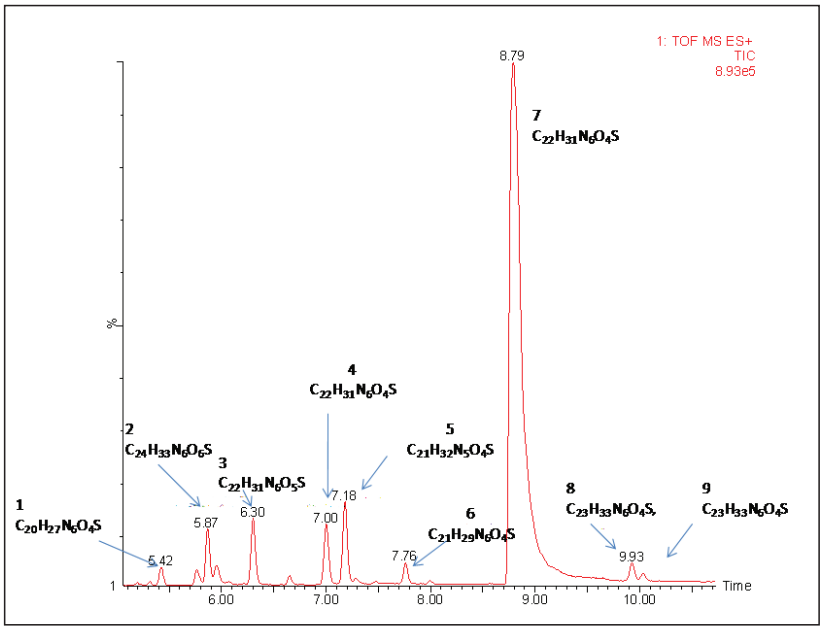

Figure 4. The elemental composition of the sildenafil related impurities found in the sample.

CONCLUSIONS

The Xevo G2 Tof operating in MS^E mode provides an excellent approach for the analysis of impurities in pharmaceutical products. The major benefits include:

- Precursor mass and fragment ion data is obtained in one simple acquisition, decreasing the need to re-inject samples for targeted ion MS/MS experiments.
- All the data is collected in accurate mass mode, allowing the elemental composition of parent and fragment ions to be derived.
- The Mass Defect Filter simplifies the task of detecting drug-related impurities by increasing the selectivity of data extraction and improving the visibility of detection for related components.
- The use of MetaboLynx and MassFragment allows rapid turnaround from data analysis to data interpretation and reporting.

References

1. Ahuja S and Alsante K. *Handbook of Isolation and Characterisation of Impurities in Pharmaceutical Compounds*. 2003; Elsevier.
2. Jones M.D et al. *Identification and Characterization of an Isolated Impurity Fraction: Analysis of an Unknown Degradant Found in Quetiapine Fumarate*. Waters Application Note. 2009; 720003079en.

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Streamlining Current Approaches for Extractable Analysis Utilizing Waters MV-10 ASFE and ACQUITY UPC^2 Systems

Baiba Čabovska, Andrew Aubin, and Michael D. Jones
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- SFE offers greater flexibility than microwave extraction and represents a substantial savings in solvent consumption and run time when compared to Soxhlet extraction
- UPC^{2}™ enhances extractables analysis by streamlining the workflow

WATERS SOLUTIONS

ACQUITY UPC^2 System configured with PDA and SQD Detection

MV-10 ASFE™ System

Empower™ 3 Software

KEY WORDS

Extractables, SFE, UPC^2, supercritical fluid, convergence chromatography

INTRODUCTION

Analysis of extractables in the pharmaceutical and food packaging industries is well established.[1-3] Analytical workflows can incorporate various techniques. Similarly, the evaluation of container closure systems can include various extraction techniques. The ACQUITY UPC^{2}™ System streamlines the analytical workflow by providing flexibility with various common solvent systems resulting from extraction procedures.[4] While supercritical fluid plays a key role in improving analytical workflow, the question is raised: "Can the sample extraction process be streamlined to utilize one technique, namely a supercritical extraction process?"

Several techniques can be used to prepare sample extracts in the extractables analysis process. Typically, either a Soxhlet, microwave, or supercritical fluid extraction (SFE) are performed. The extraction solvents must cover a wide range of polarities to ensure that non-polar and polar analytes are extracted from packaging material. The Soxhlet apparatus can be a very attractive option due to its relatively inexpensive setup. However, when the price of extraction solvents and their waste disposal is considered, microwave and SFE offer cost saving benefits including reduced solvent consumption and waste disposal, as well as valuable reduction in analysis time.

In this application, four different types of packaging material were extracted including: high density polypropylene pill bottle (HDPE), low density polypropylene bottle (LDPE), ethylene vinyl-acetate plasma bag (EVA), and polyvinyl chloride blister pack (PVC). Following extraction, the resulting solutions were rapidly screened for 14 common polymer additives using an UltraPerformance Convergence™ Chromatography (UPC^2) System with PDA and single quadrupole (SQD) mass detection. Microwave and Soxhlet were used to separately prepare IPA and hexane extracts, while different concentrations of IPA were used as the co-solvent for SFE extractions. Here, the extraction profiles of the different techniques are compared.

EXPERIMENTAL

Method conditions

UPC² Conditions

System:	ACQUITY UPC² with PDA and SQD Detection
Column:	ACQUITY UPC² BEH 2-EP 3.0 x 100 mm, 1.7 µm
Modifier:	1:1 methanol/ acetonitrile
Flow rate:	2 mL/min
Gradient:	1% B for 1 min, to 20% over 2.5 min, hold for 30 s, re-equilibrate back to 1%
Column temp.:	65 °C
APBR:	1800 psi
Injection volume:	1.0 µL
Run time:	5.1 min
Wavelength:	220 nm
MS scan range:	200 to 1200 *m/z*
Capillary:	3 kV
Cone:	25 V
Make-up flow:	0.1% formic acid in methanol, 0.2 mL/min
Data management:	Empower 3 Software

Sample description

Microwave Extractions

The samples of HDPE, LDPE, EVA, and PVC (2 g) were cut into 1x1 cm pieces and subsequently extracted in either 10 mL of isopropanol or 10 mL of hexane for 3 h at 50 °C.

Soxhlet Extractions

Soxhlet extractions were performed by placing cut pieces (roughly 1x1 cm) of material (3 g for PVC, 5 g for HDPE, LDPE, or EVA) into a Whatman 33 x 94 mm cellulose extraction thimble. The thimble was then placed in a conventional Soxhlet extraction apparatus, consisting of a condenser, a Soxhlet chamber, and an extraction flask. Approximately 175 mL of extraction solvent (either hexane or IPA) was added into the Soxhlet apparatus. All samples were extracted with the hot boiling solvent mixture for 8 h. Upon completion, the extraction solvent was reduced to near dryness and reconstituted in 15 mL of either hexane or IPA. Prior to analysis, extracts were filtered through a 0.45-µm glass fiber syringe tip filter to remove any particulates.

SFE

Supercritical fluid extraction (SFE) was performed using a Waters® MV-10 ASFE System. For each SFE experiment, cut pieces (roughly 1x1 cm) of material were loaded into 10-mL stainless steel extraction vessels (2 g for PVC, 3 g for HDPE, LDPE, or EVA). Two distinct extractions were performed on each material. The first used 5.0 mL/min carbon dioxide plus 0.10 mL/min IPA, the second used 4.0 mL/min carbon dioxide plus 1.0 mL/min IPA. All extractions were performed at 50 °C and 300 bar back pressure using a 30-min dynamic, 20-min static, and 10-min dynamic program that was repeated twice. IPA was used as a makeup solvent at 0.25 mL/min. For high IPA extractions, following the extraction process, collected solvent (a mixture of the co-solvent and make-up solvent) was reduced to near dryness and reconstituted in IPA (10 mL for PVC, 9 mL for HDPE, LDPE, and EVA). For low IPA extractions, the collected solvent was brought up to volume accordingly. Prior to analysis, extracts were filtered through a 0.45-µm glass fiber syringe tip filter to remove any particulates. Total extraction time per sample was 2 h.

RESULTS AND DISCUSSION

Comparing the duration of the extraction processes, Soxhlet extracted each sample individually for 8 h. Microwave could accommodate up to 16 samples simultaneously over a 3-hour extraction. The SFE process took 2 hours per sample with up to 10 samples loaded onto the sample tray. Even if more Soxhlet apparatus were used simultaneously, the total extraction time would still significantly exceed microwave or SFE extraction times.

In terms of solvent usage, Soxhlet required up to 175 mL of solvent, followed by evaporation to reduce sample volume. Microwave used 10 mL of solvent that could be dried down if improvements in sensitivity are needed. SFE offered the greatest flexibility in sample pre-concentration. Under low IPA extraction conditions, the final volume collected was approximately 5 mL, and brought up to volume to have the concentration of the sample comparable to microwave and Soxhlet samples. Under high IPA extraction conditions, the total volume collected was ~30 mL, which had to be evaporated to obtain the final concentration.

The fewest number of extractables were observed in the PVC and EVA samples analyzed after microwave extraction. The most extractables were observed using either hexane or IPA extract in the LDPE sample, as shown in Figure 1.

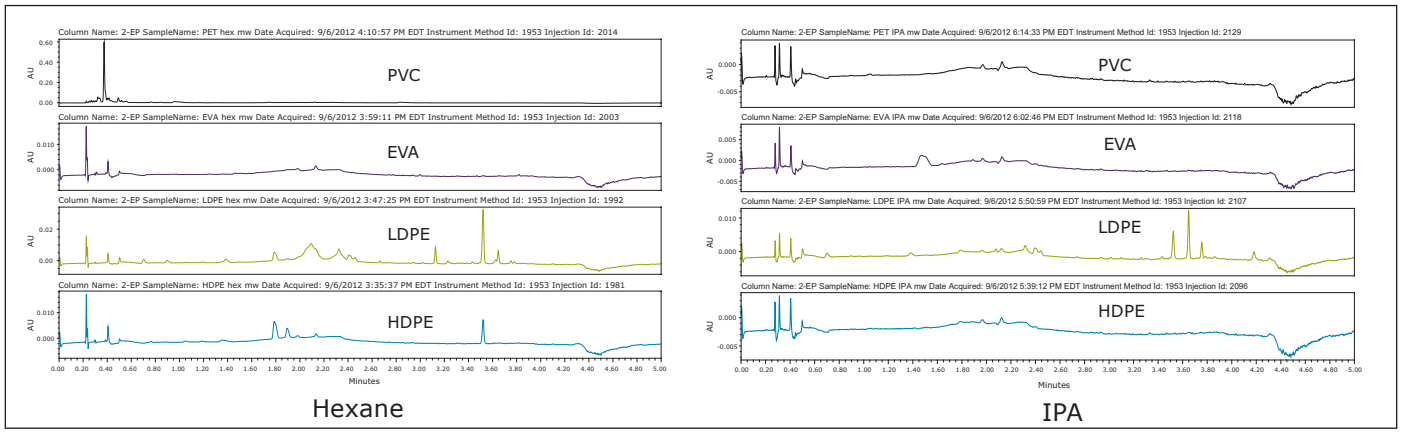

Figure 1. Hexane and IPA extracts using the microwave extraction technique.

Using Soxhlet extraction, several additional peaks were observed in the PVC chromatograms, as shown in Figure 2, which were not visible following microwave extraction. The observable differences are possibly due to the longer extraction times and higher extraction temperature used in Soxhlet extraction.

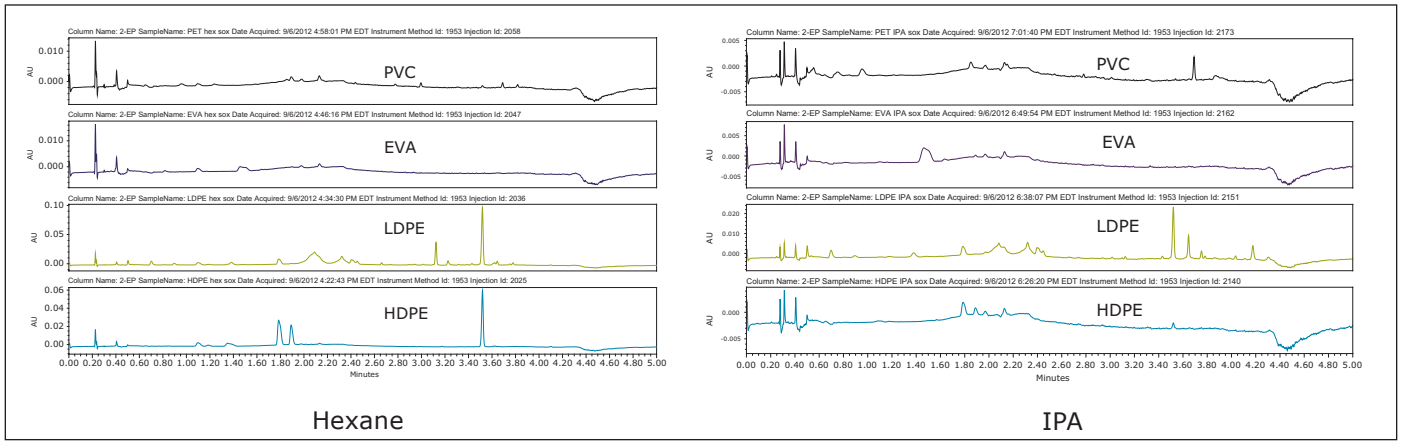

Figure 2. Hexane and IPA extracts using the Soxhlet extraction method.

Visually comparing SFE extraction profiles with the other two techniques, SFE extracted similar amounts of analytes as Soxhlet, and a greater amount than microwave extraction of PVC, as shown in Figure 3. High IPA extracted higher amounts in LDPE than the lower percentage in the IPA extraction experiment. This illustrated the flexibility and ease of adjusting to determine the optimal percentage of modifier needed for each plastic material to achieve a successful extractables analysis.

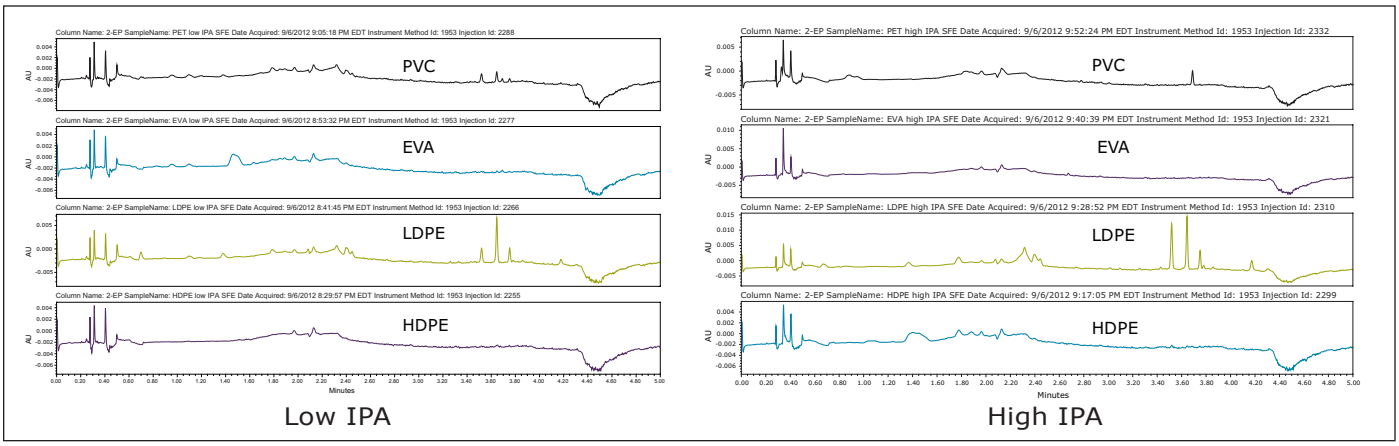

Figure 3. SFE extracts with low and high volumes of IPA co-solvent.

All extraction techniques using IPA as the solvent produced similar chromatographic profiles for the LDPE sample, as seen in Figure 4. Concentration of the extractables can be increased by extended extraction times, higher temperature in microwave and Soxhlet extractions, or a higher level of IPA in the case of SFE. Hexane extractions were not performed by SFE since CO_2 is a non-polar solvent with similar chemical properties to hexane; therefore, comparable results were expected.

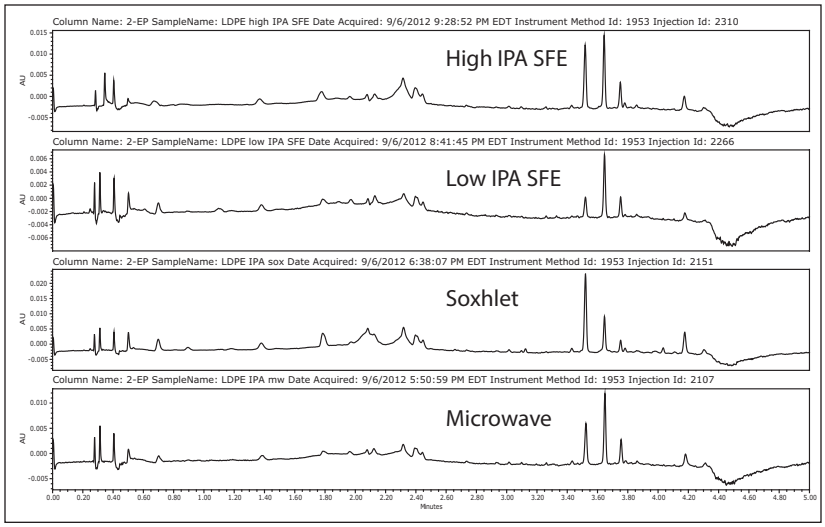

Figure 4. IPA extracts for LDPE.

Examples of identified compounds in LDPE hexane extracts are shown in Figure 5.

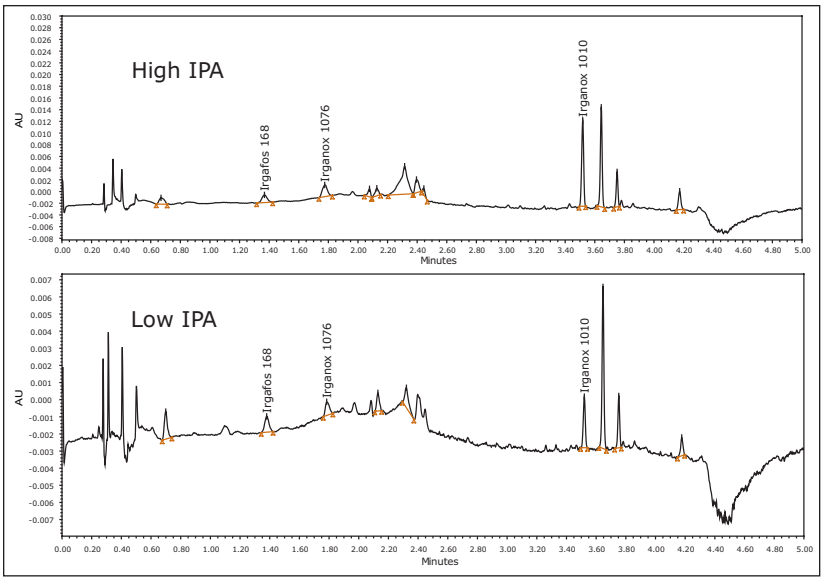

Figure 5. Identified extractables in LDPE, SFE extracts.

In summary, all of the techniques are comparable in terms of types of compounds extracted. However, it was determined that SFE offers many advantages over other extraction techniques when time and resources are important. The MV-10 ASFE System is software controlled, providing automated method development. There can be up to four co-solvents available for use, and various percentages and extraction times can be set in the methods. Soxhlet and microwave require manual solvent changes for each step in method development, which is quite time-consuming when conducting a quality by design (QbD) study.

CONCLUSIONS

SFE provided 80% to 97% savings in solvent consumption, and a 75% savings in extraction time compared to Soxhlet extraction. The software controlling SFE allowed automated method development to determine the optimal percentages and choices of extraction co-solvent. In addition, SFE provided flexibility in sample pre-concentration compared to microwave extraction.

References

1. Containers Closure Systems for Packaging Human Drugs and Biologics; Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER); Rockville, MD. 1999 May.
2. Norwood DL, Fenge Q. Strategies for the analysis of pharmaceutical excipients and their trace level impurities. *Am Pharm Rev.* 2004; 7(5): 92,94,96-99.
3. Ariasa M, Penichet I, Ysambertt F, Bauza R, Zougaghc M, Ríos Á. Fast supercritical fluid extraction of low- and high-density polyethylene additives: Comparison with conventional reflux and automatic Soxhlet extraction. *J Supercritical Fluids.* 2009; 50: 22-28.
4. Cabovska B, Jones MD, Aubin A. Application of UPC^2 in extractables analysis. Waters Application Note 720004490en. 2012 November.

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Application of UPC² in Extractables Analysis

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APPLICATION BENEFITS

- UPC²™ provides a technique for analysis of non-volatile and semi-volatile extractables, as well as polar and non-polar compounds
- Provides a turnkey single instrument approach for extractable and leachable studies
- Rapid analysis of container closure systems used for pharmaceutical, food, and clinical products

WATERS SOLUTIONS

ACQUITY UPC²™ System

ACQUITY® SQD Mass Spectrometer

Empower™ 3 Software

UPC² columns

KEY WORDS

UPC², SFC, extractables, polymer additives, UltraPerformance Convergence™ Chromatography

INTRODUCTION

Extractables from packaging materials are a concern to manufacturers and suppliers of containers for the heavily regulated pharmaceutical and food industries.[1-3] Due to these regulations, packaging material manufacturers are motivated to control and monitor their product to ensure that no potential risk exists from extractable and leachable material. Similarly, the manufacturers of supplies for industrial processes, such as plastic vessels and filters, are required to demonstrate that their products do not add any leachables in the production process.

The initial investigation, called a controlled extraction study, qualitatively and quantitatively investigates the nature of extractable profiles from critical container closure system components. It is performed early in device and packaging development. The testing involves solvent extraction techniques encompassing a range of polarity, solvent compatibility studies, and multiple analytical techniques. One of the limitations encountered in these studies involved matching the solvent extracts with the appropriate analytical technique. For example, non-polar solvent extracts can be directly injected into a gas chromatography (GC) system but must be evaporated and reconstituted with a solvent compatible with a liquid chromatography (LC) system. Likewise, water extracts must be back-extracted into a non-polar solvent for analysis by GC. UltraPerformance Convergence Chromatography (UPC²), built on the principles of supercritical fluid chromatography (SFC), allows different types of extraction solvents to be injected for separation on one system for analysis, thereby saving time and reducing sample preparation efforts.

In this application, four different types of packaging material were extracted, including a high density polypropylene pill bottle (HDPE), a low density polypropylene bottle (LDPE), an ethylene vinyl-acetate plasma bag (EVA), and a polyvinyl chloride blister pack (PVC). The extracts were screened for 14 common polymer additives. Hexane, isopropanol (IPA), and water were used as the extraction solvents. GC-MS was used to analyze hexane and IPA extracts, the ACQUITY UPLC® System was used to analyze water and IPA extracts, and the ACQUITY UPC² System was used to analyze all three solvent extracts. The UPC² analysis was compared to the GC and UPLC chromatographic profiles.

EXPERIMENTAL

Sample Description

Samples were prepared by microwave extraction. The samples of HDPE, LDPE, EVA, and PVC (2 g) were extracted in 10 mL of isopropanol or hexane for 3 h at 50 °C. Water extracts were prepared by placing 2 g of sample into 20 mL headspace vials with 10 mL of water, and keeping them in a conventional oven for 72 h at 50 °C.

GC-MS Conditions

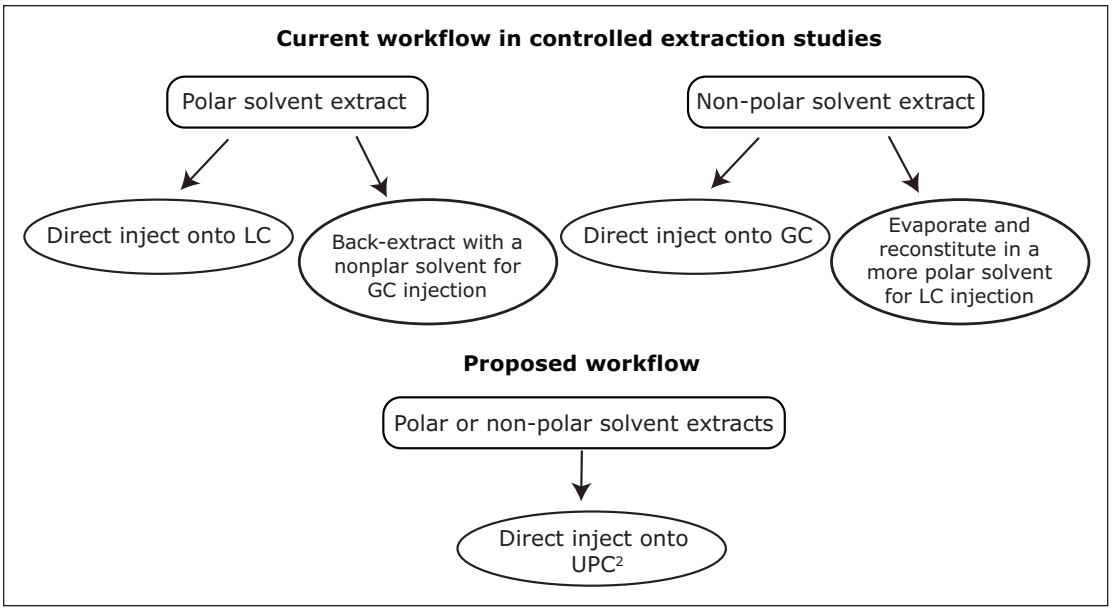
Column:	HP-5MS 30 m x 0.32 mm, 1.0 µm film
Carrier gas:	He at 2 mL/min
Temperature program:	35 °C for 5 min, 20 °C/min to 320 °C, hold 20.75 min
Injection port:	300 °C
Injection type:	1 µL splitless, 1 min purge
Makeup gas:	N_2 at 400 mL/min
Transfer line:	350 °C
Scan range:	100 to 1500 *m/z*
Run time:	40 min
Data management:	MassLynx™ *v*4.1 Software

UPC² Conditions

System:	ACQUITY UPC²
Detection:	Photodiode Array (PDA) Detector and SQD Mass Spectrometer
Column:	ACQUITY UPC² BEH 2-EP 3.0 x 100 mm, 1.7 µm
Mobile phase A:	CO_2
Mobile phase B:	1:1 methanol/acetonitrile
Flow rate:	2.0 mL/min
Gradient:	1% B for 1 min, to 20% over 2.5 min, hold for 30 s, re-equilibrate back to 1%
Column temp.:	65 °C
APBR:	1800 psi
Injection volume:	1.0 µL
Run time:	5.1 min
Wavelength:	220 nm
MS scan range:	200 to 1200 *m/z*
Capillary:	3 kV
Cone:	25 V
MS make-up flow:	0.1% formic acid in methanol, 0.2 mL/min
Data management:	Empower 3 Software

UPLC Conditions

System:	ACQUITY UPLC
Column:	ACQUITY UPLC BEH Phenyl 2.1 x 100 mm, 1.7 µm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.9 mL/min
Gradient:	50% B to 90% over 10 min, re-equilibrate back to 50% B
Column temp.:	50 °C
Injection volume:	2 µL
Run time:	12 min
Wavelength:	220 nm
MS scan range:	200 to 1500 *m/z*
Cone:	30 V
Capillary:	3 kV
Data Management:	Empower 2 Software



RESULTS AND DISCUSSION

The structures for polymer additives screened in this method are shown in Figure 1. They cover different classes of additives, such as plasticizers, antioxidants, and UV-absorbers.

Comparing the separation of the standards by each analytical technique, as shown in Figure 2, UPLC and UPC^2 were applicable to all 14 compounds chosen. The elution order was different for both methods due to orthogonal selectivity. The ACQUITY UPC^2 System provided a shorter run time compared to the ACQUITY UPLC System. It was observed that the thermal instability of some analytes, such as Irganox 1010 and Irganox 245, prevented successful chromatographic separation by GC-MS. Late eluters from Irgafos 168 to Uvitex OB produced wide peaks in GC-MS, possibly due to secondary interactions with the stationary phase or on-column degradation. The compounds selected for this screening were more compatible with liquid chromatography or convergence chromatography than with gas chromatography analysis.

Water extracts analyzed by the ACQUITY UPLC and ACQUITY UPC^2 systems did not have any peaks present (data not shown). This was expected, since water is the most common solvent present in the environment. Manufacturers avoid formulating their products to be susceptible to water solubility.

In the other two extracts, hexane and IPA, LDPE had the most extractables present, as seen in Figure 3. IPA extracts analyzed by UPLC (data not shown) produced less intense peaks than UPC^2. Prior to UPLC analysis, the hexane extracts were reduced to dryness, re-dissolved in solvent, and analyzed by UPLC (data not shown). Both the ACQUITY UPLC and ACQUITY UPC^2 systems showed the same set of extractable compounds present in the samples.

Noisy baselines were observed with the GC-MS analysis. When utilizing this technique, extracted ion chromatograms of known polymers had to be performed, thus making it difficult to screen for unknown extractables in packaging products, as shown in Figure 4. A sample pre-concentration step could have improved the intensity of the detected peaks.

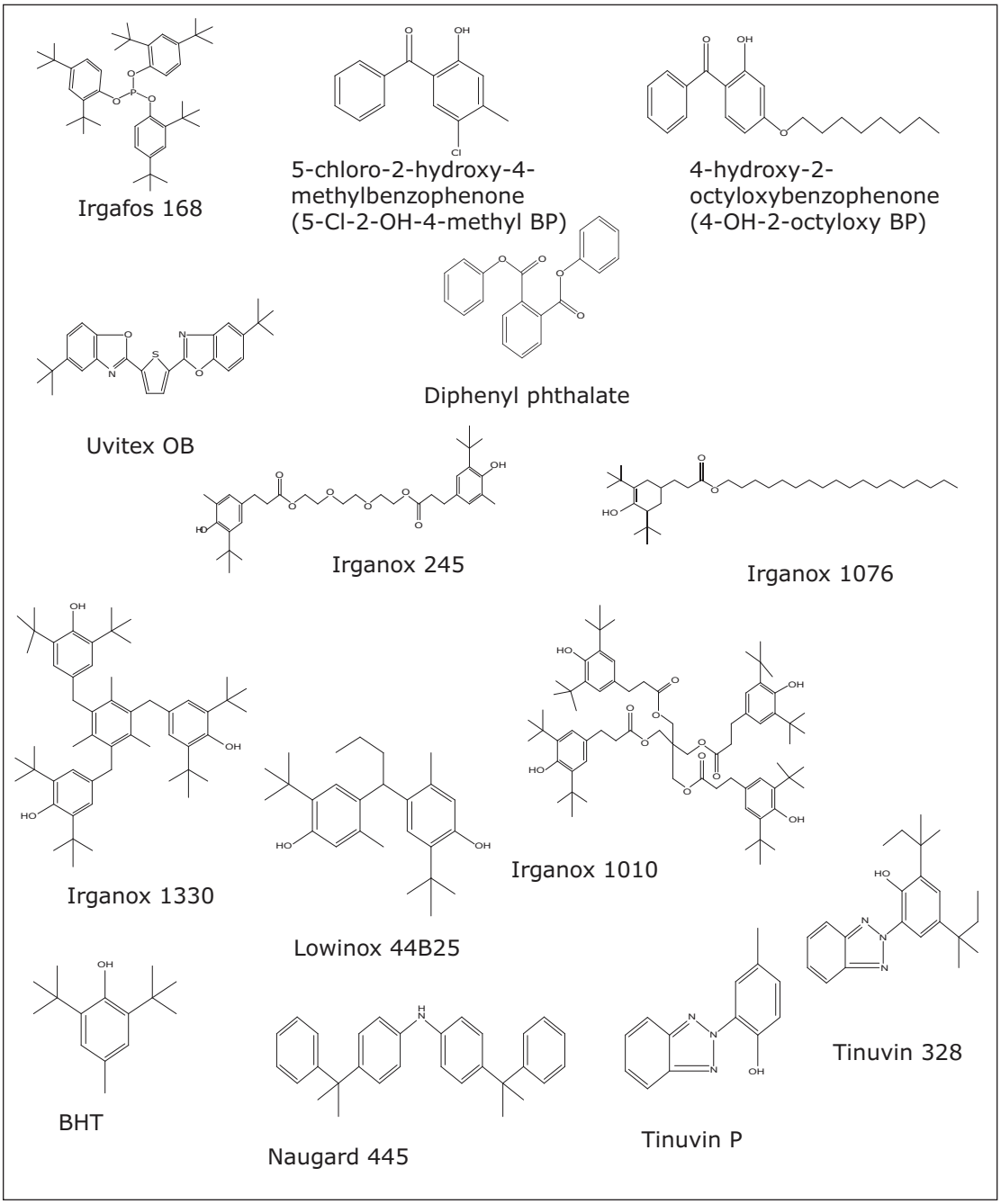


Figure 1. Polymer additives and their structures.

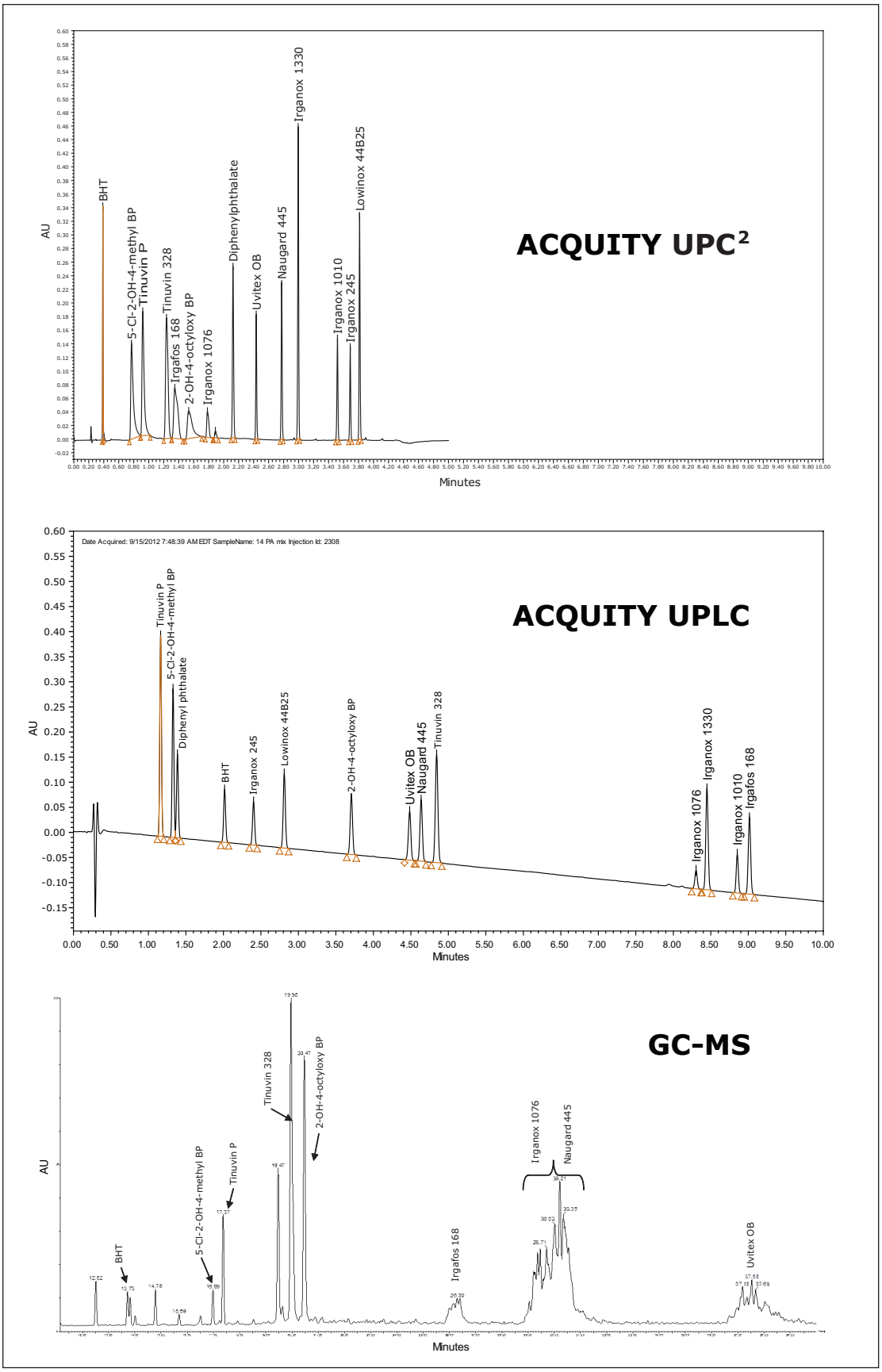


Figure 2. Chromatograms for standards separation.

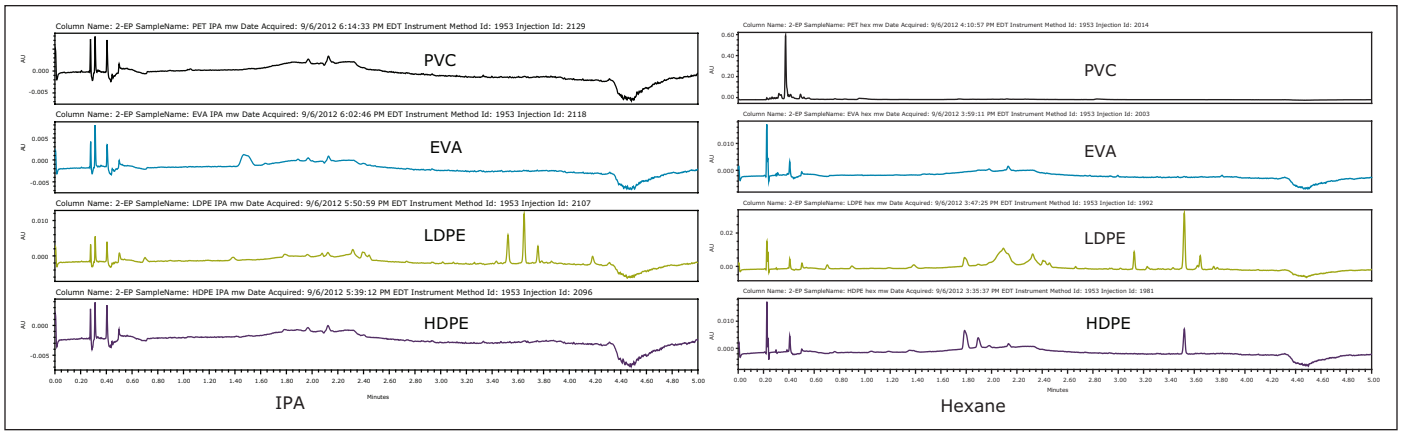


Figure 3. ACQUITY UPC² System chromatograms for IPA and hexane sample extracts.

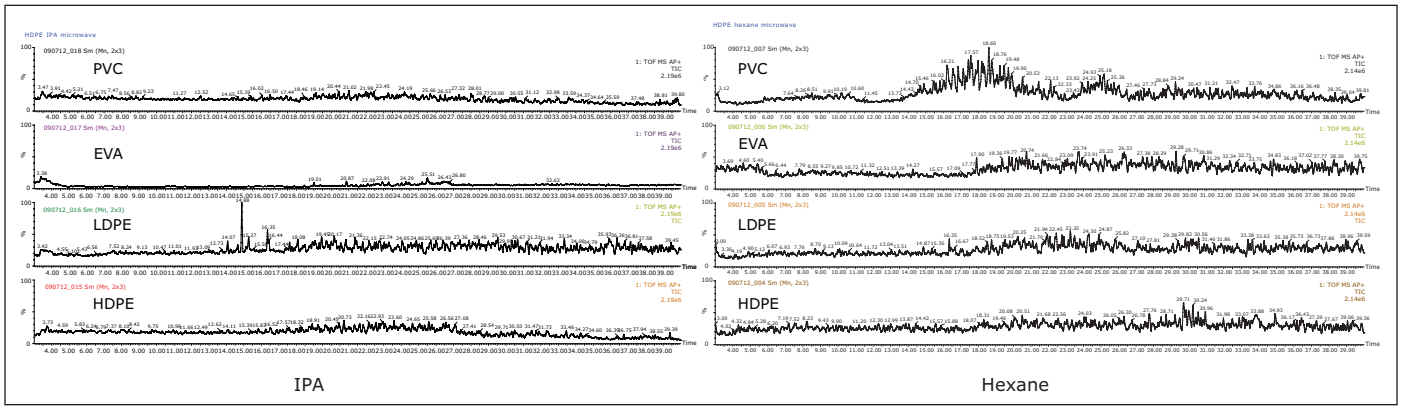


Figure 4. GC-MS chromatograms for IPA and hexane extracts.

Three known polymer additives were identified in LDPE samples by ACQUITY UPC², including Irganox 1010, Irganox 1076, and Irgafos 168, as shown in Figure 5. These are commonly used antioxidants that improve the stability of polymers. The identity of each extractable was confirmed by injection of authentic standards, comparison of the retention time, and MS data. An example for Irganox 1076 is shown in Figures 6 and 7. Each of these additives was detected in either hexane or isopropanol extracts of LDPE.

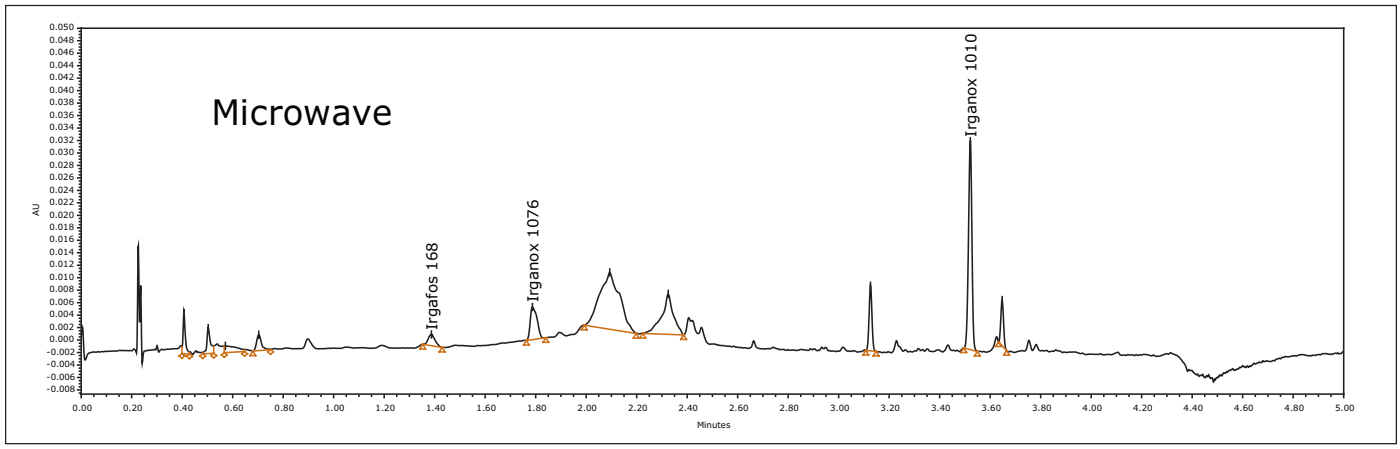


Figure 5. Identified extractables in LDPE hexane extract using ACQUITY UPC².

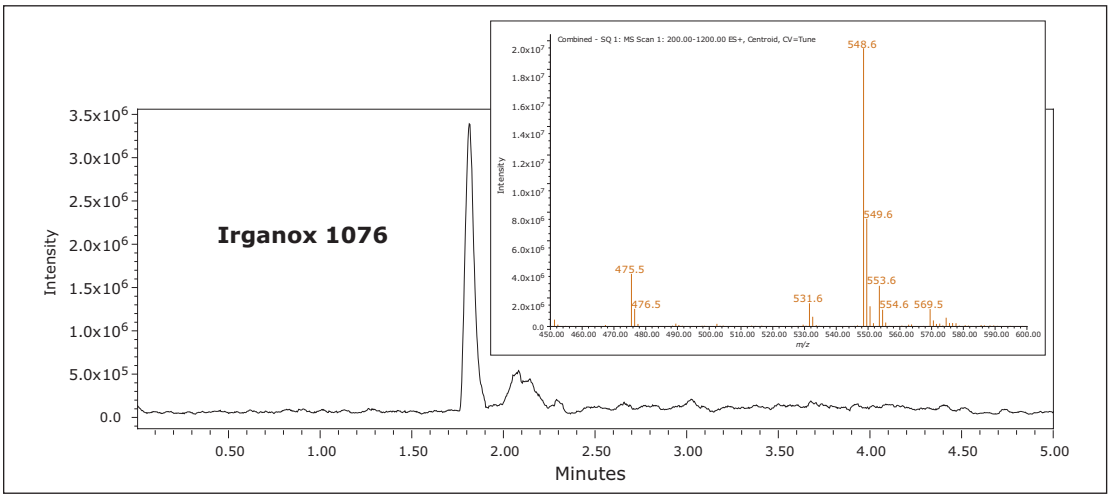


Figure 6. Irganox 1076 in LDPE hexane extract by UPC².

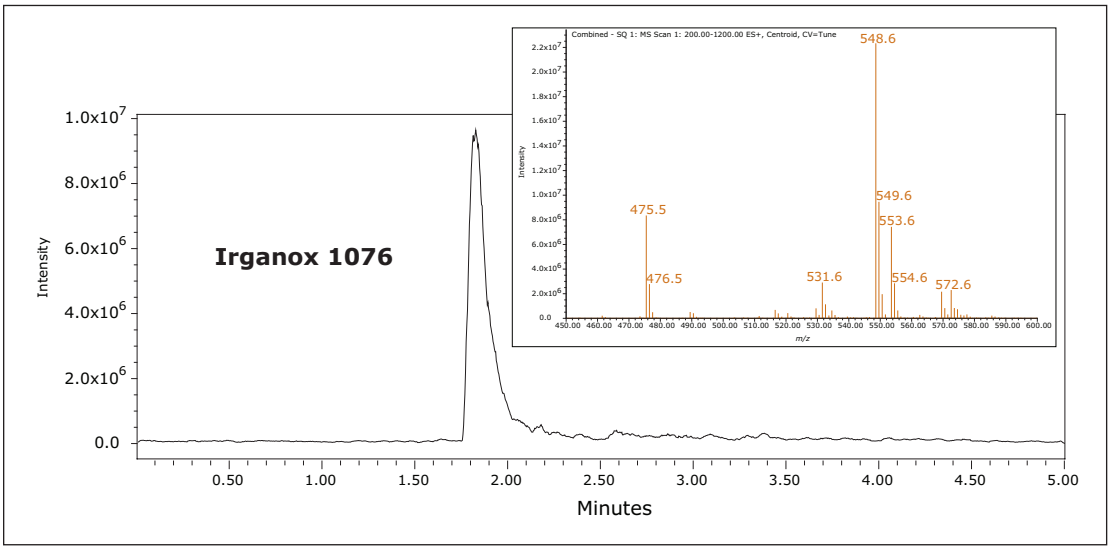


Figure 7. Irganox 1076 standard by UPC².

In GC-MS analysis, the presence of Irgafos 168 and Irganox 1076 was also confirmed using standard retention time and mass spectra.

CONCLUSIONS

In this application, a single technique was found to be compatible for all extracts of different packaging material. This capability allowed for a streamlined, simplified sample preparation workflow with better asset utilization, since all of the solvent extracts can be directly injected onto the ACQUITY UPC^2 System. Using other separation techniques, such as LC and GC, some extracts are not compatible requiring additional processing steps prior to analysis.

UPC^2 offered better information for non-volatile and thermally labile compounds than GC due to lower analysis temperatures. The UPC^2 analysis provided a two-fold improvement in run time compared to UPLC, and an eight-fold improvement in run time compared to GC.

The ease-of-use coupled with the MS detector provided quick polymer identification for known entities in the sample extracts.

References

1. Balogh MP. Testing the Critical Interface: Leachables and Extractables. *LCGC*. 2011 June.
2. Containers Closure Systems for Packaging Human Drugs and Biologics. Guidance for Industry; U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER). 1999 May.
3. Norwood DL, Fenge Q. Strategies for the analysis of pharmaceutical excipients and their trace level impurities. *Am Pharm Rev.* 2004; 7(5): 92,94, 96-99.

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[TECHNOLOGY BRIEF]

Comparing Orthogonality of Convergence Chromatography to Reversed-Phase LC

Michael D. Jones, Kate Yu, and Warren Potts

GOAL

To demonstrate the benefits of UltraPerformance Convergence Chromatography™ (UPC²®) as an orthogonal mode of separation to reversed-phase liquid chromatography (LC).

BACKGROUND

Chromatographic profiling of pharmaceutical drugs, raw materials, biologics, and even natural products is an important technique for scientists to best understand the characteristics related to their research or final products. Typically, scientists rely on analytical tools that provide orthogonal ways to analyze their solutes and assess their content.

For example, in pharmaceutical companies, LC columns with different selectivity are used to evaluate the raw material purity, refine route synthesis, determine finished drug product stability, and investigate container closure effects. For natural product research, different analytical techniques such as reversed-phase LC/MS, high performance thin layer chromatography (HPTLC), capillary electrophoresis with mass spectrometry (CE/MS), and gas chromatography with MS (GC/MS) are utilized to discover bioactive constituents in multiple sample extraction techniques. For bioanalytics, orthogonal methods of analysis are explored to determine drug safety and efficacy. Purification groups use orthogonal selectivity to better isolate and

In this study contrasting UPC² and UPLC chromatographic techniques by peak elution order, we highlight the importance of orthogonality, the challenges associated with impurity isolation and purification, and the role of profile fingerprinting.

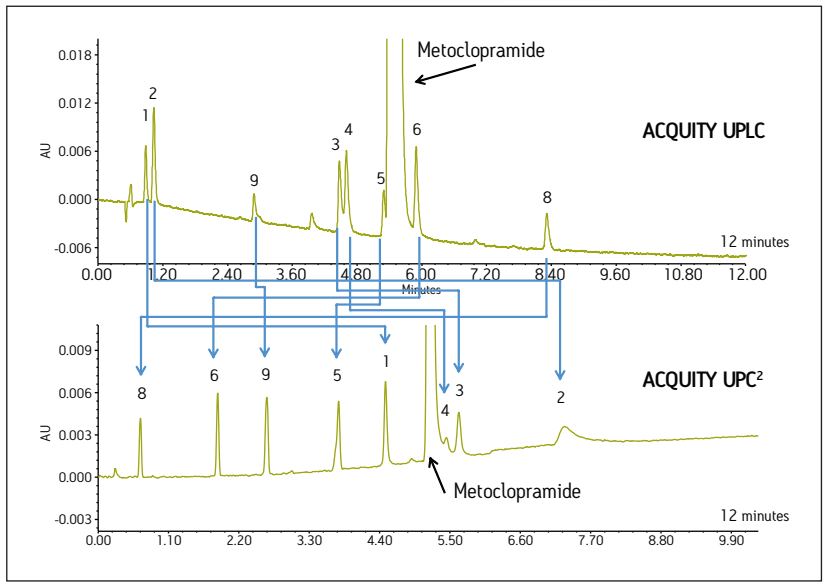

Figure 1. Comparison of UPLC and UPC² for metoclopramide and related impurities.

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purify solutes of interest. In commercialization, information from orthogonal analytical tools aids in the final decisions that aim to improve product quality.

In this investigation, pharmaceutical compounds and natural products were analyzed by convergence chromatography (UPC^2) and reversed-phase LC, using UPLC®. Evaluating the orthogonality between the two chromatographic techniques was accomplished by tracking the peak elution order of the solutes by UV and mass spectrometry. The samples were analyzed using an ACQUITY UPLC® System for the reversed-phase LC analysis and the ACQUITY® UPC^2 for the convergence chromatography analysis. Sub-2-µm particle stationary phases were used for each separation technique. Each example highlights the importance of orthogonality, the challenges associated with impurity isolation and purification, and the role of profile fingerprinting.

THE SOLUTION

An impurity profiling method was developed for metoclopramide using both RPLC and UPC^2.[1] The example shown in Figure 1 demonstrates the relative retention differences between UPC^2 and UPLC. Peaks that are not resolved by UPLC are resolved by UPC^2, and vice versa. UPC^2 also has the ability to provide longer retention for polar compounds that are difficult to retain by UPLC (e.g., peaks 1 and 2). In addition, UPC^2 can be used to resolve critical pairs (i.e., peak 5 from metoclopramide), facilitating the isolation of unknown compounds for subsequent identification and characterization.

Therefore the use of both analytical tools maximizes the ability to choose an appropriate scale-up approach, effectively aiding the drug development process.

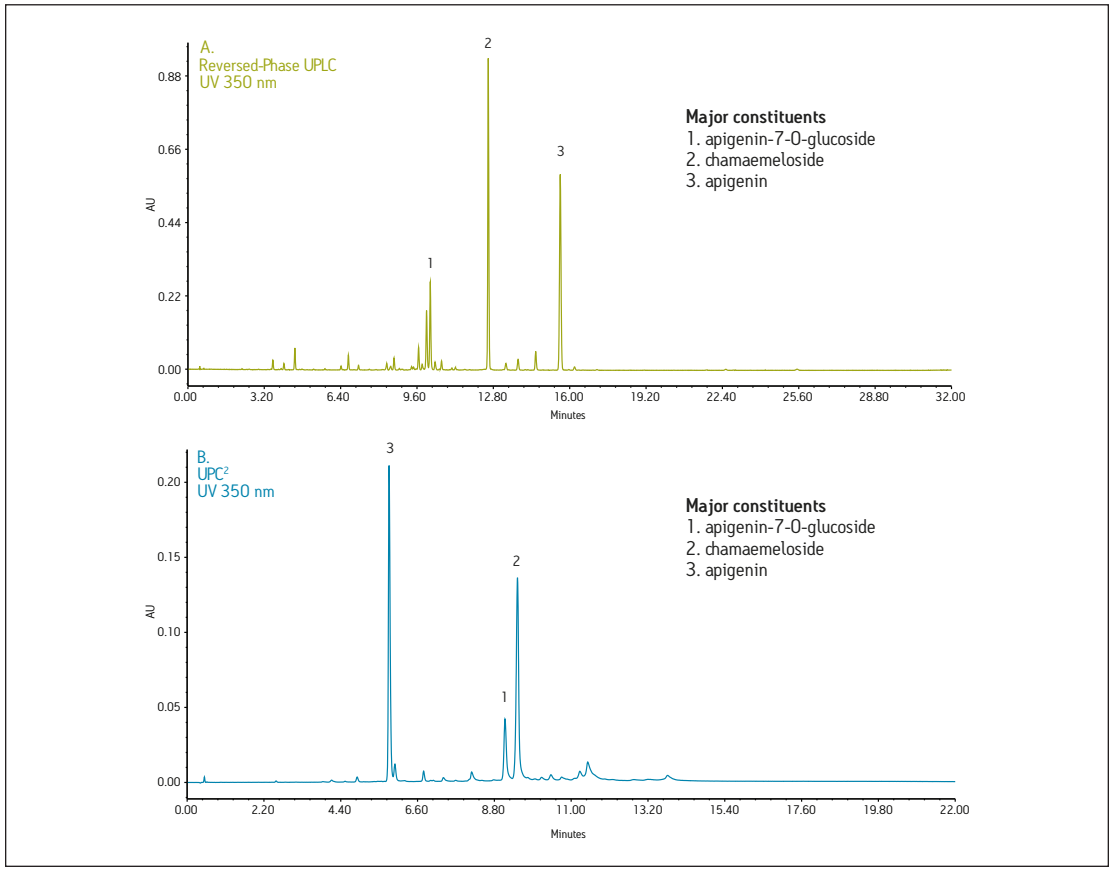

Figure 2. Comparison of UPLC and UPC^2 chromatographic traces of German chamomile methanolic extract.

The next example demonstrates the need for orthogonal modes of separation for complex natural product extracts (German and Roman chamomile methanolic extracts). For UPLC and UPC^2, peaks were identified using mass spectrometry (ACQUITY SQD System). Each peak was assigned a nominal peak I.D., whereas retention times were recorded for each UPLC and UPC^2 result. The data was plotted as retention time versus peak I.D. (not shown) to visualize the orthogonality of the separation techniques. The UPC^2 elution profile was observed as a scattered relationship rather than an inversely linear relationship to that of UPLC.

The highly non-polar analytes that eluted last by reversed-phase UPLC were observed to elute earlier by UPC^2 (Figure 2). The real importance of an orthogonal method is illustrated with the extracted chromatograms for *m/z* 475 using both UPLC and UPC^2 where the peaks are isobaric (same mass). UPC^2 resolves an additional peak that cannot be resolved in LC, and that also cannot be distinguished by MS due to the isobaric nature of the compounds. This makes UPC^2 an ideal orthogonal method for characterization of complex mixtures (Figure 3).

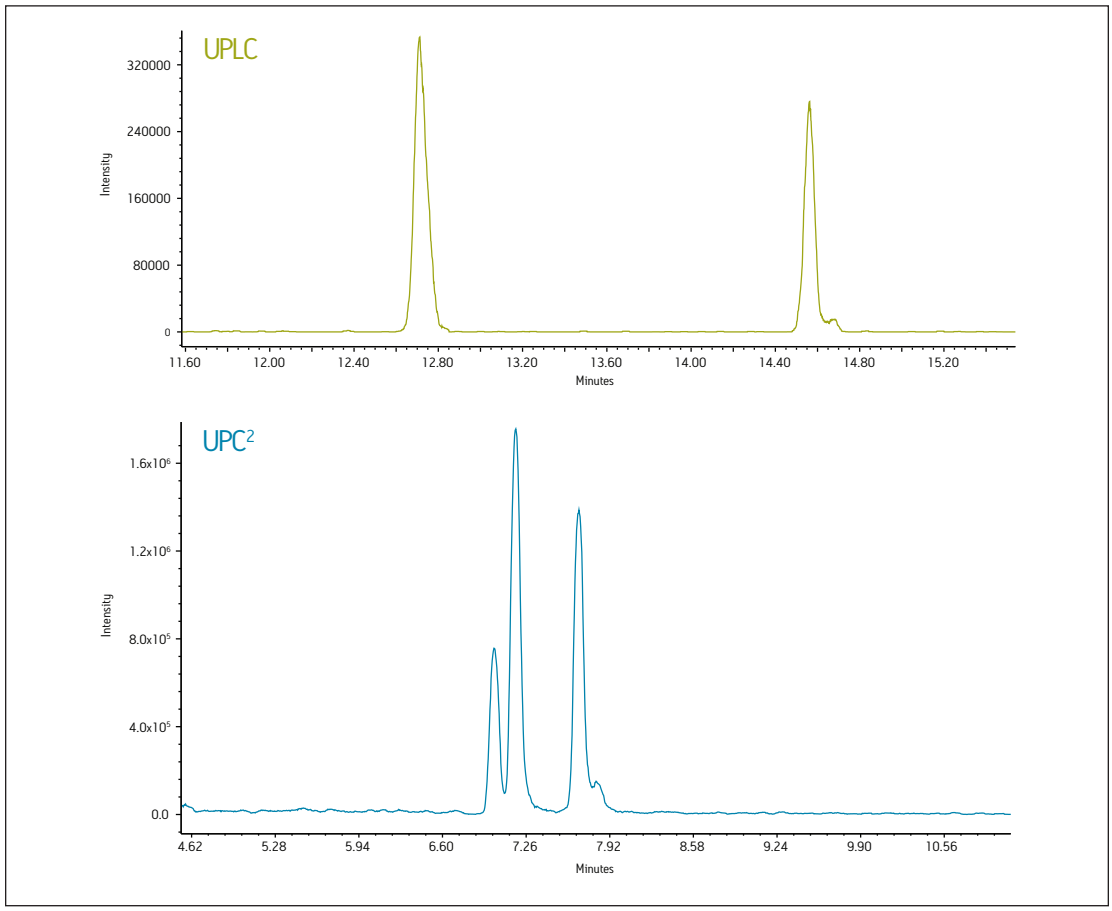

Figure 3. XIC of m/z=475. In comparing the reversed-phase UPLC and UPC^2 traces, an additional peak was found with UPC^2.

SUMMARY

In conclusion, both application examples have proven convergence chromatography to be an effective orthogonal separation technique to commonly used reversed-phase LC. UPC^2 provides a different elution order than RPLC, which is critical for resolving all peaks in a sample. From a drug development perspective, the different selectivity allows the scientist to choose a separation technique that will provide an easier path for scale-up and isolation. Unlike normal-phase LC, the separations are compatible with MS detection to give more information on unknown peaks or peak identification. The chamomile methanolic extract, directly injected for UPC^2 analysis, demonstrated compatibility with many of the extraction solvents used for complex mixture analysis such as natural products. Additionally, UPC^2 can resolve isobaric compounds, which cannot always be separated by other techniques.

Reference

1. Jones MD, Potts W. UPC^2 Method Development for Achiral Impurity Analysis. Waters Application Note 720004577EN. 2013 Jan.

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UPC² Method Development for Achiral Impurity Analysis

Michael D. Jones, Andrew Aubin, Paula Hong, and Warren Potts
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Orthogonal determination of pharmaceutical impurity profiles
- UPC² method development approaches for pharmaceutical impurity analysis
- Supercritical fluid chromatography of impurities that meet ICH guidelines and regulatory requirements

WATERS SOLUTIONS

ACQUITY UPC²™ System

ACQUITY UPC² Column Kit

Empower® 3 Software

ACQUITY® SQD Mass Spectrometer

KEY WORDS

UPC², pharmaceutical impurities, stability indicating methods, degradation profiling, method development, metoclopramide, convergence chromatography

INTRODUCTION

UltraPerformance Convergence Chromatography™ (UPC²™) exploits the benefits of a sub-2-µm particle size stationary phase with carbon dioxide as the primary mobile phase component. Convergence chromatography is an analytical tool that uses less solvent, yet provides high speed separations. Particularly for impurity analysis, convergence chromatography provides an orthogonal approach aiding discovery of unknown impurities when compared to reversed phase LC. While method development strategies in liquid and gas chromatography are well-defined, that is not the case for convergence chromatography. In order to streamline this process, a systematic approach to achiral convergence chromatography method development requires research.

It is important to understand the impurity profiles of drug products and drug substance material. Assessing the purity of the sample allows pharmaceutical companies to make decisions during the development and to move forward through commercialization of the drug. Impurity profiles dictate raw material quality from vendors, finished product shelf life, route synthesis pathways, and intellectual protection from counterfeiting. Orthogonal comparisons of the chromatography profiles provide the ability to make the best educated decisions. In this application, the ACQUITY UPC² System was used to analyze metoclopramide and related impurities. Metoclopramide, as shown in Figure 1, is an antiemetic drug used for the treatment of heartburn, healing of ulcers, and nausea resulting from chemotherapy. The method development investigated columns and solvents to determine suitable method conditions optimizing specificity and peak shape.

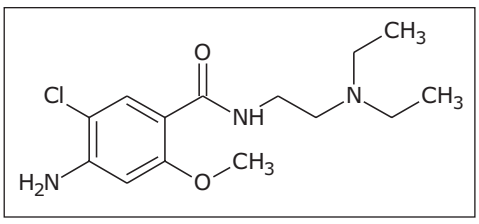

Figure 1. Chemical structure of metoclopramide.

EXPERIMENTAL

UPC² conditions

System:	ACQUITY UPC² with PDA and SQD detection
Column:	ACQUITY UPC² BEH 2-EP 3.0 x 100 mm, 1.7 µm
Mobile phase A:	CO_2
Mobile phase B:	1 g/L Ammonium formate in 50:50 methanol/acetonitrile spiked with 2% formic acid
Wash solvents:	70:30 Methanol/ isopropanol
Separation mode:	Gradient; 2% to 30% B over 5.0 min; held at 30% for 1 min
Flow rate:	2.0 mL/min
CCM Back Pressure:	1500 psi
Column temp.:	50 °C
Sample temp.:	10 °C
Injection volume:	1.0 µL
Run time:	6.0 min
Detection:	PDA 3D Channel: PDA, 200 to 410 nm; 20Hz PDA 2D Channel: 270 nm at 4.8 nm Resolution (compensated 500 to 600 nm) SQD MS: 150 to 1200 Da; ESi+ and ESi-
Make-up flow:	None required
Data management:	Empower 3 Software

Sample description

A resolution solution was prepared with metoclopramide and eight related impurities, then placed in a TruView™ Maximum Recovery Vial for injection, as shown in Table 1. The impurities were prepared at 0.1% w/w concentration of the metoclopramide standard. The resolution solution was used for the chromatographic method development.

Peak #	Name	FW	EP ref.
	METOCLOPRAMIDE (4-amino-5-chloro-N-(2-(diethylamino)ethyl)-2-methoxybenzamide	299.8	
1	4-amino-5-chloro-2-methoxybenzoic acid	201.6	(EP C)
2	4-(acetylamino)-2-hydroxybenzoic acid	195.2	(EP H)
3	4-amino-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide N-oxide	315.8	(EP G)
4	4-amino-5-chloro-N-2-(diethylaminoethyl)-2-hydroxybenzamide	285.8	(EP F)
5	4-(acetylamino)-5-chloro-N-2-(diethylaminoethyl)-2-methoxybenzamide	341.8	(EP A)
6	Methyl 4-(acetylamino)-2-methoxybenzoate	223.2	(EP D)
8	Methyl 4-(acetylamino)-5-chloro-2-methoxybenzoate	257.7	(EP B)
9	Methyl 4-amino-2-methoxybenzoate	181.1	

Table 1. List of metoclopramide impurity standards, peak designation, masses, and European Pharmacopoeia labels.

RESULTS AND DISCUSSION

Systematic screening

The method development process systematically screened columns, modifiers, and modifier additives to achieve the best separation. The initial configuration screened four UPC^2 columns with four modifiers. A "modifier" is the strong solvent mobile phase that facilitates elution of the analytes increasing in polarity. The four solvents used were methanol, methanol with 0.5% formic acid, methanol with 2 g/L ammonium formate, and methanol with 0.5% triethylamine. The screening process was performed with a generic 5% to 30% B gradient over 5 min, holding at 30% for 1 min. The total screening time was achieved in just over two hours. The methanol with ammonium formate provided the best overall peak shape compared for each column, as shown in Figure 2. The peak tracking during the method screening process was achieved by reviewing the MS spectra provided by the ACQUITY SQD. The selectivity (α) for the more polar analytes varied greatly. Since the mobile phase was held constant for these comparisons, the result of changing α are due to [stationary phase – solute] interactions.

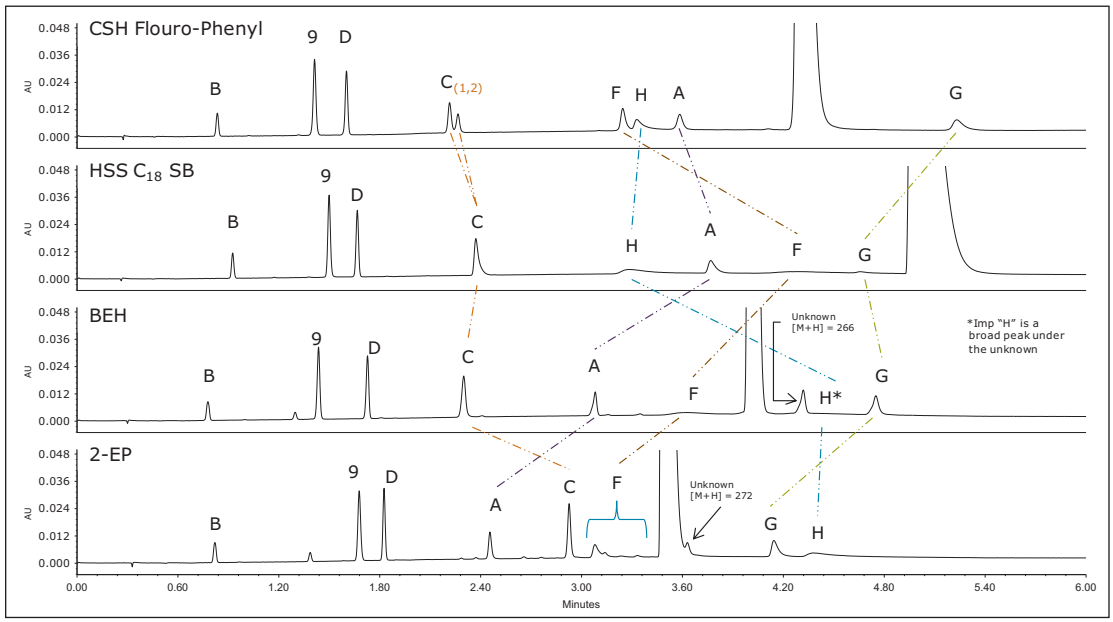

Figure 2. Column screening results. The modifier (B) was methanol with 2 g/L ammonium formate. 5% to 30% B over 5 min and held at 30% for 1 min.

Based on the results, the UPC^2 2-EP stationary phase was the optimal column of choice providing better peak shape and resolution for the majority of the analytes. The UPC^2 CSH Flouro-Phenyl column provided good selectivity and peak shape; however, impurity C unexpectedly separated into two peaks. This unknown phenomenon will be explored in another set of experiments outside the scope of this application note.[1]

Effect of gradient slope

In reversed phase LC, gradient slope is a common tool to manipulate selectivity and resolution. Using the UPC^2 2-EP stationary phase, the gradient slope was decreased by extending the overall gradient run time. The change in slope had little to no effect on the chromatographic profile with the exception of a selectivity change between peaks 6 and 7, as shown in Figure 3.

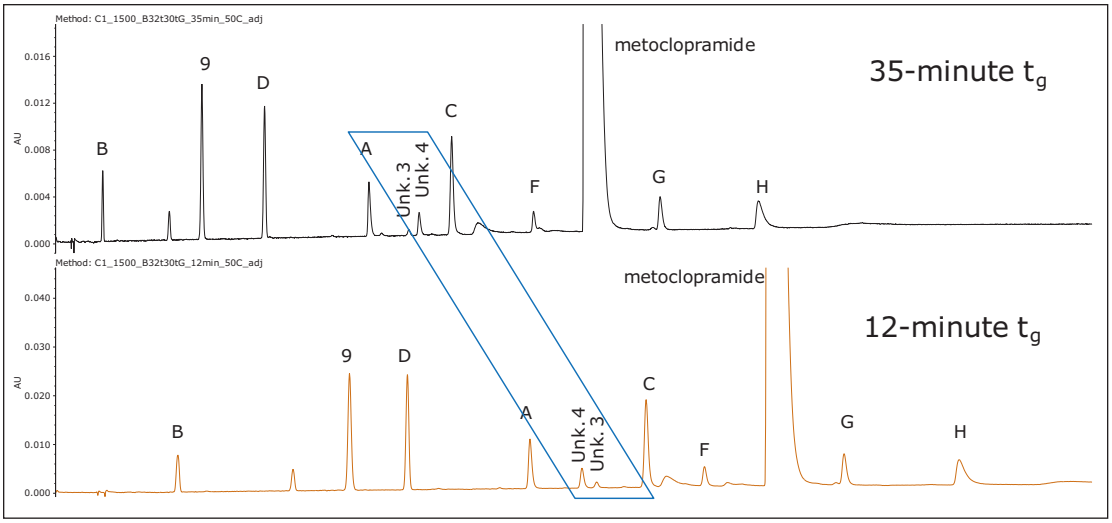

Figure 3. Normalized x-axis overlay metoclopramide analyzed with extended 12- and 35-minute gradient run times flattening the slope compared to the 6-minute screening experiments. The original gradient was used; 5% to 30% B.

Effect of different elution solvents

Inducing a shallower gradient did not increase resolution between peaks. To increase resolution, a less polar aprotic organic solvent (acetonitrile) was mixed at different compositions with methanol, the stronger elution solvent. The addition of acetonitrile increased resolution, spreading the separation space. Based on these observations, this technique proves to be a powerful tool when developing methods, as observed in previously published results.[1]

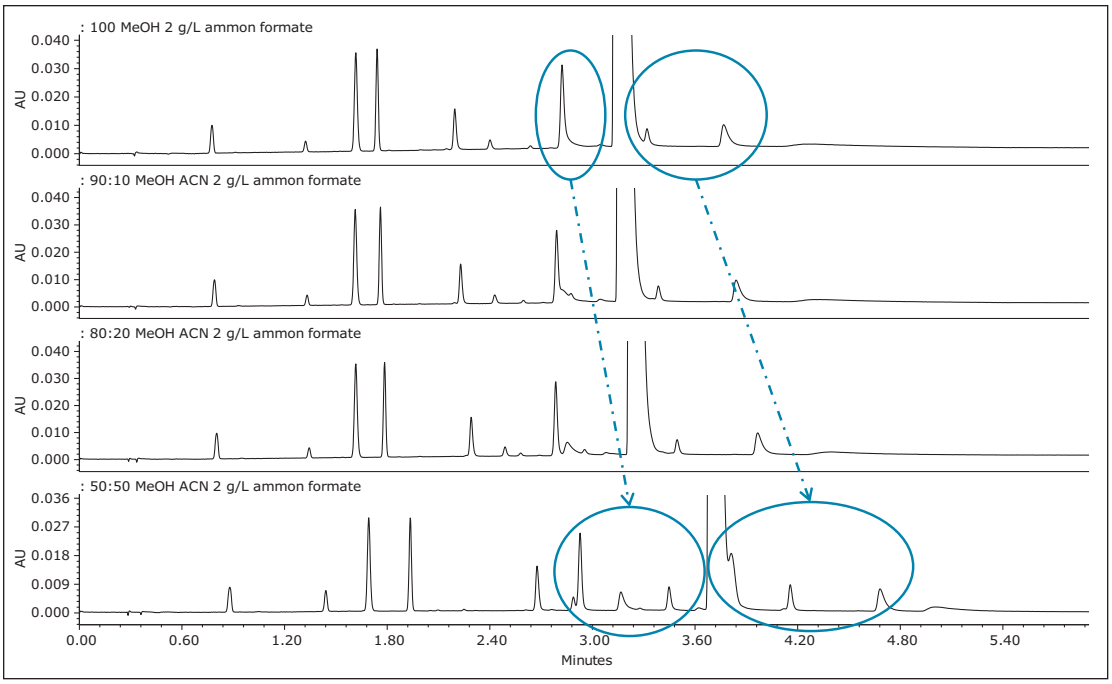

Figure 4. Highlighted in this overlay, the addition of acetonitrile to the composition of the modifier increased the resolution of the later eluting analytes.

Effect of Additive

The effect of additives can either enhance or mask analyte interactions with the stationary phase. The metoclopramide impurities have many different side group functionalities. The related impurities, shown in Table 1, indicate amines, carboxylic acids, and hydroxyl groups. Therefore, choosing a suitable additive is challenging. Ammonium formate improved peak shapes for many of the compounds in the mixture. The other additives explored during the screening process improved the peak shape of other analytes, such as impurity H; however, it affected the other components in the mixture.

Individual standards of each of the impurities were explored during the additives screening. Formic acid achieved acceptable peak shape for impurity H; however, the chromatographic performance of the other related substances were affected. The effect of additive concentration can influence peak shape. Concentrations greater than those usually practiced with reversed phase LC may be required to achieve desired peak shape. Increasing the concentration of the formic acid yielded further improvements in peak shape for impurity H, as shown in Figure 5. Unfortunately, the peak shape for impurity F was compromised, as seen in Figure 6. Combining formic acid and ammonium formate provided the benefits of each additive, resulting in optimal peak shape for the entire separation. The results of the formic acid, ammonium formate, and combination of additives in the modifier for the expired sample are shown in Figure 7. By using the expired sample in this comparison, we can better assess the selectivity and peak shape effects of the known impurities in the presence of the unknown impurities. As seen in Figure 7, addressing peak shape ultimately affects the efficiency, resolution, and sensitivity of the chromatographic separation.

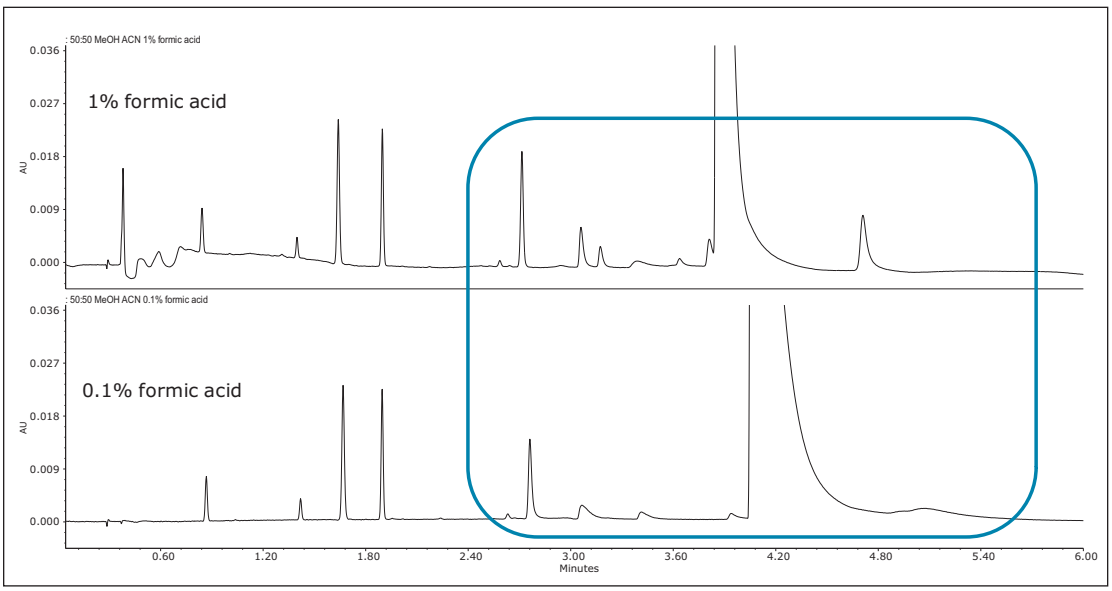

Figure 5. Comparisons of two modifiers; 0.1% formic acid in methanol versus 1% formic acid in methanol. The polar analytes (highlighted in the box) improved with increases in additive concentration, while the more neutral components were not affected. (Refer to the Experimental section for additional method parameters.)

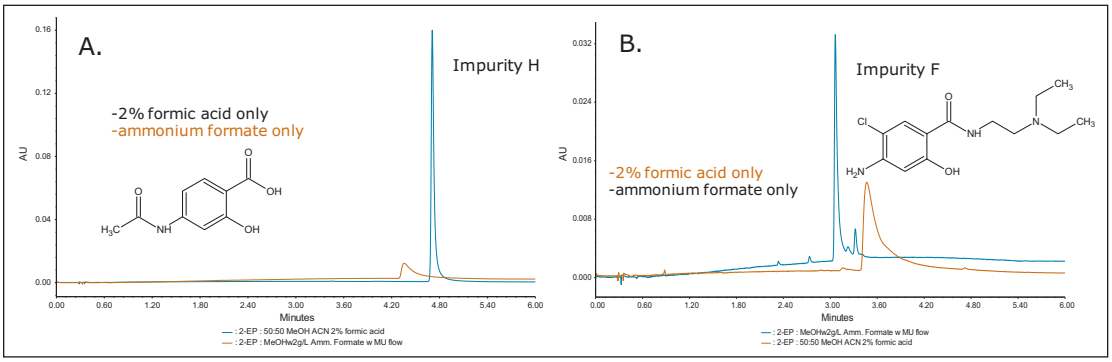

Figure 6. Peaks with hydroxyl (or polyphenols) functionality such as impurity H tend to benefit from the use of only formic acid, as shown in Figure 5A. Optimal peak shape for compounds with primary, secondary, and tertiary amine functionality trend from the use of ammonium salt-based additives as with impurity F, as shown in Figure 5B.

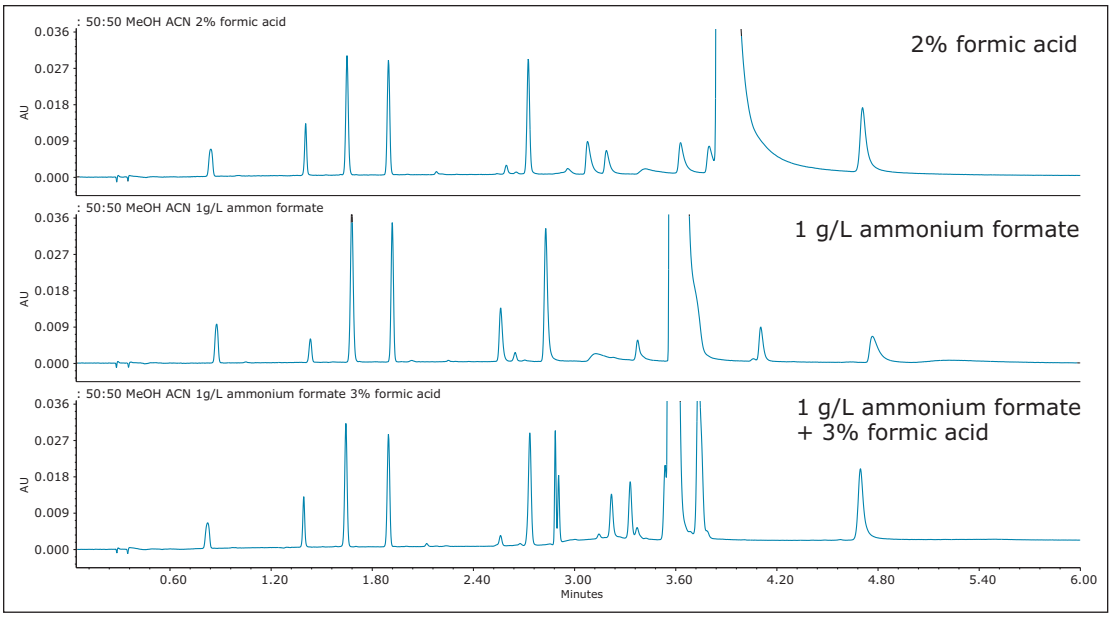

Figure 7. Injections of an expired metoclopramide sample performed with different additive compositions in the modifier. Combining ammonium formate with formic acid in terms of a "buffer-like" system provided the best peak shape for all analytes in the sample. The modifier used was 50:50 methanol/acetonitrile.

Evaluating Specificity

Once the method conditions that positively influenced selectivity, resolution, and peak shape were determined, variables were optimized. The final method was evaluated with a standard mixture of metoclopramide and impurities (control) and an expired sample mixture, as shown in Figure 8. Further interrogations of the unknown impurities are addressed in a Waters® application note.[2]

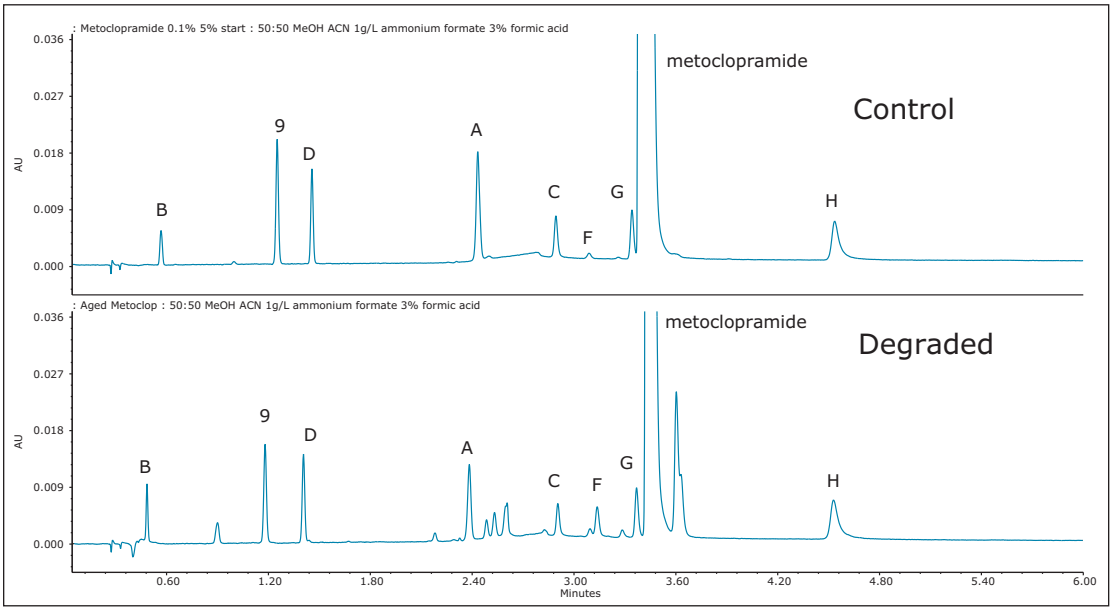

Figure 8. Comparison of a metoclopramide control mixture and a degraded mixture using the final method conditions outlined in the Experimental section.

CONCLUSIONS

An achiral analysis of metoclopramide and related substances was successfully performed using the ACQUITY UPC^2 System. The method development was facilitated by understanding the properties of the impurity structures. Many of the impurities consisted of amines, hydroxyl groups, esters, and carboxylic acids. The primary method variables that influenced selectivity, resolution, and peak integrity were stationary phase, modifier elution strength, and additive composition, respectively. The final metoclopramide related substances method demonstrated specificity for an expired metoclopramide sample.

The method development process uncovered multiple [stationary phase – analyte] interactions during the comparison of the column screening process. Further research, in addition to and guided by those previously published,[3-6] need to be explored. Understanding the influence of these interactions with method variables will help build an appropriate method development approach.

References

1. Jones MD, *et al.* Analysis of Organic Light Emitting Diode Materials by UltraPerformance Convergence Chromatography Coupled with Mass Spectrometry (UPC^2/MS). Waters Application Note 720004305EN. 2012 April.
2. Jones MD, *et al.* Impurity Profiling Using UPC^2/MS. Waters Application Note 720004575EN. 2013 Jan.
3. West C, Lesellier E. A unified classification of stationary phases for packed column supercritical fluid chromatography. *J Chromatogr A.* 2008 May; 1191(1-2):21-39.
4. West C, Khater S, Lesellier E. Characterization and use of hydrophilic interaction liquid chromatography type stationary phases in supercritical fluid chromatography. *J Chromatogr A.* 2012 Aug; 1250:182-95.
5. Lesellier E. Retention mechanisms in super/subcritical fluid chromatography on packed columns. *J Chromatogr A.* 2009 Mar; 1216(10):1881-90.
6. Zou W, Dorsey JG, Chester TL. Modifier effects on column efficiency in packed-column supercritical fluid chromatography. *Anal Chem.* 2000 Aug; 72(15):3620-6.

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[TECHNOLOGY BRIEF]

Method Development for Impurity Analysis Using ACQUITY UPLC H-Class System with an ACQUITY QDa Detector

Margaret Maziarz

GOAL

To demonstrate the use of qualitative mass spectral data in method development, using an ACQUITY UPLC® H-Class System with ACQUITY® QDa™ Detector to confirm the identity of ziprasidone HCl and related compounds.

BACKGROUND

Method development typically involves screening chromatographic parameters such as columns, organic solvents, buffers, gradient slope, flow rate, temperature, and so on. Any of these parameters may be modified to alter the resolution to achieve the required analytical quality.

Small modifications in pH often alter the relative retention (elution position) of compounds in a reversed-phase separation. As these separation variables are investigated, it is essential to track changes in chromatographic behavior for each of the sample components. At the same time, recognition of coeluting species is required.

Without accurate and complete peak tracking, development times can be prolonged and significant impurities may be unrecognized. In addition, incorrect identification or failure to identify impurities may compromise the safety and efficacy of the end pharmaceutical product. Utilizing a mass detector enables the analytical laboratory to correctly monitor peak retention by mass spectrometric detection.

The ACQUITY QDa Detector aids in the development of efficient and robust screening methods by minimizing the need for standard runs to confirm the identity of peaks by retention time.

In this study, we take advantage of qualitative mass spectral data acquired using an ACQUITY QDa Detector to track the elution of ziprasidone HCl and its USP-specified related compounds over a series of different mobile phase pH experiments. This method development process was also facilitated by using the ACQUITY UPLC® H-Class with Auto•Blend Plus™ Technology to control pH.

THE SOLUTION

Auto•Blend Plus, which is included with the ACQUITY UPLC H-Class System, was used to program the blending of acid and base stock buffers with organic and aqueous solvents to deliver a mobile phase with a constant pH. The ACQUITY QDa Detector was used to confirm identity of the ziprasidone HCl and related compounds.

In this pH screening study, 125 mM formic acid and ammonium hydroxide stock solutions, acetonitrile, and water were programmed for mixing by the quaternary pump to deliver mobile phases with pHs of 3.1, 4.0, and 5.0. The Auto•Blend Plus method, set at pH 3.1, is shown in Table 1. The impact of pH on the separation of ziprasidone HCl and the related compounds is displayed in Figure 1.

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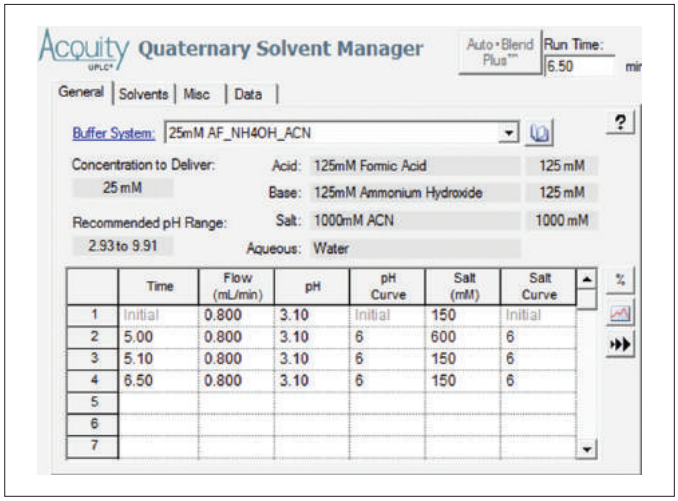

	Time	Flow (mL/min)	pH	pH Curve	Salt (mM)	Salt Curve
1	Initial	0.800	3.10	Initial	150	Initial
2	5.00	0.800	3.10	6	600	6
3	5.10	0.800	3.10	6	150	6
4	6.50	0.800	3.10	6	150	6
5						
6						
7						

Table 1. Auto•Blend Plus gradient programming for method development to deliver a mobile phase with a pH of 3.1.

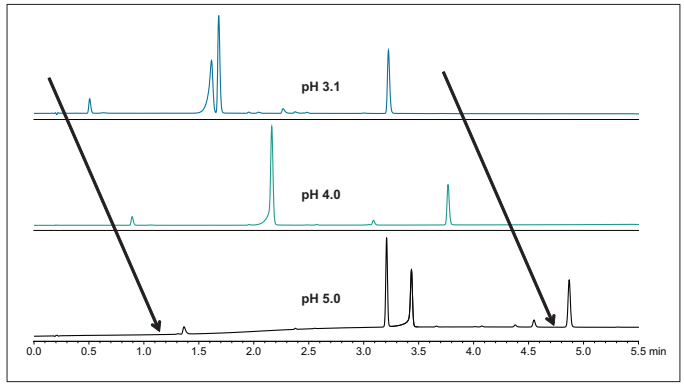

Figure 1. UV data at 254 nm. pH screening in method development using the ACQUITY UPLC H-Class System with an ACQUITY UPLC CSH C_{18} 2.1 x 50 mm, 1.7-µm Column. Column temperature and flow rates were set to 30 °C and 0.8 mL/min, respectively. Injection volume was 0.5 µL.

As shown in Figure 1, an increase in pH results in a higher retention of all the peaks. Fewer peaks were observed with pH 4.0 mobile phase than with 3.1 or 5.0. Tracking and identification of the peaks over the method developments runs with different pHs was performed using an ACQUITY QDa Detector.

Tracking the elution of the peaks by mass detection is displayed in Figure 2. The mass spectra analysis confirmed the identity of the peaks and complemented tracking the elution order of peak 2 with the UV data.

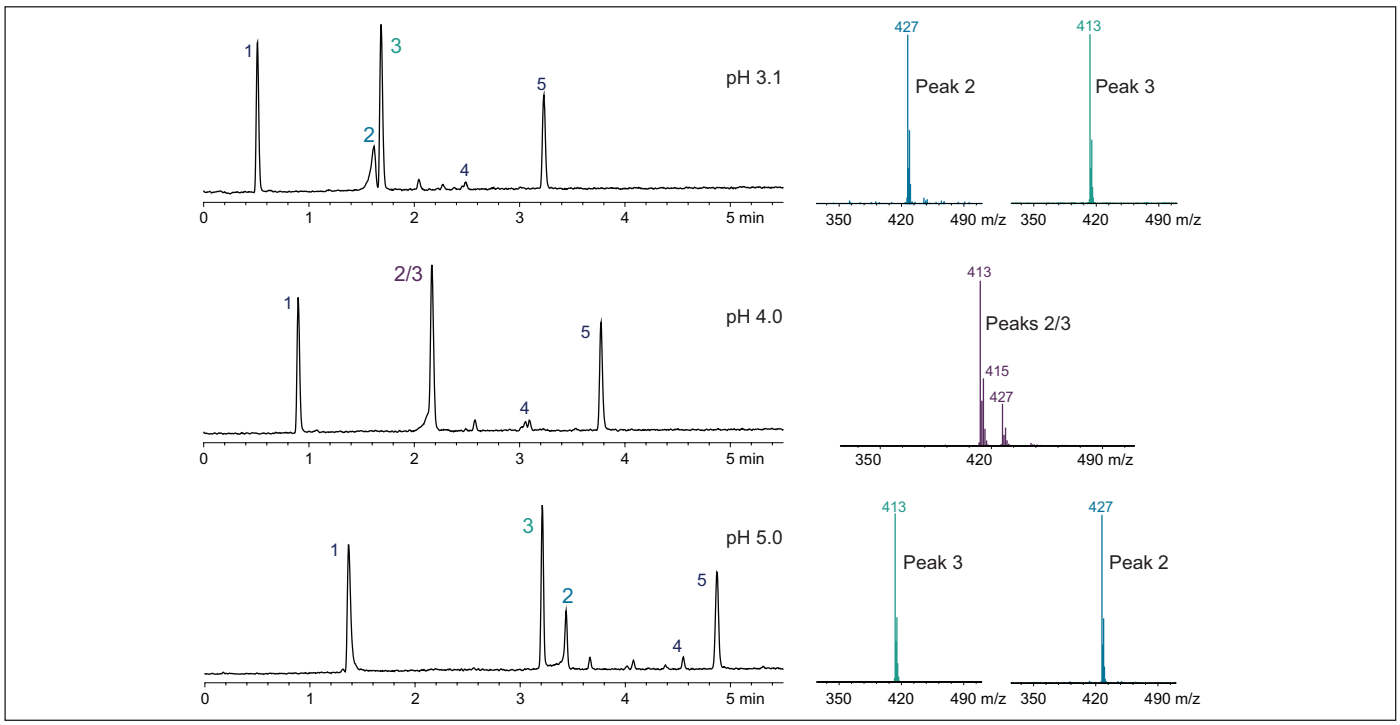

Figure 2. Peak tracking with the ACQUITY QDa Detector. Total ion chromatograms (TIC) and molecular mass determination to track retention of peaks 2 and 3 over the chromatographic runs with different pHs.

Peak 1: Related compound A

Peak 2: Related compound B (MW: 426.92 m/z)

Peak 3: Ziprasidone HCl (free base MW: 412.94 m/z)

Peak 4: Related compound C

Peak 5: Related compound D

In summary, the ACQUITY QDa Detector is a synergistic element of the chromatographic system that provides mass spectral molecular information for analytical scientists in a quick manner, without the need for high-end mass spectrometry. It streamlines development of efficient and robust methods by minimizing the need for standard runs to confirm the identity of peaks by retention times.

When used in conjunction with Empower® 3 Software, which integrates optical and mass data processing, the mass spectral data can be interrogated in the same workflow as the ACQUITY UPLC PDA Detector data.

SUMMARY

The ACQUITY QDa Detector was used to track sample components during development of the UPLC® method for the separation of Ziprasidone HCl and its USP-specified impurities. The ACQUITY QDa Detector was designed to complement the optical data with the enhanced qualitative mass spectral data to confirm the identity of components using an orthogonal detection technique.

Overall, the ACQUITY QDa Detector coupled with the ACQUITY UPLC H-Class System and and Auto•Blend Plus Technology provides complete and rapid chromatographic separation and characterization of compounds, streamlining a laboratory's workflow in the analysis of pharmaceutical products.

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A UPLC Method for Analysis of Metformin and Related Substances by Hydrophilic Interaction Chromatography (HILIC)

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APPLICATION BENEFITS

- Reduced development time facilitated by instrument flexibility
- A 6x reduction in chromatographic run time resulting in a 3.5x reduction of total required analysis time resulting in faster throughput and reduced solvent consumption in routine sample analysis
- Utilizing ACQUITY UPLC results in a savings of $5800/per 1000 injections by reducing solvent consumption

WATERS SOLUTIONS

- ACQUITY UPLC® H-Class system
- ACQUITY UPLC BEH Amide column and pre-column filter
- Empower™ 2 CDS software
- ACQUITY UPLC Columns Calculator

KEY WORDS

Method transfer, polar basic compounds, method development, melamine, cyanoguanidine, pharmaceutical impurities

INTRODUCTION

Metformin hydrochloride is an anti-diabetic drug typically administered orally while treating non-insulin dependant (type 2) diabetes mellitus.[1] It is one of the most widely-used oral antidiabetic drugs worldwide, with over 48 million generic formulation prescriptions filled in the United States alone.[2] Liquid chromatographic analysis of metformin and related impurities presents a challenging task due to the highly polar characteristics of the molecules and the low UV absorbance of the analytes. These challenges limit the options to manipulate selectivity during method development, especially with reversed-phase (RP) chromatography. Numerous methods are cited utilizing a variety of techniques such as ion chromatography, hydrophilic interaction chromatography (HILIC), and RP LC methodologies reporting run times up to 30 minutes.[1,3,4]

In this application note, a method was developed on the ACQUITY UPLC H-Class system using an ACQUITY UPLC BEH Amide sub-2 µm particle stationary phase in HILIC mode to successfully resolve and analyze metformin and six related substances. Development of the method was facilitated through the column and solvent switching capabilities of the ACQUITY UPLC H-Class which allows automated changes of stationary phase, ionic strength, cation buffer, pH and temperature. The major contributors to the successful separation of metformin and the related substances are discussed. A routine use evaluation study was performed to determine feasibility of the method for use in QC laboratories. Informatics provided visualization of trending results with intent to identify deficiencies regarding the developed methodology. The final method will provide cost reduction improvements in method robustness for routine analysis.

EXPERIMENTAL

Sample Description

Samples were provided by a pharmaceutical collaborator. Stock solutions of metformin hydrochloride, as well as impurities A, B, C, D, E and I were prepared in water. Working standards were prepared as per the previous HPLC methodology (70:30 acetonitrile:water). A working standard mixture was prepared whereas impurity concentrations were in respect to the metformin concentration: Impurity A was prepared at 0.05% of metformin and Impurities B, C, D, E and I were prepared at 0.1% of metformin. A mixture of the impurities without addition of metformin was also prepared at the same concentration as the impurities working standard. In addition to the working standard and impurity working standard, two separate preparations consisting of vials prepared with metformin with and without impurities spiked into the matrix. Individual standards were also prepared for each of the analyte constituents.

Method Conditions

Instrument:	ACQUITY UPLC H-Class configured with CM-A, CM-AUX, SSV, PDA
Buffer:	20 mM potassium phosphate, pH 2.3
Mobile Phase:	80:20 acetonitrile:buffer
Separation Mode:	Isocratic
Detection:	UV at 218 nm
Column:	ACQUITY UPLC BEH Amide, 2.1 x 150 mm, 1.7 µm, part number 186004802
Needle Wash:	90:10 acetonitrile:water
Seal Wash:	90:10 water: methanol
Sample Diluent:	70:30 acetonitrile:water
Flow Rate:	0.5 mL/min
Column Temp.:	40 °C
Injection Volume:	1.0 µL
Data Management:	Empower 2 CDS

RESULTS AND DISCUSSION

Method Transfer

The originally supplied HPLC methodology for metformin utilized isocratic conditions with a low-pH sodium phosphate buffer and acetonitrile mobile phase.[1] An Atlantis® HILIC 4.6 mm x 250 mm, 5 µm column was used with an approximate flow of 2 mL/min resulting in a run time of 30 minutes. Sample injection volume was 10 µL. The HPLC method resolves metformin and all six impurities (not shown).

The HPLC methodology was transferred to a 2.1 x 100 mm, 1.7 µm ACQUITY UPLC BEH HILIC column using the ACQUITY UPLC Columns Calculator. It should be noted that the ACQUITY UPLC BEH HILIC stationary phase does not have exactly the same selectivity as the Atlantis HILIC stationary phase due to differences in the base particle, although the Waters Column Selection application indicated that the two stationary phases have similar selectivity. In this application, there were observed selectivity differences between the Atlantis HILIC column and BEH HILIC column. The chromatogram in Figure 1 shows coelutions and a lack of overall retentivity on the BEH HILIC column using UPLC® technology. Slight changes in organic composition were not successful in resolving the impurity peaks. In some instances, as organic composition was increased, salt in the mobile phase precipitated due to mixing a high buffer concentration with a high composition of organic mobile phase. The precipitated salt resulted in increased pressure and baseline absorbance issues. It was then determined that a small amount of redevelopment would be needed to resolve the metformin and related substances by exploring suitable variables.

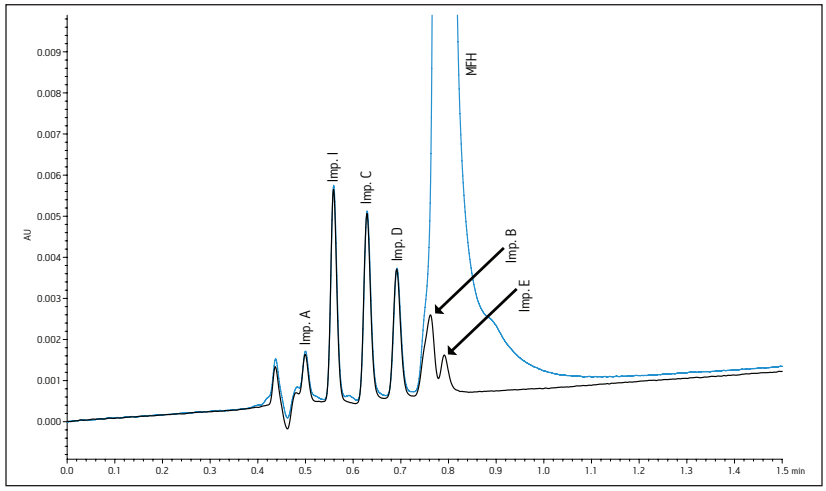

Figure 1. Working standard (blue) and Impurity mix (black) overlay. Direct scaling to a BEH HILIC column was unsuccessful in resolving two of the impurities from the API. The mobile phase was 84:16; acetonitrile: 28 mM sodium phosphate buffer pH 2.2, respectively. The flow rate was scaled to 0.736 mL/min and maintained at a temperature of 20 °C. The injection volume was 0.8 µL.

Manipulating Selectivity on HILIC

A method development scheme to analyze metformin and related substances presents a challenging task. Limitations regarding the low UV spectral absorbance of the analytes at 218 nm inhibit the use of typical MS-friendly buffers such as ammonium formate and ammonium acetate, since their UV cutoff approaches 230 nm. Reversed-phase LC is unsuccessful in retaining the analytes due to the polar basic characteristics of the compounds.

A method development scheme was employed to investigate two HILIC stationary phases: ACQUITY UPLC BEH HILIC and ACQUITY UPLC BEH Amide. Ionic strength, buffer cation selection, and temperature were determined as the remaining options to alter selectivity. Based on the poor retentivity and resolution of these compounds using the ACQUITY UPLC BEH HILIC stationary phase, the ACQUITY UPLC BEH Amide column was investigated.

The working standard was injected onto the ACQUITY UPLC BEH Amide 2.1 x 100 mm, 1.7 µm column. The resulting chromatogram in Figure 2 resolved all compounds with the exception of a slight co-elution between Impurity B and Impurity D. Desired improvements in peak shape and sensitivity were seen for Impurity E. Due to the isocratic conditions, changes in flow rate and temperature were explored individually to improve on these critical impurity peaks of interest. An experiment utilizing higher mobile-phase pH was explored but the results yielded little to no retention of many of the impurity peaks. The following relationships were observed during development of the separation on the amide column:

- Increases in organic content increased retention of Impurity D and B to co-elute with impurity E. A flow rate of 400 µL/min at 25 °C was determined to be optimal when using sodium phosphate as the aqueous mobile phase (Figure 2).
- As temperature increased, retention of impurity E decreased and co-eluted with impurity B (Figure 3).
- As flow rate increased, resolution of Impurity B, D, and E decreased.

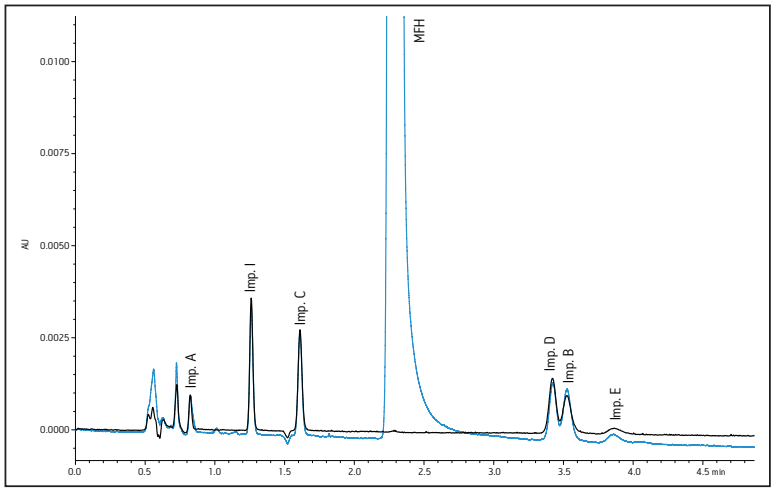

Figure 2. Injection of WS and Impurity mixture on ACQUITY UPLC BEH Amide column. A generic method was used to begin redevelopment. The conditions were derived from initial method conditions. The mobile phase was 80:20; acetonitrile: 30 mM sodium phosphate buffer pH 2.2, respectively. The flow rate was to 0.4 mL/min and maintained at a temperature of 25 °C. The injection volume was 1.0 µL.

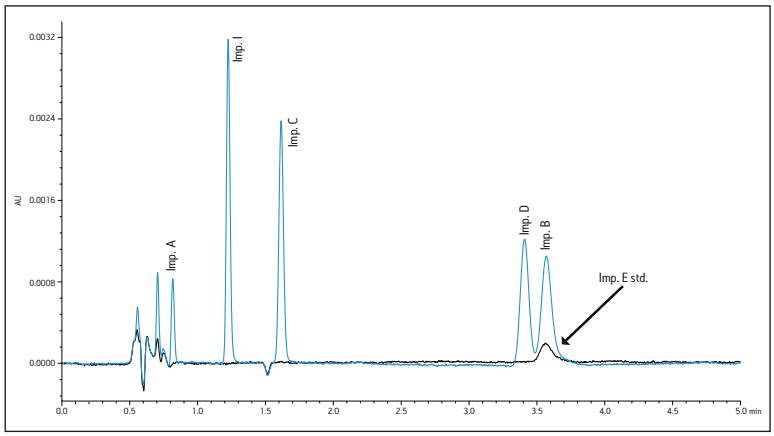

Figure 3. An Increase in temperature to 30 °C shifted retention time of Impurity E to decrease and co-elute with Impurity B. The mobile phase was 80:20; acetonitrile: 30 mM sodium phosphate buffer pH 2.2, respectively. The flow rate was to 0.4 mL/min and maintained at a temperature of 30 °C. The injection volume was 1.0 µL.

The proper selection of cation in the buffer can help control the ionic interactions on the surface of the column and in some instances, alter selectivity. The 30 mM sodium phosphate buffer was substituted with a 30 mM potassium phosphate buffer while maintaining a pH of 2.2. Optimal conditions were determined by combining the immediate improvement of changing the buffer cation to potassium. Combining the change in cation with a slight adjustment in flow rate and temperature, a desired resolution of the critical peaks was achieved. The chromatogram in Figure 4 shows better peak shape and resolution for Impurity peaks B, D, and E. Also, Impurity E shifted retention and elutes before Impurities B and D. Since the baseline noise was higher with the method in Figure 4, the buffer strength was decreased to minimize the potential for salt precipitation. The effect of decreasing the ionic strength to 10 mM resulted in co-elution of Impurities B and D. A concentration of 20 mM potassium phosphate resulted in acceptable peak shape and resolution (Figure 5).

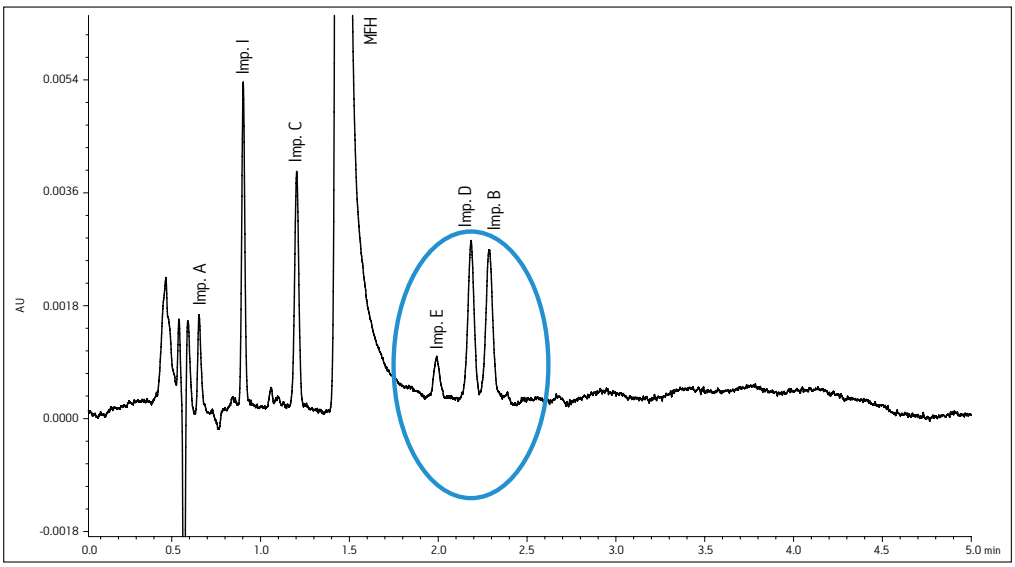

Figure 4. Changing the cation from Na+ to K+. Immediate improvement of resolution and peak shape were observed for Impurities E, D, and B. The mobile phase was 80:20; acetonitrile: 30 mM potassium phosphate buffer pH 2.2, respectively. The flow rate was to 0.5 mL/min and maintained at a temperature of 40 °C. The injection volume was 1.0 µL.

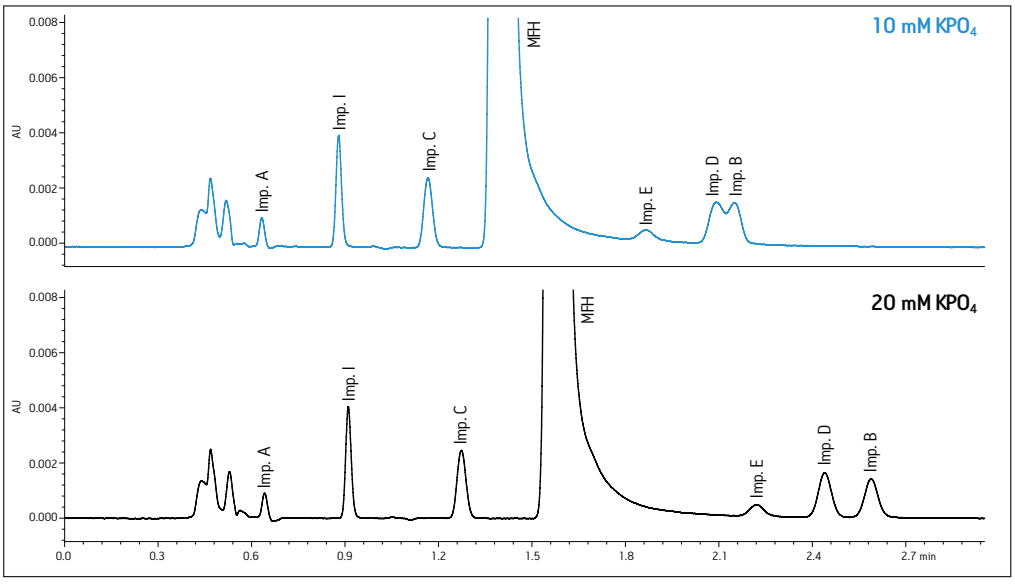

Figure 5. Effect of buffer concentration.
Note: Impurities D and B affected by ionic strength of buffer.

The effects of temperature were explored to determine the effect on selectivity when using the potassium phosphate buffer. Temperature was increased from 20 °C to 50 °C in 10 °C increments. Resolution increased for Impurities B, D, and E as temperature increased. A temperature of 40 °C was determined to provide adequate resolution of the critical pairs (Figure 6). The use of higher temperature resulted in lower column pressure, which allowed the use of a longer column to improve the resolution for the final methodology (Figure 7).

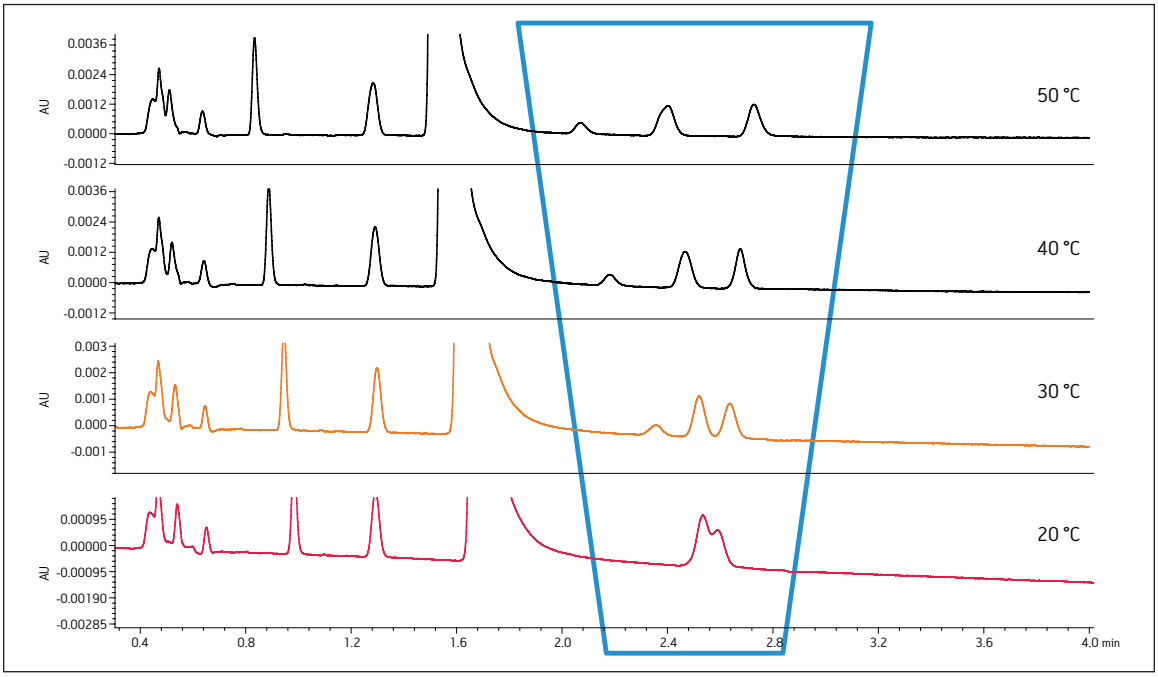

Figure 6. Effect of temperature on selectivity. As temperature increased, resolution between each of the critical pair analytes increased. Note: Impurities E, D and B resolution are affected by temperature, or perhaps linear velocity.

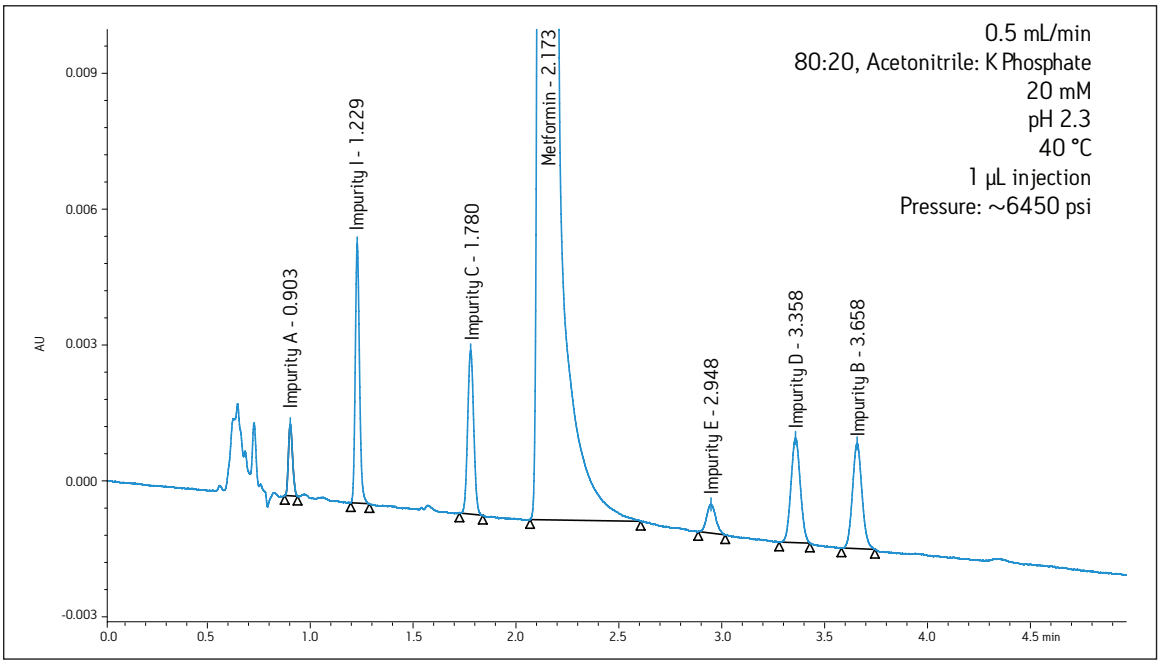

Figure 7. Final conditions. ACQUITY UPLC BEH Amide 2.1 mm x 150 mm, 1.7 µm with pre-column filter. The mobile phase was 80:20; acetonitrile: 20 mM potassium phosphate buffer pH 2.2, respectively. The flow rate was to 0.5 mL/min and maintained at a temperature of 40 °C. The injection volume was 1.0 µL of working standard.

ROUTINE USE STUDY

In order to evaluate the effects of using the potassium phosphate buffer at high organic composition, injections of the standards and samples were performed over a period of time to replicate routine use of the method in a QC laboratory. The sample set consisted of a bracketing procedure constructed with the working standard preparations, individual standard preparations, as well as the spiked and unspiked sample matrix formulations totaling over 360 injections for a given experimental run. A single bracket consisted of 30 injections, which was repeated 60 times to achieve 1800 injections to complete the study designed to replicate practices within a quality control testing laboratory. A pre-column filter was installed for preventative and investigative purposes in the event of a pressure increase over the time of the study.

In an effort to understand how the data was trending, custom calculations and custom reports were created in Empower 2 CDS, whereby processed data could be visualized in the form of trend plots without exporting to spreadsheets. Initial pressure readings were approximately 6500 psi and increased steadily to approximately 6700 psi over the first 860 injections, as indicated by the summary pressure trend plot in Figure 8a. Closer investigations of the summary trend data showed further trending within the bracketed sample set (Figure 8b). The trend plots generated in Empower 2 CDS showed increases in system pressure once the matrix samples were injected. This indicated a deficiency in the original sample preparation procedure. The sample preparation procedure was altered to include a longer centrifugation time and the use of 0.2 µm filter disks in place of 0.4 µm filter disks. The filter disks were used to filter the supernatant as it was added to the sample vial. As a result, pressure increases due to the sample preparation was eliminated and extended to a point where the method was suitable for validation.

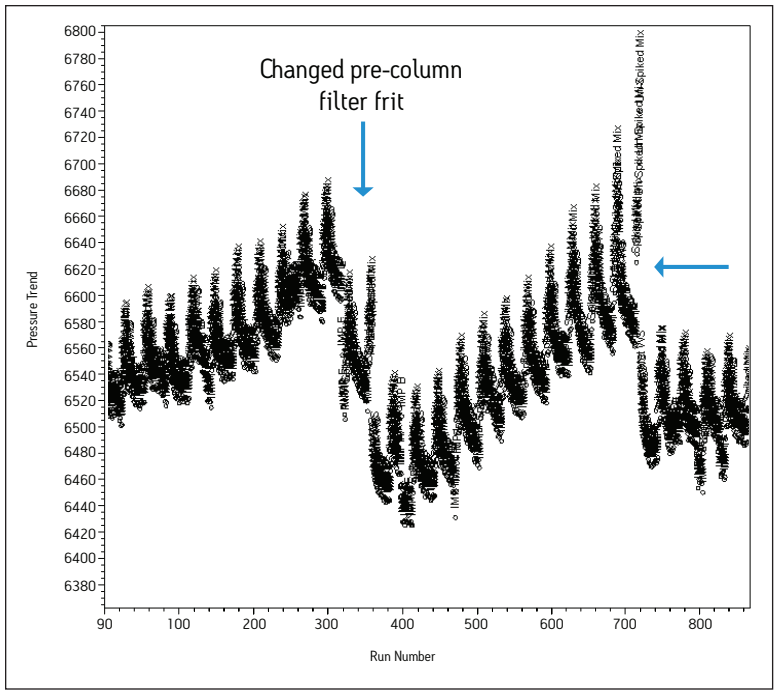

Figure 8a. Pressure trend for 860 injections. Pressure increased steadily over time. Replacement of the pre-column filter frit was successful in returning to initial pressure; however, pressure would steadily increase again.

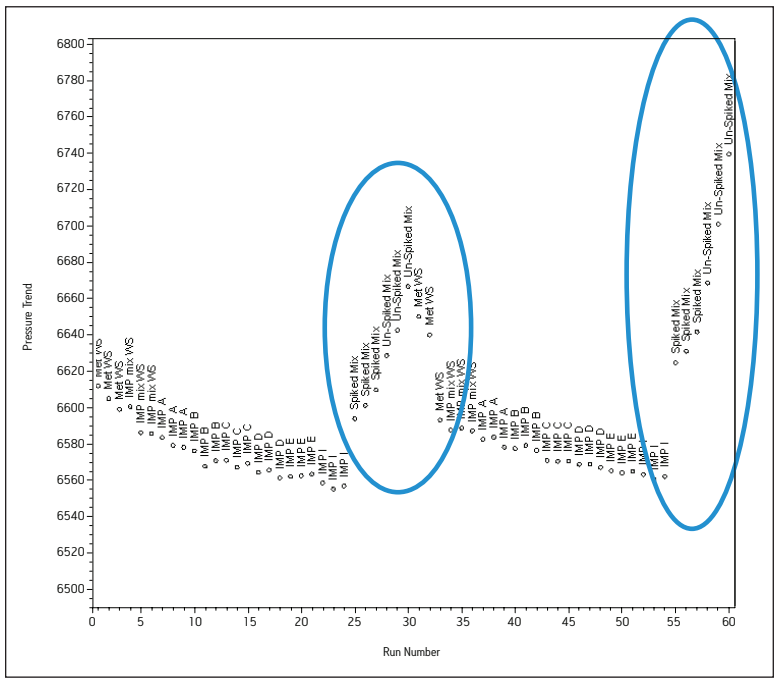

Figure 8b. Further investigation of the trend data revealed a primary trending within the bracket sample set. Injections of the matrix samples were the root cause of the total pressure increasing over time. The circled regions indicate the pressure readings of the matrix injections.

CONCLUSIONS

The complete solution consisting of informatics tools, flexible instrumentation, and a selection of chemistries resulted in a method providing a six-fold reduction in analysis time compared to the HPLC methodology. Altering the buffer cation provided a selectivity change between Impurity E and the Impurity pair; B and D. Ionic strength of the buffer influenced the retentivity of Impurities B and D. Temperature was a useful selectivity influence for HILIC method development. The informatics solutions within Empower provided trending insight to effectively troubleshoot issues relating to poor sample preparation. The use of pre-column filters also contributed towards achieving excellent column performance of over 1500 injections.

In retrospect, a Routine Use Study of the original HPLC methodology would be costly. Comparing the mobile-phase consumption during 1500 injections on HPLC versus UPLC; HPLC would utilize approximately 65 liters compared to 11 liters consumed using UPLC. At an average cost of $165 per liter acetonitrile, the resulting methodology would save approximately $8800 in solvent consumption. Implementing UPLC technology results in a time savings of 26 days per 1500 injections, or roughly an 80% reduction in analysis time in which the resources can be better utilized to increase profitability.

References

1. Ali, M.S., Rafiuddin, S., Ghori, M., Khatri, R., *Chromatographia* **67**, 517-525 [2008].
2. *The Use of Medicines in the United States: Review of 2010*. IMS Institute for Healthcare Informatics (April 2011). Retrieved on April 28, 2011.
3. Tache, F. and Albu, M., *Revue Roumaine de Chimie* **52**(6): 603-609 [2007].
4. Arayne, M.S., Sultana, N., Zuberi, M.H., *Pak J Pharm Sci* **19**(3): 231-5 [2006].

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Forced Degradation Analysis of Omeprazole Using CORTECS 2.7 µm Columns

Kenneth D. Berthelette, Thomas Swann, and Kenneth J. Fountain
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- CORTECS® C_{18}+ Columns provide superior peak shape for bases using low pH, low ionic strength pH modifiers (*e.g.* formic acid).
- CORTECS 2.7 µm, 4.6 x 150 mm Columns provide high resolution of complex mixtures while operating within the pressure limits of an HPLC system, enabling separation of structurally similar compounds.
- Combining UV detection with the ACQUITY® QDa™ Detector provides easy and reliable mass detection for simple peak identification.

WATERS SOLUTIONS

CORTECS C_{18}+ Columns

Alliance® HPLC

Empower® 3 CDS

LCMS Certified Max Recovery Vials

ACQUITY QDa Detector

KEY WORDS

Alliance HPLC, CORTECS C_{18}+, Omeprazole, ACQUITY QDa Detector, Forced Degradation

INTRODUCTION

Chemical stability testing is important when manufacturing pharmaceutical compounds. This is especially true with pharmaceuticals which are administered orally. The digestion process can alter the active pharmaceutical ingredient (API), producing potentially harmful by-products. In the development process, it is important to be able to detect these by-products and characterize them. In order to perform characterization of a compound, all degradation products and the main compound should be resolved from each other. CORTECS 2.7 µm Columns offer superior peak shape and resolution for the analysis of complex samples. The columns have 2.7 µm solid-core particles which allow for higher resolution, and lower backpressures than fully porous columns. Traditionally, the use of 150 mm sub-3-µm columns on HPLC systems is limited due to the backpressure generated. However, CORTECS 2.7 µm Columns allow an analyst to use 150 mm columns on their HPLC system, offering the highest resolution possible while operating within the pressure limits of the system (<5,000 psi). The forced degradation of Omeprazole will be shown as an example and analyzed using a CORTECS C_{18}+, 2.7 µm, 150 mm Column on an Alliance HPLC System with both UV and mass detectors.

Omeprazole is a basic compound which acts as a proton pump inhibitor used in the treatment of acid reflux and heartburn. This API is also unstable at low pH.[1] Forced degradation under acidic conditions is needed to identify/characterize by-products formed under such conditions. CORTECS 2.7 µm Columns allow for high resolution between peaks in complex mixtures such as forced degradation samples. The use of the Waters® ACQUITY QDa Detector allows for quick identification of peaks by mass. Due to the simplicity of the instrument, the ACQUITY QDa requires minimal training in order to use it effectively and can provide quick and reliable mass data. Unlike traditional mass spectrometers, the ACQUITY QDa Detector does not require regular tuning or calibration. This maximizes the ease of use for inexperienced analysts. By using the newest particle technologies and detection techniques, an analyst can quickly and reliably separate complex mixtures and easily obtain mass spectral data of the peaks. This can lead to faster decisions in method development, potentially reducing total development time.

EXPERIMENTAL

LC conditions

System:	Alliance HPLC
Column:	CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm (p/n 186007408)
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Gradient:	10–78% B in 16.5 min, return to 10% B in 0.1 min, hold for 3.4 min
Flow rate:	1.2 mL/min
Column temp.:	30 °C
Detection (UV):	280 nm
QDa setting:	ESI+ full scan from 120–420 *m/z*
QDa cone voltage:	15V
QDa capillary voltage:	0.8 kV
Injection volume:	9.6 µL
Data management:	Empower 3 CDS

Sample Preparation

Two Omeprazole tablets (20 mg Omeprazole) were separately crushed with a mortar and pestle and transferred to two 100 mL volumetric flasks. To one flask (A), 25 mL 0.1 N HCl was added and the solution was left at room temperature for 1.5 hours. 25 mL 0.1 N NaOH was added to neutralize the solution. Methanol was added to the flask to bring the sample up to 100 mL. The other solution (B) was diluted to 100 mL with 50:50 methanol:water. Both solutions were then filtered through a 0.2 µm filter. To create the sample for injection 0.66 mL of solution A and 0.34 mL of solution B were combined in an LCMS Certified Max Recovery Vial (p/n 600000749CV).

RESULTS AND DISCUSSION

The sample was injected onto an Alliance e2695 HPLC System equipped with a 2998 PDA detector and an ACQUITY QDa Detector. In the case of Omeprazole, there are at least 8 known degradation compounds found in literature searches and references to USP standards.[2,3,4] Table 1 lists the compounds and their associated masses. Two sets of isobaric compounds exist in this separation. Related Compounds F and G have the same mass (312.36 *m/z*), and Omeprazole-n-Oxide and Omeprazole Sulphone have the same mass (362.42). Figure 1 shows the separation of the acid degradation sample on a CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm Column.

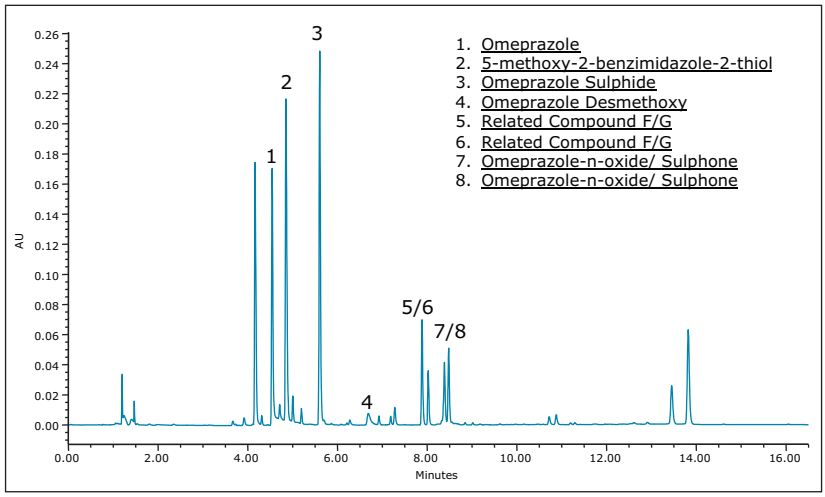

Figure 1. Separation of the forced degradation sample of Omeprazole tablets.

As seen in the above chromatography, all of the eluting peaks were sharp and well resolved. Since the backpressure on the CORTECS 2.7 µm Columns is low compared to fully porous columns with the same particle size, a 150 mm length column can be used for maximum resolution, which is critical for the two isobaric peak pairs in this separation. In addition, use of the ACQUITY QDa Mass Detector allowed matching the UV peaks to the components in Table 1, and even though the unit mass resolution does not permit isobaric pairs 5/6 and 7/8 to be distinguished, injection of pure standards for these compounds would confirm their elution order.

Table 1. Known degradation products and structurally related compounds of Omeprazole and the associated masses of each compound.

Peak ID Number	Compound	Mass (M+H)
1	Omeprazole	346.4
2	5-methoxy-2-benzimidazole-2-thiol	181.2
3	Omeprazole Sulphide	330.4
4	Omeprazole Desmethoxy	316.4
5/6	Omeprazole Related Compound F/G	312.4
7/8	Omeprazole Sulphone	362.4
7/8	Omeprazole-n-oxide	362.4

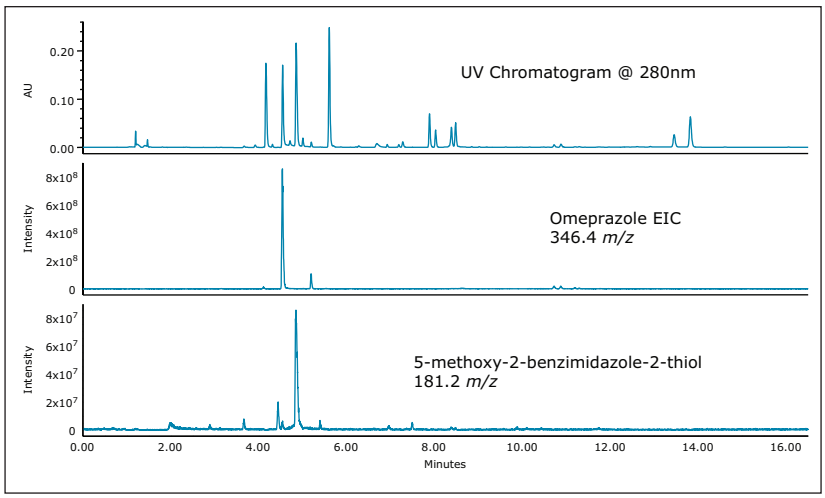

Figure 2. Extracted ion chromatograms of Omeprazole (346.4 m/z) and 5-methoxy-2-benzimidazole-2-thiol (181.2 m/z) showing positive identification of these two peaks.

Extracted ion chromatograms (EICs) were used to confirm peak identification. Figure 2 shows the EICs of Omeprazole and 5-methoxy-2-benzimidazole-2-thiol as examples. The ACQUTIY QDa Detector can also be used to generate a combined mass spectrum for a given peak. Figure 3 shows the combined spectrum for Omeprazole and Omeprazole Sulphide. Combined spectrum analysis is typically used to confirm peak identity and detect co-elution of additional compounds.

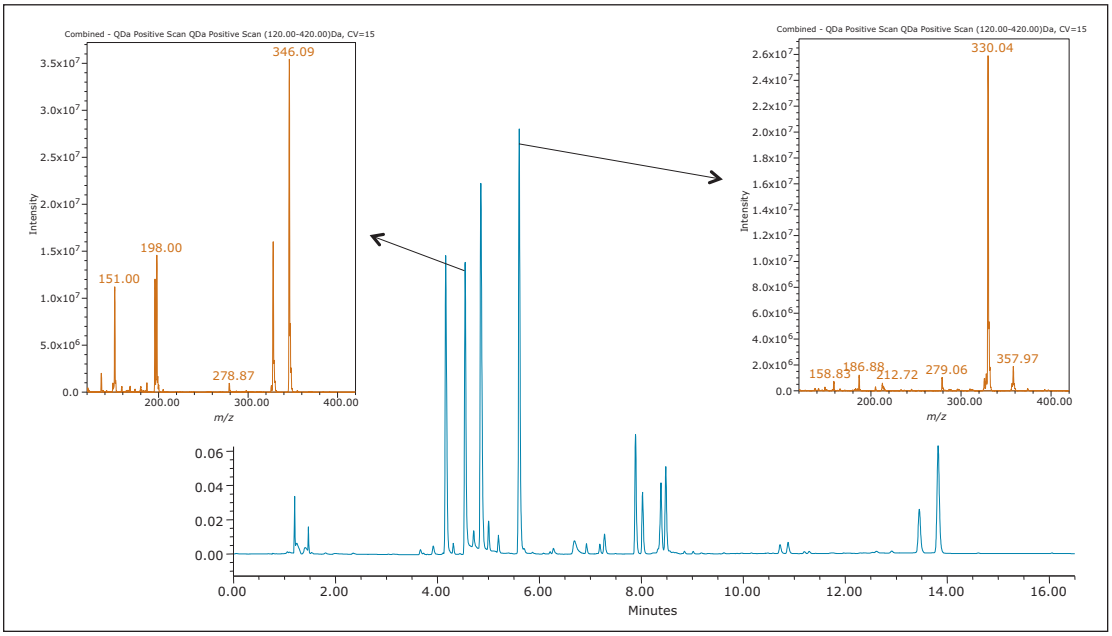

Figure 3. Combined spectrum of all masses present during the elution of Omeprazole (346.09 m/z) and Omeprazole Sulphide (330.04 m/z).

Identification of the degradants present was possible due to the high resolution separation obtained on a CORTECS 2.7 µm, 150 mm Column, and the mass data obtained on the ACQUITY QDa Detector. The use of a CORTECS 2.7 µm Column allowed for the separation of the eight separate compounds in the forced degradation sample. The high efficiency and low backpressure of these columns allows the highest possible resolution on an HPLC system.

CONCLUSIONS

A forced degradation study of Omeprazole was performed using a CORTECS C_{18}+, 2.7 µm, 4.6 x 150 mm Column on an Alliance HPLC System equipped with both a PDA and QDa Detector. This column exhibited high resolution as shown for two critical pairs of isobaric degradants, as well as backpressures compatible with traditional HPLC (<5,000 psi). The use of the CORTECS C_{18}+ stationary phase gave sharp and symmetrical peak shape for Omeprazole (a weak base) and its degradants, even when using low pH, low ionic strength pH modifiers (*e.g.* formic acid). The combination of CORTECS 2.7 µm Columns, as well as PDA and mass detection using the QDa Detector allows for the rapid separation and identification of closely eluting compounds in complex sample mixtures. On an HPLC system, 150 mm length CORTECS 2.7 µm Columns can be used at an appropriate flow rate without exceeding the pressure limits, thus maximizing the potential for increased resolution in HPLC.

References

1. Bozdag, S.; Calis, S.; Sumnu, M. Formulation and stability evaluation of enteric-coated Omeprazole formulations. *S.T.P. Pharma Sciences* 1999, 9, 321–327.
2. Seshadri, R.K.; Raghavaraju, T.V., Chakravarthy, I.E. A Single Gradient Stability-Indicating Reversed-Phase LC Method for the Estimation of Impurities in Omeprazole and Domperidone Capsules. *Scientia Pharmaceutica* 2012, 437–458.
3. Rajab, A; Touma, M.; Rudler, H.; Afonso, C.; Seuleman, M. Slow, spontaneous degradation of lansoprazole, omeprazole and pantoprazole tablets: isolation and structural characterization of the toxic antioxidants 3H-benzimidazole-2-thiones. *Pharmazie* 2013, 749–754.
4. Search Results USP Reference Standards, http://store.usp.org/OA_HTML/usp_ibeCSrdSrchResults.jsp?kw=Omeprazole.

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Increasing Efficiency of Method Validation for Metoclopramide HCl and Related Substances with Empower 3 MVM Software

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APPLICATION BENEFITS

- Automated method validation workflow
- Reduced time to complete the steps required to test and document a validated method
- Compliance with regulations on data security, different user privileges, audit trails, data traceability, and electronic signature sign-off requirements

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

Empower® 3
Chromatography Data Software

Empower 3
Method Validation Manager (MVM)

ACQUITY UPLC Columns

ACQUITY® QDa® Detector

KEY WORDS

UPLC, method validation, metoclopramide HCl, sub-2-µm particles, mass detection, ApexTrack™ peak integration

INTRODUCTION

Method validation, which demonstrates that a method is suitable for its intended purpose,[1,2] is an important regulatory requirement for pharmaceutical organizations and their supporting contract partners. A compliant laboratory must provide documented evidence and assurance that the analytical method used for testing a drug product's identity, quality, purity, and potency generates accurate and reliable results.

The validation process of an analytical method is a complex and demanding activity, consisting of many time-consuming steps. Some of these steps include creation of validation protocols, experimental work, reviewing and processing data, performing calculations, approving, and final reporting. Since some of these steps are prone to errors, a well-organized plan is essential for successful validation of an analytical method and to ensure that the appropriate regulations and guidelines are being followed.

Once validation is executed, adherence to the validation plan and specification is a critical compliance requirement. Any validation results not meeting the specifications must be clearly identified and addressed during the validation process. Raw and processed data must be appropriately stored and traceable by providing data security, audit trails, and automatic data documentation required for reviews and audits.

In this application note, we present validation of a UPLC® method for metoclopramide HCl and related substances using Empower 3 Method Validation Manager (MVM), an option for Empower 3 Chromatography Data Software.

We show how Empower 3 MVM tracked every step of the method validation process, identifying the steps and data that did not meet defined validation requirements.

Overall, we demonstrate that Empower 3 MVM automates the method validation workflow within a single software environment, reducing time and ensuring conformance to the validation requirements and acceptance criteria defined in the protocol.

EXPERIMENTAL

Method conditions

LC system:	ACQUITY UPLC H-Class
Column:	ACQUITY UPLC CSH C_{18}, 2.1 x 50 mm, 1.7 µm (Waters p/n 186005296)
Column temp.:	45 °C
Injection volume:	1.0 µL
Flow rate:	0.6 mL/min
Solvent A:	125 mM Formic acid in water
Solvent B:	Water
Solvent C:	Methanol
Separation:	Gradient

Step	Time (minutes)	Solvent A (%)	Solvent B (%)	Solvent C (%)
1	Initial	10	85.0	5.0
2	5.0	10	30.0	60.0
3	5.5	10	30.0	60.0
4	5.6	10	85.0	5.0
5	7.5	10	85.0	5.0

Purge/Sample wash:	50:50 water/methanol
Seal wash:	90:10 water/acetonitrile
UV detector:	ACQUITY UPLC PDA
UV setting:	210-400 nm (derived at 270 nm)
Mass detector:	ACQUITY QDa (Extended Performance)
Ionization mode:	ESI+, ESI-
Acquisition range:	100 – 440 *m/z*
Sampling rate:	10 pts/sec
Data:	Centroid
System control, Data acquisition, and analysis:	Empower 3 FR2 CDS Software

RESULTS AND DISCUSSION

UPLC method for metoclopramide HCl and related substances

The UPLC method validated in this study was developed using a systematic method development protocol.[3] An example of the UPLC chromatographic method for metoclopramide and related compounds is shown in Figure 1.

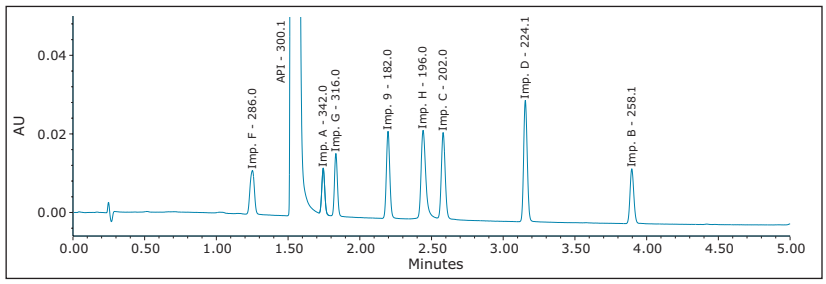


Figure 1. UPLC method for metoclopramide and related substances with UV at 270 nm.

About Empower 3 MVM

Empower 3 Method Validation Manager (MVM) is software that automates the validation process and enables efficient validation of chromatographic methods to ensure compliance to the validation requirements defined by the analytical laboratory. As shown in Figure 2, a validation workflow consists of many steps. A validation protocol (Figure 3) is created and used to execute the study. Once executed, Empower 3 MVM checks data for adherence with the validation requirements and flags any results that do not meet specifications. Validation results can be displayed in a report using validation report templates specific for each test available in Empower MVM software. The report templates can be customized as needed.

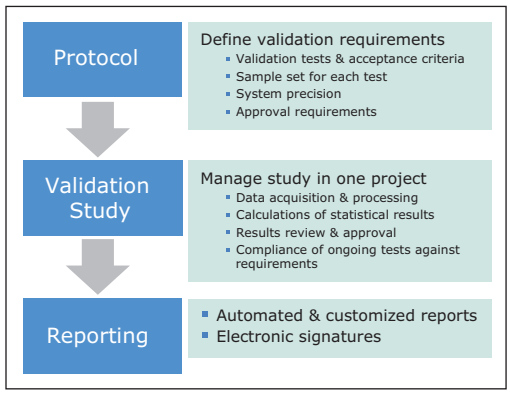


Figure 2. Workflow for validation of chromatographic methods with Empower 3 MVM.

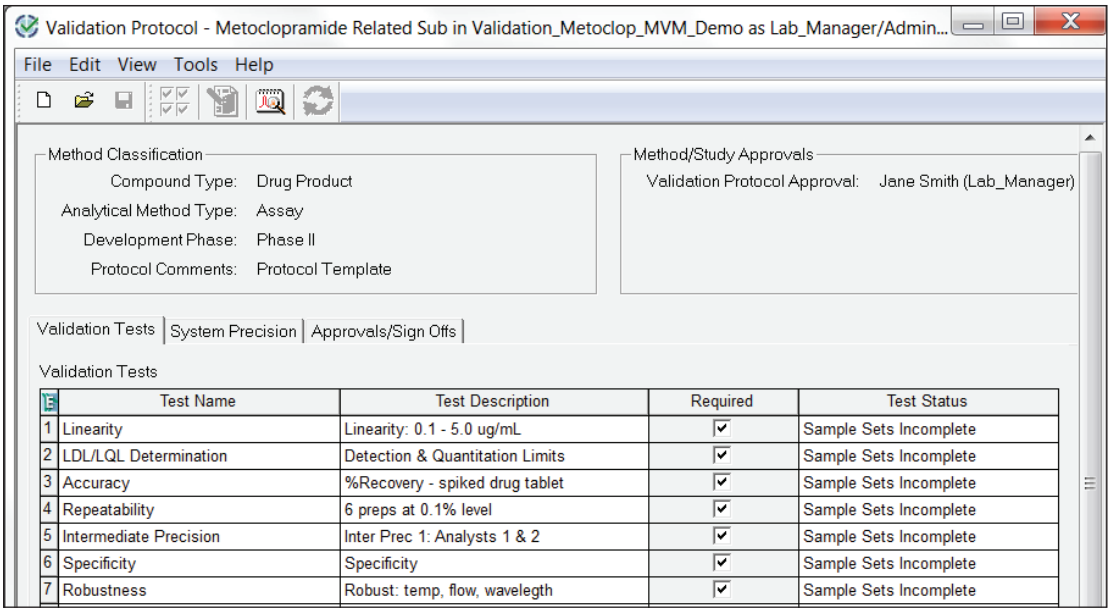


Figure 3. Validation protocol method created within Empower 3 MVM project. The validation tests, acceptance criteria for each validation test, and requirements for approval are defined in the validation protocol method. The validation protocol is approved by a lab manager via electronic signature.

Method validation

We used an established validation protocol method approved by a lab manager (via electronic signatures) to execute the validation of our UPLC method for related substances of metoclopramide HCl in drug tablet formulation. The validation tests included linearity, detection and quantitation limits, accuracy, repeatability, intermediate precision, specificity, and robustness. System precision was evaluated for each validation test using five replicate injections of the sample, as recommended in the USP General Chapter <621> on Chromatography.[4] The system precision criteria include:

- %RSD of retention times: ≤1.0%
- %RSD of peak areas: ≤2.0%
- USP resolution: ≥1.5
- Peak tailing: ≤1.5

1. Linearity

Method linearity for related substances was evaluated by analyzing seven concentrations of standard solutions ranging from 0.1 to 5.0 µg/mL. These concentrations corresponded to 0.02, 0.05, 0.1, 0.25, 0.5, 0.75, and 1.0% of the metoclopramide HCl target concentration of 0.5 mg/mL. We used Empower 3 MVM to calculate regression equation and correlation coefficients for a plot of average peak areas against the concentrations. Method linearity results generated by the software are displayed in Figure 4. The method shows linear relationship between the peak areas and concentrations for all related compounds with the correlation coefficients (r^2) greater than 0.999.

Validation_Linearity Report

Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID: 22180 Channel: PDA 270

	Comp.	Points /Level	X Value	X Value Units	Y Value	Equation	R	R^2	Intercept	Residual % RSD	Pass?
1	Imp. F	3	Amount	ug/mL	Response	Y = 4.87e+003 X - 7.11e+001	0.99990	0.99980	-71	1.39	Pass
2	Imp. A	3	Amount	ug/mL	Response	Y = 3.24e+003 X + 1.73e+002	0.99987	0.99975	173	1.51	Pass
3	Imp. G	3	Amount	ug/mL	Response	Y = 4.40e+003 X - 1.81e+001	0.99989	0.99978	-18	1.43	Pass
4	Imp. 9	3	Amount	ug/mL	Response	Y = 7.31e+003 X - 2.45e+001	0.99988	0.99976	-25	1.52	Pass
5	Imp. H	3	Amount	ug/mL	Response	Y = 9.01e+003 X - 2.23e+002	0.99988	0.99976	-223	1.52	Pass
6	Imp. C	3	Amount	ug/mL	Response	Y = 6.08e+003 X - 3.62e+001	0.99991	0.99981	-36	1.33	Pass
7	Imp. D	3	Amount	ug/mL	Response	Y = 9.04e+003 X - 1.06e+001	0.99992	0.99984	-11	1.22	Pass
8	Imp. B	3	Amount	ug/mL	Response	Y = 4.68e+003 X + 2.23e+001	0.99987	0.99974	22	1.58	Pass

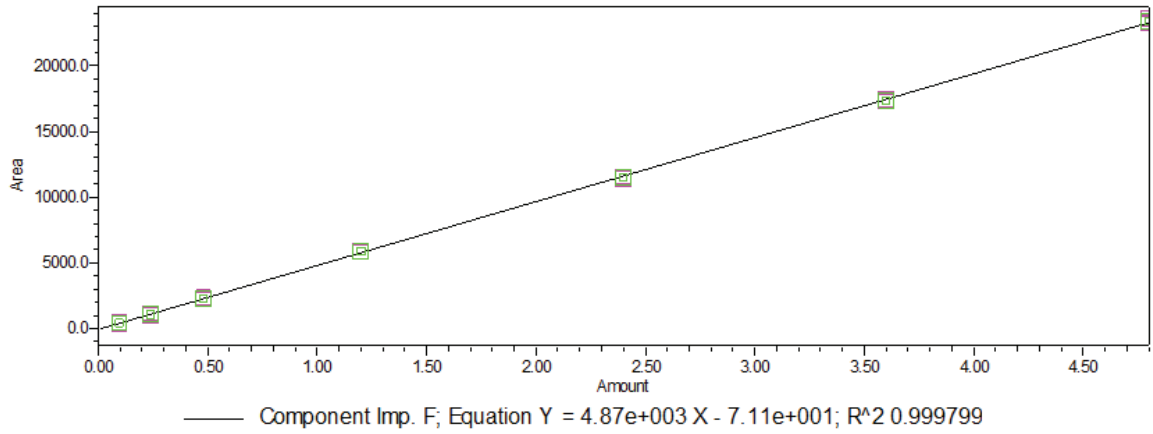


Figure 4. Method linearity results for metoclopramide related substances generated by Empower 3 MVM.

2. Detection and quantitation limits

We determined the lowest detection and quantitation limits (LDL and LQL) based on the USP signal-to-noise criteria of 3:1 and 10:1, respectively. In addition to signal-to-noise, other methods for LDL and LQL determination are available within Empower 3 MVM, such as based on linearity curve residual standard deviation and linearity curve intercept standard deviation.

As shown in Figure 5, the LDL and LQL for related substances ranged from 0.03 to 0.07 µg/mL and from 0.1 to 0.2 µg/mL, respectively.

Validation_LDL_LQL

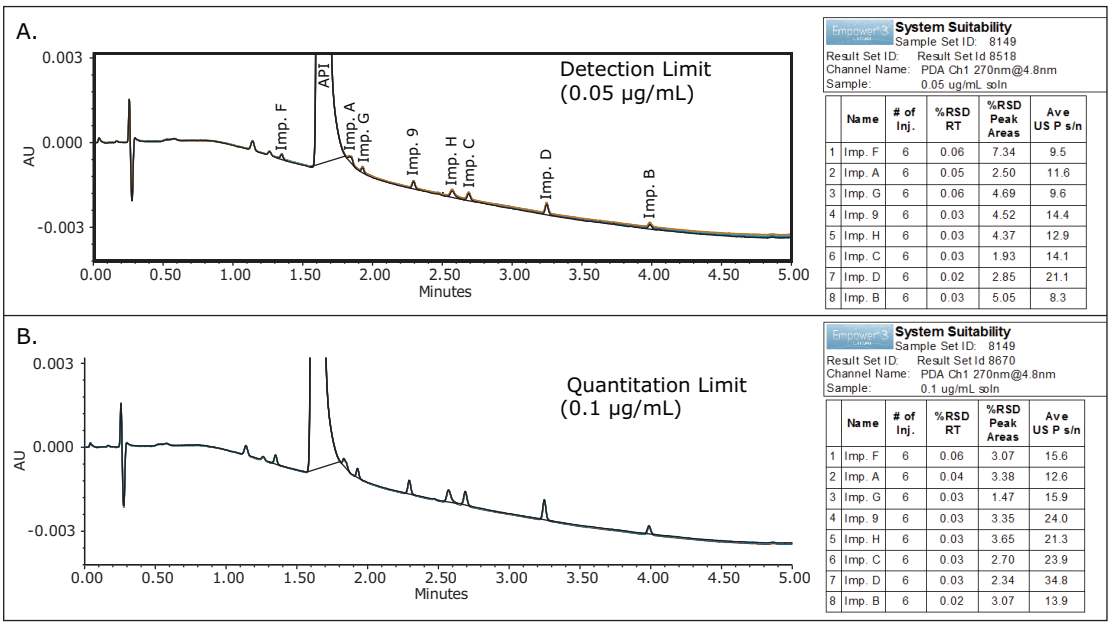
Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID: 22841 Channel: PDA 270

LDL and LQL Determination

	Component	RT Mean (min)	LDL/LQL Method	Signal/Noise Type	Lowest Detection Limit	Lowest Quantitation Limit	Units
1	Imp. F	1.305	Signal to Noise	USP s/n	0.0563	0.1706	ug/mL
2	Imp. A	1.791	Signal to Noise	USP s/n	0.0732	0.2219	ug/mL
3	Imp. G	1.889	Signal to Noise	USP s/n	0.0534	0.1618	ug/mL
4	Imp. 9	2.241	Signal to Noise	USP s/n	0.0376	0.1140	ug/mL
5	Imp. H	2.479	Signal to Noise	USP s/n	0.0436	0.1321	ug/mL
6	Imp. C	2.627	Signal to Noise	USP s/n	0.0504	0.1528	ug/mL
7	Imp. D	3.178	Signal to Noise	USP s/n	0.0312	0.0945	ug/mL
8	Imp. B	3.907	Signal to Noise	USP s/n	0.0657	0.1991	ug/mL

Figure 5. Lowest detection and quantitation concentrations determined using USP signal-to-noise criteria.

We then validated the results by analyzing six replicate injections of the solutions prepared near the LDL (0.05 µg/mL) and LQL (0.1 µg/mL) to verify the performance, Figure 6. The LDL and LQL replicates tested in this study exceeded the USP signal-to-noise criteria



System Suitability
Sample Set ID: 8149
Result Set ID: Result Set Id 8518
Channel Name: PDA Ch1 270nm@4.8nm
Sample: 0.05 ug/mL soln

	Name	# of Inj.	%RSD RT	%RSD Peak Areas	Ave US P s/n
1	Imp. F	6	0.06	7.34	9.5
2	Imp. A	6	0.05	2.50	11.6
3	Imp. G	6	0.06	4.69	9.6
4	Imp. 9	6	0.03	4.52	14.4
5	Imp. H	6	0.03	4.37	12.9
6	Imp. C	6	0.03	1.93	14.1
7	Imp. D	6	0.02	2.85	21.1
8	Imp. B	6	0.03	5.05	8.3

System Suitability
Sample Set ID: 8149
Result Set ID: Result Set Id 8670
Channel Name: PDA Ch1 270nm@4.8nm
Sample: 0.1 ug/mL soln

	Name	# of Inj.	%RSD RT	%RSD Peak Areas	Ave US P s/n
1	Imp. F	6	0.06	3.07	15.6
2	Imp. A	6	0.04	3.38	12.6
3	Imp. G	6	0.03	1.47	15.9
4	Imp. 9	6	0.03	3.35	24.0
5	Imp. H	6	0.03	3.65	21.3
6	Imp. C	6	0.03	2.70	23.9
7	Imp. D	6	0.03	2.34	34.8
8	Imp. B	6	0.02	3.07	13.9

Figure 6. Overlay of six replicate injections of LDL and LQL solutions. The ApexTrack enabled consistent integration of all the peaks.

A. All components exceeded signal-to-noise criteria of 3:1 for detection.

B. All components exceeded signal-to-noise criteria of 10:1 for quantitation.

3. Accuracy

The accuracy of an analytical method includes quantitative determination of impurities in the presence of sample matrix components. Accuracy of our method was assessed by analyzing triplicate preparations of the drug tablet solutions spiked with related substances at 0.1, 0.5, and 1.0% levels in the presence of metoclopramide HCl concentration of 0.5 mg/mL. Accuracy results are summarized in Figure 7. The % recovery for all nine determinations ranged from 97 to 101% with %RSD ≤4.21%, which passes the acceptance criteria of 90–110% and %RSD ≤10%, respectively.

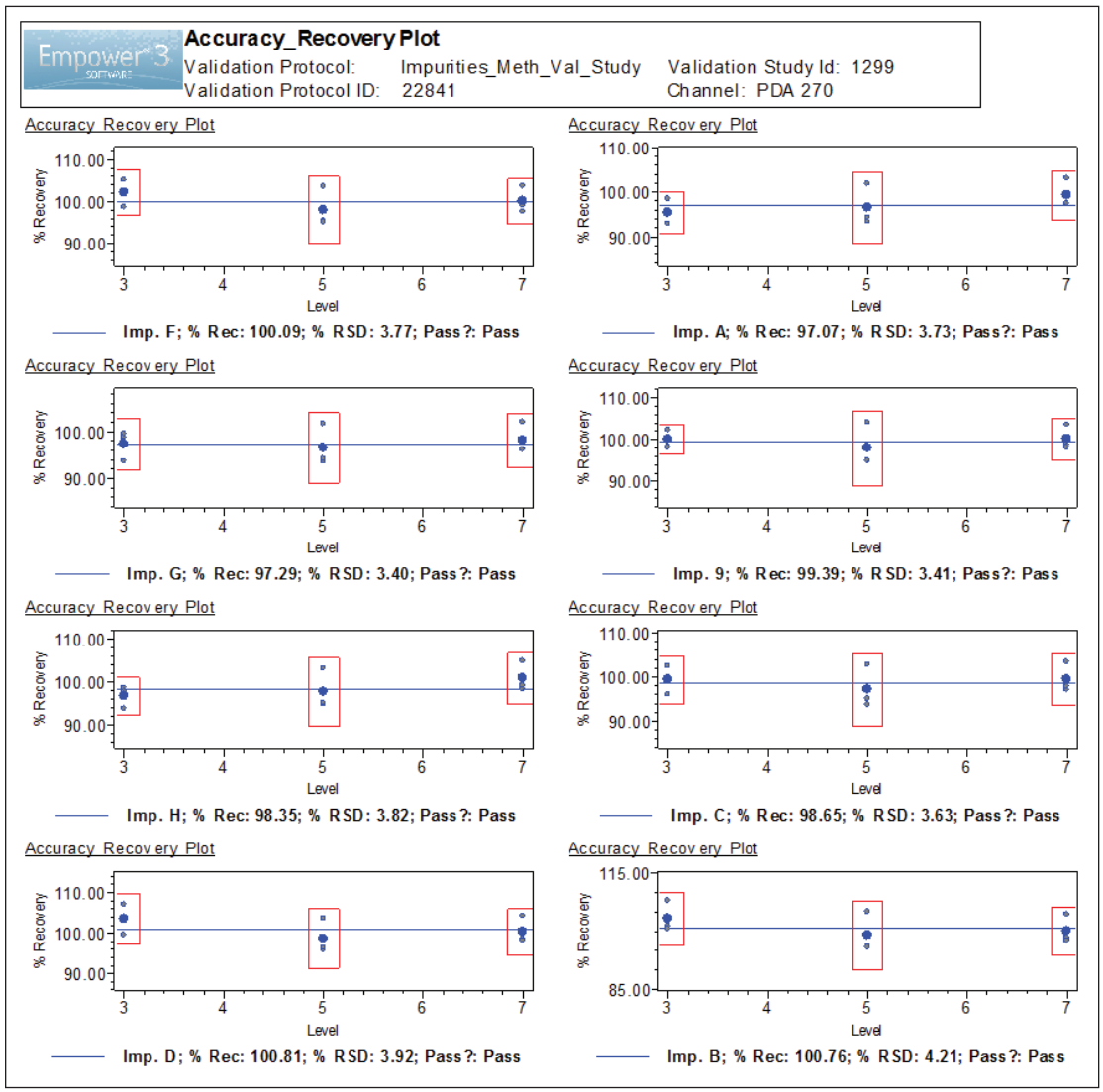


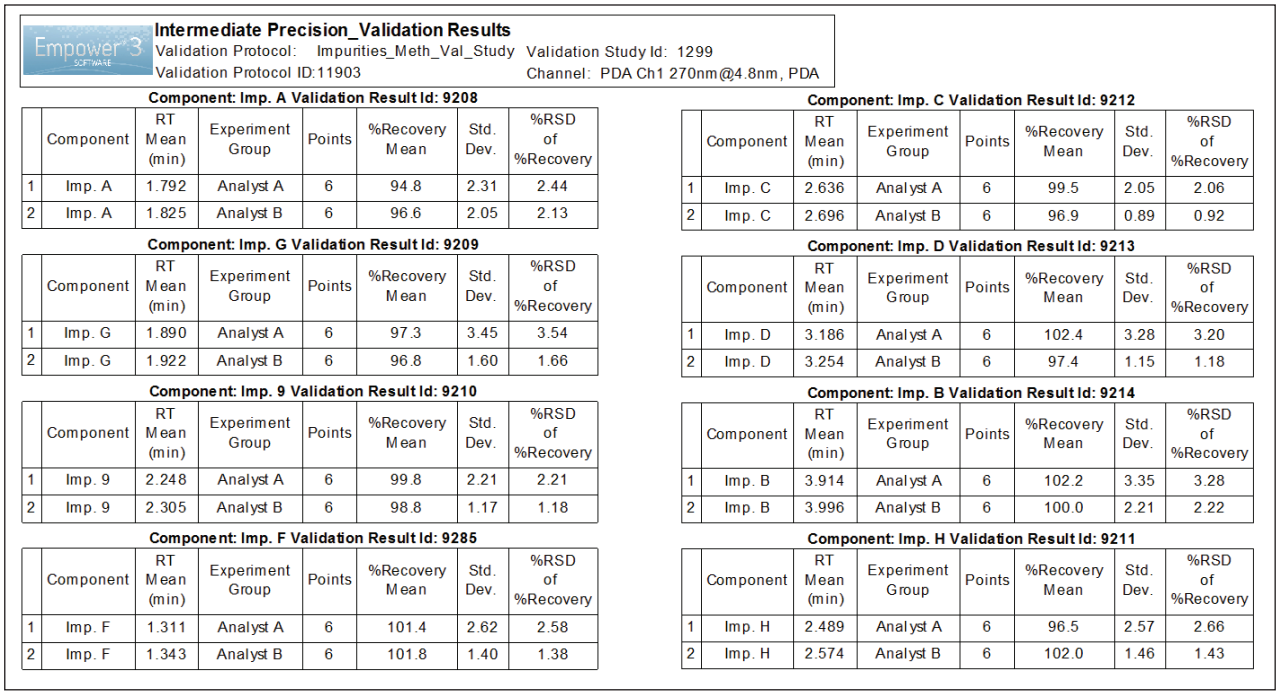
Figure 7. Accuracy results generated by Empower 3 MVM. Summary of 9 determination, 3 preparation at each levels: 0.1%, 0.5%, and 1.0%.

4. Repeatability

Method repeatability is a quantitative analysis of impurities from six independent preparations of the drug product by a single analyst. Repeatability of our method was demonstrated by spiking drug tablet sample solution with related substances at 0.1% level with respect to the metoclopramide HCl (API) concentration of 0.5 mg/mL. Repeatability results generated by analyst A (Figure 8) met the criteria for % recovery of 100 ±10% and %RSD ≤10%.

5. Intermediate precision

Intermediate precision was evaluated by a different analyst, on a different day, using different instrument and column. Summary of results from six individual spiked drug tablet sample preparations generated by analysts A and B are shown in Figure 8. Overall, the intermediate precision results met the criteria for % recovery of 100 ±10% and %RSD ≤10%.



Intermediate Precision_Validation Results
Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID:11903 Channel: PDA Ch1 270nm@4.8nm, PDA

Component: Imp. A Validation Result Id: 9208

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. A	1.792	Analyst A	6	94.8	2.31	2.44
2	Imp. A	1.825	Analyst B	6	96.6	2.05	2.13

Component: Imp. C Validation Result Id: 9212

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. C	2.636	Analyst A	6	99.5	2.05	2.06
2	Imp. C	2.696	Analyst B	6	96.9	0.89	0.92

Component: Imp. G Validation Result Id: 9209

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. G	1.890	Analyst A	6	97.3	3.45	3.54
2	Imp. G	1.922	Analyst B	6	96.8	1.60	1.66

Component: Imp. D Validation Result Id: 9213

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. D	3.186	Analyst A	6	102.4	3.28	3.20
2	Imp. D	3.254	Analyst B	6	97.4	1.15	1.18

Component: Imp. 9 Validation Result Id: 9210

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. 9	2.248	Analyst A	6	99.8	2.21	2.21
2	Imp. 9	2.305	Analyst B	6	98.8	1.17	1.18

Component: Imp. B Validation Result Id: 9214

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. B	3.914	Analyst A	6	102.2	3.35	3.28
2	Imp. B	3.996	Analyst B	6	100.0	2.21	2.22

Component: Imp. F Validation Result Id: 9285

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. F	1.311	Analyst A	6	101.4	2.62	2.58
2	Imp. F	1.343	Analyst B	6	101.8	1.40	1.38

Component: Imp. H Validation Result Id: 9211

	Component	RT Mean (min)	Experiment Group	Points	%Recovery Mean	Std. Dev.	%RSD of %Recovery
1	Imp. H	2.489	Analyst A	6	96.5	2.57	2.66
2	Imp. H	2.574	Analyst B	6	102.0	1.46	1.43

Figure 8. Repeatability (analyst A) and intermediate precision (analysts A and B) results generated by Empower 3 MVM.

6. Specificity

For the impurity test, specificity demonstrates that impurities can be separated and accurately measured in the presence of the sample matrix. This is typically done by spiking a drug substance or drug product with appropriate levels of impurities. In addition to demonstrating robust and reliable separation, it is important to identify that the desired components are not subject to interference with other species present in the sample. The UV peak purity determination is often used to show homogeneity of the chromatographic peak.

Specificity of our method was demonstrated by spiking drug tablet samples containing 0.5 mg/mL of metoclopramide HCl with related substances at 0.1% level. Accuracy and repeatability results show acceptable recoveries of each related substance. To demonstrate that the related substances are not coeluting with other components of the sample matrix, we used UV in conjunction with the MS spectral data as shown in Figure 9. Peak homogeneity was assessed using UV peak purity plot (Figure 9B). The peak purity angle is below the threshold angle, indicating the Impurity A peak is spectrally homogeneous. The mass spectral data provided additional information at the leading, apex, and tailing regions of the peak to confirm that only one mass is detected under the UV peak. The MS spectrum (Figure 9C) at the leading and tailing edge of the peak indicates the presence of an ion with mass of 342.0 m/z, which is specific to Impurity A. Overall, the UV peak purity plot and the MS spectrum shows that Impurity A is not coeluting with other peaks.

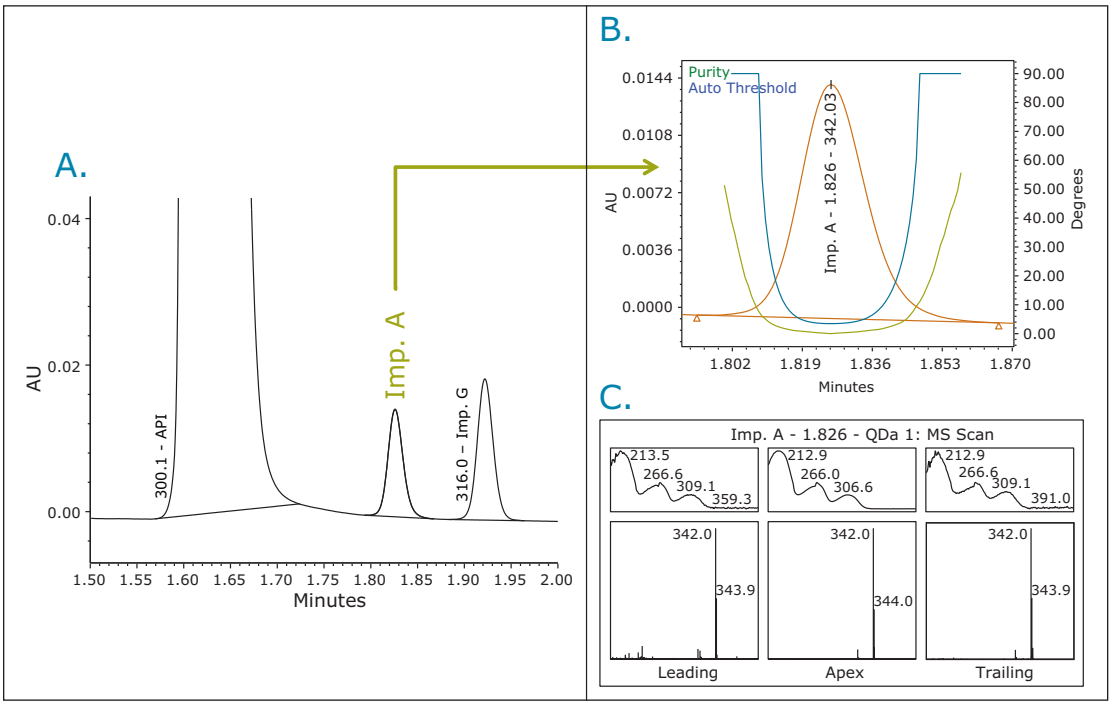


Figure 9. Peak homogeneity determination for specificity.
A. Accuracy sample with UV at 270 nm.
B. Peak purity plot of Impurity A.
C. UV and Mass profile of an Impurity A at the leading, apex, and tailing edge of the peak.

7. Robustness

Robustness is determined by the ability of the method to remain unaffected by the changes of chromatographic parameters. The parameters assessed in our study included:

- Column temperature: 45 ± 2.0 °C
- Flow rate: 0.6 ± 0.05 mL/min
- Wavelength: 270 ± 2 nm

Robustness was performed using a full factorial experimental design to investigate combination of different instrument conditions on the resolution between all the peaks, with a goal of achieving a minimum resolution of ≥2.0 for each peak. The robustness results in Figure 10 showed that the method met the criteria for resolution.

Validation_Robustness_Report

Validation Protocol: Impurities_Meth_Val_Study Validation Study Id: 1299
Validation Protocol ID: 22535 Channel: PDA Ch1 270nm@4.8nm

Assessed Field: USP Resolution

	Validation Result Id	Component	RT Mean (min)	Experiments	Assessed Field	Mean Rs	Lower Limit of Mean Rs	Upper Limit of Mean Rs	Pass /Fail
1	17451	API	1.617	8	USP Resolution	4.8	4.7	4.9	Pass
2	17487	Imp. A	1.825	8	USP Resolution	4.0	3.9	4.2	Pass
3	17489	Imp. G	1.923	8	USP Resolution	2.8	2.4	3.1	Pass
4	17491	Imp. 9	2.312	8	USP Resolution	10.3	10.1	10.5	Pass
5	17493	Imp. H	2.597	8	USP Resolution	6.2	5.5	7.0	Pass
6	17495	Imp. C	2.712	8	USP Resolution	2.4	2.0	2.8	Pass
7	17497	Imp. D	3.267	8	USP Resolution	12.9	12.3	13.4	Pass
8	17499	Imp. B	4.006	8	USP Resolution	17.1	16.6	17.6	Pass

Figure 10. Robustness results. Resolution for each component was ≥2.0.

CONCLUSIONS

We successfully validated the UPLC method for related substances of metoclopramide HCl for linearity, detection and quantitation limits, accuracy, repeatability, intermediate precision, specificity, and robustness using Empower 3 MVM. Validation results showed that the method is linear, accurate, repeatable, precise, specific, and robust for all related substances tested in this study. The quantitation limit of all related compounds was below the reporting threshold of 0.1% or 0.5 µg/mL.

Empower 3 MVM software streamlined the entire validation process, from creating a validation protocol method to acquiring, reviewing, analyzing, approving, and reporting validation data. The updated tool/message center provided status of each validation test in a study and whether the results met the acceptance criteria, and flagged any out-of-specification results. Using ApexTrack for automated peak integration enabled consistent integration of all peaks during the validation process. Using the ACQUITY QDa Detector for mass detection in conjunction with UV detection enabled quick determination of peak purity using mass and UV spectral data. Finally, the validation results and validation study were reviewed and approved via electronic signatures.

Empower 3 MVM is compliant-ready software that can be easily adapted by any analytical laboratory to improve efficiency of the chromatographic method validation process and to ensure conformance to validation requirements.

References

1. ICH Q2B, Validation of Analytical Procedures, International Conference on Harmonization, November 1996.
2. USP General Chapter, <1225>, Validation of Compendial Procedures, The United States Pharmacopeia Convention, official May 1, 2013.
3. Maziarz M, McCarthy SM, Wrona M. Improving Effectiveness of Method Development for Metoclopramide HCl and Related Substances Using a Systematic Screening Protocol. Waters Application Note; 2014: 720005026en.
4. USP General Chapter, <621>, Chromatography, USP36-NF31, The United States Pharmacopeia Convention, official December 2013.

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Improving LC-MS Analysis of Basic Impurities Using CORTECS C_{18}+, 2.7 µm Solid-Core Particle Columns

Kenneth D. Berthelette, Thomas Swann, and Kenneth J. Fountain
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Improved peak shape at low ionic strength, acidic conditions (*e.g.*, formic acid) compared to other solid-core columns
- Increased signal/noise ratio in mass spectrometry

WATERS SOLUTIONS

CORTECS® C18+, 2.7 µm Columns

ACQUITY UPLC®

ACQUITY® TQD Mass Spectrometer

Empower® 3 CDS

LCMS Certified Max Recovery Vials

KEY WORDS

ACQUITY UPLC, CORTECS C_{18}+, amitriptyline, imipramine, ACQUITY TQD

INTRODUCTION

The LC-MS analysis of trace level impurities is an important capability in numerous fields. Some chemical processes have acceptance criteria focused specifically on the presence and amount of impurities. This is especially true in the pharmaceutical industry. In such analyses, the intense and often broad main component(s) (*e.g.*, API) can obscure adjacent impurity peaks, making quantitation difficult and irreproducible. Furthermore, a significant amount of pharmaceutical compounds are basic in nature which present challenges in the chromatographic separation due to poor peak shape at low pH.

This application note describes use of two structurally similar drugs, imipramine and amitriptyline to simulate a difficult mixture of an API and a closely eluting impurity present at low levels. These compounds present a common analytical challenge. Their basic nature leads to poor peak shape in frequently employed formic acid mobile phase conditions. Such phenomena are mitigated with the CORTECS C_{18}+ columns due to the surface charge present. This technology and the solid-core particles afford superior peak shape and higher MS signal. The result is more accurate and reliable characterization of the impurity peaks using HPLC-MS and UPLC-MS instruments.

EXPERIMENTAL

LC conditions

Columns:	CORTECS C_{18}+, 2.7 µm, 3.0 x 50 mm (p/n 186007400)
	Competitor solid-core C_{18}, 2.6 µm, 3.0 x 50 mm
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in acetonitrile
Flow rate:	0.8 mL/min
Column temp.:	30 °C
Detection (UV):	254 nm
Injection volume:	10.0 µL
Method:	Start at 25% B, linear gradient to 35% B in 3.0 minutes. Return to 25% B in 0.1 minutes. Hold for 1 minute.

MS conditions

System:	ACQUITY TQD Mass Spectrometer
Ionization mode:	ESI+ Mode
Capillary voltage:	3.8 kV
Cone voltage:	30 V
Source temp.:	120 °C
Desolvation temp:	450 °C
Cone gas flow:	30 L/hr
Desolvation gas flow:	1000 L/hr
Method:	Selected Ion Recording (SIR) mode at 278.4 amu
Data Management:	Empower 3 CDS

Sample Preparation

An aqueous solution of imipramine (0.5 mg/mL, simulated API) and amitriptyline (0.5 µg/mL, simulated impurity) was prepared and placed into an LCMS Certified Max Recovery Vial (p/n 600000749CV) for analysis.

RESULTS AND DISCUSSION

Impurity analysis is an important testing method, especially in pharmaceutical manufacturing. Some validated USP methods require impurity testing as part of the overall sample analysis. Characterization and quantitation of impurities can be problematic in formulated samples as there are often other compounds besides the active pharmaceutical ingredient (API) and related impurities in the separation. Achieving a good separation between the API and the impurities is important both for characterization and quantitation of the components. Sharp, symmetrical peaks with good separation allows for more accurate analysis. CORTECS Columns allow rapid separation of compounds while displaying high efficiency leading to better separations. An example of this is the separation of imipramine and amitriptyline using a CORTECS C_{18}+, 2.7 µm Column compared to a competitor solid-core C_{18} column.

Amitriptyline and imipramine are basic compounds that belong to a group of drugs called tricyclic antidepressants. The structure of both compounds is shown in Figure 1. These compounds are structurally similar and both contain a tertiary amine on the end of a short carbon chain. These tertiary amines give the compounds their basic characteristics, which can present problems in reversed-phase LC analysis. Often, basic compounds can have poor peak shapes in low ionic strength acidic modifiers such as formic acid. However, the CORTECS C_{18}+, 2.7 µm Column provides better peak shapes for bases using these conditions than a typical C_{18} column, due to the slightly positive surface charge on the C_{18}+ stationary phase particles. This improvement is comparable to separations using TFA-modified mobile phases, but avoids the ion suppressing effect of TFA.

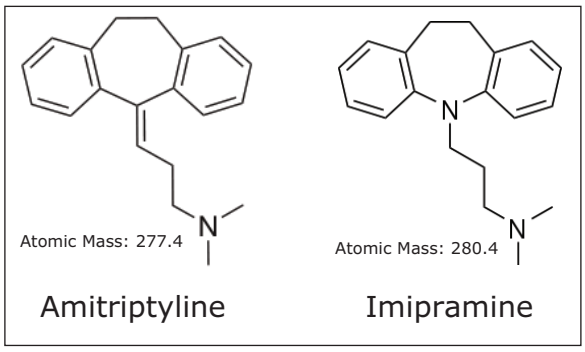

Figure 1. Chemical structures of amitriptyline and imipramine

For this analysis, an ACQUITY UPLC System was configured with a PDA and an ACQUITY TQD for UV and MS detection. A CORTECS C_{18}+, 2.7 µm Column and a competitor solid-core C_{18} column were compared for this separation. A sample containing 0.5 mg/mL Imipramine, and 0.5 µg/mL amitriptyline (0.1% the level of Imipramine) was prepared in water and 10 µL of that solution was injected onto each column. The UV chromatogram data were collected at 254 nm. The separations on both the CORTECS C_{18}+, 2.7 µm Column and the competitor solid-core C_{18}, 2.6 µm column are shown in Figure 2.

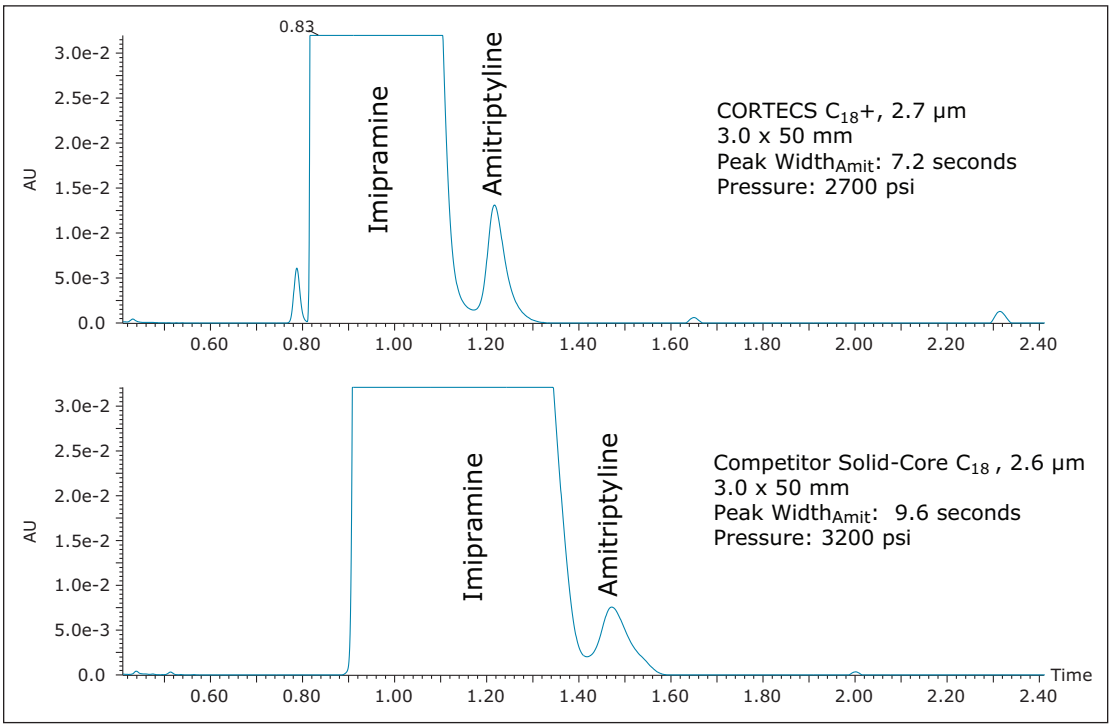

Figure 2. Separation of Imipramine (simulated API) and amitriptyline (simulated impurity at 0.1% concentration) using a CORTECS C_{18}+, 2.7 µm Column and a competitor solid-core C_{18} column, showing the decreased peak width for both peaks on the CORTECS C_{18}+ Column. Peak widths taken at 13.4% peak height.

As the UV data show, the CORTECS C_{18}+, 2.7 µm Column yields a narrower peak for the simulated impurity, amitriptyline, with increased intensity in UV as well. Additionally the simulated API peak, Imipramine, is much narrower on the CORTECS C_{18}+, 2.7 µm Column than on the competitor C_{18} column. The decreased peak width of the simulated API, imipramine, also reveals a smaller leading impurity peak that co-elutes with the main peak on the competitor solid-core column. The narrower peak shape of the Amitriptyline peak allows for better characterization of the simulated impurity, including determining peak area, and ultimately a quantitative result.

The reliable and accurate analysis of impurity peaks can also be achieved via mass spectrometry. During the above separations, the impurity peak (*m/z*) 278.4 amu, was examined. Figure 3 shows the SIR of the amitriptyline impurity on both the CORTECS C_{18}+, 2.7 µm Column and the competitor solid-core C_{18} column.

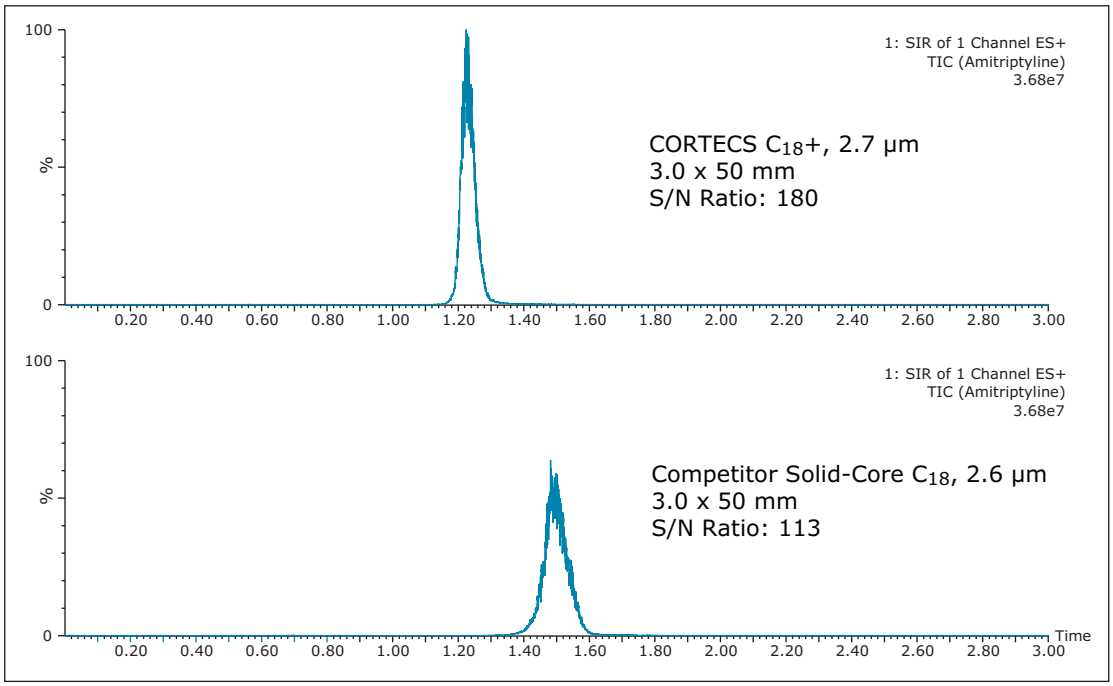

Figure 3. SIR mass spectrometry data of the amitriptyline simulated impurity with m/z 278.4 on CORTECS C_{18}+, 2.7 µm Column and competitor solid-core C_{18}, 2.6 µm columns, showing increased S/N ratio of the CORTECS C_{18}+, 2.7 µm Column. Y-Axis set to same scale to show difference in signal intensity.

As Figure 3 shows, the impurity has a 60% higher S/N ratio on the CORTECS C_{18}+, 2.7 µm Column compared to the competitor solid-core C_{18}, 2.6 µm column. A higher S/N ratio gives the analyst a better signal leading to more accurate data analysis and detection of peaks at lower concentrations. Additionally a higher signal allows an analyst to get mass information on low level, unknown impurities leading to better characterization of the sample.

CONCLUSIONS

Low level impurity testing is important in a wide variety of industries including pharmaceutical manufacturing. Some validated USP monographs require that a formulated sample have less than a certain level of impurities. These levels vary based on the analysis being performed. In order for an analyst to accurately and reliably determine the level of impurities, the individual impurity peaks must be separated. CORTECS C_{18}+, 2.7 µm Columns contain solid-core, charged surface particles which allow rapid separation of basic compounds while offering superior peak shape and MS signal when compared to competitor solid-core columns. The use of CORTECS C_{18}+, 2.7 µm Columns for routine analysis of basic compounds allows analysts to use MS compatible mobile phases leading to better MS signals, which can increase the quality of the characterization of low level impurity samples. Additionally, CORTECS C_{18}+, 2.7 µm Columns can lead to more reliable integration in UV analysis by reducing peak width and increasing resolution. The superior peak shape and enhanced signal allows better characterization of peaks with less method development effort, leading to higher analyst productivity.

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[TECHNOLOGY BRIEF]

Detection of UV-transparent Compounds by Addition of a Mass Detector to an Existing High Performance Liquid Chromatography System with Photodiode Array Detection

Aparna Chavali, Thomas E. Wheat, Patricia McConville

GOAL

Mass detection, in combination with UV detection, ensures more complete sample characterization.

BACKGROUND

Liquid chromatography is widely used to separate a broad range of analytes. While UV detection is used for qualitative analysis and quantitative analytical measurements, the compound of interest must have a UV chromophore. For compounds that have a weak or non-existent UV chromophore, pre-column or post-column derivatization of the sample, among many other alternative approaches, may be employed. However, pre- or post-column sample derivatization may not always be an attractive option. Some pre-column derivatization reactions can be slow and time-consuming, while post-column derivatization often requires additional tubing between the column and detector to provide reagent mixing and sufficient reaction time. This extra tubing increases extra-column band broadening, which deteriorates the quality of the separation. Adding a mass detector to an HPLC-UV system, as a complement to UV detection, can prove to be a more effective approach than derivatization in the analysis of non-chromophoric samples by HPLC, since mass detection does not rely on the compound having a UV chromophore. Mass detection, however, cannot be used for compounds that either poorly ionize or do not ionize under the selected conditions.

The addition of mass detection to an existing LC system with PDA detection enables detection of UV-transparent compounds.

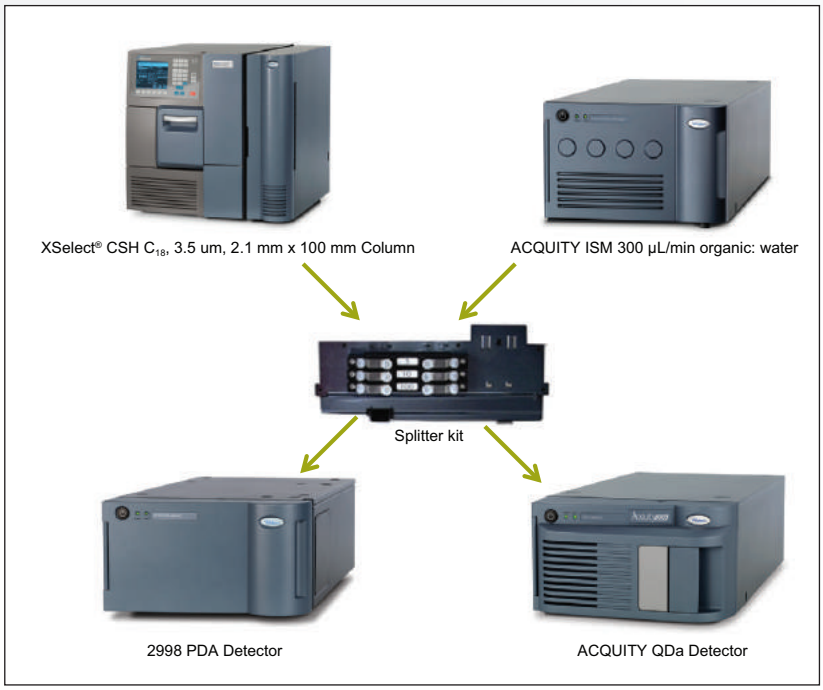

Figure 1. System configuration with Split-and-Dilute.

Therefore, using the two detectors in one system will help increase the opportunity of detecting compounds that do not share common functional groups for one mode of detection. However, the typically differing sensitivities of UV and MS detectors to the same compound may mean that the detectors are not operating in the linear dynamic ranges at a given sample concentration.

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THE SOLUTION

The addition of the ACQUITY QDa™ Detector to an existing HPLC separation monitored by UV provides an orthogonal mass detection technique to detect compounds that cannot solely be analyzed using the diode-array detector. The ACQUITY QDa Detector, with its small footprint, ease of use, and affordability, offers a convenient way to bring mass spectral information to chromatographic separations. The restrictor module in the ACQUITY® Isocratic Solvent Manager (ISM) divides the flow from the column between the Waters® 2998 Photodiode Array (PDA) Detector and the ACQUITY QDa Detector, aligning the dynamic range of both detectors in the same analysis (Figure 1).

Memantine is an orally active noncompetitive NMDA receptor antagonist used to treat Parkinson's Disease and movement disorders. While memantine and its impurities lack good UV chromophores, the ACQUITY QDa Detector achieves sensitive detection. Waters Empower® CDS enables acquisition and analysis of data acquired from the PDA and mass detectors in a single user interface (Figure 2).

Retention time and area reproducibility values for five injections prepared at 0.2 µg/mL concentration for memantine API and its impurities are shown in Figure 3. Retention time and area %RSD values are within 0.25 and 3.00, respectively.

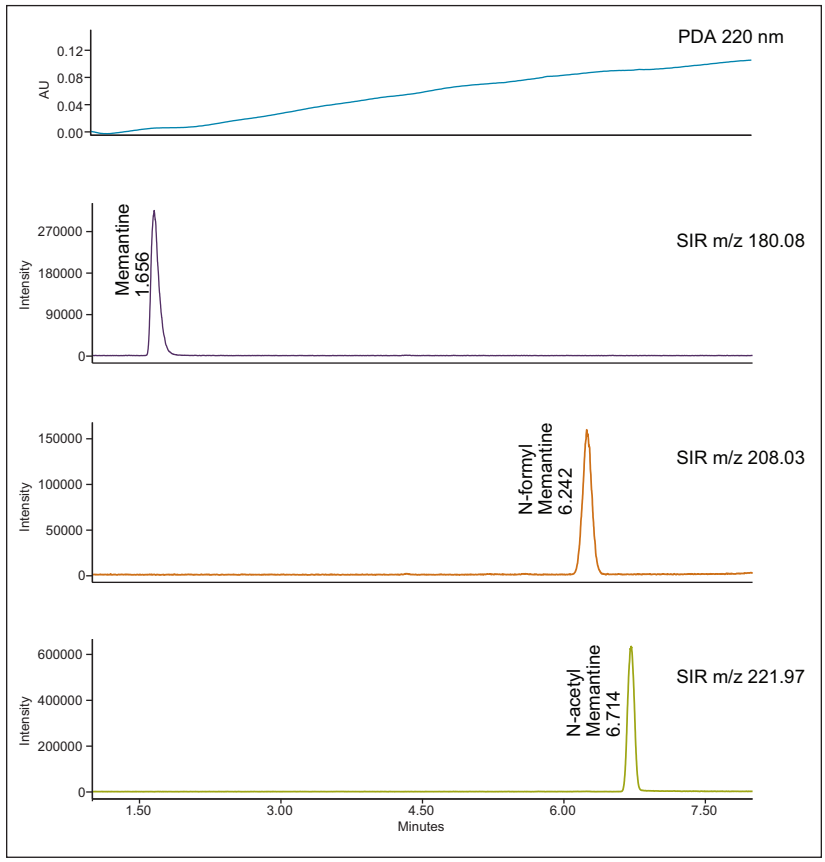

Figure 2. Memantine and impurities that lack UV chromophores show no UV trace at 220 nm and are detected with the addition of the ACQUITY QDa Detector.

%RSD (n = 5)	Retention time	Area
Memantine API	0.20	2.47
N-formyl memantine	0.12	2.50
N-acetyl memantine	0.10	2.86

Figure 3. Retention time and area %RSD for memantine and its impurities (using the ACQUITY QDa Detector). Area %RSD < 3.0 for memantine API and impurities.

SUMMARY

Mass detection allows the detection of compounds that have weak or no chromophores, such as memantine and its impurities. The combination of the ACQUITY QDa Detector and a UV detector can detect components that do not share functional groups for a single mode of detection. In this example, the retention time and area %RSD for memantine and impurities were within 0.25 and 3.00, respectively, when measured with the ACQUITY QDa Detector. The addition of the ACQUITY QDa Detector to the Alliance HPLC System with the 2998 PDA Detector and Empower CDS (software capable of combining data from orthogonal detectors) increases confidence in the analysis of non-chromophoric samples.

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Improving Effectiveness in Method Development by Using a Systematic Screening Protocol for a USP Method (Metoclopramide HCl and Related Substances)

Margaret Maziarz, Sean M. McCarthy, and Mark Wrona
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Robust UPLC® method development
- Quick and accurate identification of sample components using mass detection with the ACQUITY® QDa™ Detector
- Minimize the need for running individual injections of sample components to confirm the identity of peaks

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

ACQUITY UPLC Columns

ACQUITY QDa Detector

ACQUITY UPLC PDA Detector

Empower® 3 Software

Waters Quality Control Reference Material (QCRM) benchmarking standards

KEY WORDS

UPLC, method development, ApexTrack™ integration, Auto•Blend Plus™, metoclopramide HCl, sub-2-µm column particles, mass spectrometry

INTRODUCTION

Method development involves screening a range of chromatographic parameters to generate sufficient resolution and robust separations. While there are many approaches to method development, such as one factor at a time, systematic, and quality by design (QbD), the goals and factors used for optimizing separations are the same. The parameters that are adjusted include column chemistry, organic solvent, pH, gradient slope, flow rate, temperature, among other factors.

The impact of modifying these parameters are then systematically evaluated during development. Methods from each round of optimization are assessed using specific criteria such as the greatest number of peaks of interest with appropriate retention, resolution, and tailing values. The best method(s) from each step are then selected for further investigation until a suitable method is obtained. Throughout this development process, it is essential to ensure selection of the best conditions at each step and have demonstrable reasons for selection.

Regardless of the optimization strategy selected, it is important to identify and track critical sample components across the conditions investigated. Because peak elution order can change and UV spectra of related substances can be indistinguishable, standards (if available) are sequentially injected under the same conditions to simplify analysis. While ultimately effective, this is a time-consuming process. Using mass detection in addition to optical detection enables unambiguous identification. It also enables analysts to monitor sample components, and to rapidly identify and track coelutions and elution order changes.

In this application, we present the development of a UPLC method for the USP-defined assay for metoclopramide HCl and related substances. We combine UV (PDA) and mass detection, with the user-friendly ACQUITY QDa Detector. A systematic protocol is employed that includes scouting, screening, and optimization steps. Results for each step are analyzed and ranked using custom calculations and reported within Empower 3 Chromatography Data Software to minimize analyst bias in decision making and ensure the overall goals are achieved.

EXPERIMENTAL

Waters reference standard

Packaged in a vial:	LCMS Quality Control Reference Material (QCRM, p/n 186006963)

Method development conditions

LC system:	ACQUITY UPLC H-Class with Column Manager and Solvent Select Valve (SSV)
Columns:	All columns with dimension of 2.1 x 50 mm: ACQUITY UPLC CSH™ C_{18}, 1.7 µm (p/n 186005296) ACQUITY UPLC CORTECS C_{18}+, 1.6 µm (p/n 186007114) ACQUITY UPLC CSH Phenyl Hexyl, 1.7 µm (p/n 186005406) ACQUITY UPLC HSS Pentafluorophenyl (PFP), 1.8 µm (p/n 186005965)
Column temp.:	40, 45, and 50 °C
Injection volume:	1.0 µL
Flow rate:	0.6 mL/min
Mobile phase A:	125 mM Formic acid in water
Mobile phase B:	125 mM Ammonium hydroxide in water
Mobile phase C:	Water
Mobile phase D1:	Acetonitrile
Mobile phase D2:	Methanol

System control, data acquisition, and analysis:

Empower 3 FR2 CDS Software

Separation:	Standard gradient with 5-90% organic solvent over 5 minutes
Wash solvents:	Purge/Sample Wash: 50:50 water/methanol
Seal wash:	90:10 water/acetonitrile
PDA detector:	ACQUITY UPLC PDA
PDA settings:	210-400 nm (derived at 270 nm)
MS detector:	ACQUITY QDa (Extended Performance)
Scan mode:	100-400 *m/z*
Ionization mode:	ESI+, ESI-
Probe temp.:	600 °C
Sampling rate:	10 pts/sec
Capilllary voltage:	0.8 kV (pos/neg)
Cone voltage:	15 V
Data:	Centroid

In this application, we demonstrate how using both UV and mass data allows accurate tracking of all components during development and ensures peak purity in the final method. Overall, following a systematic protocol and utilizing mass detection enables faster and more effective development of a chromatographic method that conforms to the USP standard methodology for robustness and performance verification.[1]

Preparation of Solutions

Sample solution with APIs and related compounds

Separate stock solutions were prepared in methanol at 1.0 mg/mL. An equal volume of each stock solution was transferred to one vial and diluted with water to make a working sample with a final concentration of 0.06 mg/mL of each analyte. The compounds used in this study are listed in Table 1.

Compound	Common Name	Monoisotopic Mass (Da)
API	Metoclopramide	299.14
Imp. A	4-Acetamido-5-chloro-N-(2-(diethylamino) ethyl)-2-methoxybenzamide	341.15
Imp. B	Methyl 4-acetamido-5-chloro-2-methoxybenzamide	257.05
Imp. C	4-Amino-5-chloro-2-methoxybenzoic acid	201.02
Imp. D	Methyl 4-acetamido-2-methoxybenzoate	223.08
Imp. F	4-Amino-5-chloro-N-(2-(hydroxbenzamido)-2-hydroxbenzamide	
Imp. G	2-(4-Amino-5-chloro-2-hydroxbenzamido)-N,N-diethylethanamide oxide	315.14
Imp. H	4-Acetamido-2-hydroxbenzoic acid	195.05
Imp. 9	Methyl 4-amino-2-methoxybenzoate	181.07

Table 1. List of USP specified related substances of metoclopramide HCl for UPLC method development.

RESULTS AND DISCUSSION

Method development systematic protocol

Using a systematic protocol enables a consistent evaluation of major selectivity parameters, which ensures the development of robust and reproducible methods; here, using UPLC for faster and more sensitive analysis.

Column chemistries with different base particles and ligands were selected to reflect a wide selectivity range.

As shown in Figure 1, the protocol is built around a series of steps, each designed to address resolution systematically. The first step in our protocol involves defining our sample, success criteria, chromatographic system, and verifying system performance.

For metoclopramide and its USP-defined related substances, our goal was to separate these components to achieve a minimum USP resolution of ≥2.0 for each peak with a USP tailing of ≤1.5, and a retention factor (k*) ≥3.0. The retention factor of a peak for gradient separations is defined as k/(k+1).

For the greatest flexibility in development, we used the ACQUITY UPLC H-Class System configured with a Column Manager and Solvent Select Valve. To identify all components and possible coelutions, we used both ACQUITY PDA for optical detection and ACQUITY QDa for mass detection. We verified system performance using a LCMS Quality Control Reference Material (QCRM) to confirm system was operating properly prior initiating the study.[3]

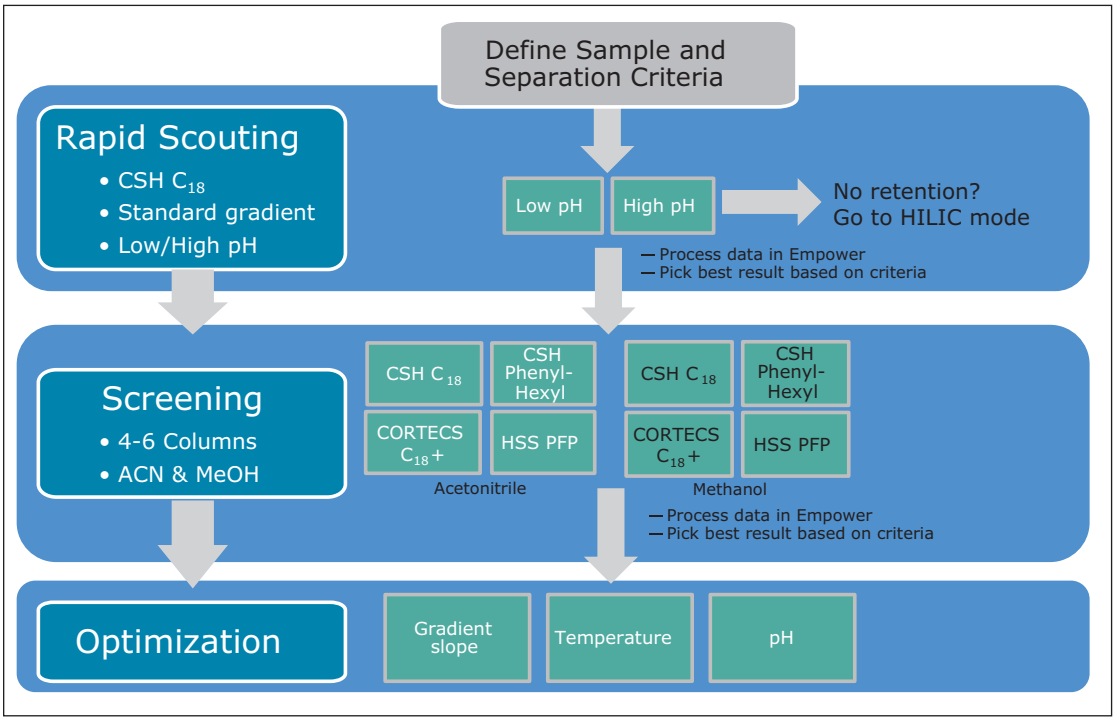


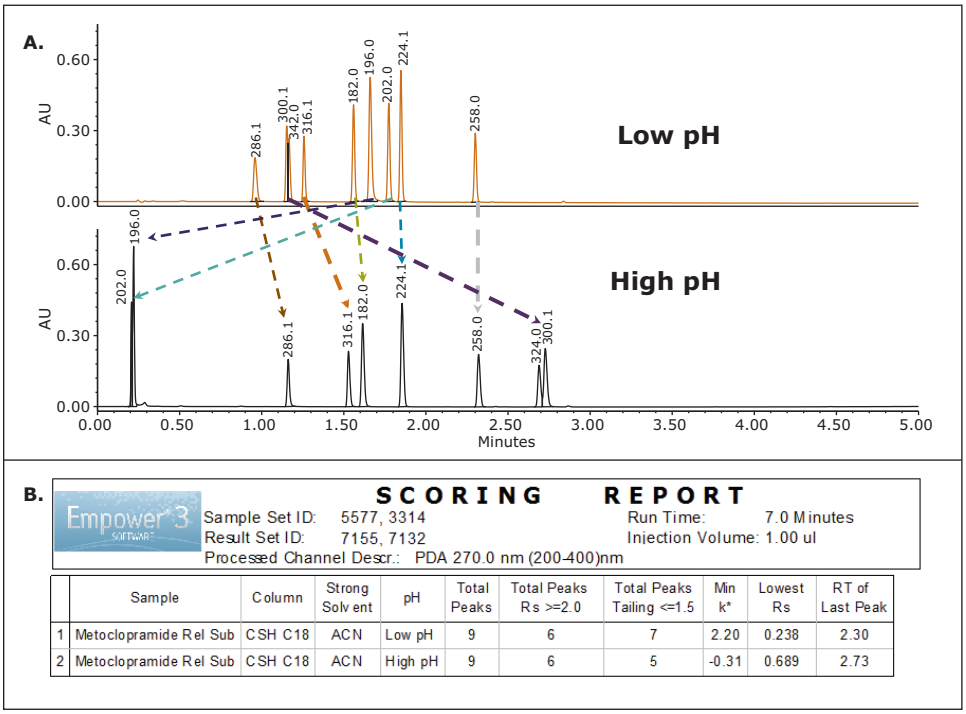
Figure 1. Systematic protocol for development of chromatographic methods.

Rapid scouting

After defining our sample, criteria, and system, we began the systematic protocol with rapid scouting to quickly screen for an acceptable separation condition. The goal of rapid scouting is to select acidic or basic conditions that provide the best retention of the sample components, as well as to identify the best separation mode (reversed-phase or HILIC).

Low and high pH separations were performed using stock solutions of 125 mM formic acid and 125 mM ammonium hydroxide, respectively. For the reversed-phase separation, we used a standard gradient of 5-90% of acetonitrile over 5 minutes. As expected for this basic sample mixture, there were dramatic changes in retention observed between the low and high pH separations (Figure 2). We were also able to track which components are most affected by the pH using the mass data. The chromatographic data was processed in Empower automatically using ApexTrack integration to detect peaks.

To determine the best conditions to move forward, we defined custom calculations and created a customized report in Empower Software. The methods were scored and selected using the best conditions by tracking the number of peaks that meet our defined goals. In this case, the best retention of all components is achieved at low pH, and for this reason, we continued our study with low pH.



	Sample	Column	Strong Solvent	pH	Total Peaks	Total Peaks Rs >=2.0	Total Peaks Tailing <=1.5	Min k*	Lowest Rs	RT of Last Peak
1	Metoclopramide Rel Sub	CSH C18	ACN	Low pH	9	6	7	2.20	0.238	2.30
2	Metoclopramide Rel Sub	CSH C18	ACN	High pH	9	6	5	-0.31	0.689	2.73

Figure 2. Rapid scouting with low and high pH. A. Chromatographic data showing impact of low and high pH on the separation of metoclopramide and related compounds. The sample components that are most affected by the pH were tracked using the mass data. B. Empower 3 scoring report. Criteria for success were defined in Empower as custom calculations, which were then used to create a report. Criteria were ranked so that best method appears first.

Screening

The conditions with best retention selected in the scouting step (low pH condition) did not fully meet our criteria for success. We moved to the screening phase of the protocol with a goal of separating all sample components. Using the Column Manager allowed us to select each column without the need for user intervention. For each separation we used the same standard gradient as in the scouting experiments, but investigated both methanol and acetonitrile eluents.

Again, we used the Empower scoring report to analyze the chromatographic data and select the best separation (Figure 3). As shown, the ACQUITY UPLC CSH C_{18} Column with methanol provides the highest number of peaks and has the highest number of peaks with resolution ≥2.0 and a tailing ≤1.5. For this reason we selected this condition for the final phase of the systematic protocol, optimization.

SCORING REPORT

Sample Set ID: 4001 Run Time: 7.0 Minutes
Result Set ID: 7073 Injection Volume: 1.00 ul
Processed Channel Descr.: PDA 270.0 nm (200-400)nm

	Sample	Column	Strong Solvent	pH	Total Peaks	Total Peaks Rs >=2.0	Total Peaks Tailing <=1.5	Lowest Rs	Min k*	RT of Last Peak
1	Metoclopramide Rel Sub	CSH C18	MeOH	Low pH	9	7	7	1.283	3.22	3.11
2	Metoclopramide Rel Sub	CORTECS C18+	ACN	Low pH	9	7	5	0.769	1.98	2.15
3	Metoclopramide Rel Sub	CSH C18	ACN	Low pH	9	5	7	2.308	2.15	2.30
4	Metoclopramide Rel Sub	CORTECS C18+	MeOH	Low pH	8	7	3	2.094	2.99	2.98
5	Metoclopramide Rel Sub	CSH Phenyl Hexyl	MeOH	Low pH	8	6	8	1.690	2.30	3.13
6	Metoclopramide Rel Sub	CSH Phenyl Hexyl	ACN	Low pH	8	5	5	0.654	0.98	2.21
7	Metoclopramide Rel Sub	HSS PFP	MeOH	Low pH	8	2	2	1.870	6.86	3.44
8	Metoclopramide Rel Sub	HSS PFP	ACN	Low pH	7	2	2	0.108	4.51	2.61

Figure 3. Empower 3 scoring report for screening different columns and organic solvents. The method using the ACQUITY UPLC CSH C_{18} Column and methanol scored highest, indicating the separation had the highest number of peaks with resolution ≥2.0 and a tailing ≤1.5.

Optimization

Although we were closer to the method development goal, the results from screening did not fully meet the criteria for success. We continued through the optimization step to improve the separation. During optimization we investigated the impact of gradient slope, column temperature, and pH. After each step we applied our scoring report to select the best conditions.

The first parameter we investigated was gradient slope by varying the gradient end point using the same gradient time. After applying our report we found that a gradient slope from 5-60% over 5 minutes provided the best separation (Figure 4). With a goal of meeting the criteria for resolution between all the peaks, we then optimized column temperature using the same system setup. Our results indicated that 45 °C yielded the greatest resolution of all components and met all of the goals we set at the start of the development process, Figure 5.

SCORING REPORT

Sample Set ID: 5181 Run Time: 7.0 Minutes
Result Set ID: 7285 Injection Volume: 1.00 ul
Processed Channel Descr.: PDA 270.0 nm (200-400)nm

	Sample	Column	Strong Solvent	pH	Total Peaks	Total Peaks Rs >=2.0	Total Peaks Tailing <=1.5	Lowest Rs	Min k*	RT of Last Peak
1	Metoclopramide Rel Sub, 60%D	CSH C18	MeOH	Low pH	9	7	9	1.727	3.56	4.01
2	Metoclopramide Rel Sub, 70%D	CSH C18	MeOH	Low pH	9	7	8	1.592	3.43	3.63
3	Metoclopramide Rel Sub, 80%D	CSH C18	MeOH	Low pH	9	7	7	1.463	3.31	3.34
4	Metoclopramide Rel Sub, 90%D	CSH C18	MeOH	Low pH	9	7	7	1.346	3.21	3.11

Figure 4. Gradient slope optimization. Different gradient slopes were explored by decreasing the % of organic at the end of the gradient from 5-90% to 80, 70, and 60% over 5 minutes. A gradient with 5-60% of methanol over 5 minutes had the highest score, indicating best separation with highest number of peaks with resolution ≥2.0 and a tailing ≤1.5.

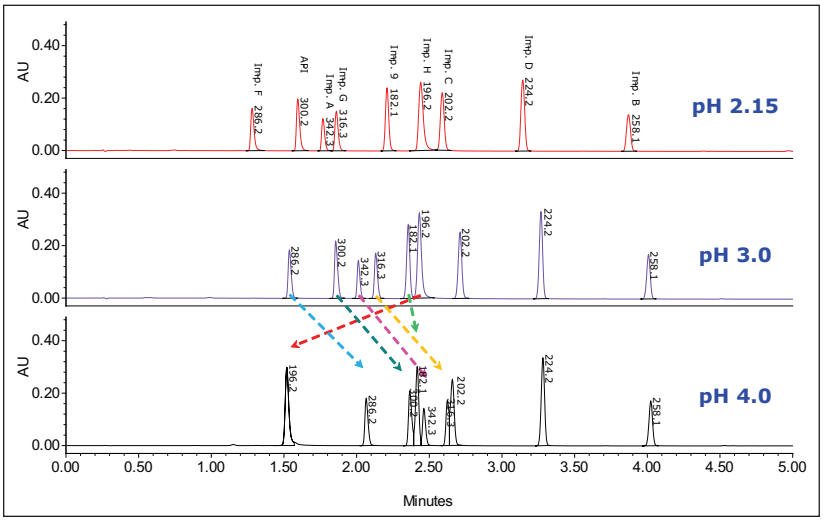
SCORING REPORT

Sample Set ID: 5325, 5232
Result Set ID: 7236, 7205
Processed Channel Descr.: PDA 270.0 nm (200-400)nm
Run Time: 7.0 Minutes
Injection Volume: 1.00 ul

	Sample	Column	Strong Solvent	pH	Total Peaks	Total Peaks Rs >=2.0	Total Peaks Tailing <=1.5	Min k*	Lowest Rs	RT of Last Peak
1	Metoclopramide Rel Sub, 45C	CSH C18	MeOH	Low pH	9	8	9	3.27	2.280	3.87
2	Metoclopramide Rel Sub, 50C	CSH C18	MeOH	Low pH	9	7	9	3.04	1.816	3.79
3	Metoclopramide Rel Sub, 40C	CSH C18	MeOH	Low pH	9	7	9	3.54	1.757	3.99

Figure 5. Column temperature optimization. The temperatures investigated included 40, 45, and 50 °C. Method at 45 °C scored highest with greatest number of peaks with a resolution of ≥2.0, indicating best separation.

At this stage, although we had met all our criteria, we also investigated impact of pH on the chromatographic separation. Often, small changes in pH can have a great impact on the retention of ionizable compounds. We performed separations at pH 2.15, 3.0, and 4.0 using the existing mobile phases defined in the protocol, Figure 6. For pH 3.0 and 4.0, we used Auto•Blend Plus Technology to blend formic acid and ammonium hydroxide solutions, methanol, and water already on the system to deliver mobile phases with constant pH. Our results showed large changes in selectivity as we moved to the higher pH and that, ultimately, pH 2.15 yielded the best separation, Figure 7.



SCORING REPORT

Sample Set ID: 5325, 6461
Result Set ID: 7263, 7273
Processed Channel Descr.: PDA 270.0 nm (200-400)nm
Run Time: 7.5, 7.0 Minutes
Injection Volume: 1.00 ul

	Sample	Column	Strong Solvent	pH	Total Peaks	Total Peaks Rs >=2.0	Total Peaks Tailing <=1.5	Min k*	Lowest Rs	RT of Last Peak
1	Metoclopramide Rel Sub	CSH C18	MeOH	Low pH	9	8	9	3.27	2.280	3.87
2	Metoclopramide Rel Sub, pH 3.0	CSH C18	MeOH	pH = 3.0	9	7	9	4.12	1.687	4.01
3	Metoclopramide Rel Sub, pH 4.0	CSH C18	MeOH	pH = 4.0	9	5	4	4.06	0.632	4.02

Figure 7. pH optimization. The method with a mobile phase pH of 2.15 scored highest, indicating best separation conditions.

Final UPLC method conditions

LC System:	ACQUITY UPLC H-Class
Column:	ACQUITY UPLC CSH C_{18}, 1.7-µm, 2.1 x 50 mm
Column temp.:	45 °C
Injection volume:	1.0 µL
Flow rate:	0.6 mL/min
Mobile phase A:	125 mM Formic acid in water
Mobile phase C:	Water
Mobile phase D2:	Methanol
Separation:	Gradient

Step	Time (minutes)	Solvent A (%)	Solvent C (%)	Solvent D2 (%)
1	Initial	10	85.0	5.0
2	5.0	10	30.0	60.0
3	5.5	10	30.0	60.0
4	5.6	10	85.0	5.0
5	7.0	10	85.0	5.0

Wash solvents:	Purge/Sample wash: 50:50 water/methanol
	Seal wash: 90:10 water/acetonitrile
PDA detector:	ACQUITY UPLC PDA
PDA settings:	210-400 nm (derived at 270 nm)
MS detector:	ACQUITY QDa (Extended Performance)
Scan mode:	100-400 *m/z*
Ionization mode:	ESI+, ESI-
Probe temp.:	600 °C
Sampling rate:	10 pts/sec
Capilllary voltage:	0.8 kV (pos/neg)
Cone voltage:	15 V
Data:	Centroid

System control, data acquisition, and analysis:

Empower 3 FR2 CDS Software

Final UPLC method

To verify performance of the developed UPLC method, we evaluated repeatability of replicate injections of the sample. The system suitability of five replicate injections was determined according to specifications defined in the USP General Chapter, <621> Chromatography.[2] Results of the method system suitability for each component are shown in Table 2.

The retention times and area repeatability were well below the USP specification of less than 2.0% RSD. The USP resolution between all the peaks was ≥2.5, which is above the general USP requirements of ≥1.5. The system suitability results of replicate injections were excellent. Further validation testing can be done automatically using Empower Method Validation Manager (MVM) Software.

Empower 3 SOFTWARE

Report Method: System Suit_Sum Report
Sample Set ID: Sample Set Id 2622
Result Set ID: Result Set Id 2660
Channel Name: PDA 270

	Name	# of Inj.	%RSD RT	%RSD Peak Areas	Ave USP Resolution	Ave USP Tailing
1	Imp. F	5	0.07	0.19		1.2
2	API	5	0.06	0.22	6.7	1.3
3	Imp. A	5	0.06	0.21	3.4	1.2
4	Imp. G	5	0.07	0.23	2.5	1.2
5	Imp. 9	5	0.06	0.19	9.2	1.1
6	Imp. H	5	0.06	0.19	4.2	1.4
7	Imp. C	5	0.06	0.31	2.5	1.1
8	Imp. D	5	0.05	0.21	9.0	1.1
9	Imp. B	5	0.04	0.21	13.0	1.1

Table 2. System suitability results for five replicate sample injections acquired using an ACQUITY UPLC H-Class System.

CONCLUSIONS

Following a systematic protocol, we have successfully developed a UPLC method for the separation of metoclopramide and related compounds. The criteria for success with a goal of separating all nine components, achieving a resolution of ≥2.0, tailing of ≤1.5, and retention factor (k*) ≥3.0, were met.

Using the ACQUITY QDa Detector in conjunction with UV detection and the ACQUITY UPLC H-Class System streamlined the method development process by removing the need for multiple chromatographic runs to confirm the identity of peaks by retention times.

Using a single injection, instead of nine individual sample injections, we were able to quickly identify components and track elution order of peaks during the method development study.

Finally, the use of ApexTrack in Empower Software enabled consistent evaluation of chromatograms for fair comparison across the development process. Empower custom calculations and reporting allowed us to generate a scoring report to easily identify the best conditions at each step in our protocol.

Overall, using a defined systematic protocol with the UPLC system, detectors, and its column chemistries enables analytical laboratories to quickly and efficiently develop chromatographic methods. Methods developed in this manner are typically more reproducible, which allows laboratories to have a higher validation success rate.

References

1. USP General Chapter, <1226>, Verification of Compedial Method, USP36-NF31, The United States Pharmacopeia Convention, official December 1, 2013.
2. USP General Chapter, <621>, Chromatography, USP36-NF31, The United States Pharmacopeia Convention, official December 1, 2013.
3. Berthelette KD, Summers M, Fountain KJ. Ensuring Data Quality by Benchmarking System Performance Using Waters Neutrals Quality Control Reference Material. Waters Corp. 2013; 720004622en.

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Analysis of Benzenesulfonic Acid and P-Toluenesufonic Acid Esters in Genotox Monitoring using UPLC/UV-MS

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Waters Corporation, Milford, MA, U.S.

APPLICATION BENEFITS

UPLC combines with PDA and mass detection provides an excellent solution to the analysis of alkyl arylsulfonate esters in drug substances and drug products for genotoxic impurity monitoring. UPLC provides both high resolution and a high-throughput analysis, reducing analysis times from as much as 30 minutes to only 5 minutes. For applications where sensitivity and selectivity are not as demanding, this UPLC/UV system allows for a simple, low cost solution to the analysis of these genotoxic impurities.

WATERS SOLUTIONS

ACQUITY UPLC System

ACQUITY UPLC PDA Detector

SQ Detector

KEY WORDS

Genotox impurities, drug monitoring, Amlodipine Besylate, Bretylium Tosylate

INTRODUCTION

Alkyl esters of sulfonic acids, particularly methanesulfonic, benzenesulfonic, and p-toluenesulfonic acid esters, are a common class of reagents used in the pharmaceutical industry as alkylating agents, catalysts, and in purification steps of the chemical synthesis of a drug substance. In addition, sulfonic acids are often used as the final salt form of the drug substance due to improved chemical properties or bioavailability. The presence of any residual alcohols from synthetic reaction or recrystalization steps may result in the formation of alkyl esters of the sulfonic acids. Many of these mesylate, besylate, or tosylate esters are known to be genotoxic, while others are potentially genotoxic, requiring monitoring in the drug substance and drug product.

The U.S. FDA draft guidance[1] and the EMEA guidelines[2,3] require that any possible genotoxic impurities in a drug substance or drug product that have not been shown to be removed during early synthesis steps be monitored to ensure that the levels are below the Threshold for Toxicological Concern (TTC) of 1.5 µg/day based upon the maximum daily dosage of the pharmaceutical compound.

Depending upon the particular active pharmaceutical ingredient (API), the sensitivity requirements for the analytical method can be quite challenging. For a drug product with a maximum daily dose of 1000 mg, any genotoxic impurity must be analyzed to be less than the TTC level:

$$[1.5\ \mu g/day] / 1.000\ gm = 1.5\ ppm$$

Therefore, the genotoxic impurity must be at a level less than 1.5 ppm in the drug product or drug substance.[4,5]

The most common analytical techniques for monitoring alkyl sulfonate esters have been GC/MS or HPLC/UV/MS with derivatization using pentafluorothiophenol. More recently, HPLC/MS has been shown to give good results without the need for a complicated derivatization step; however, run times on the order of 20 to 30 minutes are required to achieve sufficient resolution from the API.6

This paper demonstrates the improvements obtained using sub-2-µm UltraPerformance LC® (UPLC®) column packing materials UPLC/UV/MS for the analysis of benzenesulfonic acid, p-toluenesulfonic acid, and their alkyl esters. Analysis times of less than 5 minutes with sufficient sensitivity to meet FDA and EMEA requirements are demonstrated without the need for pre- or post-column derivatization.

EXPERIMENTAL

LC conditions

LC system:	Waters ACQUITY UPLC System
Column:	ACQUITY UPLC BEH Phenyl Column 2.1 x 50 mm, 1.7 µm
Column temp.:	50 °C
Flow rate:	600 µL/min
Mobile phase A:	5 mM Ammonium Formate, pH 9.0
Mobile phase B:	Methanol
Gradient:	10 to 98% B/5 min
PDA detection:	210 to 300nm, 1.2 nm resolution, 40 pts/sec

MS conditions

MS System:	Waters SQ Mass Detector
Ionization Mode:	ESI positive
Capillary Voltage:	3200 V
Cone Voltage:	24 V
Desolvation temp.:	400 °C
Source temp.:	130 °C
Desolvation gas:	800 L/Hr
Cone gas flow:	50 L/Hr
MS method:	- ESI+ / SIR

RESULTS AND DISCUSSION

A method scouting approach was utilized to quickly develop a chromatographic method for the analysis of methyl benzenesulfonate, ethyl benzenesulfonate, methyl p-toluenesulfonate, and ethyl p-toluenesulfonate utilizing an ACQUITY UPLC System with an ACQUITY UPLC PDA detector and a SQ Mass Detector. The MS data was collected in ESI+ mode using timed SIR functions to maximize the dwell time and therefore sensitivity of the analysis. A simple 5-minute gradient from 10% to 98% methanol resulted in a rapid separation with resolution of 1.9 or better for all analytes with sufficient sensitivity to meet FDA and EMEA Genotoxic Impurities Guideline requirements.

UV detection

A mixture of 1 ppm each of the alkylsulfonate ester standards was analyzed in less than 3 minutes and the UV chromatograms (220 nm) for three replicate injections are shown (Figure 1). All of the alkylsulfonate esters analyzed exhibited good linearity with correlation coefficients greater than 0.9997 for calibration curves covering the concentration range from 0.01 to 10 ppm (Figure 2). This is sufficiently sensitive to monitor these genotoxic and potentially genotoxic impurities (PGIs) in drug substances and drug products at the levels required by FDA and EMEA guidelines as long as the analytes can be well resolved from the active pharmaceutical ingredient and excipients.

Using the conditions described here with 10 µL injections, the retention time reproducibility, and the limits of detection and quantitation determined for standards with UV detection are listed in Table 1. An LOQ of 0.04 ppm corresponds to a quantitation limit of 4 ppm in a 1% solution of a drug substance or a drug product. This is sufficiently sensitive to meet regulatory guidelines for the analysis of these PGIs in pharmaceutical products with a maximum daily dose of 375 mg or less, which is adequate for a number of commercially-available pharmaceuticals. If additional sensitivity is required, preparation of the samples at higher concentrations (~5%) and/or injecting larger volumes (~20 µL) may achieve the desired LOQs, however, the use of MS detection may be necessary to meet the highest sensitivity applications.

Component	Mean RT*	%RSD RT*	LOD (3X)	LOQ (10X)
MBS	1.775	0.05%	0.01 ppm	0.04 ppm
EBS	2.175	0.05%	0.01 ppm	0.04 ppm
MTS	2.250	0.04%	0.01 ppm	0.04 ppm
ETS	2.577	0.03%	0.01 ppm	0.04 ppm

**All conc., N=30*

Table 1. Limits of detection and quantitation and reproducibility of retention times for methyl and ethyl benzenesulfonates and methyl and ethyl toluenesulfonates standards with this method using UV detection at 220 nm.

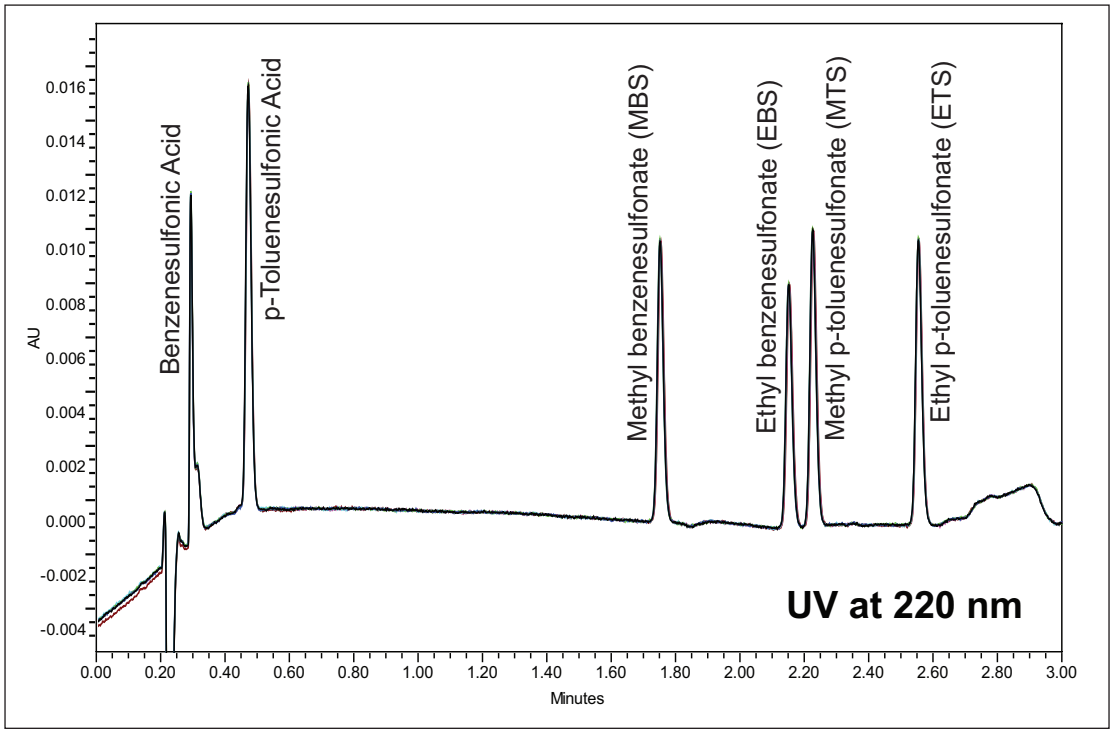

Figure 1. Overlay of three replicate injections (10 µL) of 1 ppm alkylsulfonate ester standards with UV detection at 220 nm. Benzenesulfonic acid and p-toluenesulfonic acid standards (1 ppm) are shown for reference.

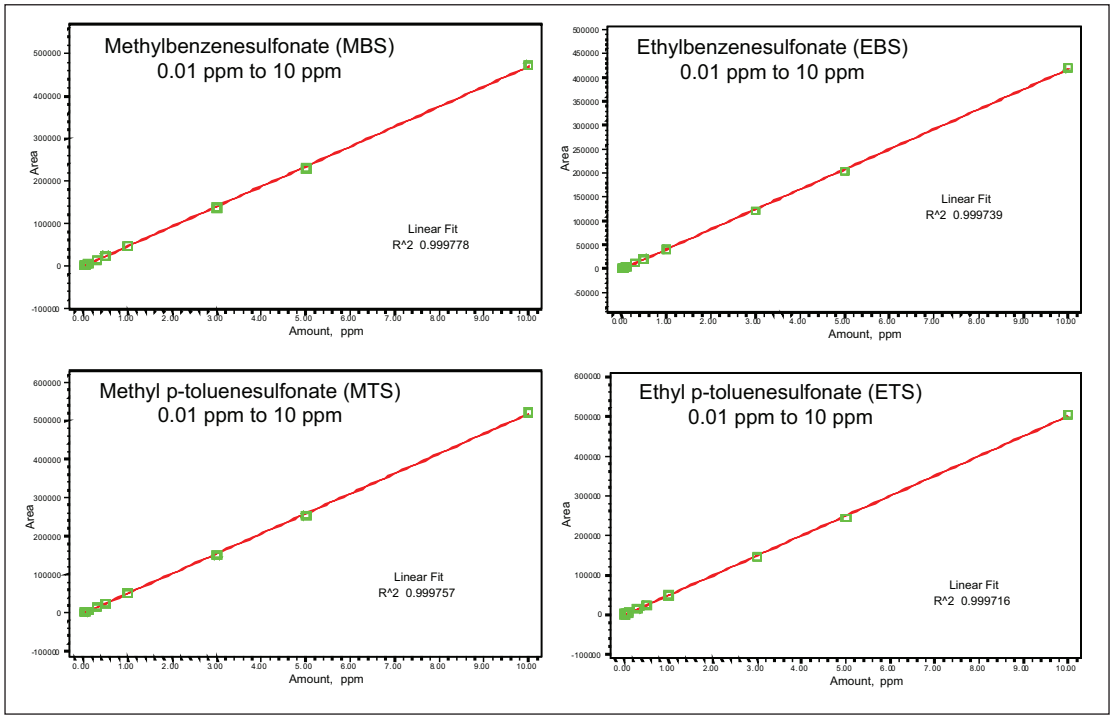

Figure 2. UV results at 220 nm demonstrates good linearity over a range of approximately 0.01 ppm to 10 ppm with correlation coefficients greater than 0.9997 for all analytes.

MS detection

Mass spectrometry data (SIR mode) collected for the same 1 ppm standards of the alkylsulfonate esters is shown (Figure 3). The narrow peaks observed with UPLC, which are typically 1 to 3 seconds wide, require rapid MS scanning to obtain 15 to 20 data points across the peaks necessary for accurate and reproducible integration and quantitation. The use of timed SIR functions allows for the optimization of dwell time and sensitivity for each component while maintaining sufficient data points for good integration.

MS data for all of the alkylsulfonate esters analyzed exhibited good linearity with correlation coefficients greater than 0.9960 for calibration curves covering the concentration range from 0.01 to 3 ppm (Figure 4). Using the conditions described here, the retention time reproducibility and the limits of detection and quantitation obtained are listed in Table 2. An LOQ of 6 ppb (MTS) corresponds to a quantitation limit of 0.6 ppm in a 1% solution of a drug substance or a drug product and meets the sensitivity level required for a pharmaceutical product with a maximum daily dose of 2500 mg or less, which is adequate for most commercially available pharmaceuticals.

Higher sample concentrations and larger injection volumes may be employed to achieve additional sensitivity for particularly difficult samples.

Component	Mean RT*	%RSD RT*	LOD (3X)	LOQ (10X)
MBS	1.775	0.05%	20 ppb	60 ppb
EBS	2.175	0.05%	5 ppb	15 ppb
MTS	2.250	0.04%	2 ppb	6 ppb
ETS	2.577	0.03%	~0.3 ppb	~1 ppb

**All conc., N=30*

Table 2. Limits of detection and quantitation and reproducibility of retention times for methyl and ethyl benzenesulfonates and methyl and ethyl toluenesulfonates with this method.

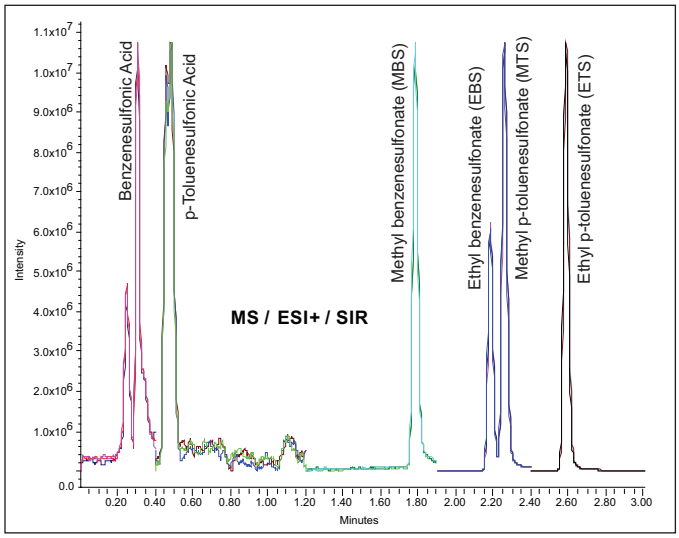

Figure 3. Overlay of three replicate injections (10 µL) of 1 ppm alkylsulfonate ester standards with MS detection using ESI+ and SIR mode. Benzenesulfonic acid and p-toluenesulfonic acid standards (1 ppm) are shown for reference.

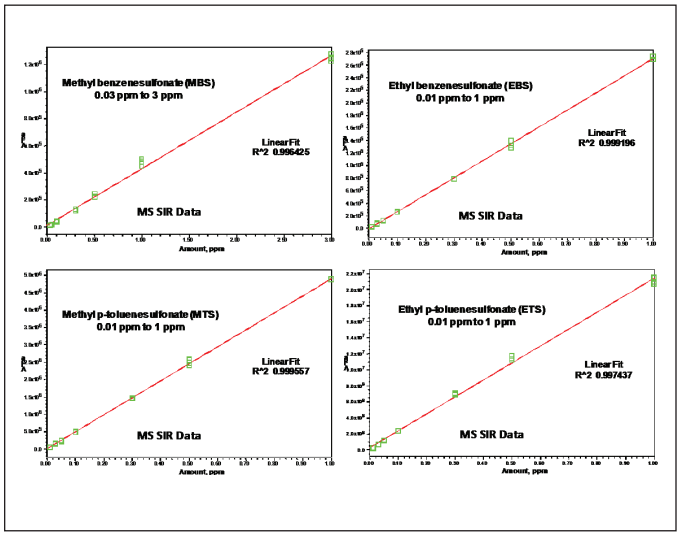

Figure 4. MS results using ESI+ / SIR mode demonstrates good linearity over a range of approximately 0.01 to 3.0 ppm for all analytes.

Amlodipine Besylate

The first example shown uses the drug substance Amlodipine Besylate, which is the benzenesulfonic acid salt of Amlodipine. If this material is exposed to residual alcohols from purification/recrystalization steps or any other source, the potential exists for the formation of the potentially genotoxic alcohol esters of the benzenesulfonic acid. This drug substance must therefore be monitored for the presence of these compounds.

Amlodipine Besylate is a long-acting calcium channel blocker use for the treatment of high blood pressure. It is usually administered orally and has a maximum daily dose of 10 mg, which corresponds to a TTC level of 150 ppm for each of the genotoxic impurities. Amlodipine Besylate was obtained from U.S. Pharmacopeia (Rockville, MD) and was analyzed as a 1% solution. Methyl benzenesulfonate (MBS) and ethyl benzenesulfonate (EBS) were not detected in this sample (less than 0.2 ppm) as demonstrated in Figure 5 (MS data).

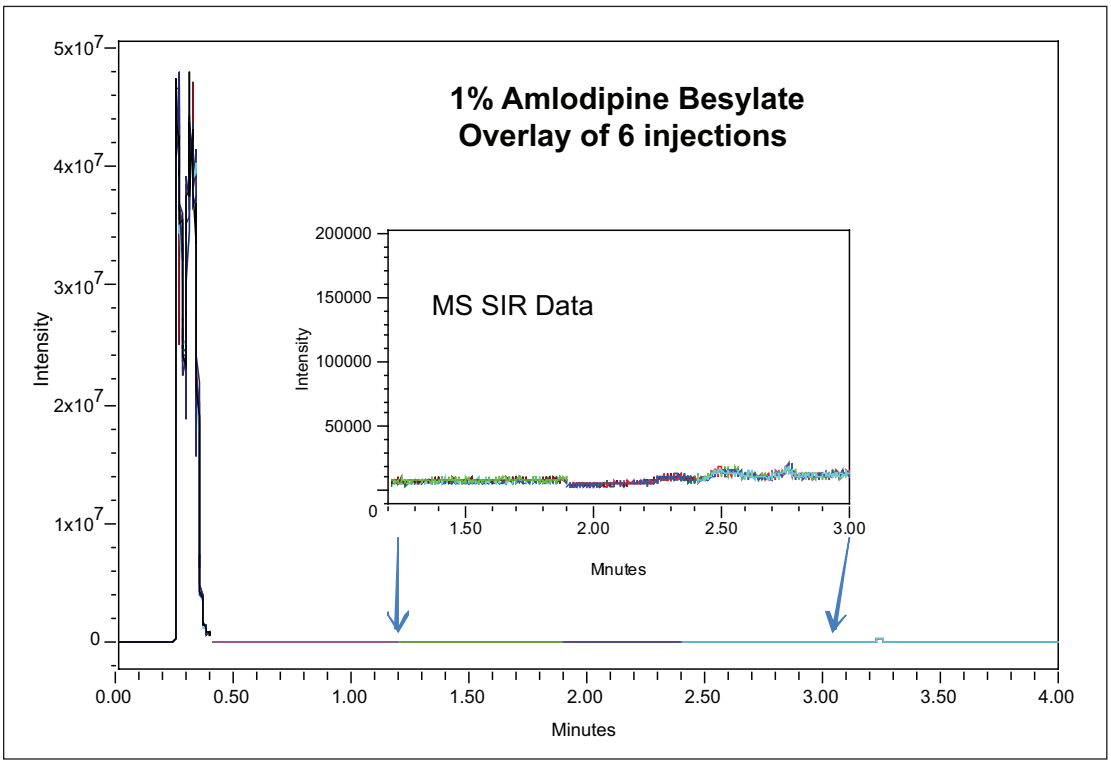

Figure 5. MS analysis of alkyl toluenesulfonates in Amlodipine Besylate.

A second sample was prepared and spiked with methyl benzenesulfonate and ethyl benzenesulfonate at 15 ppm each relative to the solid Amlodipine Besylate representing 1/10th the regulatory requirements for the limits of these genotoxins in this particular drug substance. This sample was analyzed and the resulting MS chromatograms for six replicate injections (Figure 6) demonstrate the excellent sensitivity and reproducibility of the chromatographic method. The separation of the analytes of interest away from the free sulfonic acids ensures that ionization of the sulfonate esters will not be suppressed by the excess of the benzenesulfonic acid. The quantitative MS results (Table 3) display the accuracy and precision of the method with RSDs of 3.5 to 8% and recoveries of 103% and 122% for the spiked MBS and EBS components respectively.

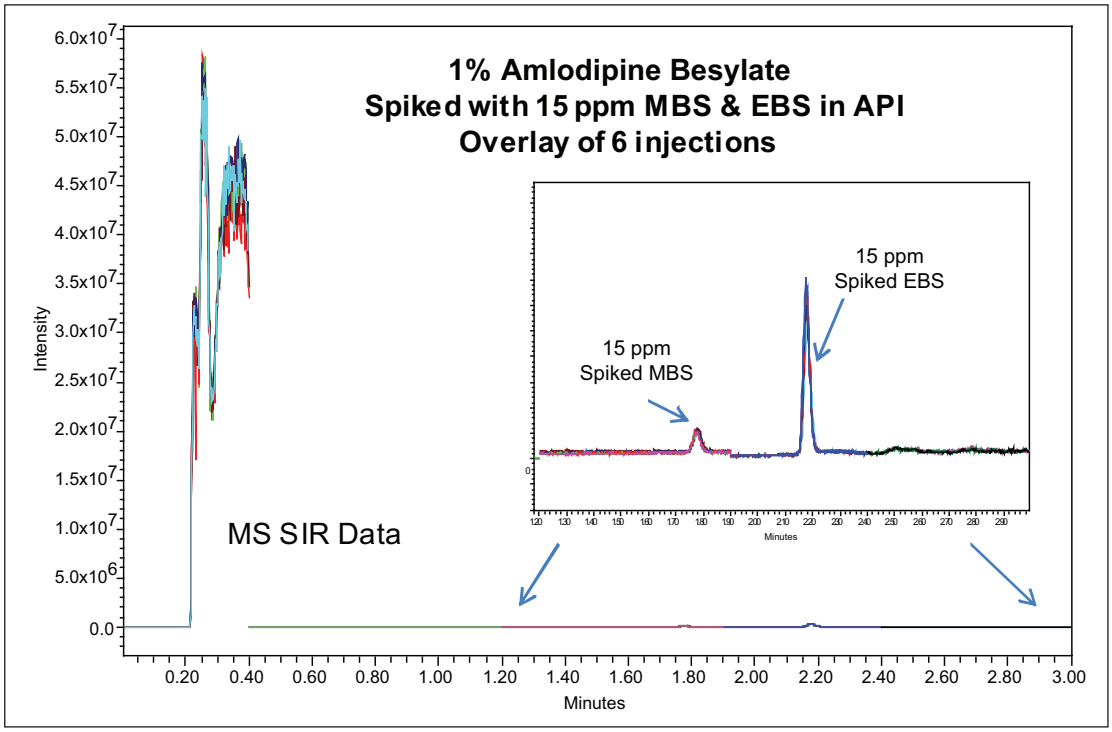

Figure 6. MS analysis of Amlodipine Besylate spiked with 15 ppm methyl benzenesulfonate and ethyl benzenesulfonate.

Sample	Mean MBS*	%RSD	Mean EBS*	%RSD
Amlodipine Besylate	N/D	——	N/D	——
Amlodipine Besylate spiked with 15 ppm (in API)	15.4 ppm	3.4%	18.3 ppm	8.1%

**Average of 6 inj.*

Table 3. Analytical MS results for Amlodipine Besylate sample and sample spiked with 15 ppm of methyl benzenesulfonate and ethyl benzenesulfonate.

UV detection has adequate sensitivity to analyze these PGIs in many drug products providing that the TTC levels are sufficiently high and that the analytes of interest are well resolved from the API, the free acid, and any excipients present in the drug product. The analysis using UV detection at 220 nm of the Amlodipine Besylate sample spiked with 15 ppm of MBS and EBS (Figure 7) demonstrates the resolution of the analytes from the benzenesulfonic acid, the late eluting Amlodipine, and other impurities present in the sample. These results illustrate the capability of UV detection to achieve the detection levels required by the FDA and the EMEA for MBS and EBS in this particular sample.

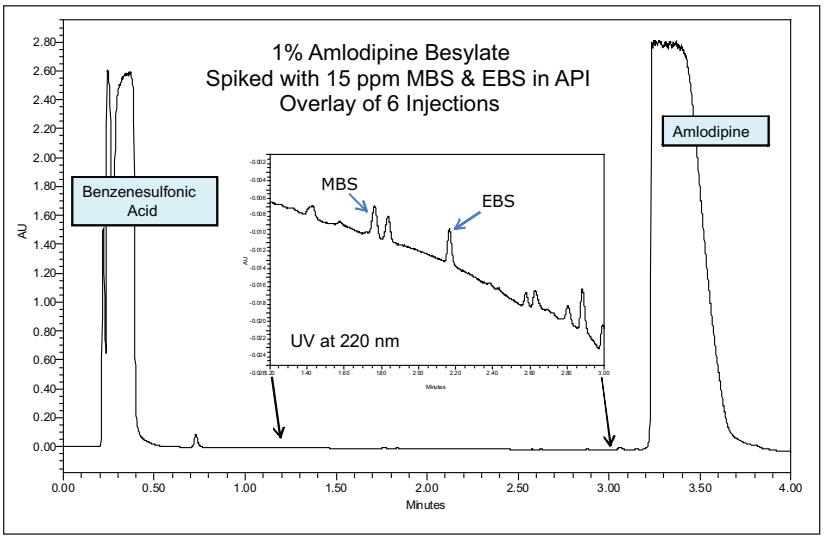

Figure 7. UV Analysis of Amlodipine Besylate spiked with 15 ppm methyl benzenesulfonate and ethyl benzenesulfonate.

Bretylium Tosylate

The second example shown uses the drug substance Bretylium Tosylate, which is the p-toluenesulfonic acid salt of Bretylium. Bretylium Tosylate is an antifibrillatory and antiarrhythmic agent that is normally administered by IV and has a maximum daily dose of approximately 2 to 3 gm, which corresponds to a TTC level of 0.5 ppm (for 3 gm dose) for each of the genotoxic impurities. Bretylium Tosylate was obtained from U.S. Pharmacopeia (Rockville, MD) and was analyzed as a 1% solution. Methyl toluenesulfonate (MTS) and ethyl toluenesulfonate (ETS) were not detected in this sample (less than 0.2 ppm) as demonstrated in Figure 8.

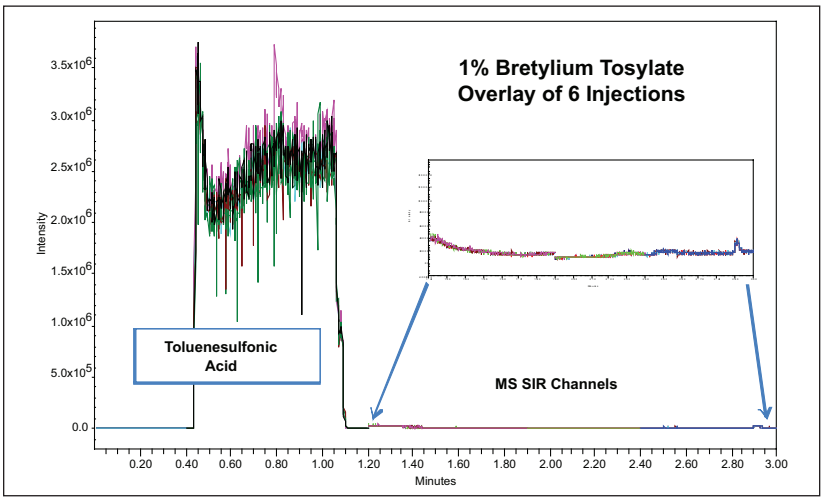

Figure 8. Analysis of alkyl toluenesulfonates in Bretylium Tosylate.

A second sample was prepared and spiked with methyl toluenesulfonate and ethyl toluenesulfonate at 0.5 ppm each relative to the solid Bretylium Tosylate representing the regulatory requirements for the limits of these genotoxins in this particular drug substance. This sample was analyzed and the resulting chromatograms for six replicate injections (Figure 9) demonstrate the excellent sensitivity and reproducibility of the chromatographic method. The separation of the analytes of interest away from the free sulfonic acids ensures that ionization of the sulfonate esters will not be suppressed by the excess of the toluenesulfonic acid. The quantitative results (Table 4) display the accuracy and precision of the method with RSDs of 4 to 7% and recoveries of 94% and 104% for the spiked MTS and ETS components respectively.

Sample	Mean MTS*	%RSD	Mean ETS*	%RSD
Bretylium Tosylate	N/D	——	N/D	——
Bretylium Tosylate spiked with 0.5 ppm *Average of 6 inj.	0.47 ppm	7.3%	0.52 ppm	4.1%

Table 4. Analytical MS results for Bretylium Tosylate sample and sample spiked with 0.5 ppm of methyl toluenesulfonate and ethyl toluenesulfonate.

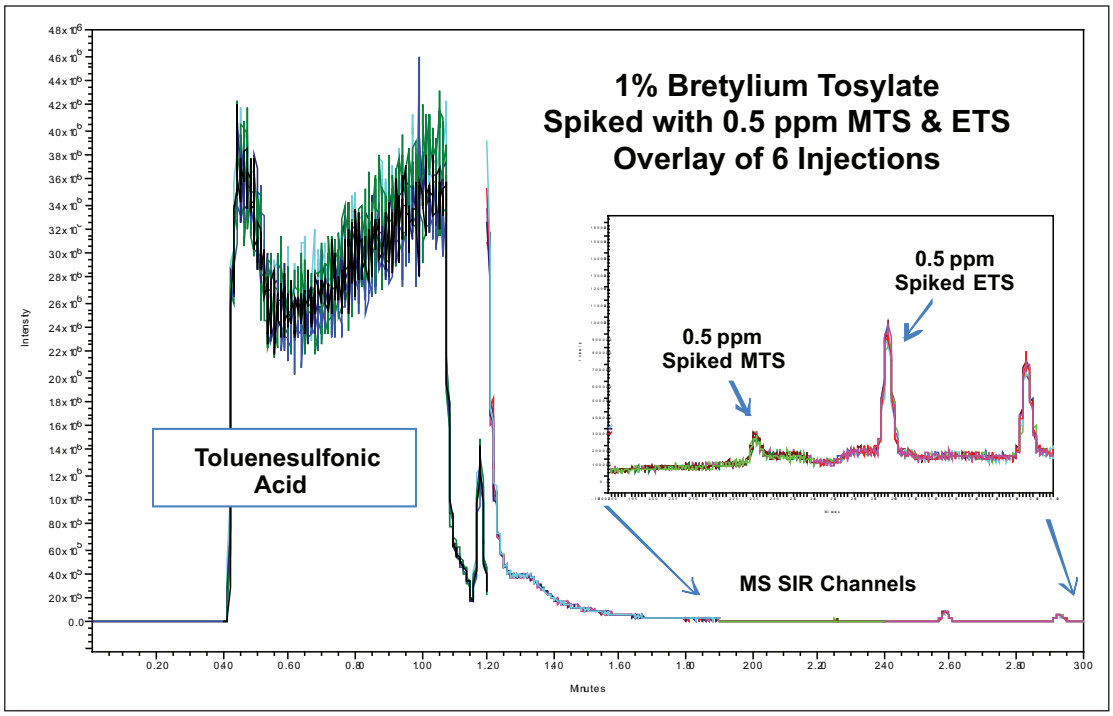

Figure 9. Analysis of Bretylium Tosylate spiked with 0.5 ppm methyl toluenesulfonate and ethyl toluenesulfonate.

UV data obtained for this sample (Figure 10) demonstrates the limitations of UV detection for many of these types of genotoxic impurity analyses. Due to the inherent combination of lower sensitivity and specificity of UV detection relative to MS detection, UV detection may be unsuitable for a number of drug products or drug substances with high daily doses that require very high sensitivity or those that are not separated chromatographically from the analytes of interest. Bretylium elutes much earlier in the chromatogram than Amlodipine and tails into the region where the MTS and ETS peaks elute swamping out the analytes.

In addition, the low TTC levels required for this analysis (0.5 ppm in API) are below the quantitation limits of the UV method. Sensitivity could be improved somewhat by injecting a larger volume (20 µL) of a sample concentration of 5%, however, interference of the analytes with the Bretylium peak would still be an issue.

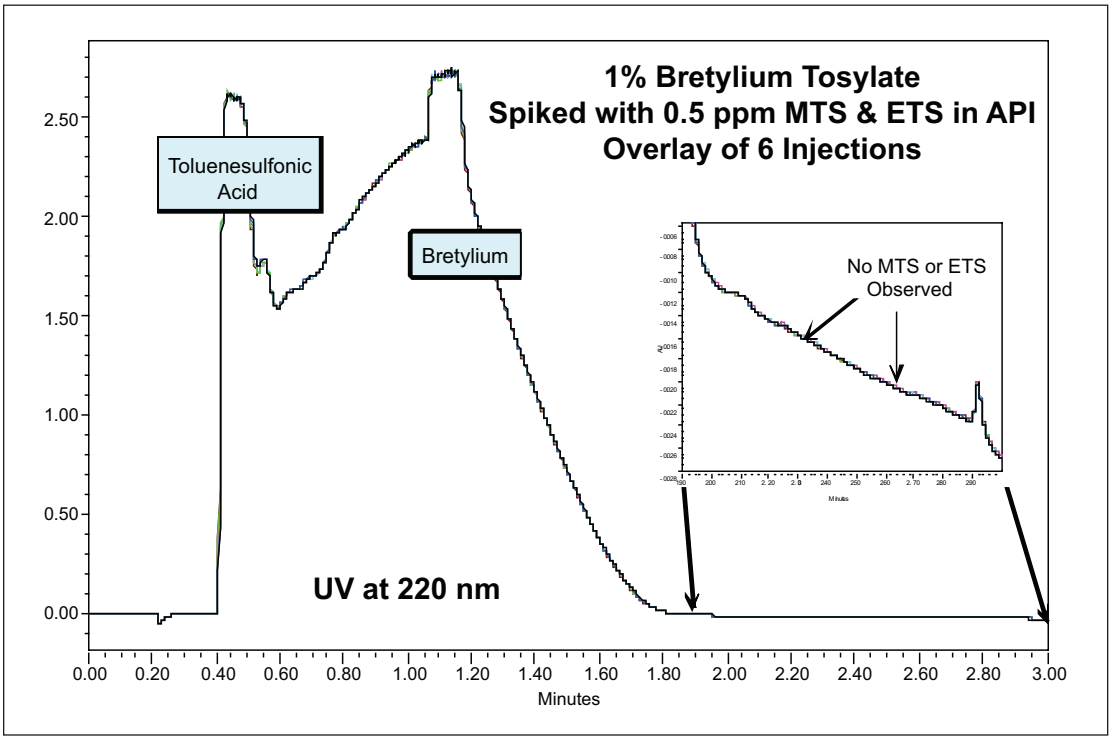

Figure 10. Analysis of Bretylium Tosylate spiked with 0.5 ppm methyl toluenesulfonate and ethyl toluenesulfonate.

Overall, UPLC with MS detection using SIR mode offers the most sensitive method with the best specificity for the analysis of alkyl arylsulfonates in drug substances and drug products. Despite the limitations of UV detection, UPLC with UV may be used successfully for the analysis of GTIs in a number of pharmaceutical compounds, particularly those with low maximum daily doses that do not require the utmost sensitivity afforded by MS detection.

CONCLUSION

The ACQUITY UPLC System paired with the ACQUITY UPLC PDA Detector and the SQ Mass Detector provides an excellent system solution to the analysis of alkyl arylsulfonate esters in drug substances and drug products for genotoxic impurity monitoring. UPLC allows for a high resolution and high throughput analysis, reducing analysis times from as much as 30 minutes to only 5 minutes, increasing laboratory productivity while decreasing solvent consumption and the generation of waste solvents.

The use of a single quadrupole mass spectrometer with single ion recording (SIR) methods achieves the specificity and sensitivity necessary to analyze very low levels of impurities in the presence of 1 to 10% concentrations of drug substances or drug products, to meet today's demanding regulatory requirements for genotoxic impurity analysis. For particular applications where sensitivity and selectivity are not as demanding, UPLC combined with UV detection allows for a simple, low cost solution to the analysis of these genotoxic impurities.

References

1. US Food and Drug Administration, Guidance for Industry, Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches, Draft Guidance. Dec. 2008.
2. The European Medicines Agency [EMEA], Committee for Medicinal Products for Human Use [CHMP], Guidelines on the Limits of Genotoxic Impurities, CPMP/SWP/5199/02.
3. The European Medicines Agency [EMEA], Committee for Medicinal Products for Human Use [CHMP], Questions and Answers on the CHMP Guideline on the Limits of Genotoxic Impurities, CPMP/SWP/431994/07.
4. Glowienke S, Frieauff W, Allmendinger T, Martus H, Suter W, Mueller L. Mutat. Res. 581 (2005) 23.
5. Muller L, et al. Regulatory Toxicology and Pharmacology. 2006; 44: 198-211.
6. Taylor GE, Gosling M, Pearce A. J. Chromotogr A. 2006; 1119: 231-237.

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Quantitative Determination of Genotoxic Impurities Using Xevo TQD

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APPLICATION BENEFITS

This work demonstrates how the enhanced sensitivity of the Xevo TQD with fast positive and negative switching, along with tools that facilitate instrument setup and method development, allows for faster analysis of genotoxic impurities.

WATERS SOLUTIONS

Xevo® TQD

KEY WORDS

ADAR, IntelliStart,™
genotoxins, impurities

INTRODUCTION

Alkyl sulfonic acids, particularly methanesulfonic acid, benzenesulfonic acid, and p-toluenesulfonic acid, are a common class of alkylating agents used in the pharmaceutical industry as alkylating reagents, catalysts, and in purification steps in the chemical synthesis of an API. In addition, these sulfonic acids are often used as the final salt form of the drug due to improved chemical properties or bioavailability.

The presence of any residual alcohols from synthetic reaction or recrystalization steps may result in the formation of alkyl esters of the sulfonic acids. Many of these mesylate, besylate, or tosylate esters are known to be genotoxic while others are potentially genotoxic, requiring monitoring in the drug substance and drug product.

Typical methods utilized in the past for the analysis of these akyl sulfonate esters have been based on GC/MS or HPLC/UV/MS, with derivitization typically using run times in the order of 20 to 30 minutes We have previously demonstrated how good results can also be achieved using UPLC®/MS with run times of less than 5 minutes.

In this application note, we show how the latest advances in instrumentation provide us greater power and ease the analysis of these genotoxic impurities. We demonstrate how the enhanced sensitivity of the Xevo TQD with positive and negative switching allows for faster analysis of these impurities. Additionally, the ability to reduce matrix interference using RADAR™ helps speed up the method development process.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC
Column:	ACQUITY UPLC HSS T3 Column, 2.1 x 50 mm, 1.8 µm
Column temp.:	45 °C
Injection vol.:	15 µL (20 µL loop partial loop mode)
Mobile phase A:	98 H_2O 2% MeOH 0.1% Formic acid
Mobile phase B:	98 MeoH 2% H_2O 0.1% Formic acid

Gradient table

Time (min)	Flow Rate	%A	%B	Curve
Initial	0.600	95.0	5.0	
2.50	0.600	2.0	98.0	6
3.00	0.600	2.0	98.0	6
4.00	0.600	95.0	5.0	1
4.50	0.600	95.0	5.0	1

Negative ion mode

Capillary voltage: 0.8 kV

Function type: MRM of 3 channels

Chan reaction	Cone voltage	Col. energy	Compound
95.00 > 79.90	40.0	15.0	methanesulfonic acid
157.00 > 79.90	45.0	24.0	benzenesulfonic acid
171.00 > 79.90	48.0	26.0	toluenesulfonic acid

Positive ion mode

Capillary voltage: 0.5 kV

Chan reaction	Cone voltage	Col. energy	Compound
173.10 > 77.00	25.0	16.0	methylbenzene sulfonate
187.00 > 77.00	25.0	22.0	ethyl benzenesulphonate
187.00 > 155.00	30.0	10.0	methyl p-toluenesulfonate
201.00 > 173.00	25.0	10.0	ethyl p-toluenesulfonate
229.10 > 91.00	40.0	20.0	2S glycidyl tosylate

RESULTS AND DISCUSSION

Standard solutions of a number of known genotoxic impurities were prepared at 1 mg/mL in acetonitrile and then diluted to concentrations from 0.1 to 500 ng/mL in 5% acetonitrile. In addition, a solution prepared by crushing and dissolving a 10 mg amplodipine besylate tablet, spiked with 1.5 µg genotoxic impurities, was also prepared.

The individual standard solutions were used to help tune the MS using IntelliStart.

Even under the best chromatographic performance coelution can occur. Implementing an approach whereby qualitative MS scan data obtained from the matrix is simultaneously acquired with quantitative multiple reaction monitoring (MRM) MS data can aid in the monitoring of potential interfering compounds, ensuring assay robustness and reproducibility.

In RADAR mode, MRM data can be collected in parallel to the collection of spectral MS data, in both positive and negative ion modes. This can be done with little or no impact on the quality of the MRM data. As a result, you can accurately quantify target compounds while at the same time track other sample matrix components, arming you with a greater depth of knowledge about your sample.

It is important to recognize that RADAR is only possible because of the Xevo TQD's ability to rapidly alternate between MS, MS/MS, positive, and negative ion modes without compromising performance.

RADAR was used to assist in the method development for the LC/MS/MS method for the analysis of the impurities in the spiked tablet solution. The final method developed was used to demonstrate the linearity and sensitivity the system for the genotoxic impurities.

Figure 1 shows how RADAR was used to aid in the method development of the LC/MS/MS method for the analysis of the impurities in the tablet formulation solution.

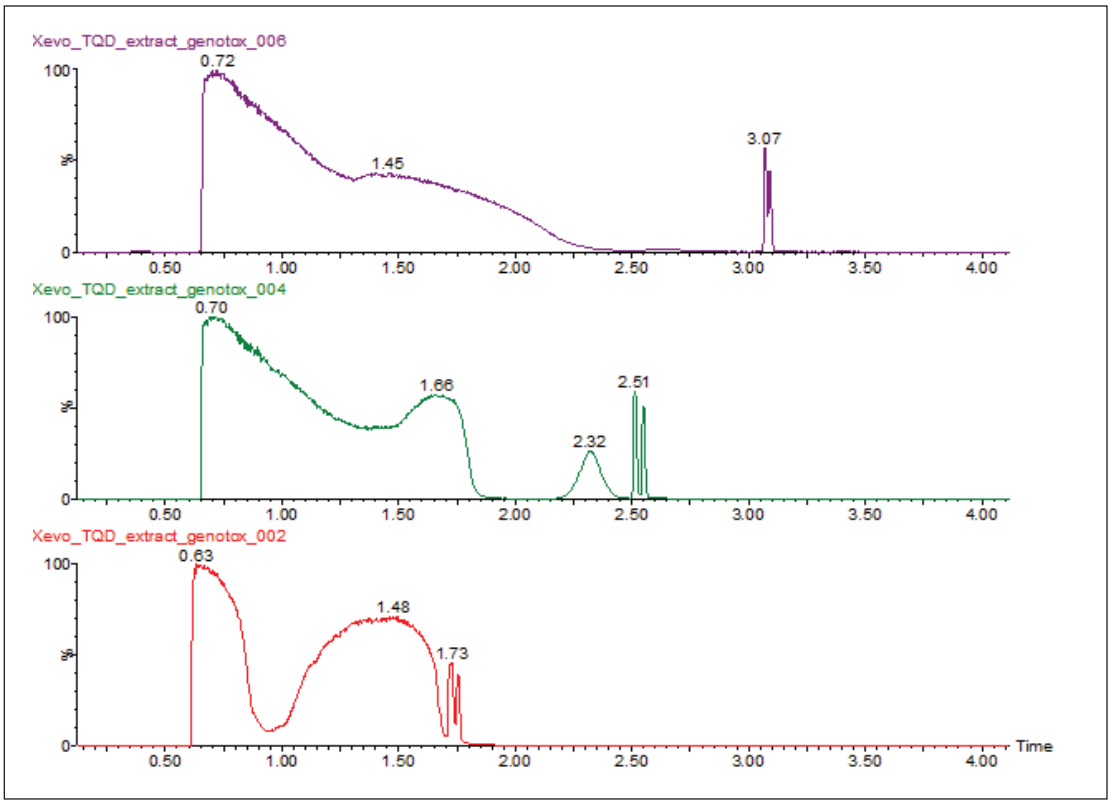

Figure 1. The method is developed using the Xevo TQD in RADAR mode.

Figure 2 shows the linearity plot obtained for toluene sulfonic acid in the range of 0.1 to 500 ng/mL.

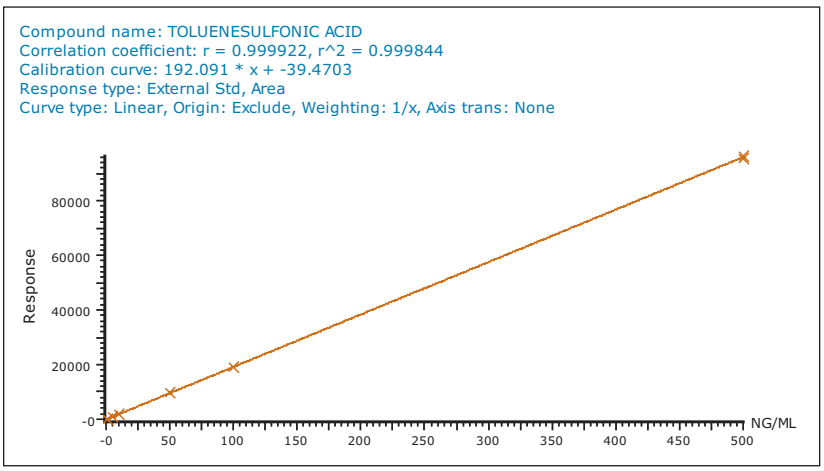

Figure 2. Linearity plot.

An example chromatogram of methane sulfonic acid at 0.1 ng/mL is shown in Figure 3, and glycidyl tosylate in Figure 4.

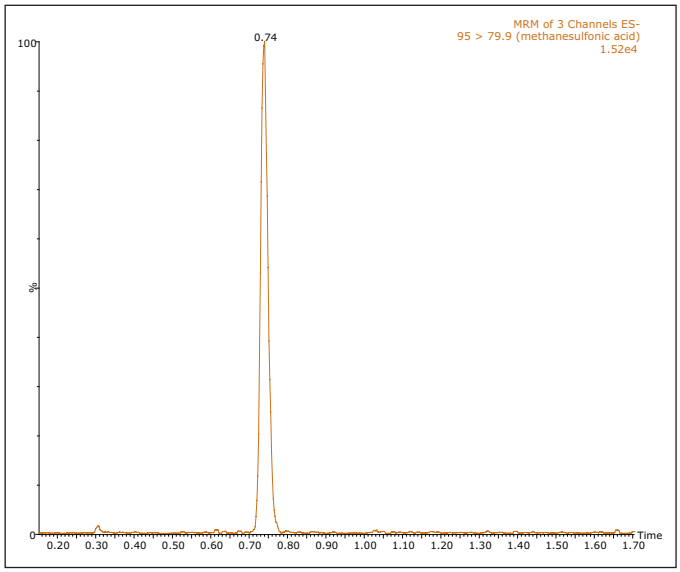

Figure 3. Methane sulfonic acid at 0.1 ng/mL.

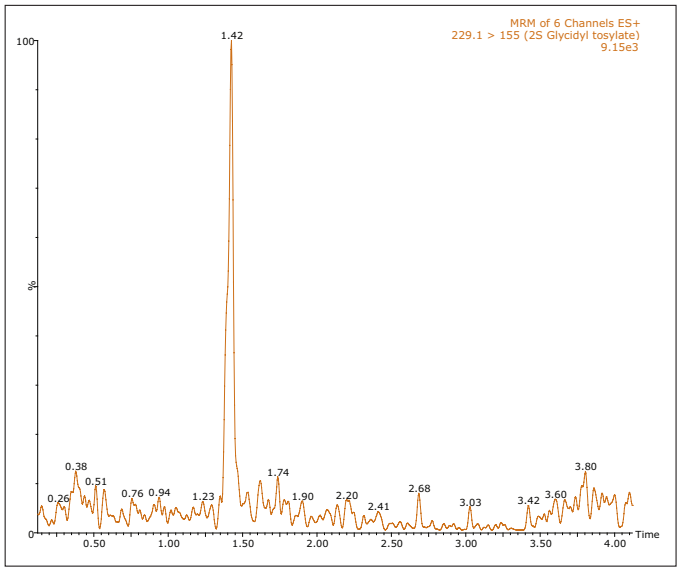

Figure 4. Glycidyl tosylate.

CONCLUSIONS

The introduction of the tandem quadrupole detector Xevo TQD has added an extra tool for the analysis of genotoxic impurities when paired with UPLC. It offers sensitive and rapid methods that can be developed quickly and easily through the use of multiple features such as IntelliStart and RADAR, positive/negative switching, and the generation of "all of the data all of the time." This allows for significant improvements in laboratory productivity at a time when scientific and regulatory demands are increasing.

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Identifying Leachables and Extractables from Packaging Materials

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APPLICATION BENEFITS

- Facilitates the daunting task of identifying unknown compounds in any field that deals with structural elucidation, such as Pharmaceutical, Chemical Material, and Food industries.
- Provides a workflow for the systematic identification of extractables.
- The same workflow applies to either GC or UPLC with QTof.

WATERS SOLUTIONS

Xevo® G2 QTof Mass Spectrometer

Atmospheric Pressure Gas Chromatography (APGC)

MassLynx™ Software

MS^E Technology

MassFragment™ Sofware

KEY WORDS

Extractables, leachables, resins, monomers and oligomers, plasticizers, stabilizers, fillers, coloring agents, antioxidants, antistatic agents, elemental composition

INTRODUCTION

The Pharmaceutical industry is required by the U.S. FDA to demonstrate that no toxic or harmful substances migrate from packaging materials into a drug during its expected product shelf life.[1-5] Similarly, in the Food and Cosmetics industries, there is significant interest in the investigation of packaging leachables present in their products. By definition, extractables are compounds that are extracted from packaging or device components under controlled extraction conditions. Leachables are compounds that migrate from the packaging into the product during its normal shelf life. In the ideal case, leachables are a subset of extractables. If a thorough and accurate identification – or at least compound class identification of all potential contaminants is not performed, it can lead to product recall, financial losses, and/or brand alienation for the company.[6]

The initial investigation, called a controlled extraction study, involves some type of solvent extraction, typically a reflux, microwave, or supercritical fluid extraction.[7] The solvents chosen must cover a wide range of polarities to ensure that non-polar and polar analytes are extracted. The analytical techniques employed for analyzing extracts must be comprehensive to cover as many analytes as possible including GC-FID-MS (volatiles) and LC-UV-MS (non-volatiles).[5]

The challenge with the compounds observed in a controlled extraction study is their identification. Resin manufacturers rarely provide a complete list of all the additives in polymers used for packaging. The original ingredients can degrade or undergo chemical changes during the manufacturing process. Also, the resin manufacturer may not be aware of possible contaminants present within the compounds. Typical extractables include monomers and oligomers from incomplete polymerization reactions; plasticizers, stabilizers, fillers, coloring agents, antioxidants, and antistatic agents, as well as their degradants. Additionally, residues from detergents and mold release agents that can be present on the resin after the molding process.

EXPERIMENTAL

Sample preparation

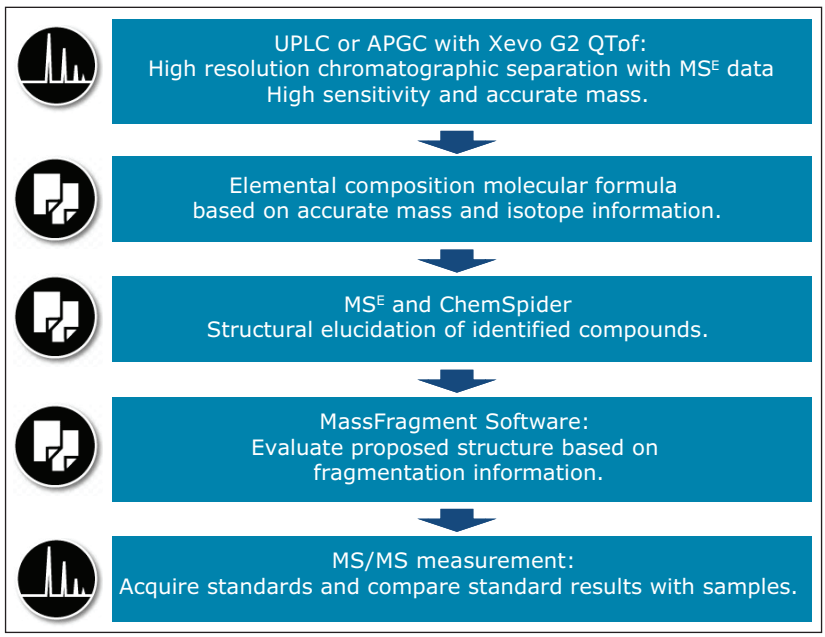
Samples were prepared by microwave extraction. The samples of polypropylene and nylon (2 g) were extracted in 10 mL of isopropanol for 3 h at 70°C. After the extraction the supernatant was transferred to the GC vials.

MS CONDITIONS

MS System:	Xevo G2 QTof with 7890A GC
Column:	HP1-MS, 30 m x 0.32 mm, 1.0 µm film
Carrier gas:	He at 2 mL/min
Temp.:	35 °C for 5 min, 20 °C/min to 320 °C, hold 20.75 min
Injection port:	300 °C
Injection type:	1 µL splitless, 1 min purge
Makeup gas:	N_2 at 500 mL/min
Scan range:	50 to 1,000 Da
Collision ramp for MS^E:	15 to 25 eV
Data management:	MassLynx v. 4.1 Software

Many of the analytes obtained from single quadrupole GC/MS data can be identified using commercially available libraries, such as NIST. However, a difficulty arises for volatiles analysis when the compound of interest is not listed in the library, or when the sensitivity of a single quadrupole MS is not sufficient for a positive identification. Therefore, additional techniques, such as Atmospheric Pressure Gas Chromatography (APGC) and Quadrupole Time-of-Flight (QTof) described in this application note, are beneficial.[8] Due to the absence of libraries for LC/MS data accurate mass data would vastly facilitate the non-volatile analysis. For both volatile and semi-volatile analysis performed here, MS^E data, acquisition on a quadrupole time of flight mass spectrometer, with commercially available structural elucidation tools proves to be valuable for identification of the unknown compounds.

Workflow



RESULTS AND DISCUSSION

Two widely available polymer materials were chosen for this study: polypropylene and nylon. In this application note, the identification of three different types of extractables is shown: an antioxidant, a monomer and a degradant of a monomer.

In the polypropylene sample, a peak (Peak A) was observed at a retention time of 26.3 min, as shown in Figure 1. Performing elemental composition analysis on the accurate mass APGC spectrum, shown in Figure 2, suggested a molecular formula of $C_{43}H_{63}O_3P$, as shown in Figure 3. The elemental composition software calculates the possible molecular formulas for the observed mass and also uses the isotope pattern algorithm to match the observed pattern with the theoretical one for each candidate molecular formula. In this case, there are two choices shown for the ion with the second being a closer match if only mass difference is considered. However, the combination of mass difference and isotope fit brings the correct one to the top of the list.

The APGC analysis was performed under dry source conditions,[9] which promotes molecular ion ($M^{\cdot+}$) formation ahead of the protonated adduct ($[M+H]^+$). It is interesting to note that under high energy collision conditions the molecular ion fragments more easily than the protonated adduct; therefore the difference in the base peak was observed (646.4 versus 647.4) between the two channels, shown in Figure 2.

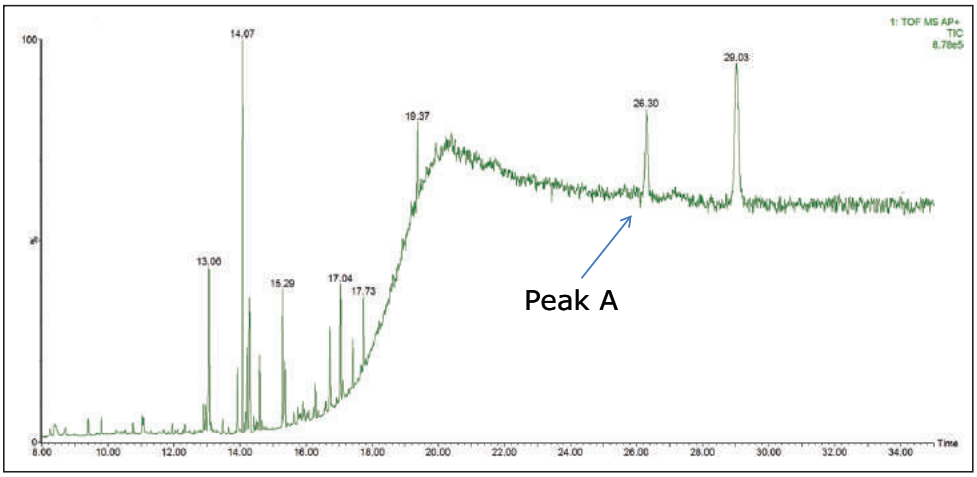


Figure 1. Polypropylene TIC.

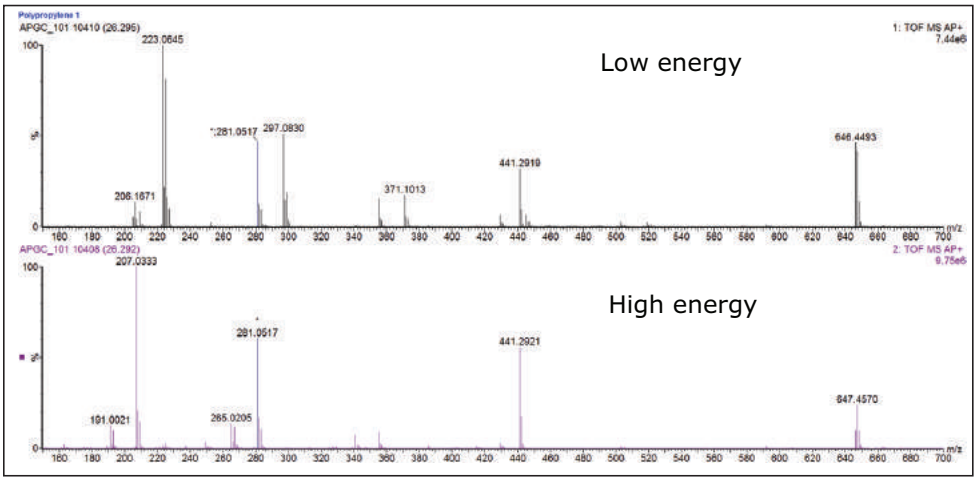


Figure 2. High and low energy spectra for Peak A.

Single Mass Analysis
Tolerance = 3.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3
Monoisotopic Mass, Odd and Even Electron Ions
265 formula(e) evaluated with 2 results within limits (up to 50 best isotopic matches for each mass)
Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	i-FIT Norm	Fit Conf %	C	H	O	P
646.4493	646.4515	-2.2	-3.4	12.0	C42 H63 O3 P	224.4	0.182	83.35	42	63	3	1
	646.4503	-1.0	-1.5	-1.0	C31 H66 O13	226.1	1.793	16.65	31	66	13	

Figure 3. Elemental composition data for Peak A.

Performing a search of the proposed elemental composition formula in ChemSpider gave Irgafos 168, shown in Figure 4, as the top answer when sorted by "# of References", as described by Little, *et al.*[10] Irgafos 168 is a trisarylphosphite processing stabilizer and protects the resin polymer, such as polypropylene, against oxidation during resin synthesis.

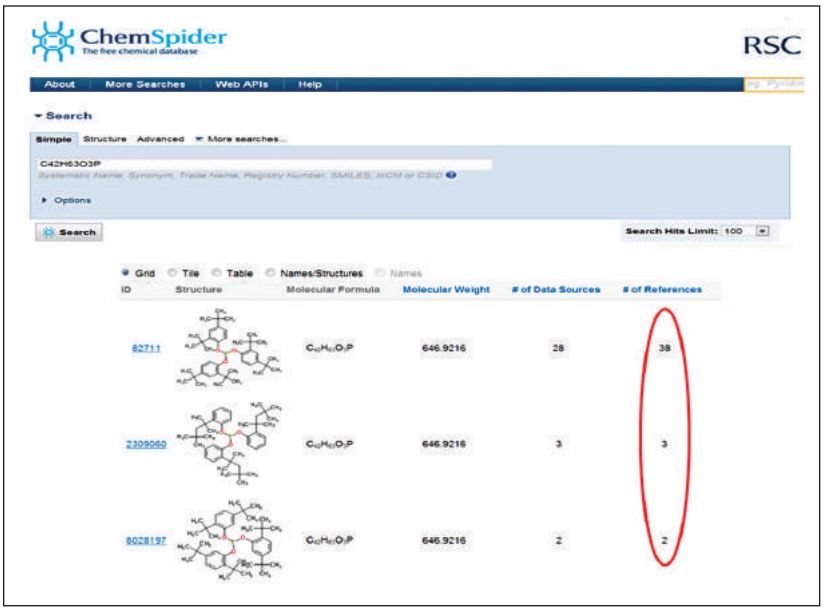


Figure 4. ChemSpider search for $C_{42}H_{63}O_3P$, first match is Irgafos 168. The search hits are ordered by number of references and data sources.

Confidence in the identification was increased when another structural elucidation tool, Waters® MassFragment Software, was able to match several fragments observed in the high and low energy spectra to major fragment ions of Irgafos 168, as shown in Figure 5. MassFragment identifies bonds in precursor structure and then assigns a score based on the type and likelihood of the bond breakage. In addition, the number of bonds broken is listed. The lower the score (*e.g.* S:1.0, B:1.0 vs. S:4.5, B:2.0) the more probable the appearance of the fragment substructure.

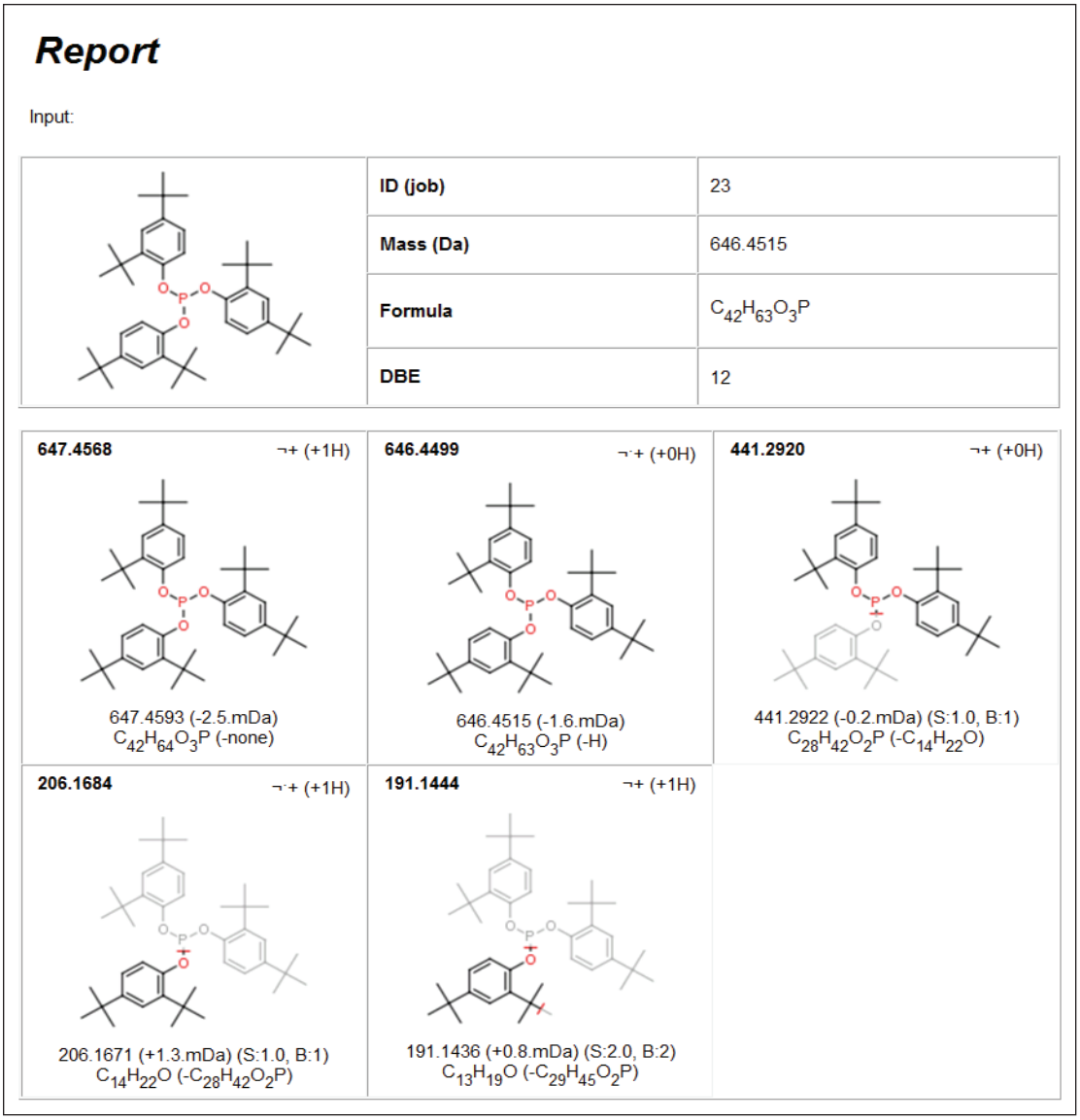


Figure 5. MassFragment Software report for confirmation of Irgafos 168.

The next step in this workflow is to purchase a standard and compare the retention time and fragmentation pattern with the sample.

Laurin lactam is a known starting material for the manufacturing of nylon. In the nylon extract the laurin lactam monomer (Peak B) is observed at a retention time of 15.93 minutes, as shown in Figure 6. The identity of the peak was confirmed by molecular formula and MassFragment following the workflow described in the previous example. A smaller peak is observed at a retention time of 16.07 minutes (Peak C). The measured mass is consistent with a molecular formula of $C_{12}H_{21}NO$, shown in Figure 7, which indicated that the peak was likely a laurin lactam degradant with an extra double bond in the molecule (laurin lactam monomer is $C_{12}H_{23}NO$). The parent ions in each spectra were confirmed by the presence of the in-source dimers (2M+H). For laurin lactam the observed dimer has *m/z* 395.3652 and for the degradant it is *m/z* 391.3324.

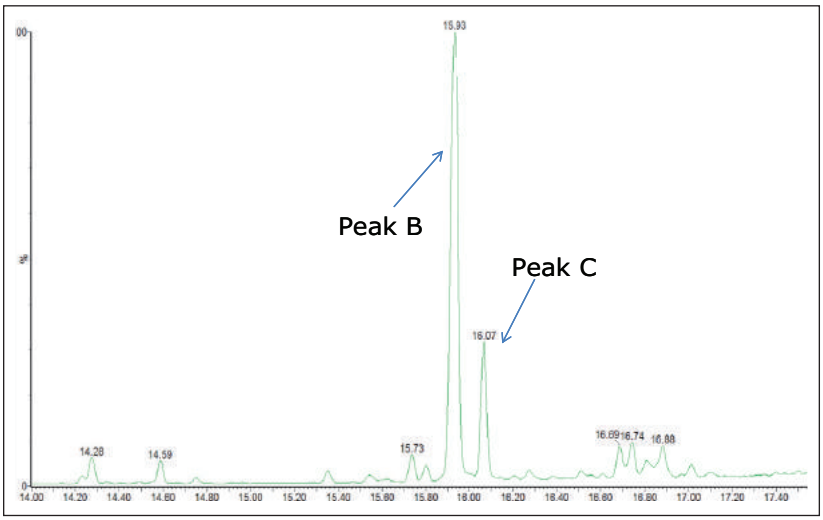


Figure 6. TIC for nylon extract.

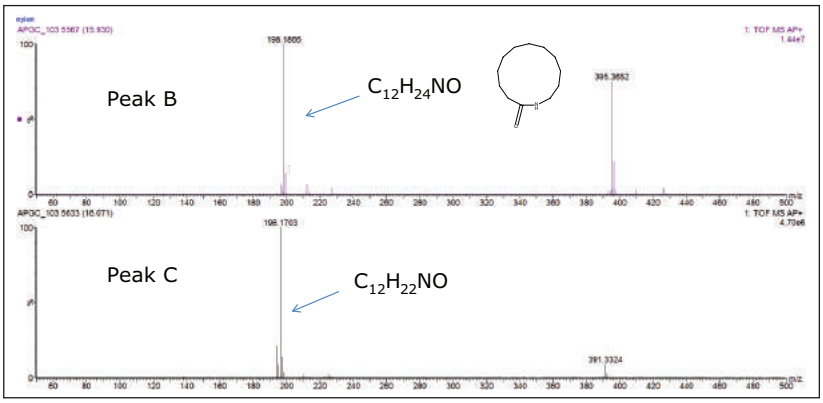


Figure 7. Spectra and molecular formula $[M+H]^+$ for Peaks B and C.

The ChemSpider search for $C_{12}H_{23}NO$ showed laurin lactam as the second top choice. The search of $C_{12}H_{21}NO$ did not provide any appropriate match based on the known compounds in the polymer.

Since a standard of this degradant is not likely to be available, the Xevo G2 QTof data allowed the assignment of a structure to this compound. It is not possible to determine the exact location of the double bond on the laurin lactam ring. However, in these types of studies it is not always necessary to determine an exact structure. It is sufficient if the compound's class has been identified. It was clear that the degradant is related to laurin lactam, therefore its toxicological profile was expected to be similar.

CONCLUSIONS

- Xevo G2 QTof is a valuable tool in the identification and structural elucidation of extractables. MS^E functionality allows simultaneous acquisition of precursor and fragment ions. Accurate mass and fragmentation information assists in the assignment of structures for many unknown compounds.
- Elemental composition and Mass Fragment Software provide the analyst with additional resources in cases when compounds of interest are not found in commercially available libraries.
- The workflow described can facilitate the daunting task of identifying the unknowns in any field that deals with structural elucidation, such as Pharmaceutical, Chemical Material, and Food industries.
- The fragments, the most likely molecular formula, and some chemical intuition based on ingredients known to be present can often provide a likely structure. In the extractable field a likely structure is often sufficient since the goal is to establish a safety threshold.

References

1. M P Balogh. "Testing the Critical Interface: Leachables and Extractables". LCGC, June 1, 2011.
2. F Mofatt. Extractables and Leachables in Pharma-Serious Issue. http://www.solvias.com/sites/default/files/solvias_whitepaper_web.pdf
3. Containers Closure Systems for Packaging Human Drugs and Biologics; Guidance for Industry. U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) and Center for Biologics Evaluation and Research (CBER), Rockville, MD. May 1999.
4. D L Norwood, Q Fenge. Strategies for the analysis of pharmaceutical excipients and their trace level impurities. Am Pharm Rev. 7(5): 92, 94, 96-99, 2004.
5. Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, Leachables and Extractables Working Group. Product Quality Research Institute (PQRI), 2006. (WWW.PQRI.ORG).
6. http://www.webmd.com/pain-management/news/20110629/new-tylenol-recall-due-to-musty-odor
7. M Ariasa, I Penichet, F Ysambertt, R Bauza, M Zougaghc, Á Ríos. Fast supercritical fluid extraction of low- and high-density polyethylene additives: Comparison with conventional reflux and automatic Soxhlet extraction. J. of Supercritical Fluids, 50: 22-28, 2009.
8. Detection and Identification of Extractable Compounds from Polymers. Waters Technology Brief, no. 720004211en, January, 2012.
9. Determination of High Molecular Weight Phthalates in Sediments Using Atmospheric Pressure Chemical Ionization GC/MS. Waters poster, no. PSTR134667160.
10. J L Little, A J Williams, A Pshenichnov, V Tkachenko. Identification of "known unknowns" utilizing accurate mass data and ChemSpider. JASMS. 23(1): 179-185, 2012.

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Routine Trace-Level Contamination Testing in High-Quality Manufactured Parts and Assemblies

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APPLICATION BENEFITS

- Provides a simple and rapid method for the detection of organic and ionic contaminants as part of the quality control of manufactured 'clean' parts.
- Streamlines workflow, from instrument setup and sample analysis, to data processing.
- Facilitates fast automated data processing and interpretation with MarkerLynx™ XS Software, providing quick access to information about clean and contaminated parts.

WATERS SOLUTIONS

ACQUITY UPLC® System

Xevo® G2 QTof MS System

MassLynx™ Software

MarkerLynx XS Application Manager

KEY WORDS

Raw material QC testing, chemical contaminants, electronics, packaging, manufacturing, QC, impurities testing

GOAL

To provide an easy-to-use methodology for trace level monitoring of contaminants in manufactured 'clean' parts and assemblies.

INTRODUCTION

The cleanliness of parts and assemblies is critical to many modern high technology industries, and impacts not only on yield, but also the viability of the products. As surfaces define many of the properties of materials, even trace surface contaminants may dramatically alter the properties and performance characteristics of a material. Low level contamination can therefore be extremely detrimental to the processing or application of the components downstream.

It is vital for these industries to quickly recognize that a component has become contaminated, identify the contaminant, and trace the contamination to the specific part of the process affected. Failure to quickly identify and respond to contamination may lead to product hold, major recall, or loss of revenue and reputation.

Typically the primary source of contamination is from the industrial manufacturing process. Contaminants may originate from moulding, machining, stamping, polishing, etc. which require the use of coolants, lubricants, dispersants, chemical etching, and cleaning agents; or as by-products from chemical reactions used to modify the properties of the material. Once manufactured, the parts undergo rigorous cleaning before entering assembly. Secondary contamination may be from the environment, packaging, or through handling. A final stage of cleaning is therefore required to remove loose particles, organic and ionic contaminants which might be introduced during storage, handling or shipping.

To optimize the cleaning process and troubleshoot problems in the manufacturing process, a rapid and easy sampling method and measurement is required. Here, the Waters® ACQUITY UPLC system has been modified to provide direct injection without any separation, which greatly enhances the sensitivity of the method for the detection of low-level contaminants. For these analyses, adopting a chemometric approach can quickly provide information about the similarities and differences within large mass spectral datasets, and may be used to highlight sudden changes, such as the appearance of a new contaminant. Automation of this process greatly reduces the analysis time required and the probability of

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC (modified with capillary assembly to provide back pressure)
	No column
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid
Flow rate:	0.03 mL/min, isocratic, 50:50 mobile phase A:B
Total run time:	3.0 min
Injection volume:	20 µL, full loop, overfill factor 2.0 times

MS conditions

MS system:	Xevo G2 QTof MS
Ionization:	ESI positive and negative
Scan mass range:	*m/z* 50 to 1200
Capillary voltage:	3 kV
Sample cone:	35 V
Source temp.:	120 °C
Desolvation gas:	Nitrogen, 1000 L/hr, 500 °C
Cone gas:	Nitrogen, 25 L/hr
Lock mass compound:	Leucine enkephalin (*m/z* 556.2771 and 278.1141)

Acquisition and processing methods

The samples were analyzed using an ACQUITY UPLC System with Xevo G2 QTof MS, and data were acquired using MassLynx Software, v. 4.1. MarkerLynx XS Application Manager was used to process the data.

error in this assessment, while increasing sensitivity. A database of possible contaminants can also be built, so that identified contaminants can be traced to a particular source or process.

Such a workflow may be applicable to many fields where surface contamination and impurities may be an issue, for example in the electronics and packaging industries.

Figure 1. ACQUITY UPLC System with Xevo G2 QTof MS.

Sample Preparation

A dedicated ACQUITY UPLC System and fluidics were used for trace contaminant analysis to ensure that there was no contamination arising from the system or previously run samples. All glassware must be ultraclean, and all solvents and reagents used must be of the highest quality and purity – minimum LC/MS grade solvents were used. In addition, all glassware used were rinsed five times with a soak solution immediately prior to use, and the entire UPLC®/MS system was flushed for an hour before use.

For this analysis, sample parts were treated with a detergent containing polyethylene glycol (PEG), a ubiquitous polymer, then underwent complete and incomplete passivation and rinsing.

Samples were prepared in triplicate by soaking individual parts in appropriately sized glass sampling jars (minimizing the volume of solvent used, while completely submerging the component part) for eight hours. The soak solution used was a 50:50 mixture of acetonitrile and water with 0.1% (v/v) formic acid. Sampling jars without parts were prepared in the same manner and analyzed in parallel with the parts as reference blanks. One mL aliquots of the soak solutions were transferred to Waters LC/MS Certified Vial (P/N: 60000668CV), and analyzed by direct-flow injection.

Process workflow

The comprehensive detection and chemical component information obtained using UPLC/ToF-MS can be effective for supporting quality control and the discovery and identification of contaminants. This method allows the cleanliness of otherwise challenging irregular shaped parts and assemblies, and their internal surfaces to be measured. The chemical composition information obtained is processed and visualized by multivariate analysis in order to simplify the interpretation of the results. Any contaminating organic and ionic species identified can then be elucidated using fragment ion information. The workflow for this process is shown in Figure 2.

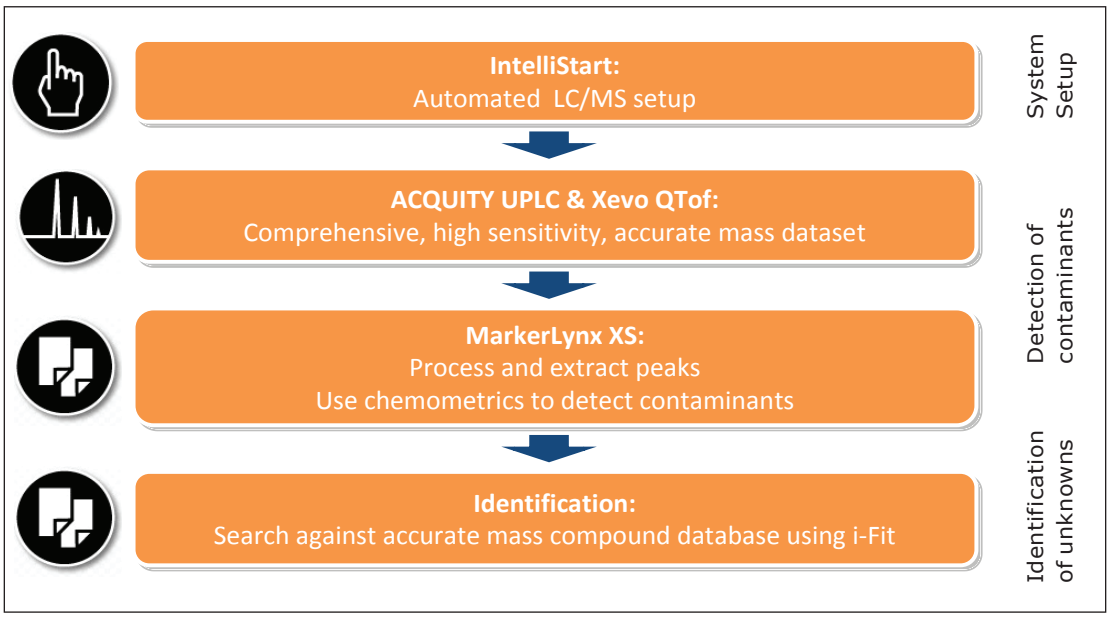

Figure 2. ToF screening workflow for trace contaminant analysis.

In monitoring product quality, the material from different production lots, including non-conforming material are measured. Samples are measured in triplicate and accurate masses are automatically extracted and stored. MarkerLynx XS performs Principal Component Analysis (PCA) on the extracted data and separates out the non-conforming material based on differences in their scores.

IntelliStart automated LC/MS test and verification

Incorporated into MassLynx Software is Waters IntelliStart™ Technology, which automates calibration and LockSpray™ setup. IntelliStart also provides a simple and automated system check procedure to ensure that the LC/MS system is operating optimally. An LC/MS system check, shown in Figure 3, is performed prior to analysis using a series of user-defined criteria, such as mass accuracy, retention time, peak height, etc. for a particular concentration, and injection volume of a reference compound. These can be used for the long-term monitoring of the system's performance.

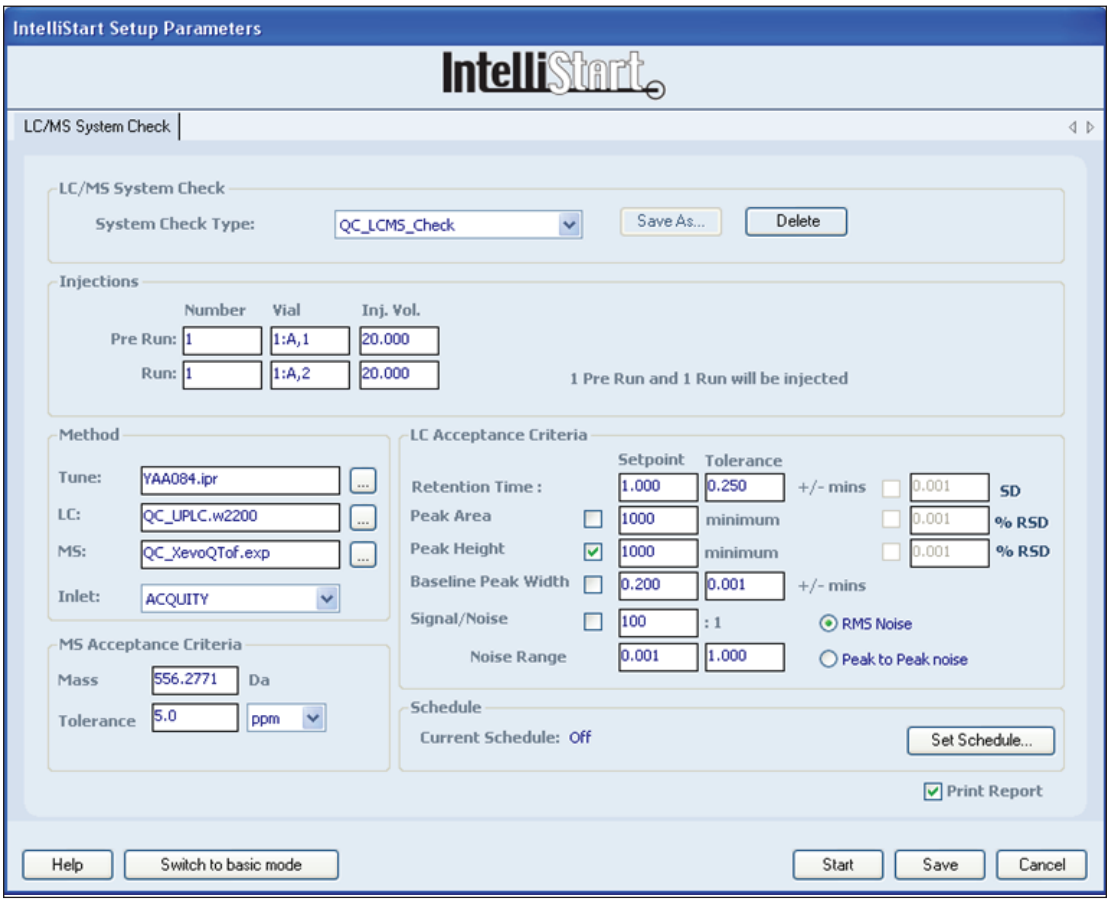

Figure 3. Using the analytical method, the LC/MS system check ensures that both the LC and MS are working within acceptance criteria set by the user prior to analysis.

A novel feature of Xevo G2 QTof MS is its ability to run methods with multi-point lock mass. For the trace analysis, a two-point lock mass (*m/z* 278.1141, 556.2771) was utilized to correct for environmental changes in the laboratory over time, which would otherwise result in loss of mass accuracy. Good mass accuracy allows the elemental composition of both the precursor and its fragment ions in MS/MS to be determined with greater confidence and precision, aiding the elucidation of unknown compounds.

MarkerLynx approach

Trace contaminant analysis typically relies on manual processing and analysis of data which is time consuming and tedious. The MarkerLynx and Extended Statistics software enable users to overcome the time-consuming problem of identifying patterns in the in LC/MS data sets by showing the similarities and differences between detected MS traces.

Use of MarkerLynx XS Application Manager eliminates the need to manually process (strip and background subtract) each and every spectrum individually. A 20 µL isocratic LC injection is used to enhance the sensitivity of the method to low-level contaminants, as shown in Figure 4. MarkerLynx XS automatically combines spectra over the selected scan range, subtracting background components present in the mobile phase, as shown in Figure 5.

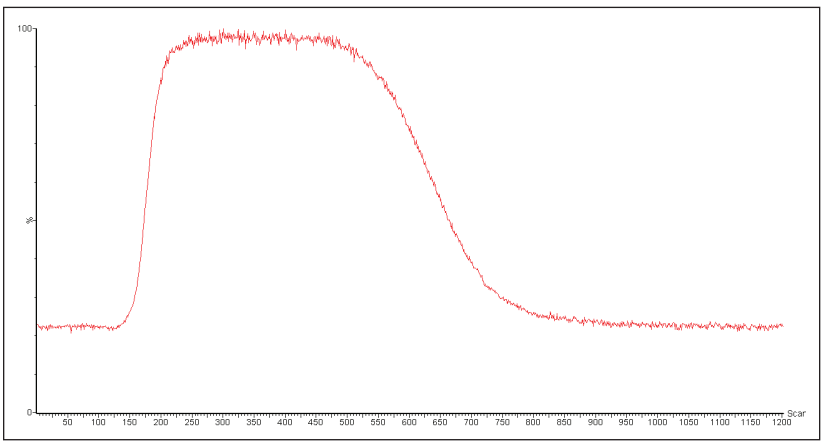

Figure 4. An isocratic LC injection enhances the sensitivity of the method to analyze low level contaminants.

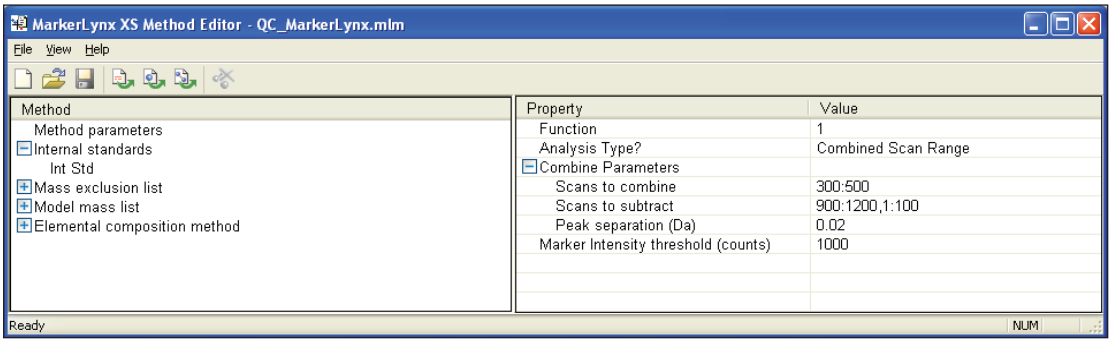

Figure 5. MarkerLynx XS automatically processes and extracts components over the selected scan range.

The processed and extracted data are then interrogated using the Extended Statistics chemometrics-based software tool included in the MarkerLynx XS Application Manager. Principal Component Analysis is performed, and the data set is displayed on a scores plot which shows the possible presence of atypical observations, such as contaminated parts and patterns in the data, as shown in Figure 6.

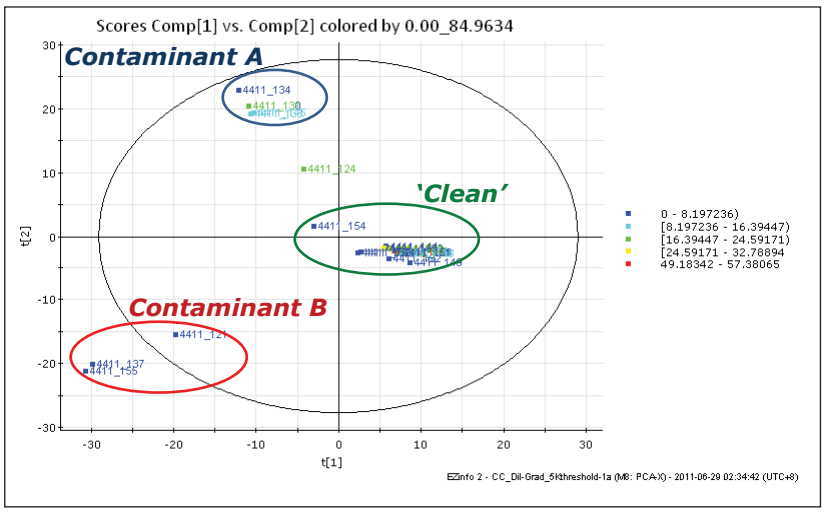

Figure 6. PCA score plot showing differences among the sample sets.

An Orthogonal Partial Least Squares (OPLS) model can then be applied to compare and separate the two groups by their components, enabling extraction of those components responsible for the differences between them. Using the conforming (Clean) group and non-conforming (Contaminant B) group, an S-Plot is obtained, as shown in Figure 7.

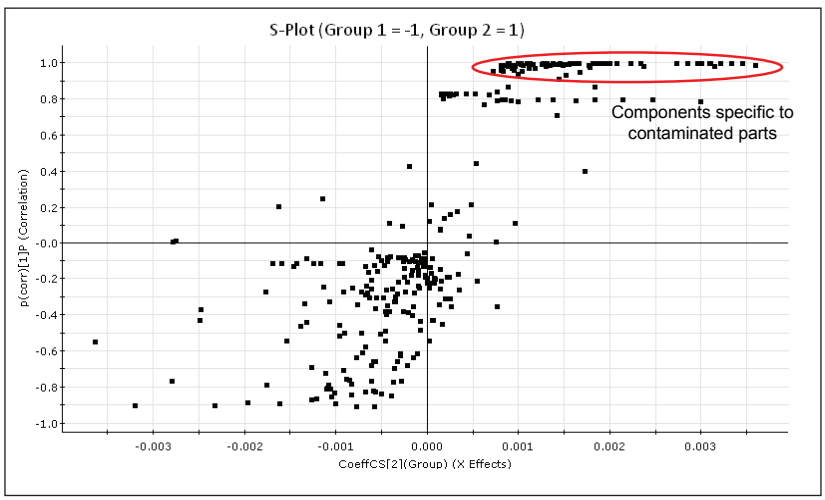

Figure 7. OPLS Model: components showing the greatest difference between the 'clean' (Group 1) and Contaminant B affected parts (Group 2) appear at the extremes of the S-Plot.

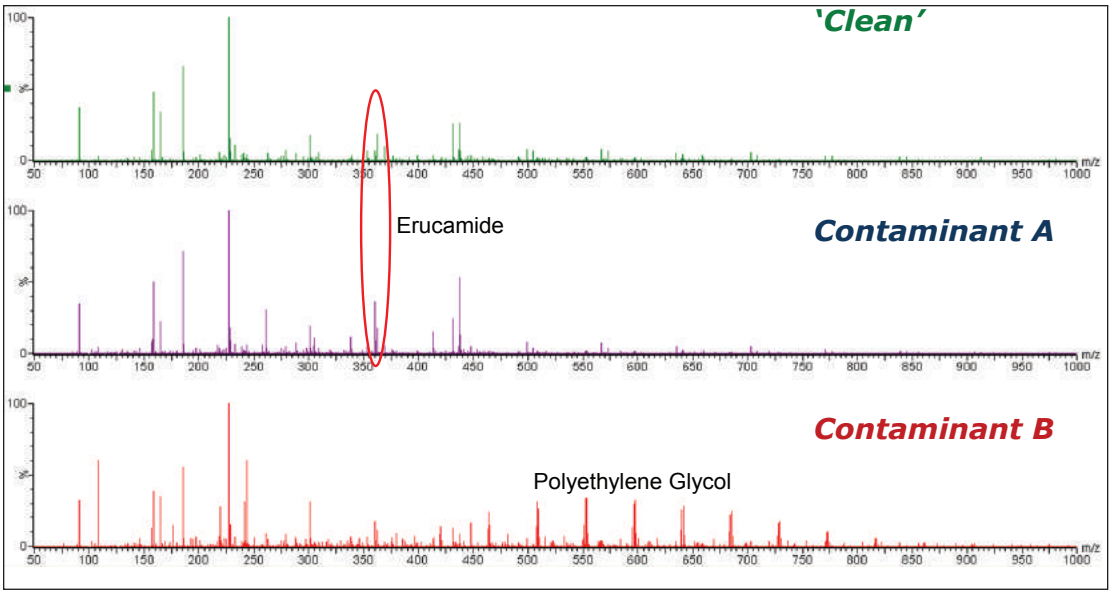

Figure 8. Representative combined spectra for the 'clean' and contaminated samples.

Once the non-conforming components responsible for differences with the clean samples are determined, candidate contaminants can be determined by searching against internal or external databases of known contaminants. In this case, the components specific to Contaminant B were shown to correspond to PEG, in the combined spectrum, shown in Figure 8. The peaks present in both the 'clean' and Contaminant A samples were mainly due to sodium formate, formed by formic acid in the soak solution reacting with sodium from the glassware. However, MarkerLynx XS found that the component which differentiated Contaminant A from the 'Clean' samples was *m/z* 360.32, corresponding to erucamide $[M+Na]^+$, a common slip agent used in plastics manufacturing.

The high mass accuracy and accurate isotope patterns provided by Time-of-Flight mass spectrometry (ToF MS) allow elemental compositions to be generated with high confidence for unknown compounds. MS/MS can also be used to provide structural information in order to help identify and confirm contaminants. After contaminated components and contaminants have been identified, they can be used to trace the source (manufacturing or processing step) where the contaminant(s) may have been introduced, and the issue can be addressed. MarkerLynx XS is sufficiently selective to separate identify differences in manufacturing and processing contaminants on the different cleaning validation piece parts.

CONCLUSIONS

- The ACQUITY UPLC System with Xevo G2 QTof MS, combined with MarkerLynx XS Application Manager can be used for chemical contamination analysis and quality monitoring in component parts and assemblies used in the manufacturing process.
- The LC/MS System Check functionality within IntelliStart ensures that the UPLC/MS system performs to specifications before the start of cleanliness analyses, and allows long-term monitoring of the performance of the system.
- Lock mass correction allows mass accuracy to be maintained over a long period of time, ensuring consistency over large numbers of samples typically required for routine quality control operations, and it facilitates identification of unknowns by reducing the numbers of possible elemental compositions.
- The MarkerLynx XS Application Manager removes time-consuming manual processing and interpretation of data. It enables processing the data into an easily understandable format, with calculations and comparisons performed automatically. Information about contaminated parts can be quickly extracted, allowing a targeted analysis of the contaminants.
- The solution proposed would be useful for raw material QC testing across many manufacturing industries.

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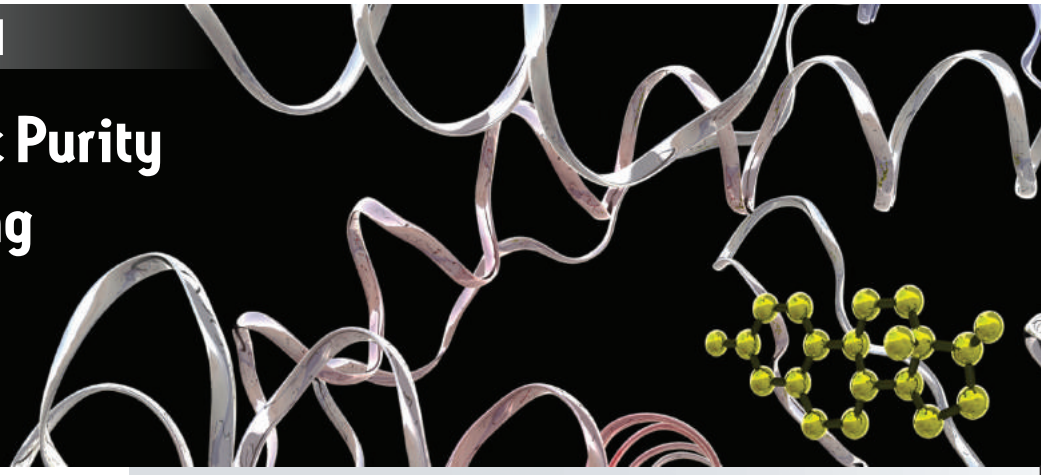
[TECHNOLOGY BRIEF]

Chromatographic Purity of Estradiol Using the ACQUITY UPC^2 System



GOAL

To demonstrate the results obtained from the impurity analysis of estradiol with the ACQUITY UPC^{2}™ System are equal to or better than those achieved using the current USP method.

BACKGROUND

Currently, the United States Pharmacopeia (USP) method for the estimation of chromatographic purity of estradiol utilizes a 4.6 x 250 mm silica column and a mobile phase consisting of 2,2,4-trimethylpentane, *n*-butyl chloride, and methanol 45:4:1 at 2 mL/minute. Since many laboratories have a desire to limit the use of aliphatic hydrocarbons and chlorinated solvents, alternative chromatographic techniques, like supercritical fluid chromatography (SFC) must be evaluated.

The Waters® ACQUITY UPC^2 System was used to develop a method for the evaluation of the chromatographic purity of estradiol. Results obtained from the UltraPerformance Convergence Chromatography™ (UPC^{2}™) method were directly compared to results obtained for the current USP method to detect estradiol impurities. The results from both techniques were similar with the UPC^2 method showing adequate sensitivity to detect impurities in estradiol equal to those obtained from the normal phase HPLC USP method. In addition, when using UPC^2, sample run time is reduced and overall cost per analysis (based on solvent usage and waste disposal costs) is significantly reduced.

The UPC^2 method used for the estimation of the chromatographic purity of estradiol was three times faster than the current normal phase HPLC method and reduced cost per analysis by more than 100 times.

THE SOLUTION

A sample of estradiol was prepared and analyzed using the current USP method as shown in Figure 1. The results of this analysis were used to compare with the results obtained in the method developed on an ACQUITY UPC^2 System as seen in Figure 2 using the identical sample preparation.

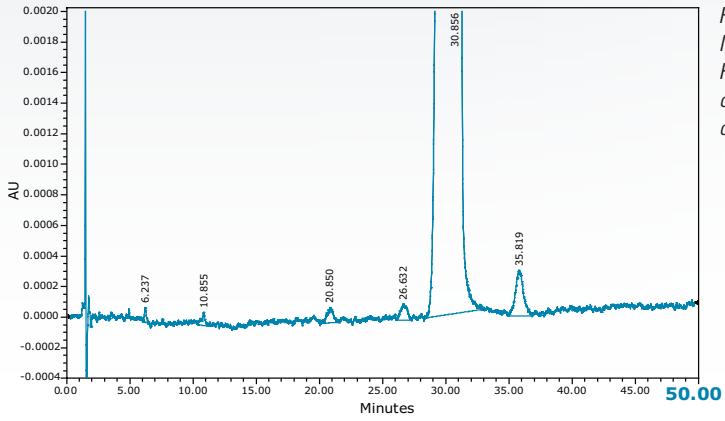


Figure 1. Normal phase HPLC separation of estradiol and impurities.

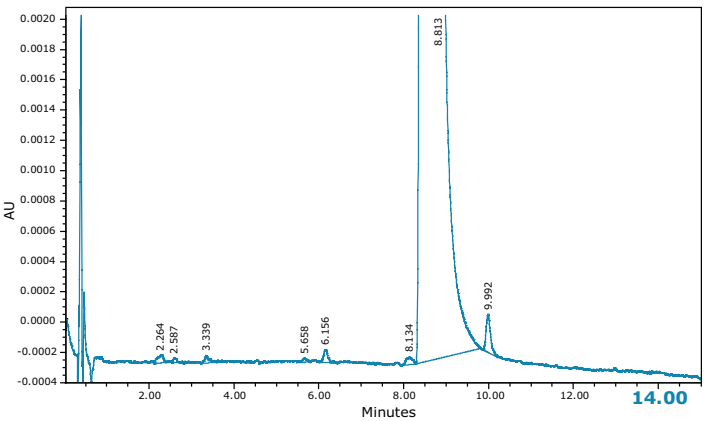


Figure 2. ACQUITY UPC^2 separation of estradiol and impurities.

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The UPC^2 method conditions were as follows:

Column: ACQUITY UPC^2 BEH, 2.1 x 150 mm, 1.7 µm
Mobile phase: A=CO_2 B=1:1 Methanol/2-Propanol
Back pressure: 130 bar/1880 psi
Temp.: 45 °C
Detection: UV/PDA at 280 nm

Time (min)	Flow (mL/min)	%A	%B	Curve
0.0	1.2	97	3	–
15.0	1.2	93	7	8
15.1	1.2	97	3	6
20.0	1.2	97	3	6

A comparison of results from the two methods is shown in Table 1. Both the normal phase HPLC and UPC^2 methods detected at least five impurities below 0.1% (based on area). Signal to noise values for peaks in the range of 0.01% were all approximately 3:1 for both methods with the UPC^2 results giving slightly higher values. The largest impurity (approximately 0.05% based on area) gave a signal to noise value of 16:1 for UPC^2 and 9:1 for normal phase HPLC. These results clearly show that the ACQUITY UPC^2 System can be used to successfully analyze minor impurities from estradiol. The run time of the UPC^2 method was considerably shorter than the normal phase HPLC method (20 minutes compared to 60 minutes) resulting in an increase in lab productivity. An analysis of cost per run showed that the cost of solvent for the normal phase HPLC method was $5.89 compared to less than $0.05 per run using UPC^2. The normal phase HPLC method generated, as mixed chlorinated waste for disposal, 108 mL of 2,2,4-trimethylpentane, 9.6 mL of *n*-butyl chloride, and 2.4 mL of methanol. The UPC^2 method generated disposal waste of 0.60 mL each of methanol and 2-propanol. The CO_2 used in the separation was vented through the laboratory exhaust. Waste disposal costs were reduced by more than 150 times using the UPC^2 method.

Compound	UPC^2 RT	%Area	S/N	Normal phase HPLC RT	%Area	S/N
Unk. impurity	2.26	0.012	3.4	6.24	0.006	2.9
Unk. impurity	2.59	0.004	1.9	Not Found		
Unk. impurity	3.34	0.010	3.1	10.86	0.010	2.7
Unk. impurity	5.66	0.006	1.7	Not Found		
Unk. impurity	6.15	0.016	5.5	20.85	0.018	3.0
Unk. impurity	8.13	0.013	3.1	26.63	0.021	3.2
Estradiol	8.81	99.890	–	30.86	99.87	–
Main impurity	9.99	0.046	16.0	36.81	0.077	9.2

Table 1. Comparison of estradiol impurity detection using UPC^2 vs. normal phase HPLC.

SUMMARY

A method for the estimation of the chromatographic purity of estradiol was developed, using the ACQUITY UPC^2 System. This UPC^2 method was three times faster than the current normal phase method from the USP. In addition to speed, this method reduced the cost per analysis by more than 100 times, primarily by reducing the need for aliphatic hydrocarbons and chlorinated solvents. Required sensitivity levels were achieved in the UPC^2 method with impurities as low as 0.01% of the main peaks being easily detected. The ACQUITY UPC^2 System is an ideal choice for laboratories looking for an alternative to conventional normal phase HPLC.

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Improving Resolution Using e*X*tended *P*erformance (*XP*) Columns

Mia Summers, Kenneth Berthelette, and Kenneth J. Fountain

GOAL

To demonstrate improved resolution of ***XP*** 2.5 µm Columns over traditional HPLC particle size columns for challenging separations.

BACKGROUND

It is widely accepted that transferring methods to smaller particle sizes can result in faster analysis time. By transferring a method directly to a smaller particle size, there may also be improvements in resolution. As the particle size gets smaller, however, the back pressure across the column will increase. While the use of sub-2-µm columns may necessitate the use of a UPLC® System, HPLC users can still realize significant benefits in resolution by transferring their HPLC methods to a e***X***tended ***P***erformance (***XP***) 2.5 µm Column. This may be particularly beneficial for the separation of complex mixtures, where the added resolution from a smaller particle size column may help to identify impurities or target compounds without resorting to increasing column length and run times.

Improving the resolution of a related-compounds separation using e*X*tended *P*erformance (*XP*) columns.

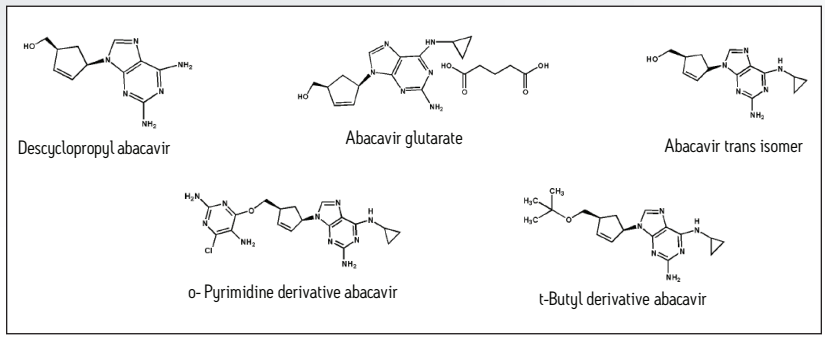

Figure 1. Abacavir components in the USP related compounds mixture.

An example of improved resolution using an ***XP*** 2.5 µm Column is demonstrated using a related compounds mixture of abacavir. Abacavir is a nucleoside reverse-transcriptase inhibitor that is used in anti-HIV therapy. The mixture of related compounds contains five compounds, including the main component, abacavir, shown here in glutarate form (Figure 1). The separation of abacavir from its trans-isomer is particularly challenging. Here, the overall improved separation of abacavir from its related compounds is demonstrated, comparing the use of a 3.5-µm column to a high efficiency ***XP*** 2.5 µm Column.

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THE SOLUTION

To properly separate related compounds while minimizing extensive method development in HPLC, a highly efficient column with higher resolving power should be used. ***XP*** Columns contain 2.5-µm particles packed at high pressures in UltraPerformance hardware. The back pressure allowances of the ***XP*** 2.5 µm particle column still allow for use on an HPLC system.

To demonstrate the improvement in performance using ***XP*** Columns, the related compounds mixture for abacavir was tested on a 100-mm 3.5-µm, XSelect® CSH™ C_{18} Column. The same method was run using the same column chemistry and dimensions with ***XP*** 2.5 µm Columns. The comparative separations are shown in Figure 2.

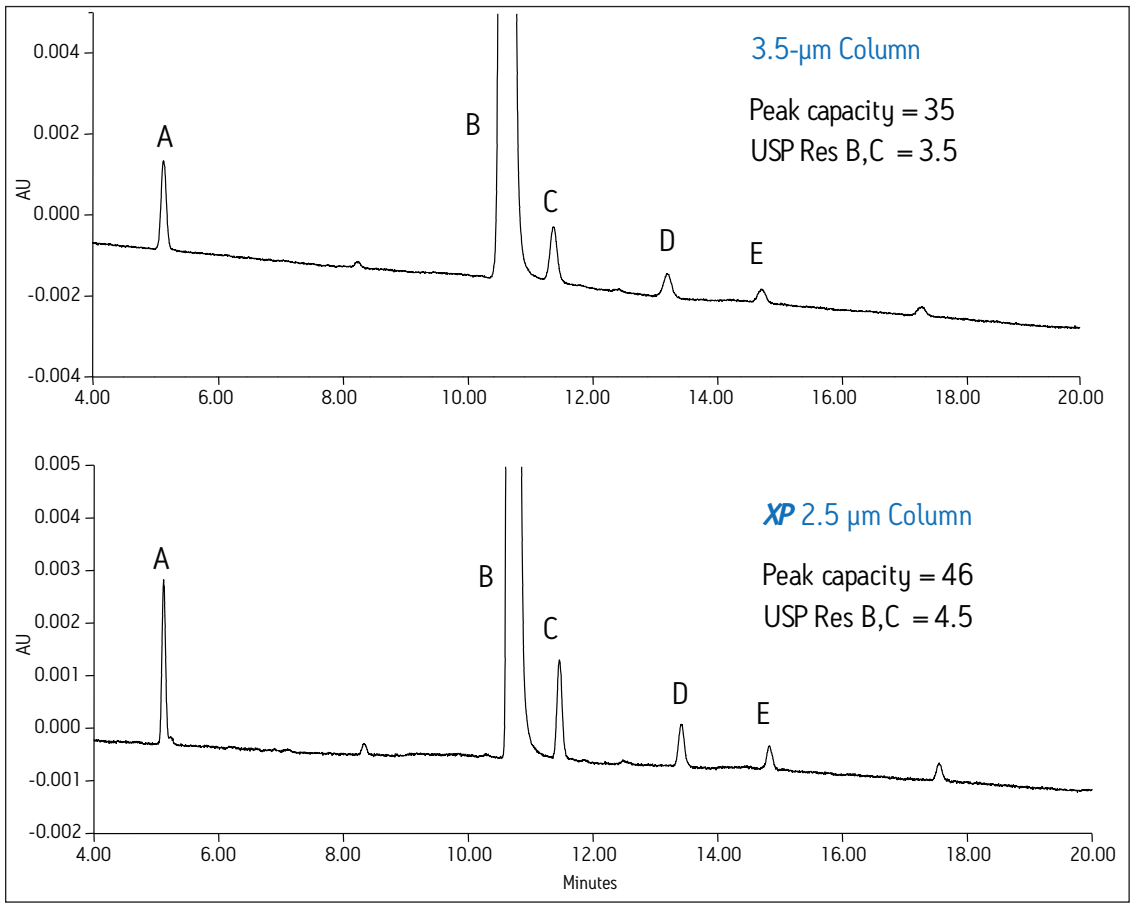

*Figure 2. Separation of abacavir related compounds, demonstrating improved assay performance using an e**X**tended **P**erformance (**XP**) Column. A) Descyclopropyl abacavir, B) Abacavir glutarate, C) 1R,4R Trans abacavir, D) o-Pyrimidine abacavir, E) t-Butyl abacavir.*

In this case, by simply changing the 3.5-µm column to an ***XP*** 2.5 µm Column, significant performance improvements are seen as the overall peak capacity for the separation increases 31%, and the peak heights increase up to 42%. A 28% increase in resolution demonstrates the improved separation between closely eluting compounds abacavir and trans-abacavir. This example illustrates the capability to increase sensitivity and resolution by using an ***XP*** Column, which can result in more accurate identification and quantification of target compounds such as impurities.

SUMMARY

By transferring HPLC methods to an ***XP*** 2.5 µm Column, improvements in resolution and sensitivity can be achieved. This was demonstrated with a related compounds method for abacavir, in which an overall improvement of 31% in peak capacity, up to 42% in peak height, and 29% in resolution were observed by changing the 3.5-µm column to an ***XP*** 2.5 µm Column. The use of ***XP*** Columns allows HPLC users to maximize the separation performance on their HPLC systems, lessening the need for further method development, and promoting cost-effective asset utilization.

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Transfer of USP Methods for Impurities Analysis of Ziprasidone HCl between HPLC Systems and to UPLC

Margaret Maziarz, Michael D. Jones, Sean M. McCarthy, Warren B. Potts, and Frederic Forini
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Method transferability between different systems and different sites
- 63% reduction in run time when using UPLC®
- 93% savings in mobile phase consumption when using UPLC
- Reduced cost for solvent and waste disposal

WATERS SOLUTIONS

Alliance® HPLC System

ACQUITY UPLC® H-Class System

Method Transfer Kits

ACQUITY UPLC Columns

XBridge™ HPLC Columns

Empower® 3 Software

KEY WORDS

ACQUITY UPLC Columns Calculator, Waters® Column Selectivity Chart, method transfer, sub-2-µm particle technology, ziprasidone HCl, USP methods

INTRODUCTION

U.S. Pharmacopeia (USP) compendial methods are routinely adopted by pharmaceutical companies for testing raw materials and finished products. Successful implementation of the USP methods and transferability between instruments are key steps to enhance throughput for routine analysis. Effective method transfer generates identical results for the same analysis independent of the laboratory, instrument, and the resources for a specific method.

By ensuring successful lab-to-lab method transferability, companies can replicate methods at additional sites or with partners such as contract research or manufacturing organizations (CROs and CMOs). Transferring an HPLC-based USP method to UPLC Technology offers such organizations the additional opportunity to achieve productivity goals by reducing analysis time while ensuring reliable, high-quality chromatographic separations that are the basis for decisions about product quality. UPLC Technology offers QC and manufacturing facilities significant advantages in terms of increased throughput, improved quality, and reduced costs.

Transferring compendial HPLC methods between systems can be challenging. Many of the USP monographs utilize diluents with a high concentration of organic solvents for the preparation of standard and sample solutions. While strong solvent diluents are common for HPLC applications, they often prove unsatisfactory for analyses performed on modern systems that feature minimized dispersion.

In this study, the transfer of two USP compendial methods for impurities analysis of ziprasidone HCl[1] from HPLC to UPLC is demonstrated. The use of strong solvent as a sample diluent can be a barrier when transitioning a method to a low-volume system, depending on the injection volume. Presented here is a straightforward way to address this potential issue.

The success of the method transfer between different HPLC systems and conversion to UPLC was measured by evaluating the system suitability requirements listed in the USP monograph for ziprasidone HCl. Success of the transfer was further demonstrated by running the methods on the same instrument configurations in a laboratory located in a different country, where identical results were achieved.

EXPERIMENTAL

Sample description

All solutions (Table 1) were prepared in methanol/water/concentrated HCl at a composition of 20:5:0.01 to comply with the impurities methods defined in the USP monograph for ziprasidone HCl. Since the USP does not list a sample preparation protocol for the ziprasidone HCl capsules, the drug substance sample preparation was used with one modification. Sample solutions were filtered through 0.2-µm PTFE syringe filters to remove any particulates.

Solutions	Early-eluting peaks	Late-eluting peaks
Standard solutions	**System suitability solution:** 0.24 mg/mL of ziprasidone HCl 0.5 µg/mL of related compound A 0.8 µg/mL of related compound B	**System suitability solution:** 0.24 mg/mL of ziprasidone HCl 0.8 µg/mL of related compound C 0.8 µg/mL of related compound D
	Standard solution: 0.5 µg/mL of related compound A 0.8 µg/mL of related compound B	**Standard solution:** 0.8 µg/mL of related compound C 0.8 µg/mL of related compound D
Sample solutions	**Sample solution:** 0.4 mg/mL of capsule content	**Sample solution:** 0.45 mg/mL of capsule content

Table 1. Standard and sample solutions composition for impurities analysis of ziprasidone HCl.

System control, data acquisition, and analysis

Empower 3 Software

Method conditions

HPLC conditions for early-eluting impurities method

LC systems:	Alliance 2695 HPLC with 2489 UV/Visible Detector ACQUITY UPLC H-Class with TUV Detector
Column:	XBridge C_8 4.6 x 150 mm, 5 µm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	20 µL
Flow rate:	1.5 mL/min
Mobile phase:	2:3 methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 3.0 adjusted with phosphoric acid
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

HPLC conditions for late-eluting impurities method

LC systems:	Alliance 2695 HPLC with 2489 UV/Visible Detector ACQUITY UPLC H-Class with TUV Detector
Column:	XBridge C_8 4.6 x 150 mm, 5 µm
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	20 µL
Flow rate:	1.0 mL/min
Mobile phase:	11:1:8 acetonitrile/methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 6.0 adjusted with 5N potassium hydroxide
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

UPLC conditions for early-eluting impurities method

LC system:	ACQUITY UPLC H-Class with TUV Detector
Column:	ACQUITY UPLC BEH C_8, 2.1 x 50 mm, 1.7 µm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	1.4 µL
Flow rate:	0.313 mL/min
Mobile phase:	2:3 methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 3.0 adjusted with phosphoric acid
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

UPLC conditions for late-eluting impurities method

LC system:	ACQUITY UPLC H-Class with TUV Detector
Column:	ACQUITY UPLC BEH C_8, 2.1 x 50 mm, 1.7 µm
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	1.4 µL
Flow rate:	0.208 mL/min
Mobile phase:	11:1:8 acetonitrile/methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 6.0 adjusted with 5N potassium hydroxide
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

USP system suitability criteria for early-eluting method:

For five replicate injections of system suitability solution

- Resolution between ziprasidone and ziprasidone related compound B: Not less than (NLT) 1.5
- Relative standard deviation (RSD) for ziprasidone related compound B: Not more than (NMT) 10%

USP system suitability criteria for late-eluting method:

For five replicate injections of system suitability solution

- Resolution between ziprasidone and ziprasidone related compound C: NLT 6.0
- RSD for ziprasidone related compound C: NMT 10%

RESULTS AND DISCUSSION

Managing system volume differences

One of the challenges of migrating a USP monograph from legacy to modern LC technologies is the impact of technology improvements in managing system volume and dispersion that may cause distorted peaks. The volume of an LC system in front of the injector will impact the time required to equilibrate the method to the initial conditions. The system volume after the injector is related to the system dispersion, which mixes sample with the mobile phase. In this work, we investigate these relationships and determine an effective way to adjust for different volumes.

As an example, injecting sample dissolved in a strong organic diluent onto systems with a small post-injector volume can yield a less focused injecton, often evidenced by fronting. Peaks with lower retention factor (k') eluting isocratically are typically observed to be more distorted compared to the later eluting peaks with larger k'. Methods with a gradient elution are also susceptible but less affected by the strong organic diluents, due to peak focusing at the head of the column.

Method transfer of USP HPLC method

The USP monograph designates using an L7 column for the ziprasidone HCl early and late impurities methods and suggests using a Zorbax RX-C_8 Column. Using the Waters Reversed-Phase Column Selectivity Chart (www.waters.com/selectivitychart), a Waters XBridge C_8 Column was chosen. The standard and sample solutions were prepared in diluent containing 80% methanol. The compendial method for the early-eluting impurities was run as described on the Alliance HPLC System (Figure 1A), and on the ACQUITY UPLC H-Class System (Figure 1B) using the same XBridge C_8 Column and the same mobile phase. Distortion of the peaks was observed when the system suitability solution was injected on the ACQUITY UPLC H-Class System.

Several experiments were designed to investigate and solve the issue of observed peak distortion. It was hypothesized that the peak distortion was due to a high concentration of organic solvent. These experiments included the following:

1. Reducing the injection volume.
2. Reducing the concentration of organic solvent in sample diluent.
3. Increasing the post-injector volume of the UPLC system.

The USP allows reduction of the injection volume as long as the precision and the detection limits are acceptable.[2] While reducing the injection volume improved peak symmetry and resolution, it decreased sensitivity (Figure 2). For this reason, injection volume could not be decreased to mitigate the distorted peaks.

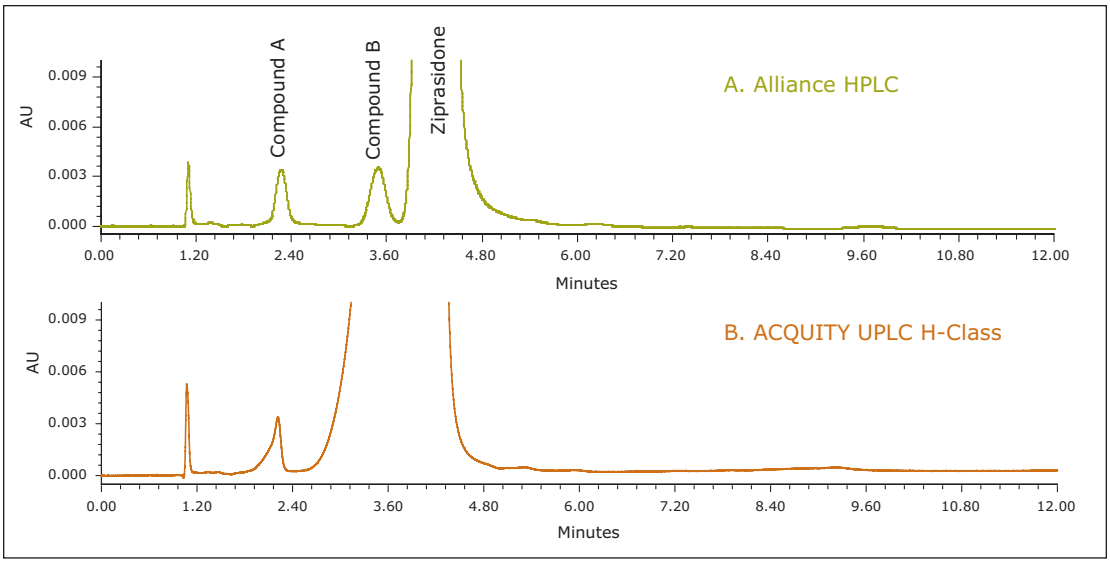

Figure 1. HPLC data of the system suitability solution for ziprasidone HCl early-eluting impurities analysis.

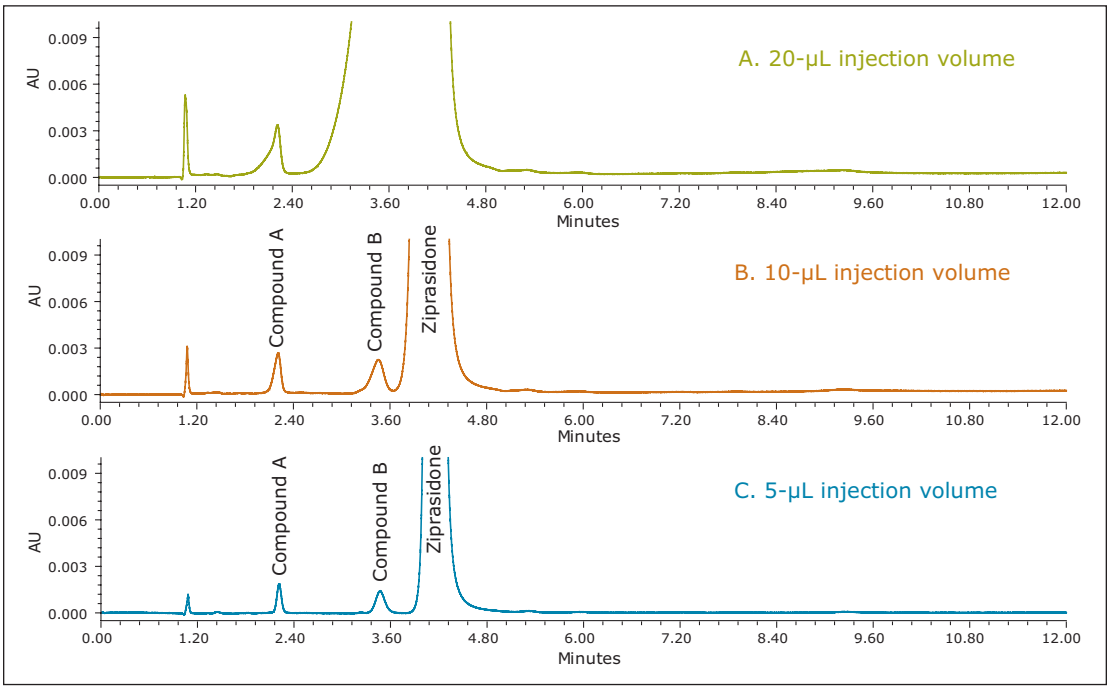

Figure 2. Injection volume study to investigate HPLC analysis performed on an ACQUITY UPLC H-Class System. System suitability solution was prepared in diluent containing 80% methanol.

Reducing the composition of methanol in the diluent to 70% enhanced the chromatographic separation (Figure 3). However, modification of the sample diluent is not recommended by the USP.

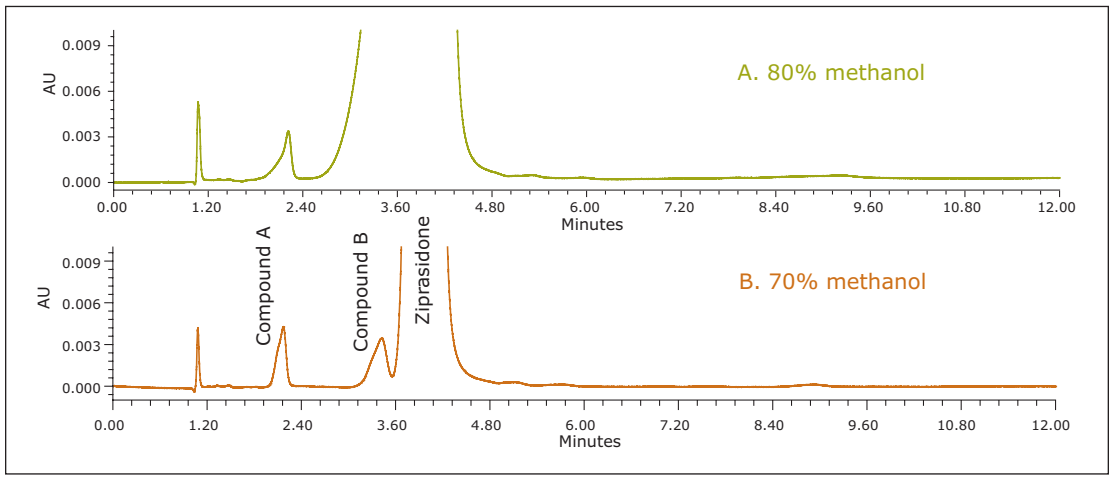

Figure 3. Reducing solvent concentration in sample diluent to investigate HPLC analysis performed on an ACQUITY UPLC H-Class System. The injection volume was 20 µL.

Increasing system volume between the injector and the column inlet with 50-µL tubing significantly improved peak shape and resolution between all the peaks, conforming to allowable modifications documented by the USP (Figure 4).

Finally, increasing the volume of the UPLC system after the injector allows the diluent to mix with the mobile phase before it enters the column, improving peak symmetry and resolution.

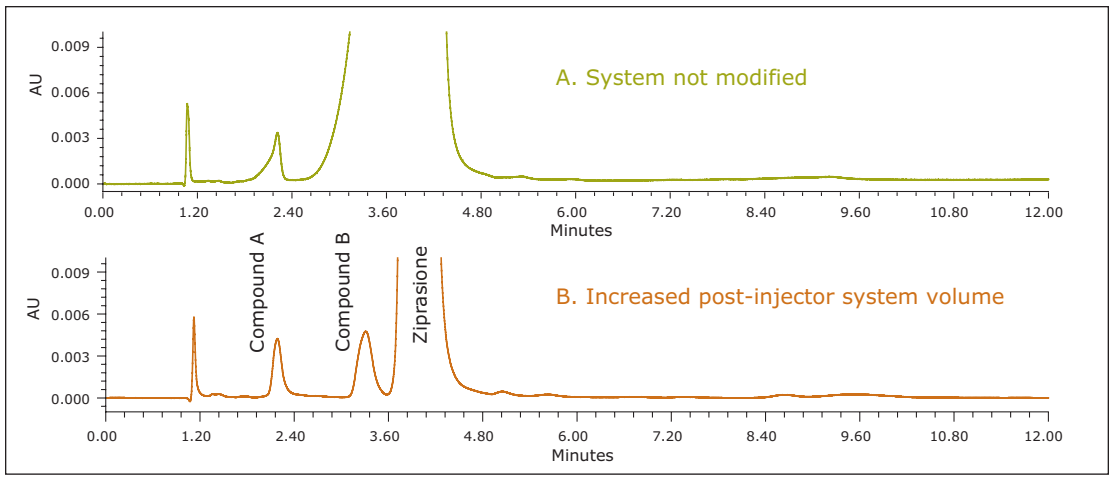

Figure 4. Increasing post-injector volume of an ACQUITY UPLC H-Class System to investigate mitigation of the strong solvent effects. System suitability solution was prepared in diluent containing 80% methanol and injected at 20 µL.

By increasing the post-injector volume, the compendial HPLC methods for the early- and late-eluting impurities could be successfully run on the ACQUITY UPLC H-Class System. Overall, transferability of the compendial methods between LC systems with different extra-column volume, specifically the Alliance HPLC and ACQUITY UPLC H-Class systems, was successful (Figures 5 and 6).

Finally, the system suitability results for the five replicate injections of the system suitability solution met the requirements listed in the USP monograph for ziprasidone HCl (Tables 2 and 3).

Method transfer from HPLC to UPLC

Next, the HPLC methods were converted to UPLC on the ACQUITY UPLC H-Class System. Three steps involved in this transfer process include:

1. Select an equivalent Waters HPLC column with the appropriate "L" designation, and run the USP monograph.
2. Using the ACQUITY UPLC Columns Calculator, select an equivalent ACQUITY UPLC Column and transfer the HPLC method to UPLC.
3. Verify performance of the UPLC method by measuring method's suitability.

As stated, an XBridge C_8 Column was chosen using the Waters Reversed-Phase Column Selectivity Chart. We then used the Waters ACQUITY UPLC Columns Calculator (www.waters.com/myuplc) to scale the method to UPLC, as described in a Waters application note.[3]

The run times of the early- and late-eluting impurities methods for ziprasidone HCl were reduced to four and five minutes, respectively (Figures 5 and 6).

The sample set sequence included five replicate injections of the system suitability solutions, samples, and blanks. The system suitability results for the early- and late-eluting impurities methods are summarized in the Tables 2 and 3, respectively. The UPLC results were well within the USP requirements listed in the USP monograph for ziprasidone HCl, and were comparable to the HPLC results.

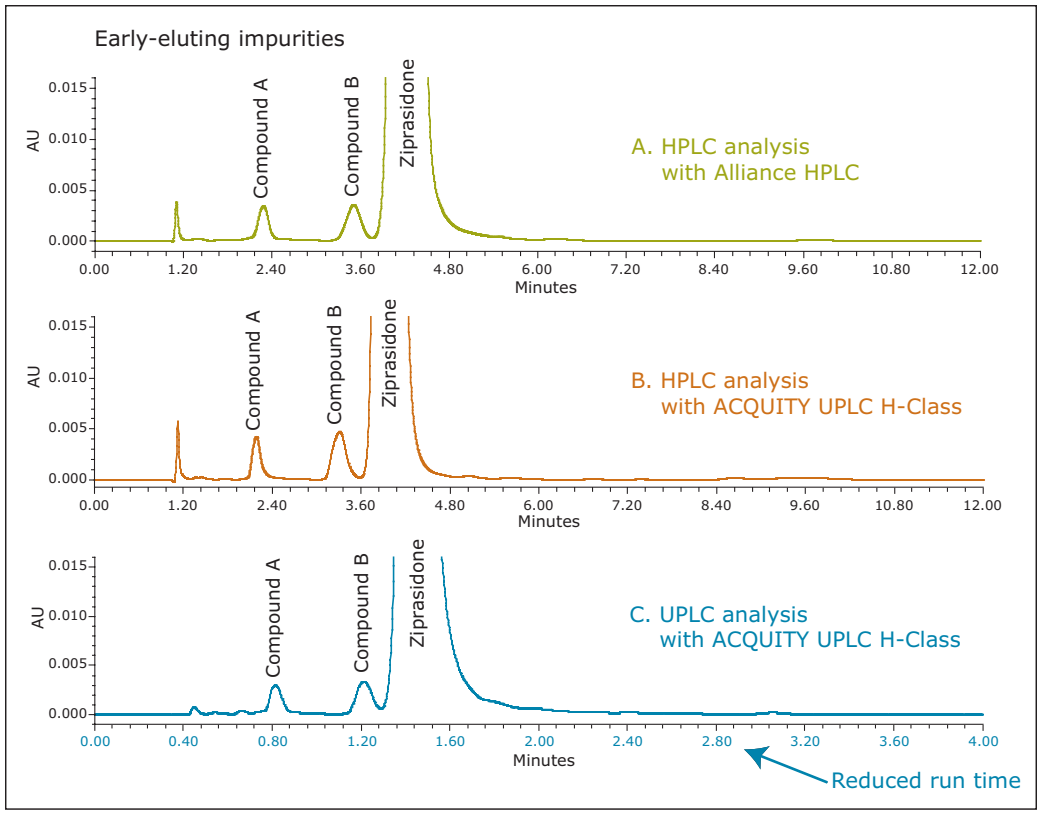

Figure 5. Chromatographic data of ziprasidone HCl early-eluting impurities method.

A. HPLC analysis acquired using Alliance HPLC System.

B. HPLC analysis acquired using ACQUITY UPLC H-Class System with an increased post-injector volume.

C. UPLC analysis acquired using ACQUITY UPLC H-Class System.

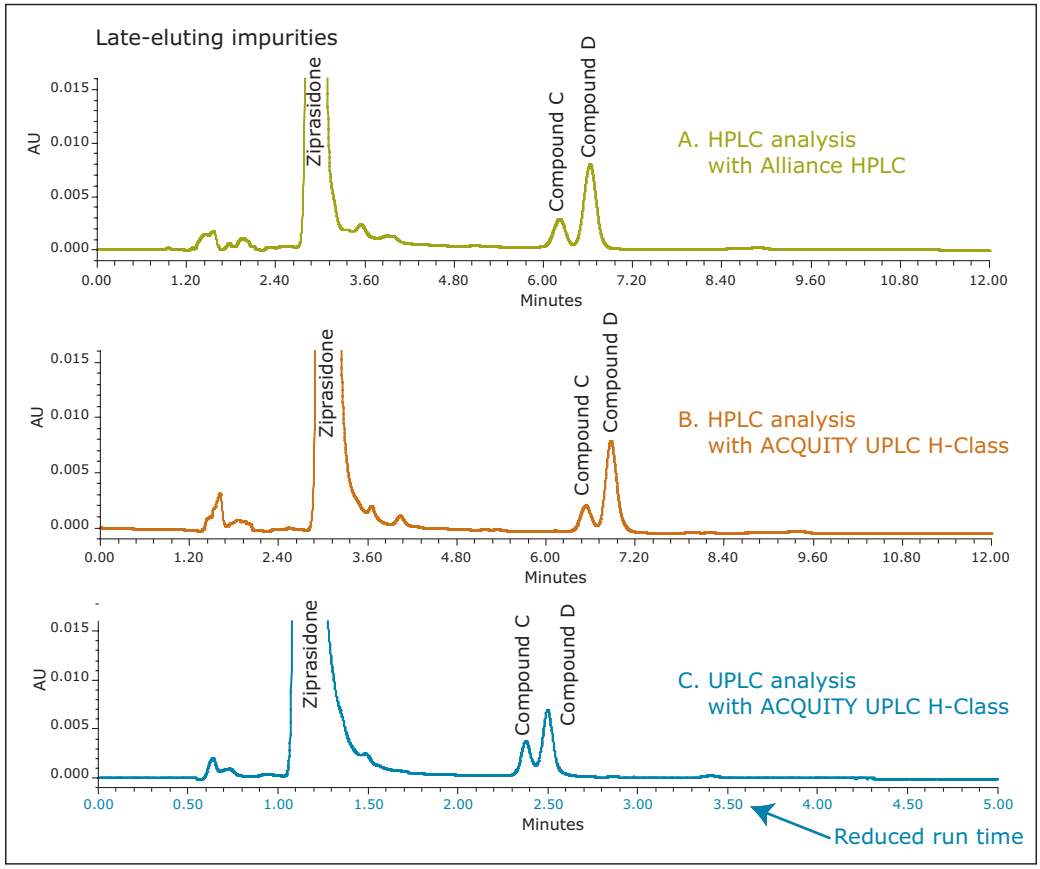

Figure 6. Chromatographic data of ziprasidone HCl late-eluting impurities method.

A. HPLC analysis acquired using Alliance HPLC System.

B. HPLC analysis acquired using ACQUITY UPLC H-Class System with an increased post-injector volume.

C. UPLC analysis acquired using ACQUITY UPLC H-Class System.

Parameter	USP criteria	HPLC analysis		UPLC analysis
		Alliance HPLC	ACQUITY UPLC H-Class	
Resolution between ziprasidone and related compound B	NLT 1.5	1.8	1.9	1.7
%RSD for related compound B	NMT 10%			
■ Retention times		0.2%	0.0%	0.1%
■ Peak areas		0.4%	0.1%	0.3%

Table 2. System suitability results for USP method transfer of the ziprasidone HCl early-eluting impurities method from Alliance HPLC to the ACQUITY UPLC H-Class System.

Parameter	USP criteria	HPLC analysis		UPLC analysis
		Alliance HPLC	ACQUITY UPLC H-Class	
Resolution between ziprasidone and related compound C	NLT 6.0	14.1	18.4	16.3
%RSD for related compound C	NMT 10%			
■ Retention times		0.0%	0.1%	0.0%
■ Peak areas		0.1%	0.7%	0.2%

Table 3. System suitability results for the USP method transfer of the ziprasidone HCl late-eluting impurities method from Alliance HPLC to the ACQUITY UPLC H-Class System.

Lab-to-lab method transfer

Finally, a lab-to-lab method transfer study was conducted using the compendial HPLC method for the early-eluting impurities on the ACQUITY UPLC H-Class System. Two different laboratories performed the test, including Waters laboratories in Milford, MA, USA and Guyancourt, Yvelines, France.

Reproducibility of the HPLC method acquired by both laboratories was demonstrated by comparing the system suitability results (Table 4). System suitability results acquired by both laboratories were comparable and met the requirements defined in the USP monograph for ziprasidone HCl.

Parameter	USP criteria	HPLC analysis on ACQUITY UPLC H-Class	
		Milford, MA (USA)	Guyancourt, Yvelines (France)
Resolution between ziprasidone and related compound B	NLT 1.5	1.9	1.6
%RSD for related compound B	NMT 10%		
■ Retention times		0.0%	0.1%
■ Peak areas		0.1%	0.9%

Table 4. System suitability results for lab-to-lab transfer of the HPLC method for ziprasidone HCl early-eluting impurities on the ACQUITY UPLC H-Class System.

CONCLUSIONS

Two compendial HPLC methods for impurities of ziprasidone HCl were tested on two different HPLC systems and successfully transferred to UPLC. Increasing post-injector volume of the ACQUITY UPLC H-Class System was required to duplicate the chromatographic separation and meet the USP requirements for system suitability.

The ACQUITY UPLC H-Class System successfully replicated the analytical quality of the HPLC compendial methods. Excellent performance of both the HPLC and the UPLC methods demonstrate that the ACQUITY UPLC H-Class System is suitable for HPLC and UPLC applications.

- Implementing the ACQUITY UPLC H-Class System within quality control laboratories can reduce the costs of operation and maintenance by maximizing asset utilization and eliminating the need for multiple systems to perform HPLC and UPLC analyses.
- Conversion to UPLC technology can decrease overall costs and improve laboratory throughput and productivity for release testing of finished products.
- The UPLC methods for the required early- and late-eluting impurities tests provided a 67% and 58% reduction in run times over the corresponding HPLC methodologies.
- In addition to reduced analysis time, UPLC technology provides cost savings related to solvent and waste disposal. Consumption of mobile phase per UPLC injection, for the early and late impurities methods, is reduced by 93% compared to the HPLC injection.

The reproducibility of the compendial HPLC method on the ACQUITY UPLC H-Class System during the lab-to-lab study was excellent, which is the final key to successful method transferability between sites.

References

1. USP Monograph, Ziprasidone HCl, USP35-NF29, The United States Pharmacopeia Convention, official May 1, 2012.
2. USP General Chapter, <621> Chromatography, USP35-NF30, The United States Pharmacopeia Convention, official December 1, 2012.
3. Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721en. 2010 Sept.

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Transfer of Two USP Compendial Methods for Impurities of Ziprasidone HCL to a Single UPLC Method

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APPLICATION BENEFITS

- Method transferability between different systems and different sites
- Faster assay run times
- Savings in mobile phase consumption
- Reduced cost for solvent and waste disposal

WATERS SOLUTIONS

Alliance® HPLC System

ACQUITY UPLC® H-Class System

Method Transfer Kits

ACQUITY UPLC Columns

XBridge™ Columns

Empower® 3 Software

KEY WORDS

ACQUITY UPLC Columns Calculator, Waters Column Selectivity Chart, method transfer, sub-2-µm particle technology, ziprasidone HCl, USP methods

INTRODUCTION

The majority of today's compendial methods are considered outdated by both industry and regulators, and do not take advantage of the recent advances in recent chromatographic instrumentation and stationary phase solutions. The U.S. Pharmacopeia (USP) launched an initiative in May 2010 to modernize monographs for small molecule drug products and excipients, identified as a priority by the U.S. Food and Drug Administration, that use outdated technology, have safety or environmental concerns, or are missing key aspects.

HPLC methods listed in the United States Pharmacopeia (USP) National Formulary (NF) monographs are typically long and consume a high volume of solvents. With the evolution of new technology, many companies want to migrate to UPLC® technology with sub-2-µm particle columns to reduce analysis time and solvent usage, improve chromatographic performance, and maintain quality. Adopting UPLC technology streamlines laboratory processes by improving efficiency, productivity, and profitability of pharmaceutical manufacturing facilities.

Ziprasidone HCl is an anti-psychotic drug administered orally to treat acute manic or mixed episodes associated with bipolar disorder. The USP specifies two HPLC methods[1] to analyze impurities of ziprasidone HCl. In this study, we combine two compendial methods into one UPLC method for the separation of ziprasidone HCl impurities to reduce analysis time and improve chromatographic separation. Performance of the UPLC method is measured by evaluating five replicate injections of the system suitability solution against the requirements listed in the USP monograph for ziprasidone HCl.

EXPERIMENTAL

Sample description

All solutions, shown in Table 1, were prepared in diluent (methanol/water/HCl at 20:5:0.01) as per the impurities methods defined in the USP monograph for ziprasidone HCl.[1] Since the USP does not list a sample preparation protocol for the Ziprasidone HCl capsules, the drug product sample preparation was used with one modification. Sample solutions were filtered through 0.2-µm PTFE syringe filters to remove any particulates.

Solutions	Early-eluting peaks	Late-eluting peaks
Standard solutions	**System suitability solution:** 0.24 mg/mL of ziprasidone HCl	**System suitability solution:** 0.24 mg/mL of ziprasidone HCl
	0.5 µg/mL of related compound A	0.8 µg/mL of related compound C
	0.8 µg/mL of related compound B	0.8 µg/mL of related compound D
	Standard solution: 0.5 µg/mL of related compound A	**Standard solution:** 0.8 µg/mL of related compound C
	0.8 µg/mL of related compound B	0.8 µg/mL of related compound D
Sample solutions	**Sample solution:** 0.4 mg/mL of capsule content	**Sample solution:** 0.45 mg/mL of capsule content

Table 1. Standard and sample solutions composition for impurities analysis of ziprasidone HCl.

System control, data acquisition, and analysis

Empower 3 Software

Method conditions

HPLC conditions for early-eluting impurities method

LC system:	Alliance 2695 HPLC with 2489 UV/Visible Detector
Column:	XBridge C_8 4.6 x 150 mm, 5 µm
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	20 µL
Flow rate:	1.5 mL/min
Mobile phase:	2:3 methanol/buffer
	Buffer: 50 mM potassium phosphate monobasic, pH 3.0 adjusted with phosphoric acid
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

USP system suitability criteria for early-eluting impurities method

For five replicate injections of the system suitability solution:

- Resolution between ziprasidone and ziprasidone related compound B: Not Less Than (NLT) 1.5
- Relative standard deviation (RSD) for ziprasidone related compound B: Not More Than (NMT) 10%

HPLC conditions for late-eluting impurities method

LC system:	Alliance 2695 HPLC with

	2489 UV/Visible Detector
Column:	XBridge C_8 4.6 x 150 mm, 5 µm
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	20 µL
Flow rate:	1.0 mL/min
Mobile phase:	11:1:8 acetonitrile/methanol/buffer Buffer: 50 mM potassium phosphate monobasic, pH 6.0 adjusted with 5N potassium hydroxide
Separation mode:	Isocratic
Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

USP system suitability criteria for late-eluting impurities method

For five replicate injections of the system suitability solution:

- Resolution between ziprasidone and ziprasidone related compound C: NLT 6.0
- Relative standard deviation (RSD) for ziprasidone related compound C: NMT 10%

UPLC conditions

LC system:	ACQUITY UPLC H-Class with ACQUITY UPLC TUV Detector
Column:	ACQUITY UPLC BEH C_8 2.1 x 50 mm, 1.7 µm
Column temp.:	35 °C
Sample temp.:	10 °C
Injection volume:	1.4 µL
Flow rate:	0.5 mL/min
Mobile phase:	Solvent A: 50mM Potassium phosphate monobasic, pH 6.0
Solvent B:	Methanol
Separation mode:	Gradient

Step	Time (minutes)	%A	%B
1	Initial	65.0	35.0
2	0.60	35.0	65.0
3	2.70	25.0	75.0
4	3.90	25.0	75.0
5	3.96	65.0	35.0
6	6.50	65.0	35.0

Wash solvents:	50:50 water/methanol
Detection:	UV, 229 nm

RESULTS AND DISCUSSION

Two compendial methods for the early and late eluting impurities of ziprasidone HCl were analyzed as described using the Alliance 2695 HPLC System equipped with a 2489 UV/Visible Detector. The USP designates an L7 column, specifically a Zorbax RX-C_8 column, for the impurities testing. Using the Waters Reversed-Phase Column Selectivity Chart (www.waters.com/selectivitychart), an equivalent Waters XBridge C_8 Column was chosen. Chromatographic data for both HPLC methods are displayed in Figure 1.

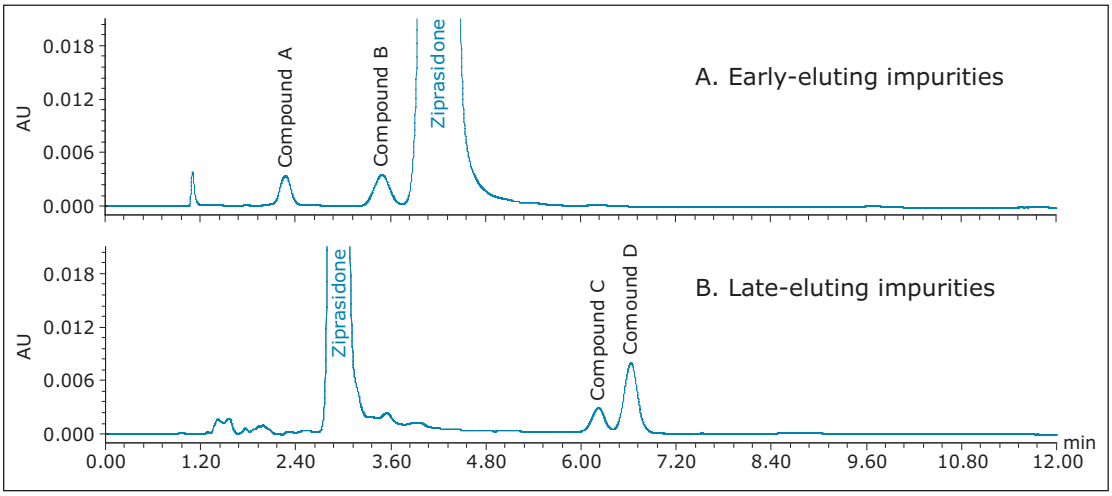

Figure 1. HPLC data of the system suitability solutions acquired on the Alliance HPLC System
A. Early-eluting impurities method
B. Late-eluting impurities method

UPLC method development

Method development of a UPLC-based separation of ziprasidone HCl and all the specified related compounds was conducted by a gradient elution using a potassium phosphate monobasic buffer with pH of 6.0 and methanol. The mobile phase with a neutral pH 6.0, specified in the late-eluting impurities method, was investigated due to the neutral characteristics of the ziprasidone HCl related substances.

A Waters ACQUITY UPLC BEH C_8 Column has the same stationary phase as the Waters HPLC XBridge C_8 Column, hence it was selected for method development. The dimensions of the UPLC Column were decided by the column length (L) to the particle size (dp) ratio (L/dp). The L/dp value for the HPLC column is 30,000. The L/dp value for the UPLC Column with a dimension of 50 mm in length and 1.7-µm particle size is 29,412. Injection volume was scaled down to UPLC using the Waters ACQUITY UPLC Columns Calculator, as previously described.[2]

A generic scouting gradient from 5% to 95% methanol over 15 minutes was performed to investigate the elution and separation between the peaks. Increasing the starting percent of the organic solvent to 35% lowered the run time, providing an adequate separation between all the peaks. Examples of the chromatographic data for the gradient elution study are displayed in Figure 2.

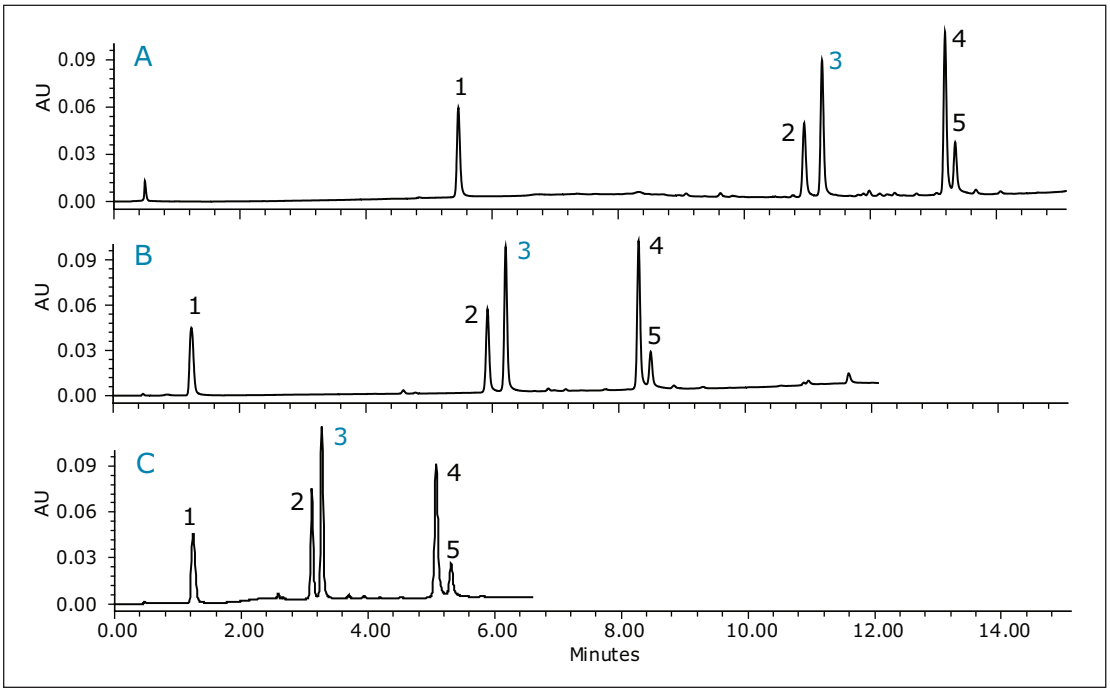

Figure 2. Gradient elution study for UPLC method development.
Peak 1: Related compound A
Peak 2: Related compound B
Peak 3: Ziprasidone
Peak 4: Related compound D
Peak 5: Related compound C

A. 5% to 95% methanol (Solvent B) over 15 minutes
B. 35% to 65%B over 5.0 minutes, 65% to 90%B over 5.0 minutes, hold at 90% for 2.0 minutes
C. 35% to 65%B over 1.0 minute, 65% to 75%B over 3.5 minutes, hold at 75% for 2.0 minutes

Different flow rates were also explored to further reduce run time and determine the effect on resolution. The flow rate was increased from 0.3 to 0.5 mL/min, as shown in Figure 3. It was observed that an increase in flow rate lowered the resolution between peaks 2 (related compound B) and 3 (ziprasidone), but still passing the USP criteria for the system suitability, shown in Table 2. In addition, a flow rate of 0.5 mL/min reduced run time from 6.6 to 4.0 minutes and decreased peak width by 32%.

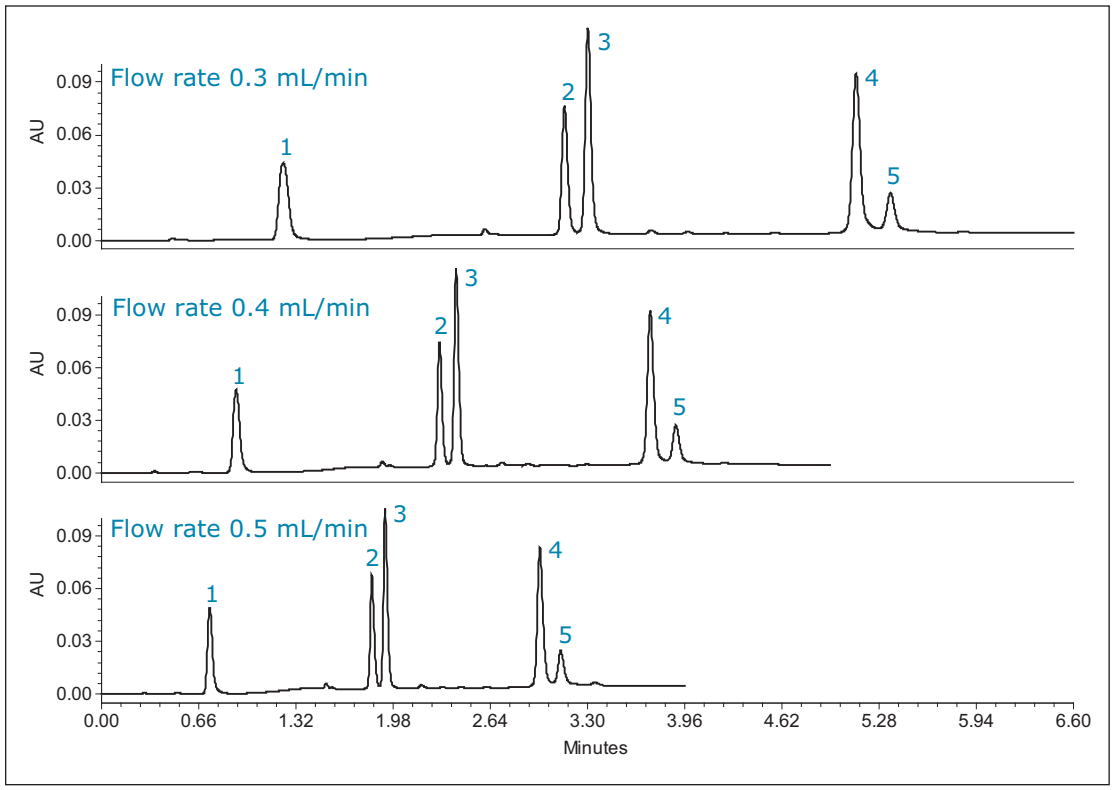

Figure 3. Flow rate study for UPLC method development.
Peak 1: Related compound A
Peak 2: Related compound B
Peak 3: Ziprasidone HCl
Peak 4: Related compound D
Peak 5: Related compound C

The resulting UPLC method successfully resolved ziprasidone and all the specified related compounds, shown in Figure 4.

Performance of the UPLC method was determined by comparing the system suitability results of the five replicate injections of the system suitability solutions against the acceptance criteria defined in the USP monograph for ziprasidone HCl. System suitability results met the USP requirements for the early and late impurities methods listed in the USP monograph for ziprasidone HCl, shown in Table 2.

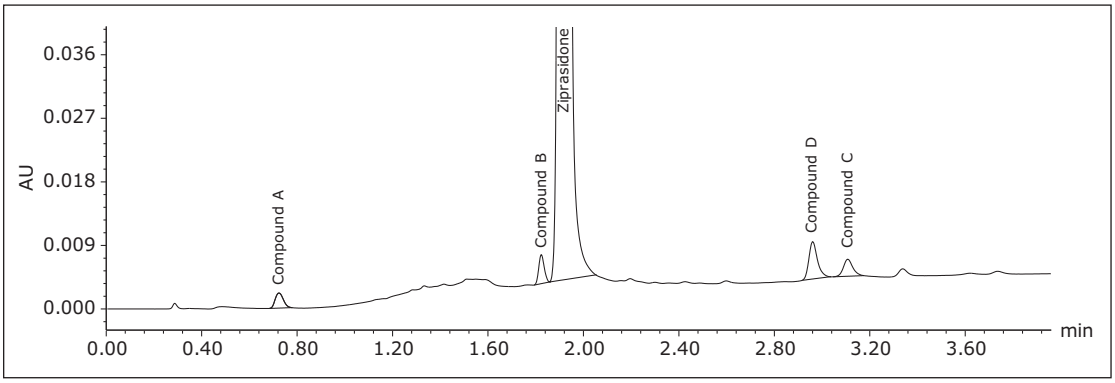

Figure 4. Final UPLC method. System suitability solution was injected onto ACQUITY UPLC BEH C_8, 2.1 x 5 0 mm, 1.7 µm Column with an amount of 1.4 µL. Column temperature was maintained at 35 °C. Mobile phase consisting solvent A: 50 mM potassium phosphate monobasic with pH of 6.0 and solvent B: methanol was delivered via a gradient elution with a flow rate of 0.5 mL/min.

System suitability parameters	USP criteria	UPLC results
Early-eluting impurities method		
• Resolution between ziprasidone and related compound B	• NLT 1.5	• 2.02
• %RSD for related compound B	• NMT 10%	• RT: 0.1% • Area: 0.2%
Late-eluting impurities method		
• Resolution between ziprasidone and related compound C	• NLT 6.0	• 19.1*
• %RSD for related compound C	• NMT 10%	• RT: 0.1% • Area: 0.2%

*Note: Elution order for related compounds C and D has changed with analysis on UPLC.
Value reported in the table reflects resolution between ziprasidone and related compound D

Table 2. System suitability results for five replicate injections of the system suitability solution on an ACQUITY UPLC H-Class System.

CONCLUSIONS

- UPLC methodology successfully separated ziprasidone HCl and all related compounds indicated by the USP monograph for ziprasidone HCl.
- The UPLC method provides a 75% reduction in run time compared to the two compendial procedures, while meeting the USP criteria for system suitability.
- The amount of mobile phase per UPLC injection is 3.25 mL compared to 30.00 mL for the two compendial HPLC methodologies, which represents an 89% savings in mobile phase consumption.
- Implementing UPLC technology provides improvements in laboratory throughput and productivity by reducing analysis time for release testing of manufacturing batches.

References

1. USP Monograph, Ziprasidone HCl, USP35-NF29, The United States Pharmacopeia Convention, official May 1, 2012.
2. Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721en. 2010 Sept.

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USP Method Transfer of Donepezil Tablets from HPLC to UPLC

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Waters Corporation, 34 Maple St., Milford, MA, USA

APPLICATION BENEFITS

- Updating USP Methods from HPLC to UPLC® to increase analysis throughput and reduce solvent cost
- Increasing method robustness for complex formulations
- 80% decrease in analysis time, faster throughput for routine sample analysis
- 92% reduction in solvent usage and sample injected

WATERS SOLUTIONS

ACQUITY UPLC® H-Class system

XBridge™ C_{18} e***X***tended ***P***erformance [***XP***] 2.5 µm columns

Empower™ 3 CDS

Waters Donepezil Analytical Standard

KEY WORDS

Method transfer, USP methods, HPLC, UPLC, donepezil, tablet, XP, ACQUITY UPLC Columns Calculator

INTRODUCTION

Compendial methods are often used in the analysis of generic drugs. Typically, methods are developed and submitted based on a specific formulation. However when the excipients are changed, the method specified in a USP monograph may be inadequate in eluting all components off of the column. The result can be deteriorating peak shape and premature method failure due to a build-up of poorly soluble excipients on the column.

Donepezil is a drug used to treat symptoms of dementia in Alzheimer's patients and is formulated in a tablet preparation. Here, we demonstrate the transfer of the USP compendial method for donepezil from HPLC to UPLC. Methods for assay and impurities analyses are sometimes combined to minimize the number of methods developed and submitted for regulatory approval. In this application, the transfer of the organic impurities method, which is a more complicated gradient separation, is demonstrated. Since impurity standards were not readily available, the transfer and routine use study is demonstrated using donepezil standard and tablets. A routine use study using conditions typical of a QC laboratory protocol is performed with the UPLC method and assay suitability criteria are evaluated to assess the long-term robustness of the method.

EXPERIMENTAL

Alliance 2695 HPLC Conditions

Column:	XBridge C_{18}, 4.6 x 250 mm, 5 µm (L1), part number 186003117
Gradient:	25% B to 60% B over 10 min, hold for 30 min, return to 25% B in one min and re-equilibrate for 9 min
Flow Rate:	1.5 mL/min
Run Time:	50 min
Injection Volume:	20 µL

ACQUITY UPLC H-Class Conditions

Column:	ACQUITY UPLC BEH C_{18} 2.1 x 100 mm, 1.7 µm (L1), part number 186002352
Gradient:	25% B to 60% B over 2.5 min, hold for 7.5 min, return to 25% B in 0.25 min and re-equilibrate for 2.25 min
Flow Rate:	0.5 mL/min
Run Time:	12.5 min
Injection Volume:	1.7 µL
Column:	XBridge C_{18} ***XP***, 2.1 x 100 mm, 2.5 µm (L1), part number 186006031
Gradient:	25% B to 60% B over 2 min, hold for 6 min, return to 25% B in 0.2 min and re-equilibrate for 1.8 min
Flow Rate:	0.63 mL/min
Run Time:	10 min
Injection Volume:	1.7 µL

RESULTS AND DISCUSSION

The USP method for donepezil describes sample preparation as 'dissolve in diluent and sonicate if necessary'. When preparing the tablet sample according to the USP method, the final product is a solution with visible fine particulates. In order to render the sample clear and suitable for injection, the sample was further filtered through a 0.2 µm PTFE membrane and then centrifuged at 12,000 rpm for 5 minutes. Upon centrifugation, a small pellet was visible and the supernatant was carefully pipetted into a sample vial for injection.

The USP organic impurities method for donepezil describes the use of an L1 column and the suggested column is Kromasil® C18. Using the Waters Column Selectivity Chart, a more modern L1 column, XBridge C_{18}, was selected since this column chemistry is also available in smaller particle sizes. The USP compendial method was first run as described on an Alliance® HPLC system using five replicate injections of donepezil standard and donepezil prepared tablet. System suitability criteria described in the monograph were monitored and found to be within specifications (Table 1).

The USP method was then transferred from HPLC to UPLC using the ACQUITY UPLC Columns Calculator. Scaling was performed accounting for particle size and the column was scaled to an ACQUITY UPLC BEH C_{18} 1.7 µm column, which has the identical stationary-phase chemistry to the HPLC column (XBridge C_{18}). Five replicate injections of both donepezil standard and donepezil tablet were analyzed separately. System suitability criteria for donepezil, including %RSD for peak area, USP tailing and USP plate count were compared between HPLC and UPLC. A comparison of both systems is shown in Table 1, where the UPLC transferred method passes all criteria specified in the monograph. The run time of the UPLC method is 12.5 minutes compared to the 50-minute HPLC method (Figure 1), resulting in a 75% savings in analysis time and 92% savings in solvent consumption and sample injected.

	USP	HPLC	UPLC
Retention Time(min)	none	9.44	2.70
% RSD Area*	NMT 2.0	0.40	0.26
USP Tailing	NMT 1.5	0.71	0.74
USP Plate Count	NLT 40,000	57638	134196

* 5 replicate standard injections

Table 1. System suitability results comparing HPLC to UPLC for five replicate injections of donepezil standard.

HPLC and UPLC Conditions

Mobile Phase:	A: 0.1% phosphoric acid in water, adjust to pH 6.5 with triethylamine B: acetonitrile
Diluent:	water:acetonitrile (3/1)
Needle Wash:	60:40 water:acetonitrile
Sample Purge:	60:40 water:acetonitrile
Seal Wash:	50:50 methanol:water
Column Temp.:	50 °C
Detection:	UV at 286 nm
Data Management:	Empower 3 CDS

USP System Suitability Criteria

Tailing Factor:	Not More Than (NMT) 1.5 USP Plate Count: Not Less Than (NLT) 40,000 plates
Replicate Injections:	NMT 2.0% RSD for donepezil peak (5 replicates)

Sample Preparation

Donepezil (1 mg/mL) in diluent:
Waters Analytical Standard

Donepezil Tablets (1 mg/mL):
Crushed tablets were weighed in a 50 mL volumetric flask and 25 mL diluent was added. The sample was sonicated for 15 minutes and made up to volume with diluent. The sample was mixed well, filtered though a 0.2 µm PTFE filter and centrifuged at 12,000 rpm for 5 minutes.

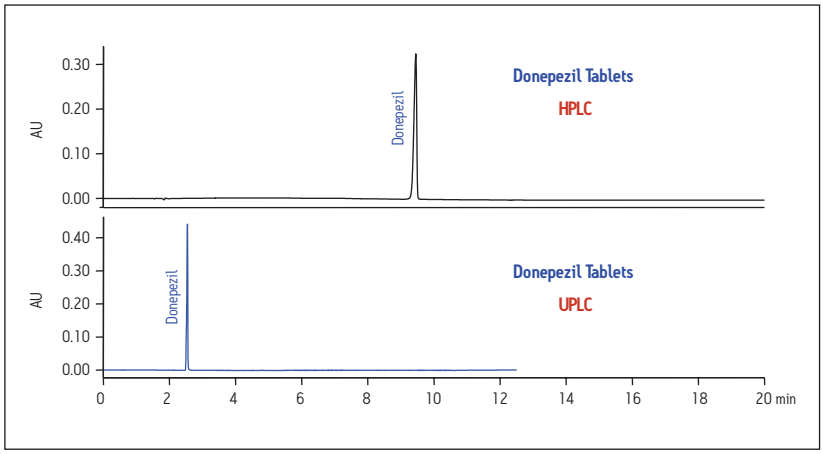

Figure 1. Chromatograms of donepezil tablet comparing HPLC to UPLC per the USP method. (Note: the full 50-minute HPLC run time is not shown.)

ROUTINE USE STUDY

Evaluation #1

In order to evaluate the effects of repeatedly analyzing a tablet drug formulation using a UPLC transferred USP method, a routine use evaluation was performed with the ACQUITY UPLC BEH C_{18}, 1.7 µm column using donepezil tablets.

Donepezil tablet samples were injected repeatedly using donepezil standard as a bracketing standard to emulate the process in a typical quality control (QC) laboratory. Mobile phase was prepared fresh daily to prevent bacterial growth. Twenty injections of donepezil tablet samples were made, along with five replicate injections of donepezil standard, and this cycle was repeated continuously until assay suitability criteria no longer passed. Pressure, retention time, peak area, USP tailing and USP plate count were monitored throughout the study.

USP tailing remained within assay criteria throughout the study, however, after approximately 700 injections, the USP plate count began rapidly decreasing (Figure 2). At approximately 850 injections, the donepezil peak shape began to split (Figure 3) and the routine use study was stopped. Pressure remained stable throughout the study. The system was cleaned, mobile phases replaced and the column was washed. The column inlet and outlet frits were also replaced to determine if the poor peak shape could be attributed to particulate contamination, but the USP plate count still did not pass system suitability. These troubleshooting measures indicated that the UPLC column stationary phase had become contaminated by the poorly soluble excipients in the donepezil tablet formulation.

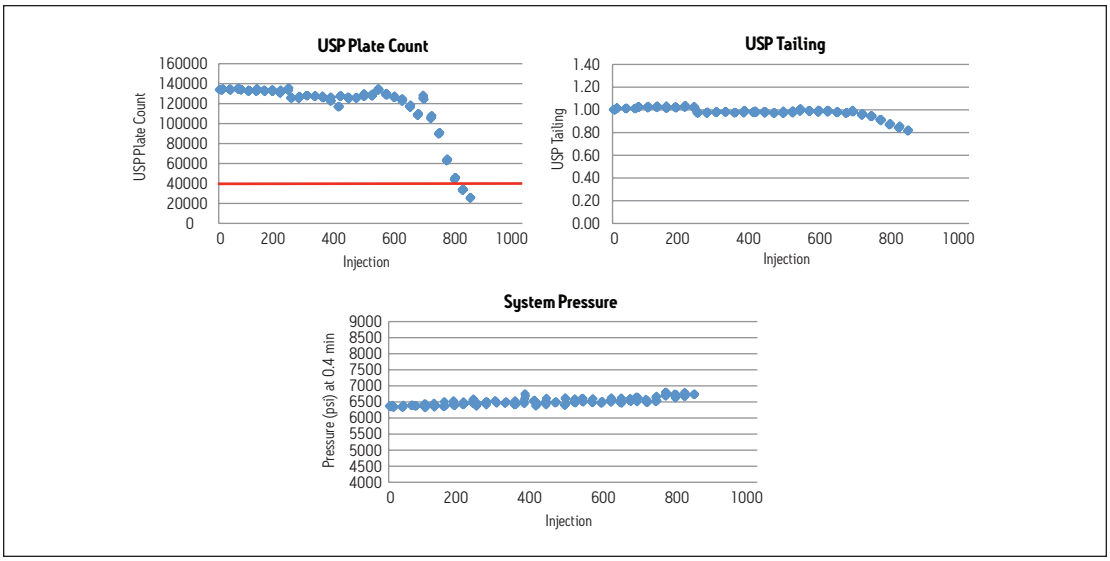

Figure 2. System suitability trend plots from routine use evaluation #1 (UPLC 1.7 µm column).

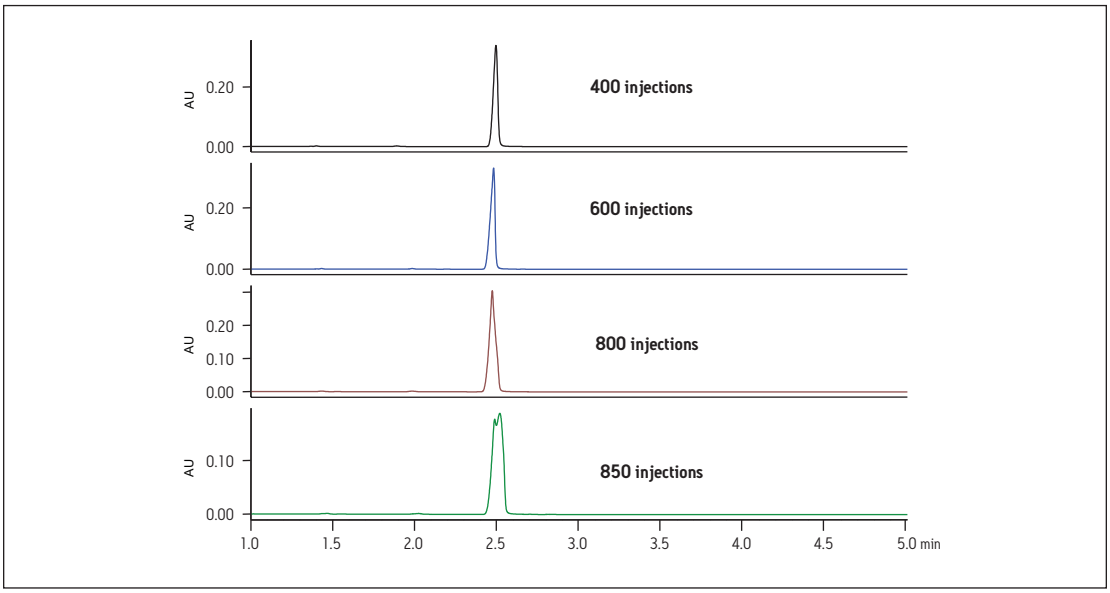

Figure 3. Chromatograms of donepezil standard from routine use evaluation #1 (UPLC 1.7 µm column).

Evaluation #2

Sample preparation for formulated drug tablets is often limited due to time, cost and recovery concerns. For this reason, additional sample preparation steps were not pursued. Instead, the UPLC method using the ACQUITY UPLC 1.7 µm column was transferred to an XBridge C_{18} ***XP*** 2.5 µm column using the ACQUITY UPLC Columns Calculator. Due to the larger particle size and subsequently lower backpressure, the method was run at a higher linear velocity, which resulted in a 10-minute method, an 80% savings in analysis time compared to the original 50-minute HPLC method.

A second routine use study was performed with the same repeating sample set containing donepezil standards and tablets, using the ***XP*** 2.5 µm column. The results of this routine use study show USP plate count and USP tailing factor failing criteria after about 1600 injections (Figure 4). The routine use study was stopped at approximately 1700 injections, when the peak shape deterioration became evident (Figure 5).

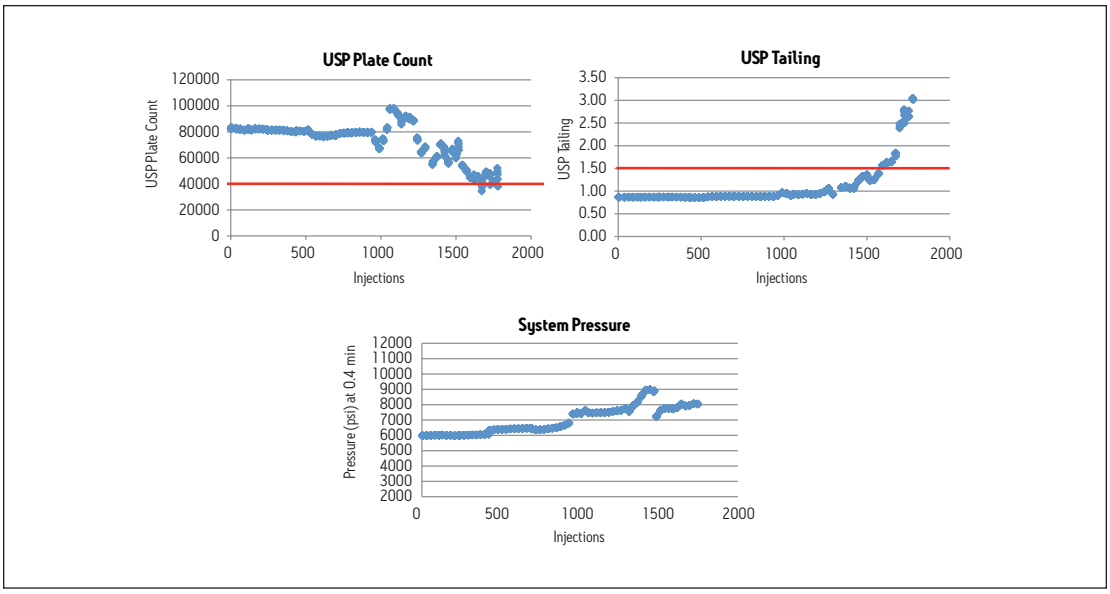

*Figure 4. System suitability trend plots from routine use evaluation #2 (**XP** 2.5 µm column).*

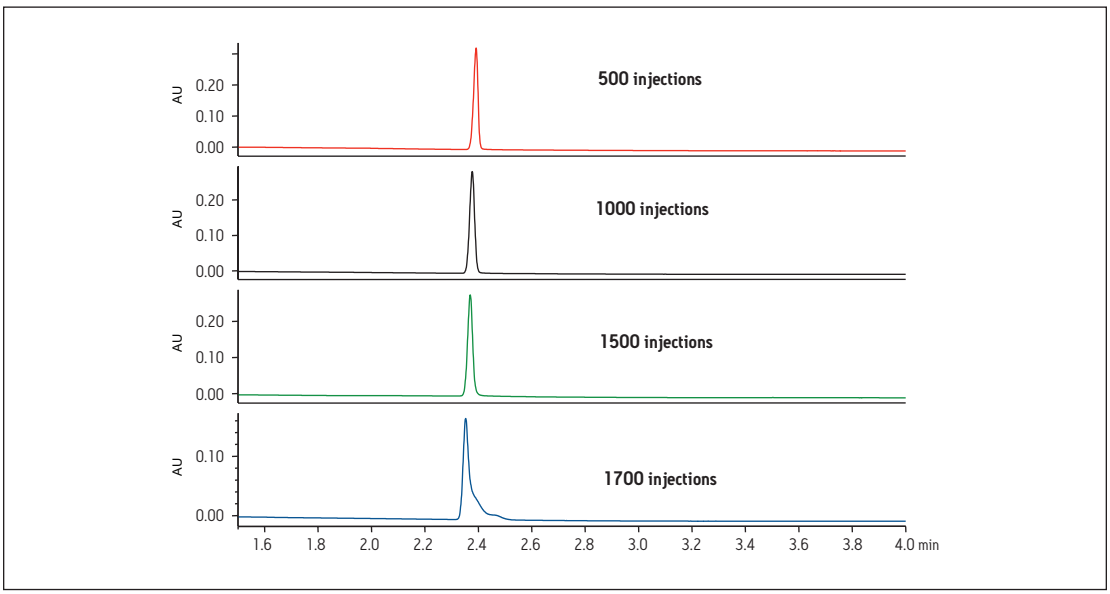

*Figure 5. Chromatograms of donepezil standard from routine use evaluation #2 (**XP** 2.5 µm column).*

Although the same formulation was analyzed, the ***XP*** 2.5 µm column resulted in twice as many injections compared to the 1.7 µm column. At 1600 injections, all system suitability criteria were still within specification (Table 2). In the analysis of complex formulation matrices, there is limited removal of insoluble excipients using simplistic sample preparation procedures. This often results in an unavoidable build-up of sample components on the column. In the case of donepezil tablets, the use of an ***XP*** 2.5 µm column allowed for a greater number of injections, while still meeting the USP monograph system suitability requirements.

	Routine Use Evaluation		
	USP Criteria	Start	1600 Injections
Retention Time(min)	none	2.35	2.27
%RSD Area*	NMT 2.0	0.49	0.32
USP Tailing	NMT 1.5	0.87	1.38
USP Plate Count	NLT 40,000	82472	44951

* 5 replicate standard injections

*Table 2. System suitability results before and after 1600 injections from routine use evaluation #2 (**XP** 2.5 µm column).*

CONCLUSIONS

The USP compendial method for donepezil tablets was successfully transferred from HPLC to UPLC using the Waters Column Selectivity Chart and ACQUITY UPLC Columns Calculator. Routine use, typical of a QC laboratory analyzing a formulated donepezil tablet sample, was evaluated on the ACQUITY UPLC BEH C_{18} 1.7 µm column. Approximately 850 injections were achieved before peak splitting was evident and the method failed system suitability. Troubleshooting steps attributed the cause of failure to sample build-up on the column bed. Due to practical limitations in changes to the sample preparation, the method was transferred to an XBridge C_{18} ***XP*** 2.5 µm column, where approximately 1600 injections were achieved. The method using the ***XP*** 2.5 µm column is approximately 80% faster than the HPLC method and results in a 92% savings in sample injected and solvent consumption. In cases where formulated sample is not directly compatible with the original compendial method, the use of ***XP*** 2.5 µm columns can provide method ruggedness while still providing significant sample throughput and solvent cost savings.

References

1. Jones M.D., Alden P., Fountain K.J., Aubin A. *Implementation of Methods Translation between Liquid Chromatography Instrumentation*, Waters Application Note [2010], Part Number 720003721EN.
2. USP Monograph. Donepezil Hydrochloride, USP35-NF30. The United States Pharmacopeial Convention, official from May 1, 2012.

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USP Method Transfer of Levonorgestrel and Ethinyl Estradiol Tablets from HPLC to UPLC

Mia Summers and Kenneth J. Fountain, Waters Corporation, 34 Maple St., Milford, MA, USA

APPLICATION BENEFITS

- Updating USP methods from HPLC to UPLC® using sub-2 µm columns
- 85% decrease in analysis time, faster throughput for routine sample analysis
- 92% reduction in solvent usage and sample injected

WATERS SOLUTIONS

- ACQUITY UPLC® H-Class and Alliance® systems
- ACQUITY UPLC BEH C_8 and XBridge™ C_8 columns
- Empower™ 2 CDS
- Method Transfer kit
- Waters Levonorgestrel-Ethinyl Estradiol USP standard

KEY WORDS

Method transfer, USP methods, HPLC, UPLC, levonorgestrel, ethinyl estradiol, birth control, ACQUITY UPLC Columns Calculator, quality control

INTRODUCTION

Chromatographic assays for the analysis of generic drugs are frequently based on USP compendial methods. The HPLC methods described in the USP are not routinely updated and do not take advantage of sub-2 µm particle technology, which provides faster run times and increased productivity of analysis. Additionally, routine analysis of drug formulations can result in more frequent and costly replacement of columns due to the chemical diversity of excipients and other formulation agents. USP methods developed on HPLC are often isocratic to eliminate the need for re-equilibration, thereby increasing sample throughput. However, if the sample is not properly eluted off of the column, it can build up on the column bed, resulting in increased backpressure and potentially premature failure of the column.

This application note will first compare the USP method for levonorgestrel and ethinyl estradiol tablets using two different L7 HPLC columns. The method is then transferred to UPLC using a UPLC column with the same stationary phase. The use of UPLC and sub-2µm particle columns allows for a significantly faster analysis while still meeting the system suitability criteria specified in the USP monograph. Finally, suggestions are offered to alleviate the increased backpressure that may be observed when repeatedly analyzing formulated levonorgestrel-ethinyl estradiol tablet samples in a typical quality control laboratory.

EXPERIMENTAL

Alliance 2695 HPLC Conditions

Mobile Phase:	7:3:9 acetonitrile: methanol:water
Separation Mode:	Isocratic
Detection:	UV at 215 nm
USP Column:	Zorbax® C_8, 4.6 x 150 mm, 5 µm (USP designation: L7); XBridge C_8, 4.6 x 150 mm, 5 µm (USP designation: L7), part number 186003017
Needle Wash:	acetonitrile
Sample Purge:	acetonitrile
Seal Wash:	50:50 methanol:water
Flow Rate:	1 mL/min
Injection Volume:	50 µL

ACQUITY UPLC H-Class Conditions

Mobile Phase:	7:3:9 acetonitrile: methanol:water
Separation Mode:	Isocratic
Detection:	UV at 215 nm
USP Column:	ACQUITY UPLC BEH C_8, 2.1 x 50 mm, 1.7 µm (USP designation: L7), part number 186002877
Needle Wash:	acetonitrile
Sample Purge:	acetonitrile
Seal Wash:	50:50 methanol:water
Flow Rate:	0.61 mL/min
Injection Volume:	3.5 µL

Data Management

Empower 2 CDS

USP System Suitability Criteria

USP Resolution:	NLT 2.5
Peak Area RSD:	NMT 2.0%

Sample Preparation

Standard:
Levonorgestrel, 15 µg/mL and ethinyl estradiol, 3 µg/mL in mobile phase (Waters Analytical Standard).

Sample:
Dissolve levonorgestrel and ethinyl estradiol commercially-available tablets in mobile phase to a final concentration of 15 µg/mL levonorgestrel and 3 µg/mL ethinyl estradiol. Sonicate for 5 minutes, shake mechanically for 20 minutes. Centrifuge at 4000 rpm for 10 minutes. Collect supernatant and re-centrifuge at 12,000 rpm for 30 minutes, pipet clear supernatant for injection.

RESULTS AND DISCUSSION

Samples were prepared according to the USP compendial assay method for levonorgestrel and ethinyl estradiol tablets.[1] Samples were first centrifuged at 4000 rpm for 10 minutes and yielded a pale cloudy solution. Next, an aliquot of sample was filtered through a 0.2 µm PTFE filter, but the filtrate remained cloudy due to the extremely fine nature of the particulates in the sample and further filtration was not pursued. Samples were instead centrifuged at 12,000 rpm for 30 minutes and the supernatant was collected, yielding a clear solution for injection.

The USP method for levonorgestrel and ethinyl estradiol tablets requires the use of an L7 column and suggests an Agilent Zorbax C_8 column. This column was tested per the USP assay method on an Alliance HPLC system, with five replicate injections of both levonorgestrel-ethinyl estradiol standard and tablets. The samples were also run on the HPLC system using a Waters XBridge C_8 column. This column was chosen since it has similar selectivity to the Zorbax C_8 column and it has an equivalent UPLC column chemistry (ACQUITY UPLC BEH C_8), facilitating direct method transfer to UPLC. Chromatograms comparing the USP method using the Zorbax and XBridge C_8 HPLC columns are compared in Figure 1. The columns show similar selectivity, but the Zorbax C_8 column shows greater overall retention of the two active ingredients compared to the XBridge C_8 column. However, the faster elution of analytes using the XBridge column allows for a much shorter analysis time, while sacrificing only a small amount of resolution. All of the USP assay suitability results were well within the specified criteria limits for both columns (Table 1). The effect of retentivity on productivity in the high throughput analysis of generic drugs is important to consider, even within the same USP column designation categories.

Next, the USP assay method was transferred from HPLC to UPLC using the ACQUITY UPLC Columns Calculator.[2] Scaling calculations were performed accounting for particle size and the method was scaled from the XBridge C_8 HPLC column to an ACQUITY UPLC BEH C_8, 1.7 µm column. Both columns have the same stationary phase chemistry and only differ in particle size. Five replicate injections of both levonorgestrel and ethinyl estradiol tablets and standard were analyzed separately. Assay suitability criteria including %RSD for peak area, and USP resolution between ethinyl estradiol and levonorgestrel peaks were compared between HPLC and UPLC. A comparison of both systems is shown in Table 1, where the UPLC transferred method clearly passes all system suitability criteria. The run time of the UPLC method is 1.5 minutes compared to a 10-minute HPLC method, resulting in an approximate 85% savings in analysis time and 92% savings in solvent consumption and sample injected (Figure 1).

System	USP Method	Column (L7 designation)	Sample	Peak Area % RSD		USP Resolution	Runtime (min)
				ethinyl estradiol	levonorgestrel		
HPLC	Original	Zorbax C_8	standard tablet	0.22 0.58	0.60 0.65	8.5 8.6	20
HPLC	Original	XBridge C_8	standard tablet	0.24 0.31	0.19 0.05	6.1 6.1	10
UPLC	UPLC Transferred	ACQUITY UPLC BEH C_8	standard tablet	0.16 0.09	0.39 1.12	5.3 5.3	1.5

Assay Suitability Criteria

USP Resolution (between 2 peaks): NLT 2.5

Peak Area RSD: NMT 2.0%

Table 1. Assay suitability results comparing HPLC to UPLC for five replicate injections of levonorgestrel and ethinyl estradiol standard and tablet samples.

ROUTINE USE STUDY

In order to evaluate the performance of the UPLC transferred USP method with high-throughput analysis of formulated tablet samples, a routine use study was performed using the ACQUITY UPLC BEH C_8, 1.7 µm column.

Levonorgestrel and ethinyl estradiol tablet samples were injected along with a standard mixture of levonorgestrel and ethinyl estradiol as a bracketing standard to simulate routine use in a typical quality control (QC) laboratory. Five replicate injections of standard were followed by twenty replicate injections of formulated tablet sample and this cycle of injections was repeated continuously until assay suitability criteria no longer passed. Pressure, peak area, retention time and USP resolution between the two peaks (levonorgestrel and ethinyl estradiol) were monitored throughout the study.

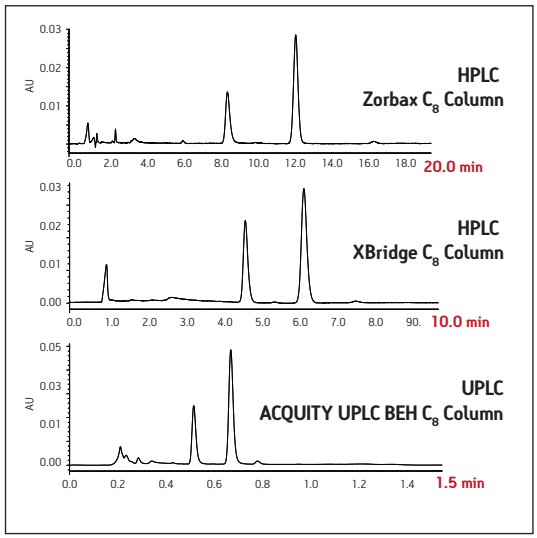

Figure 1. Chromatograms of levonorgestrel and ethinyl estradiol tablet sample comparing HPLC to UPLC.

Peak area RSD remained within 2.0% for levonorgestrel and ethinyl estradiol standard injections, and USP resolution remained greater than 2.5 throughout the study, which is within assay specifications (Figure 2). Backpressure increased steadily throughout the study (Figure 3a), and increased approximately 36% from 7200 psi to 9800 psi over the first 1000 injections. The overall pressure trend for the UPLC routine use study was compared to a routine use evaluation for the original USP method run on an Alliance HPLC system using the Zorbax C_8 column. On the HPLC system (Figure 3b), the pressure began near 1350 psi and rose to approximately 2350 psi over 1000 injections, a 74% increase. Both the original USP method on HPLC, and the UPLC transferred method for levonorgestrel and ethinyl estradiol exhibited a gradual increase in pressure using different columns and different systems. For this reason, the pressure increase is attributed to the sample, likely due to poor aqueous solubility of the hydrophobic steroids and sample formulation components in the aqueous mobile phase, which contains only about 50% organic solvent. While the pressure increase on the ACQUITY UPLC system is noticeable, the relative pressure increase is far below that of the HPLC system using the Zorbax column. Despite the observed pressure increases throughout the study, both systems were well within the running pressure limits of the instrumentation and all system suitability criteria were met.

The routine use evaluation on the ACQUITY UPLC system was stopped at 2200 injections. The assay suitability criteria still passed all requirements, as shown in Table 2, demonstrating robust column performance for levonorgestrel and ethinyl estradiol on the ACQUITY UPLC BEH C_8 column, even after 2000 injections.

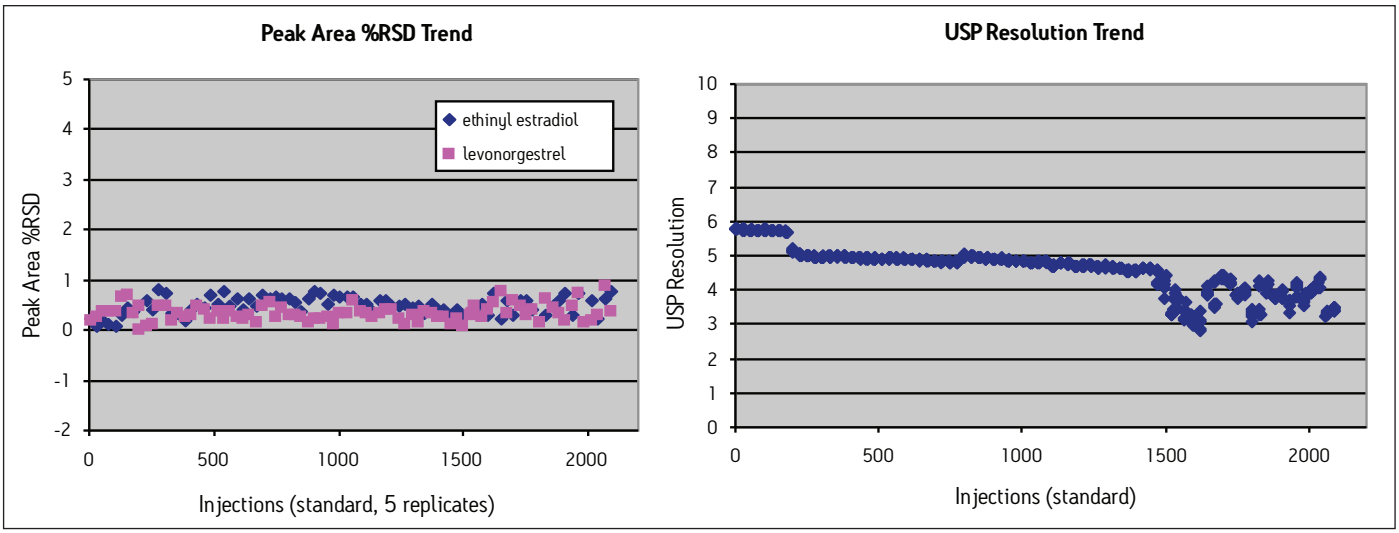

Figure 2. USP assay suitability trend plots from the routine use evaluation for levonorgestrel and ethinyl estradiol using the UPLC transferred method.

Levonorgestrel and Ethinyl Estradiol Standard			
	%RSD Peak Area* (NMT 2.0%)		USP Resolution (NLT 2.5)
	ethinyl estradiol	levonorgestrel	
Routine Use Study: start	0.21	0.20	5.7
Routine Use Study: 2200 injections	0.57	0.22	3.3

*from 5 replicate standard injections

Table 2. USP assay suitability results before and after 2200 injections from the routine use evaluation using the UPLC transferred method.

Although the UPLC method meets assay suitability criteria for more than 2000 injections, a modification to the method was made in an attempt to alleviate the pressure increase over time, which is thought to be caused by sample build–up on the column bed. A second routine use evaluation was performed on a new ACQUITY UPLC BEH C_8 column, this time adding a gradient wash at the end of the isocratic USP method after each sample injection. After the 1.5 minute isocratic portion of the separation for the UPLC method, a gradient to 100% acetonitrile in 0.3 minutes was added, with a 1-minute hold at 100% acetonitrile (wash step) and then re-equilibration at 100% mobile-phase A. While the addition of a gradient wash lengthened the total cycle time to 4 minutes (including re-equilibration), this high organic washing step after each injection helps to elute the hydrophobic sample components and prevent build-up of sample on the column, thereby stabilizing the pressure throughout repeated injections from routine use (Figure 3c).

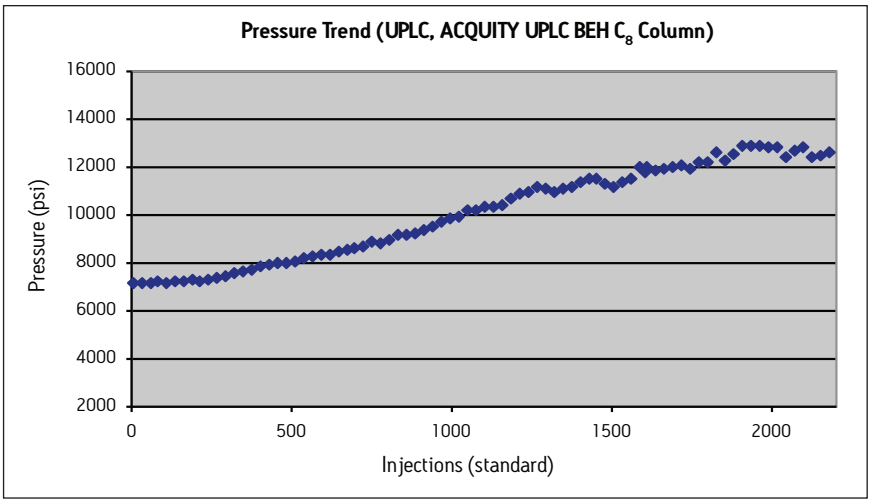

Figure 3a. Pressure trend plot from the routine use evaluation on a UPLC system with an ACQUITY UPLC BEH C_8 column (UPLC transferred USP method).

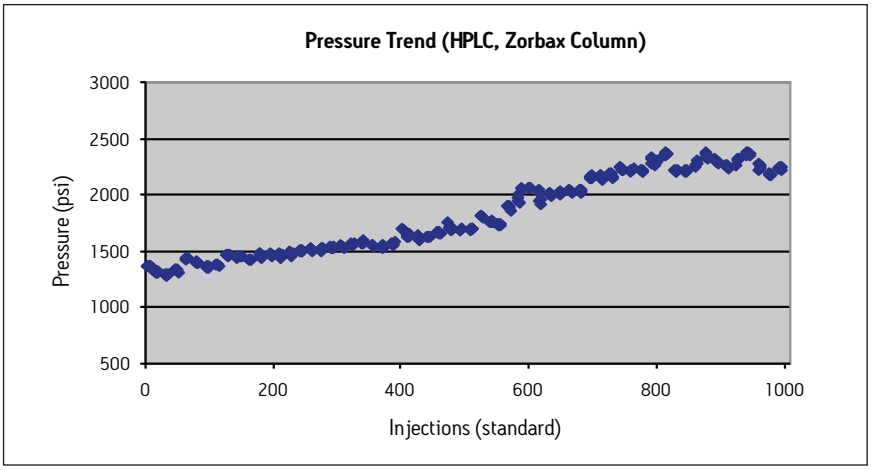

Figure 3b. Pressure trend plot from the routine use evaluation on an HPLC system with a Zorbax C_8 column (original USP method).

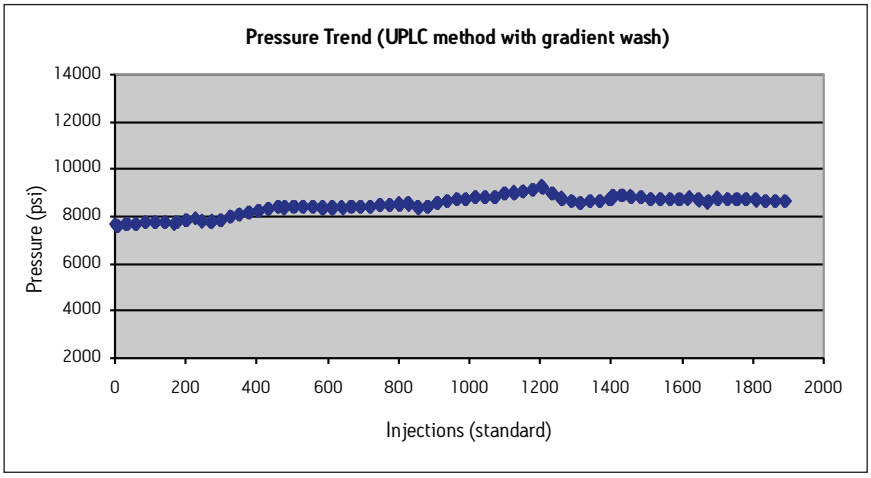

Figure 3c. Pressure trend plot from the routine use evaluation on an UPLC system with an ACQUITY UPLC BEH C_8 column (UPLC transferred method with gradient wash added).

CONCLUSIONS

The USP compendial method for levonorgestrel and ethinyl estradiol tablets was successfully transferred from HPLC to UPLC using scalable column chemistries and the ACQUITY UPLC Columns Calculator. The UPLC method is approximately 85% faster than the HPLC method and results in a 92% savings in sample amount injected and mobile-phase solvent consumption. While extended centrifugation of the tablet sample was helpful in preparing a better quality sample for injection, it did not fully alleviate the increased pressure seen in both HPLC and UPLC during a routine use study. Instead, incorporation of a gradient wash to the isocratic method aided in preventing sample build-up on column, thereby stabilizing the pressure during routine use evaluations. Routine use of the UPLC transferred USP method was evaluated using formulated tablet samples on an ACQUITY UPLC BEH C_8, 1.7 µm column. After 2200 injections, the column still passed all USP assay suitability specifications for levonorgestrel and ethinyl estradiol tablets, demonstrating that extended column performance is achievable for high throughput analysis of generic tablet formulations using an isocratic USP monograph method.

References

1. USP Monograph. *Levonorgestrel and Ethinyl Estradiol Tablets*, USP34-NF29, 3299. The United States Pharmacopeial Convention, official from August 1, 2011.
2. Jones M.D., Alden P., Fountain K.J., Aubin A. *Implementation of Methods Translation between Liquid Chromatography Instrumentation*, Waters Application Note [2010], Part Number 720003721EN.

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Online Monitoring of Process Column Effluents in Purification by UPLC

Tanya Tollifson
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

The PATROL UPLC® Process Analysis System, with automated online and atline analyses, aids in manufacturing throughput and yield by providing real-time information regarding the process effluent. The creation of multi-point calibration curves from a single standard vial allows for real-time decision making on effluent fraction collection based on quantitative data of target product as well as product and process impurities.

WATERS SOLUTIONS

UPLC®

PATROL UPLC Process Analysis System

Empower® Software

ACQUITY UPLC Column Chemistries

Connections INSIGHT®

NuGenesis® SDMS

KEY WORDS

Real-TIME™ LC, online, atline, PAT, purification, effluent monitoring, fraction collection, real-time analysis, in-process API

INTRODUCTION

For pharmaceutical and biopharmaceutical companies, among other industries, Process Analytical Technology (PAT) is a critical component of the overall manufacturing process, relied upon to provide richer process understanding and consistent product quality at maximum yields with minimal waste.

PAT involves taking timely measurements throughout the production process to verify the quality of in-process batches and to understand performance in each of the critical steps of that process. Many different sensor technologies are employed throughout the manufacturing process to measure the attributes of the in-process batches. Deployment of appropriate sensors to monitor identified critical quality attributes (CQAs) can aid in maintaining process control and functioning well within the established design space of the operation.

Typically, process steps such as purification are assessed by spectroscopic sensors, which include near-infrared spectroscopy (NIR) or Raman spectroscopy. These techniques have the ability to provide real-time information about the purification process but lack the ability to effectively resolve and quantify multiple components in the effluent stream.

Performance of these sensors needs to be benchmarked against a reference standard, which in most instances is high performance liquid chromatography (HPLC) because it is a more selective and sensitive technique, with a broader linear dynamic range, and has the ability to quantify multiple components within complex samples.

HPLC is the most widely-used technique in pharmaceutical QC laboratories. However, its long run times and complex system operation have prevented it from being routinely used for atline or online analysis.

With the introduction of Waters® UltraPerformance LC® (UPLC) technology, it is now possible to achieve near real-time chromatographic analysis for in-process samples. UPLC is delivered in a system that includes integrated hardware and software, offering a simple design that requires little to no user input.

The PATROL UPLC Process Analysis System brings reference-standard methodology to process development and is directly scalable through commercial operations, eliminating the need to calibrate spectroscopic sensors or to send suspect samples to an off-line QC laboratory. This application note discusses the use of UPLC for monitoring effluent from a process purification column.

EXPERIMENTAL

The PATROL UPLC Process Analysis System monitored the simulated process column effluent under the following conditions:

Column:	Waters ACQUITY UPLC® BEH 2.1 mm x 50 mm, C_{18}, 1.7 µm
Eluents:	A: 0.1% Formic acid in water
	B: 0.1% Formic acid in acetonitrile
Gradient:	10% to 25% over 1 minute; Curve 4
Flow rate:	1.0 mL/min
Temp.:	50 °C
Inj. volume:	5 µL
Detection:	243 nm; 40 Hz; Time Constant 0.025 sec
Wash:	70:15:15 Acetonitrile/ water/isopropanol
Purge:	1 mL (4x volume of transfer line)
Run time:	1 min
Cycle time:	2 min, 40 sec

RESULTS AND DISCUSSION

Methods

The purification steps of an in-process API determine the purity and yield of the final product. Spectroscopic techniques are not as selective or sensitive as LC for assessing process column effluent during the purification process. By employing UPLC to monitor the process column effluent, the quality of the final product API can be controlled and optimized with greater confidence.

To demonstrate the utility of online monitoring with the PATROL UPLC Process Analysis System, a process chromatography effluent was simulated using a quaternary gradient pump.

To simulate the process column effluent, a gradient profile was developed to mimic the effluent generated by the purification of an API from two impurities. The profile measured with a UV/Vis detector is shown in Figure 1. The separation profile has one impurity that is well resolved from the API and a second impurity that is not completely resolved from the API.

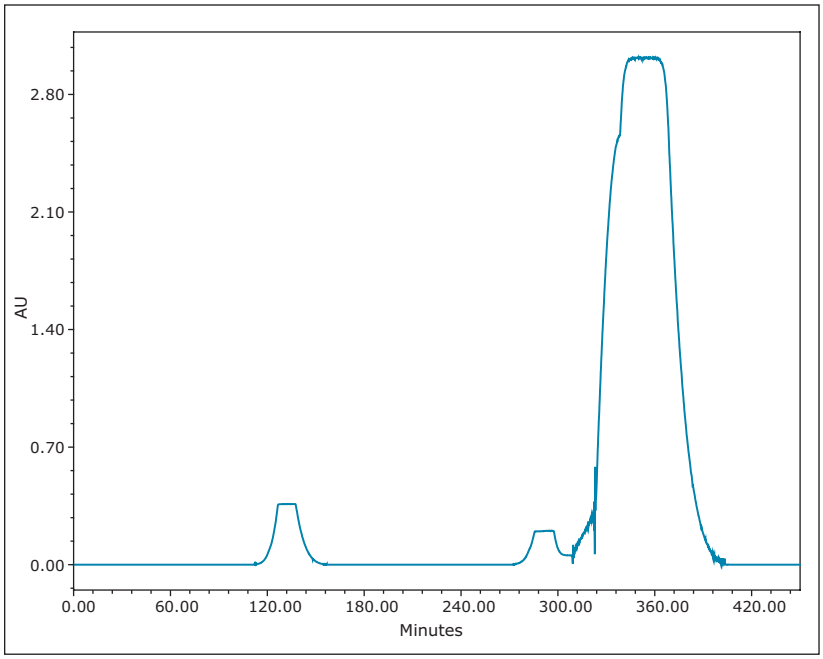

Figure 1. UV/Vis trace of simulated process column effluent (243 nm).

UPLC Technology

UPLC is based upon the use of sub 2-µm column particles and system technology that takes advantage of the benefits of these particles. Since its introduction, many users have transferred their HPLC QC methods to UPLC with great success, realizing tremendous improvements in both throughput, sensitivity, and resolution.

The PATROL UPLC Process Analysis System brings these significant improvements to the manufacturing floor in a manner that allows LC to be used as a real-time sensor. It is a holistically-designed system that integrates UPLC technology, control software, and a ruggedly-engineered sample management module for managing the samples and workflow in an in-process environment. The system's components, along with all solvents, waste, and standards, are contained within an enclosed case that is compatible with all requirements of a manufacturing environment.

The system is designed to be compatible with both online (direct automatic sampling from a process) and atline (manual sample drawn from a process) analysis. The ACQUITY UPLC Process Sample Manager (PSM) can be interfaced with process streams or reactors to provide real-time analysis and quantification without the need for user intervention. Data can be sent to distributed control systems (DCS) or LIMS for completely automated monitoring. For atline applications the system features a walk-up interface with barcode scanning capabilities that eliminates the need for information input from the technician. It also provides sample chain of custody with 21 CFR Part 11 compliant-ready Empower Software.

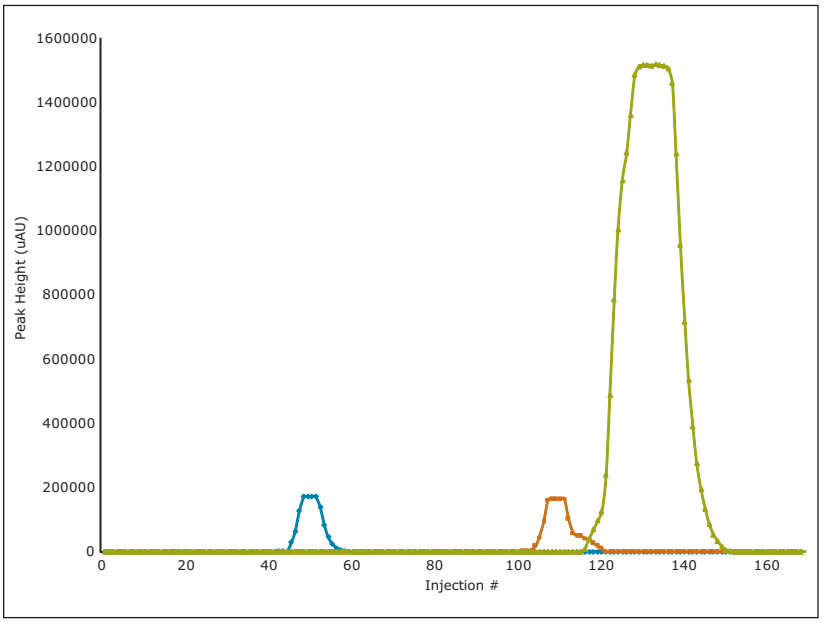

Figure 2. A summary of the peak heights generated by UPLC monitoring models the trace generated by UV/Vis, but also allows for the quantification of individual peaks when there is not baseline resolution.

Application

A system suitability standard (API) was injected from a vial prior to the beginning of the process column simulation. The repeatability of 10 replicate injections (Table 1) was well within the method requirements. A tee was placed in the effluent line to draw sample through a transfer line to the injection valve of the ACQUITY UPLC PSM.

The PATROL UPLC Process Analysis System was able to monitor the effluent off the simulated process column and successfully quantify all three peaks to generate the same profile as was observed by the UV/Vis detector (Figure 2).

Three extracted chromatograms from different time points during the simulation (Figure 3) demonstrate the ability to quantify each of the components throughout the progression of the purification process. The most significant benefit of monitoring by UPLC over another spectroscopic method is the ability to determine the optimal time to begin collection of the API.

Figure 4 overlays all of the injections from the time point of the apex of the impurity 2 peak on the process column simulation to the time point of the apex of the API. The peaks are well resolved on the UPLC column and can be easily quantified, even at very different levels. Table 2 summarizes the peak areas that were calculated for each of the individual injections on the PATROL UPLC Process Analysis System. The point at which all of the impurity has eluted off the process column can be determined even when it is in very low levels compared to the API (Figure 5).

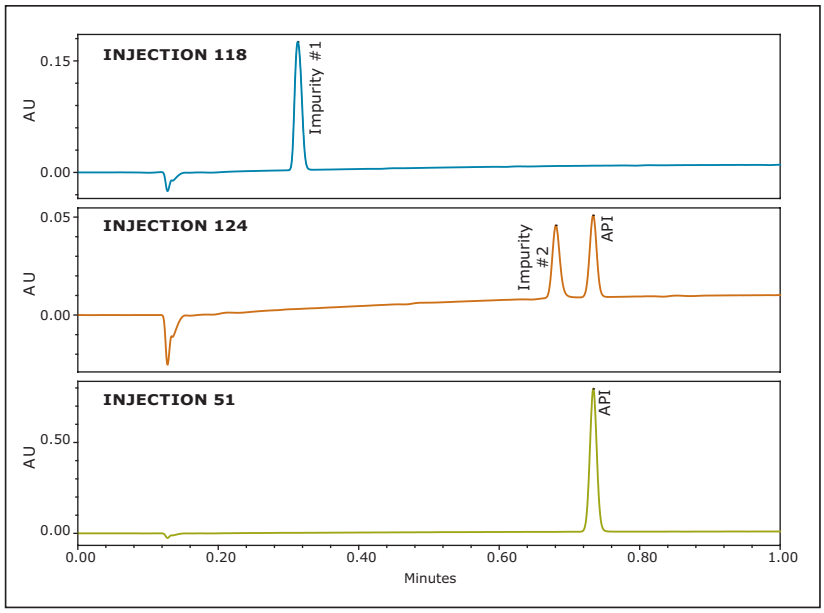

Figure 3. Individual chromatographic traces collected from the simulated process column effluent allow for purity assessment of the API and are used to trigger its collection.

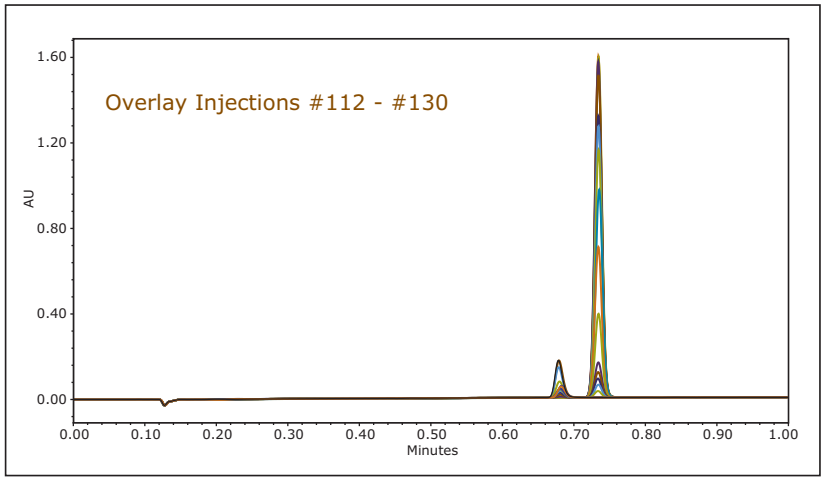

Figure 4. Overlay of all injections from the time point of the apex of the impurity 2 peak on the process column simulation to the time point of the apex of the API.

	Retention time %RSD	Peak area %RSD	Peak height %RSD
API (phenacetin)	0.08%	0.06%	0.14%

Table 1. Repeatability of 10 replicate injections.

Injection #	Peak area salicylic acid	Peak area phenacetin	Peak area salicylic acid ——————X 100% Peak area p henacetin
112	131278	–	
113	107101	–	
114	57779	–	
115	42602	–	
116	40202	–	
117	33420	420	7957%
118	24705	21240	116%
119	17451	42089	41.5%
120	10129	62435	16.2%
121	3442	83320	4.13%
122	440	114626	0.38%
123	–	273784	
124	–	493515	
125	–	682528	
126	–	818540	
127	–	901826	
128	–	939711	
129	–	1072950	
130	–	1112468	

Table 2. Summary of peak areas that were calculated for each of the individual injections.

CONCLUSIONS

- Monitoring of process column effluent by UPLC allows for the simultaneous quantification of APIs and process impurities for maximum product yields and purity.
- Deployment of the PATROL UPLC Process Analysis System on the manufacturing floor promotes process efficiency, while maintaining rich process understanding.
- The PATROL UPLC Process Analysis System was designed with ease-of-use in mind to meet the challenging and diverse applications of in-process samples.
- The fully automated integration with online analysis and walk-up atline analysis reduces risk associated with user handling errors.

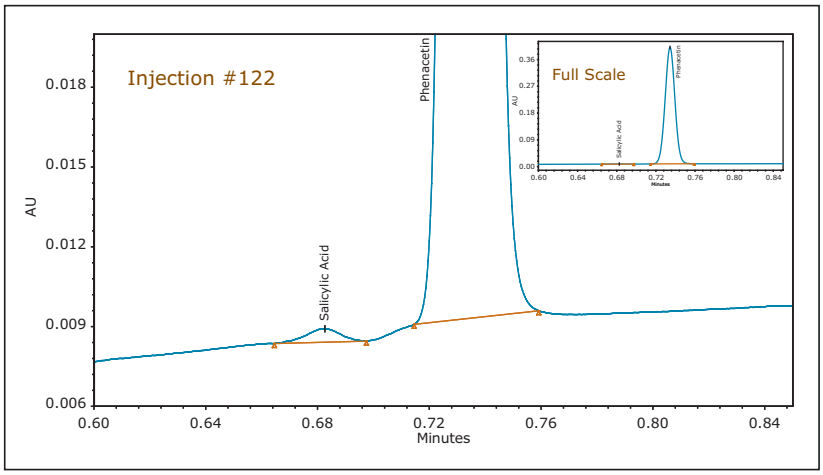

Figure 5. Determining the point at which all of the impurity has eluted off the process column, even when it is in very low levels compared to the API.

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Automating the Creation of Chromatographic Methods for Method Validation Using Empower Sample Set Generator

Margaret Maziarz, Mark Wrona, Paul Rainville, Jade Byrd, Mia Summers, and Diane Diehl

GOAL

To demonstrate the use of Empower® 3 Software's Sample Set Generator to automate the creation of chromatographic methods and sample set methods, used in the validation of a UPLC® method for metoclopramide HCl and related substances.

BACKGROUND

The validation process of an analytical method is a complex and demanding activity, consisting of many time-consuming steps. Some of these steps include acquiring, reviewing and processing data, performing calculations, approving, and final reporting of the validation results. One critical task is robustness testing, during which the effects of minor changes in chromatographic parameters on method performance are investigated to establish tolerance limits. Multiple chromatographic methods must be carefully designed and created to acquire data for each validation test. Designing, creating, and verifying these methods manually can be tedious and prone to errors.

Empower 3 Sample Set Generator simplifies the creation of instrument methods, method sets, and sample set methods by defining ranges of variables needed for testing. By automating these tasks, chromatographic method and the sample sequence generation is streamlined and transcription errors eliminated. As the tedious tasks are minimized, laboratory efficiency and productivity increases.

Empower 3 Sample Set Generator simplifies the creation of instrument methods, method sets, and sample set methods by defining ranges of variables needed for testing.

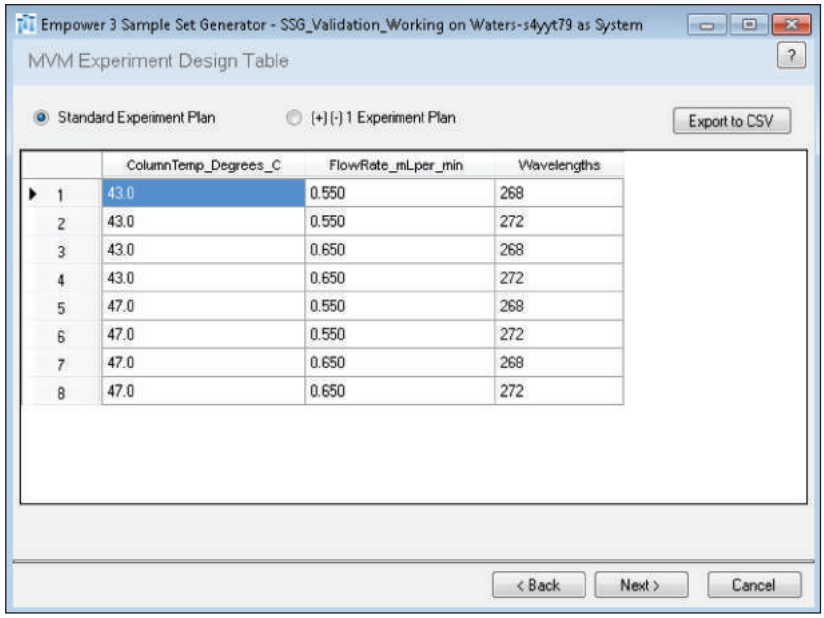

Figure 1. Design of Experiments (DoE) for robustness test loaded to the Empower 3 Sample Set Generator from the Empower 3 MVM protocol.

Here we illustrate the use of Empower 3 Sample Set Generator to automatically create chromatographic methods for robustness testing in the validation of a UPLC method for metoclopramide HCl and its USP-specified related substances.

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THE SOLUTION

The UPLC method for Metoclopramide HCl and its USP-specified related substances was validated using Empower 3 Software's Method Validation Manager (MVM).[1] MVM software streamlines the entire validation process in one application, from creating validation protocol method to acquiring data, reviewing, analyzing, approving, and reporting validation data.

Empower 3 Sample Set Generator was used to streamline robustness testing for creating chromatographic methods. For robustness, we assessed these parameters:

- Column temperature: 45 ± 2.0 °C
- Flow rate: 0.6 ± 0.05 mL/min
- Wavelength: 270 ± 2 nm

In the Empower 3 MVM project, we opened Sample Set Generator and loaded the robustness validation test as shown in Figure 1. The MVM experimental design, with a combination of eight different instrument conditions, was imported to Empower 3 Sample Set Generator.

Next, we used the Sample Set Generator to create instrument methods, method sets, and a sample set method to run the robustness experiments by completing the following steps:

1. Map factors for column temperature, flow rate, and detection wavelength to the desired settings
2. Define settings for gradient separation
3. Configure requirements for blanks/standards solutions and equilibration time
4. Generate sample set method

Using Empower 3 Sample Set Generator, we were able to automatically create instrument methods with different chromatographic conditions, method sets, and sample set method for the robustness study. The sample set method for the robustness test (Figure 2) is designed according to the experimental plan for the robustness validation test, with injections of blanks/standard solutions, experiment name, and method sets for each run. The equilibration steps are added between sample lines when there is a change in instrument condition, such as flow rate or column temperature. The instrument methods are automatically built into the methods sets.

Robustness_SSG1_1 in SSG_Validation_Working as System/Administrator - Sample Set Method Editor

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Apply Table Preferences | Sample Set Method

	Plate/Well	# of Injs	SampleName	Experiment Name	Method Set / Report Method	Function	Run Time (Minutes)	Column Temp. Degrees C	Flow Rate (mL/min)	Wavelengths (nm)
1					Robustness_SSG1_1	Equilibrate	60.00			
2	1:A,1	2	Blank		Robustness_SSG1_1	Inject Samples	7.50	43.0	0.550	268
3	1:A,2	1	Metoclopramide_1	Experiment 1	Robustness_SSG1_1	Inject Samples	7.50	43.0	0.550	268
4	1:A,2	1	Metoclopramide_2	Experiment 2	Robustness_SSG1_2	Inject Samples	7.50	43.0	0.550	272
5					Robustness_SSG1_3	Equilibrate	20.00			
6	1:A,2	1	Metoclopramide_3	Experiment 3	Robustness_SSG1_3	Inject Samples	7.50	43.0	0.650	268
7	1:A,2	1	Metoclopramide_4	Experiment 4	Robustness_SSG1_4	Inject Samples	7.50	43.0	0.650	272
8					Robustness_SSG1_5	Equilibrate	60.00			
9	1:A,2	1	Metoclopramide_5	Experiment 5	Robustness_SSG1_5	Inject Samples	7.50	47.0	0.550	268
10	1:A,2	1	Metoclopramide_6	Experiment 6	Robustness_SSG1_6	Inject Samples	7.50	47.0	0.550	272
11					Robustness_SSG1_7	Equilibrate	20.00			
12	1:A,2	1	Metoclopramide_7	Experiment 7	Robustness_SSG1_7	Inject Samples	7.50	47.0	0.650	268
13	1:A,2	1	Metoclopramide_8	Experiment 8	Robustness_SSG1_8	Inject Samples	7.50	47.0	0.650	272

Figure 2. Sample set method for robustness test generated using Empower 3 Sample Set Generator.

This automated generation allowed us to quickly start the chromatographic run with confidence that all the methods are correctly created. In addition, it reduced the time needed to create chromatographic methods by about 95% compared to a manual process. The chromatographic data acquired for the robustness testing is shown in Figure 3.

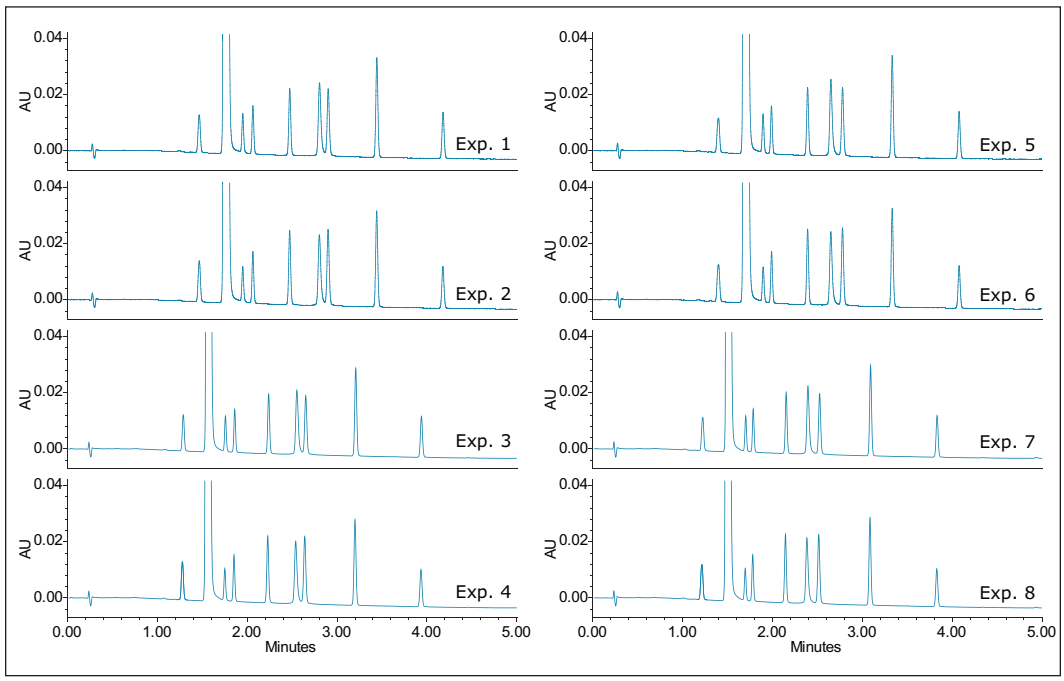

Figure 3. Separation of metoclopramide and USP-specified related substances according to the experimental design for robustness.

SUMMARY

By using the Empower 3 Sample Set Generator, users are able to simultaneously and automatically create instrument methods, method sets, and sample set methods to perform chromatographic runs. As a result of automation, transcription errors that may arise during the manual process are eliminated and the time associated with generation of chromatographic methods is reduced. This improves laboratory efficiency, hence enabling increase in productivity.

Empower 3 Sample Set Generator can be adapted by any analytical laboratory to automate creation of chromatographic methods for wide range of applications performed on Waters ACQUITY UPLC® Systems, including method development and validation.

References:

1. Maziarz M, McCarthy SM, Wrona M. Increasing Efficiency of Method Validation for Metoclopramide HCl and Related Substances with Empower MVM Software. Waters Application Note, 2014: 720005111en.

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Transfer of an Isocratic USP Assay from an Agilent 1100 Series LC System to a ACQUITY UPLC H-Class System: Analysis of Tioconazole and Related Impurities

Paula Hong and Patricia R. McConville
Waters Corporation, Milford, MA

GOAL

To transfer an HPLC USP method from an Agilent 1100 Series LC System to an ACQUITY UPLC® H-Class System, meeting all USP method criteria.

BACKGROUND

Many historical USP monographs were developed and designed for HPLC instrumentation and columns. However, with goals to modernize equipment, laboratories often need to ensure the transferability of established methods from existing HPLC instrumentation to newer or alternative UHPLC or UPLC® instrumentation. These HPLC methods typically require higher flow rates, larger column dimensions, and larger injection volumes than typical UPLC analyses. The USP monograph for tioconazole and its organic impurities is one such example.[1] This USP assay requires a 5 mm x 250 mm column, and a 20-µL sample injection volume. While there is no specified flow rate, the retention time window for tioconazole is between 12–17 minutes, which is achievable using flow rates of between 0.5–1 mL/min. Although this method is typically run on HPLC instrumentation, the compatibility of these methods with UHPLC and UPLC instrumentation enables the laboratory to utilize new equipment with the goal of modernizing the laboratory.

USP methods can be successfully transferred from an Agilent 1100 Series LC System to an ACQUITY UPLC H-Class System.

THE SOLUTION

The USP monograph for tioconazole and organic impurities[1] was performed using an Agilent 1100 Series LC System (Table 1). To meet the retention time criteria for the assay, a flow rate of 0.75 mL/min was used on a XBridge® C_{18}, 5 µm, 4.6 mm x 250 mm Column. The assay was then transferred to an appropriately configured ACQUITY UPLC H-Class System with a 50-µL extension loop (Table 1). Both systems were run with mobile phase temperature control. The Agilent 1100 Series LC System was configured with a passive pre-heater (3 µL), while the ACQUITY UPLC H-Class System was configured with an active pre-heater in the standard configuration.

Agilent 1100 Series LC System		ACQUITY UPLC H-Class System	
Module	Part #	Module	Part #
Degasser	G1322A		
Quaternary pump	G1311A	Quaternary Solvent Manager	186015018
Autosampler	G1313A	Sample Manager FTN	186015017
Column compartment	G1316A	Column Heater (30 CH-A)	186015045
DAD detector	G1315B	PDA detector	186015032

Table 1. System modules and part numbers.

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The USP monograph for tioconazole includes two HPLC tests: the assay, and the organic impurities analysis. Both tests were performed with standards purchased from USP (p/n 1667439). Figure 1 shows overlays of the standard chromatograms on each system. Both systems produced comparable separations for the assay and organic impurities analysis. For six replicate injections, the retention times and relative retention times varied by less than 2% deviation (Table 2). These values are within the generally accepted criterion for retention time variance of 3-5% for method transfer from one manufacturer's system to another.[2] Furthermore, equivalent USP resolution of 1.4 (n=6) was obtained for the critical pair (related compound C and related compound B). The retention time repeatability for each system was within 0.2% RSD, however, lower variability was observed on the ACQUITY UPLC H-Class System.

Although equivalency in methods transfer is desired, for USP methods, specific system suitability requirements must be met for each analysis. For the assay of tioconazole, system suitability includes column efficiency of not less than (NLT) 1000 and tailing of not more than (NMT).[2] Both sets of analyses met these criteria (Table 3). The relative standard deviation for USP tailing was less than 2.0% on each system.

Testing for organic impurities of tioconazole was also performed according to the USP monograph. The standard for organic impurities test contained a mixture of related compound A (USP p/n 1667450), related compound B (USP p/n 1667461), and related compound C (USP p/n 1667472). The same standard was tested on both systems, and six replicate injections were performed. A representative chromatogram is shown in Figure1, as previously described. For quantitation of a sample, the tioconazole standard was prepared according to the USP monograph. The results (Figure 2, Table 4) produced impurity percentages within 0.01% on both systems. According to USP guidelines, "In such tests the limit at or below which a peak is disregarded is generally 0.05%," therefore, Related compound A was disregarded (Table 4).[3] Both related compound B

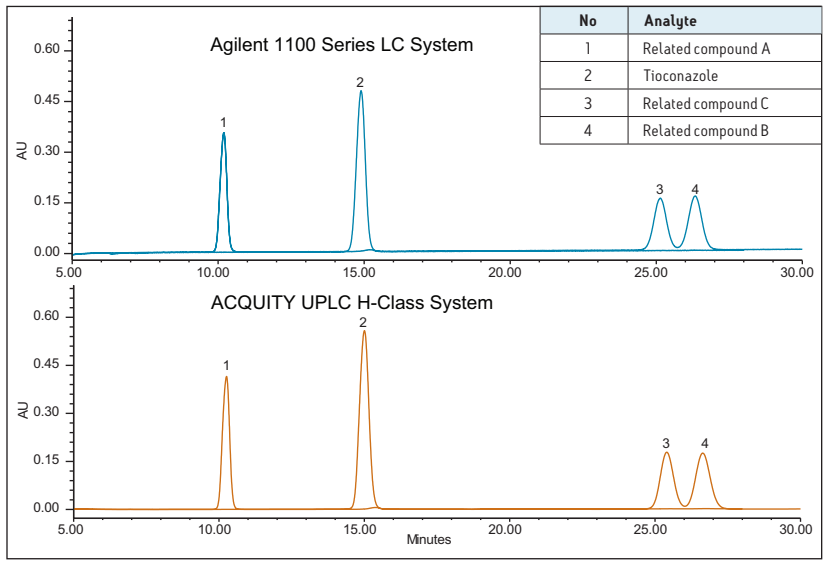

Figure 1. Assay and organic impurities analysis for tioconazole. The USP monograph was performed on both an Agilent 1100 Series LC System (top chromatogram) and an ACQUITY UPLC H-Class System (bottom chromatogram). Each chromatogram is an overlay of the standards for both the assay and organic impurities analysis.

Compound	Retention time (min)			Relative retention time		
	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	Percent deviation	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	Absolute deviation
Assay						
Ticonazole	14.91 (0.07)	15.01 (0.03)	0.67	N/A	N/A	N/A
Organic impurities						
Related compound A	10.18 (0.11)	10.26 (0.02)	0.79	0.683	0.684	0.001
Related compound C	25.11 (0.15)	25.42 (0.03)	1.23	1.684	1.694	0.010
Related compound B	26.3 (0.15)	26.65 (0.03)	1.33	1.764	1.775	0.011

Table 2. Comparison of average retention times and peak areas for tioconazole and related substances on an Agilent 1100 Series LC System and an ACQUITY UPLC H-Class System. Six replicate injections were performed. Retention times and relative retention times on both systems were within 2% deviation. %RSD for retention time of each analyte are in parentheses.

and related compound C were within USP acceptance criteria of NMT 1.0% of the API. While in this example, greater baseline noise was observed with the Agilent 1100 Series LC System, the disparity was consistent with instrument specifications[4, 5] and did not affect the analysis.

SUMMARY

For many laboratories, there is often a need to run existing HPLC methods on UHPLC or UPLC equipment while maintaining the separation and meeting system suitability requirements and acceptance criteria. This can be accomplished in method transfer from an Agilent 1100 LC Series System to an ACQUITY UPLC H-Class System. The USP assay for tioconazole, an isocratic separation, was successfully transferred across the systems from different manufacturers. Both systems met all acceptance and system suitability requirements for the USP monograph assay and organic impurities tests and demonstrated comparable separations. These results illustrate the ability to successfully transfer USP methods from HPLC instrumentation to properly configured ACQUITY UPLC H-Class System.

References

1. Official Monographs, USP 37 NF32 S2. United States Pharmacopeia and National Formulary (USP 37-NF 32 S2) Baltimore, MD: United Book Press, Inc.; 2014. p. 4968.
2. Easy Transfer of Standard HPLC Methods to the Agilent 1200 Series Rapid Resolution System. Agilent Technologies, Inc; 2006.
3. Chapter <621> CHROMATOGRAPHY United States Pharmacopeia and National Formulary (USP 37-NF 32 S1) Baltimore, MD: United Book Press, Inc.; 2014. p. 6376–85.
4. 1100 Series DAD and MWD Reference Manual. Germany: Agilent Technologies; 2004.
5. ACQUITY UPLC Photodiode Array (PDA) Detector Instrument Specifications. Milford, MA: Waters Corporation; 2014.

	USP plate count			USP tailing		
Compound	USP system suitability requirements	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	USP system suitability requirements	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System
Tioconazole	**NLT 1000 Theoretical plates**	10,780	10,009	**NMT 2.0**	0.998 (0.66)	1.02 (1.07)

Table 3. System suitability results for assay of tioconazole performed on an Agilent 1100 Series LC system and an ACQUITY UPLC H-Class System. Six replicate injections were performed. Testing performed on both systems met all system suitability criteria. Relative standard deviations in parentheses.

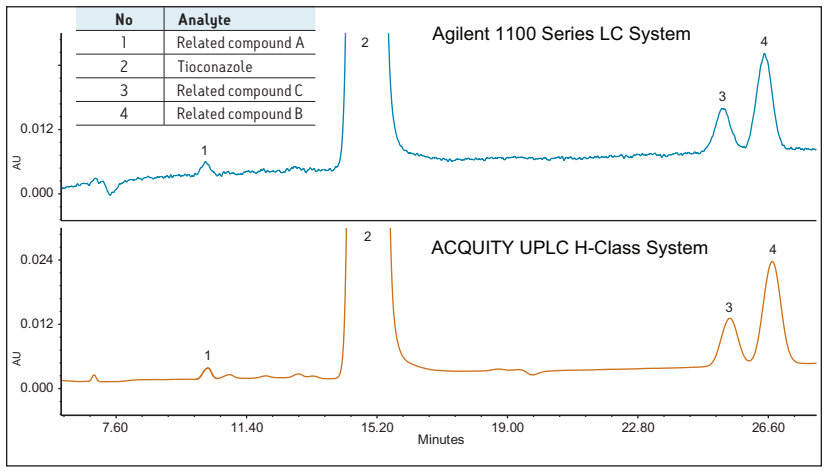

Figure 2. Oragnic impruities analysis for tioconazole. The USP monograph was performed on both an Agilent 1100 Series LC System (top chromatogram) and an ACQUITY UPLC H-Class System (bottom chromatogram).

Compound	% Impurity		
	Agilent 1100 Series LC System	ACQUITY UPLC H-Class System	**Absolute deviation**
Related compound A	0.01	0.005	**0.005**
Related compound C	0.06	0.05	**0.01**
Related compound B	0.12	0.11	**0.01**

Table 4. Results of USP monograph for organic impurities of tioconazole. The sample, a tioconazole standard, was purchased from USP and prepared according to the monograph. Six replicate injections were performed and the average was reported. All values (n=6) were within 0.01% on Agilent 1100 Series LC System and ACQUITY UPLC H-Class System. The calculated percent of each organic impurity was within the acceptance criteria of NMT 1.0%.

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Detection and Identification of Synthetic Phosphodiesterase Type-5 Inhibitors in Adulterated Herbal Supplements Using UPLC and Data-Directed Analysis by Mass Spectrometry

Marian Twohig, Andrew Aubin, Michael Jones, and Robert S. Plumb
Waters Corporation, Milford, MA, USA

INTRODUCTION

There are currently three synthetic phosphodiesterase type 5 (PDE5) inhibitors that have been approved by the U.S. Food and Drug Administration for the treatment of erectile dysfunction (ED): sildenafil citrate (brand name Viagra), vardenafil hydrochloride (brand name Levitra), and tadalafil (brand name Cialis). Their chemical structures are shown in Figure 3. These medications can be obtained legally by prescription from a licensed physician and they should be used under medical supervision.

It has been extensively reported that there have been adulterations to herbal dietary supplements (HDS) with synthetic drugs.[1-4] Natural aphrodisiacs are heavily advertised on the Internet. Some claim to enhance sexual function as natural alternatives to the three approved, synthetic PDE-5 inhibitors. Recently, there have been reports that these supposed natural alternatives have actually been illicitly adulterated with one of the pharmaceutical ED drugs or their structurally-modified analogues.[5-16]

When an HDS product is labelled as natural, there is also a sense of security that it is safe to use. Given that an HDS could contain undeclared synthetic drugs and that it can easily be obtained over the Internet, without prescription, there is the potential for a threat to public health.

It is therefore very important that analytical methods be able to rapidly detect both known PDE5 adulterants and potentially new analogues that have not yet been reported.

In this paper, we evaluate the use of the Xevo® TQ MS tandem quadrupole mass spectrometer, equipped with a novel collision cell design, for the detection and identification of PDE5 adulterants in herbal supplements.

ACQUITY UPLC System with Xevo TQ MS.

Survey scanning with the Xevo TQ MS

On a conventional tandem quadrupole mass spectrometer, the search for unknowns generally requires multiple injections: one injection in full-scan LC/MS mode, followed by a second injection for targeted LC/MS/MS experiments. This increases the time required to obtain necessary data, in addition to the time the analyst needs to construct MS/MS methods.

The Survey Scan feature of the Xevo TQ MS allows intelligent switching from LC/MS to LC/MS/MS data modes in a single run, thus improving productivity. Conventional MS or ScanWave™ MS scanning experiments can be used to trigger MS/MS experiments in real time as the peaks are eluting from the LC column. Conventional product ion or enhanced product ion spectra (ScanWave) data can be generated for all the components present in these complex samples.

In ScanWave mode, duty cycle improvements result in signal enhancement in scanning acquisition modes, which facilitates the detection of low-level adulterants.[17] A more targeted screen can also be performed, using parent ion or neutral loss spectral acquisition, to screen for compounds that have common structural features.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC®
Solvent delivery:	ACQUITY UPLC Binary Solvent Manager
Sample delivery:	ACQUITY UPLC Sample Manager
Column:	ACQUITY UPLC BEH C_{18}, 2.1 x 100 mm, 1.7 µm (P/N 186002352)
Column temp.:	60 °C
Sample temp.:	4 °C
Injection volume:	5 µL
Flow rate:	550 µL/min
Mobile phase A:	10 mM Ammonium acetate in water, pH 6.7
Mobile phase B:	50:50 Methanol/acetonitrile
Gradient:	0 to 9 min, 20 to 52% B, hold at 52% B until 10.5 min, 10.5 to 12.5 min 85% B, hold at 85% B until 15 min, then return to initial conditions

MS conditions

MS system:	Xevo TQ MS
Ionization mode:	ESI positive
Capillary voltage:	3.0 kV
Cone voltage:	35 V
Collision energy:	20 to 40 eV
Desolvation temp.:	400 °C
Desolvation gas:	800 L/Hr
Collision gas:	0.15 mL/min
Source temp.:	150 °C
Acquisition range:	100 to 700 amu
Scan speed:	5000 amu/sec

PDA conditions

Range:	210 to 400 nm
Sampling rate:	20 points/sec

Sample extraction

The crushed tablet sample or powdered capsule contents were transferred quantitatively to a 100 mL volumetric flask, and sonicated in 50:50 methanol/water for 20 minutes. A representative aliquot was transferred to a centrifuge tube and centrifuged at 3000 RPM for 10 minutes. A portion of the supernatant was placed in a vial and used for analysis by LC/MS.

A summary of the samples

Five products were obtained over the Internet (Figure 2) and analyzed by UPLC/MS/MS using data-directed analysis on the Xevo TQ MS. Four capsules and one tablet supplement were purchased. All five were found to be adulterated, containing sildenafil and/or tadalafil, or analogues of these drugs.

- Sample 1, a capsule, was found to be adulterated with tadalafil only.
- Sample 2, a capsule, contained sildenafil, although it shared the same product name as Sample 1 but was shipped from a different geographical location.
- Sample 3, the tablet, contained sildenafil, tadalafil, and other compounds we believe are related to the API.
- Sample 4, a capsule, contained sildenafil and tadalafil.
- Sample 5, a capsule, contained what we suspect to be analogues of the known synthetic PDE5 inhibitors.

Figure 2. Some of the adulterated herbal supplement products obtained over the Internet.

Survey scan of sildenafil, vardenafil, and tadalafil standards

A survey scan of a standard solution of sildenafil, vardenafil, and tadalafil is shown in Figure 4. The primary function is ScanWave MS, which switches to ScanWave DS when a peak is detected to acquire a full MS/MS spectrum from 50 to 700 amu at a collision energy of 30 eV.

In the spectrum for tadalafil (Figure 5) from the peak eluting at retention time (RT) 6.21 min, *m/z* 390, several diagnostic fragments are seen: *m/z* 268, *m/z* 262, *m/z* 169, and *m/z* 135.

In the spectrum for sildenafil, RT 8.52 min, fragments *m/z* 377, *m/z* 311, *m/z* 283, and *m/z* 99 are seen.

The spectrum for vardenafil, at RT 8.91 min, shows MS/MS fragments of *m/z* 312, *m/z* 299, *m/z* 169, and *m/z* 99. These ions can be used to confirm peak identity.

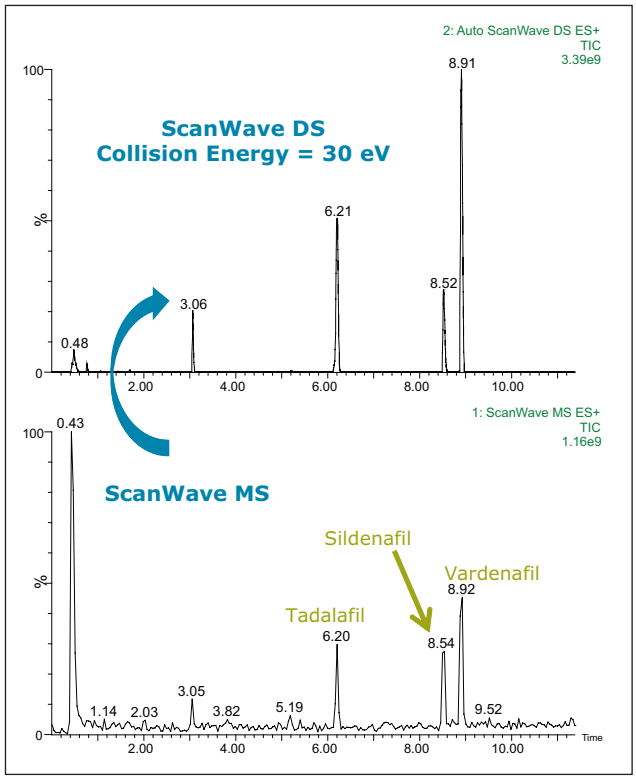

Figure 4. Survey ScanWave MS (lower trace) switching to ScanWave DS for a standard mix of tadalafil, RT 6.21 min, sildenafil, RT 8.52 min, and vardenafil, RT 8.91 min.

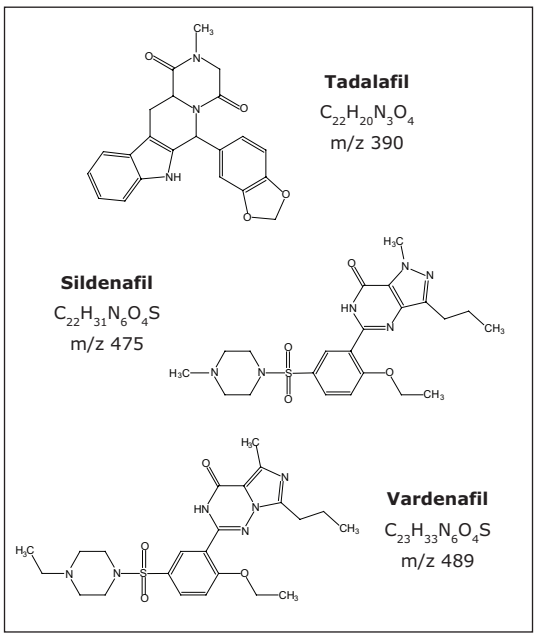

Figure 3. Structures of tadalafil, sildenafil, and vardenafil.

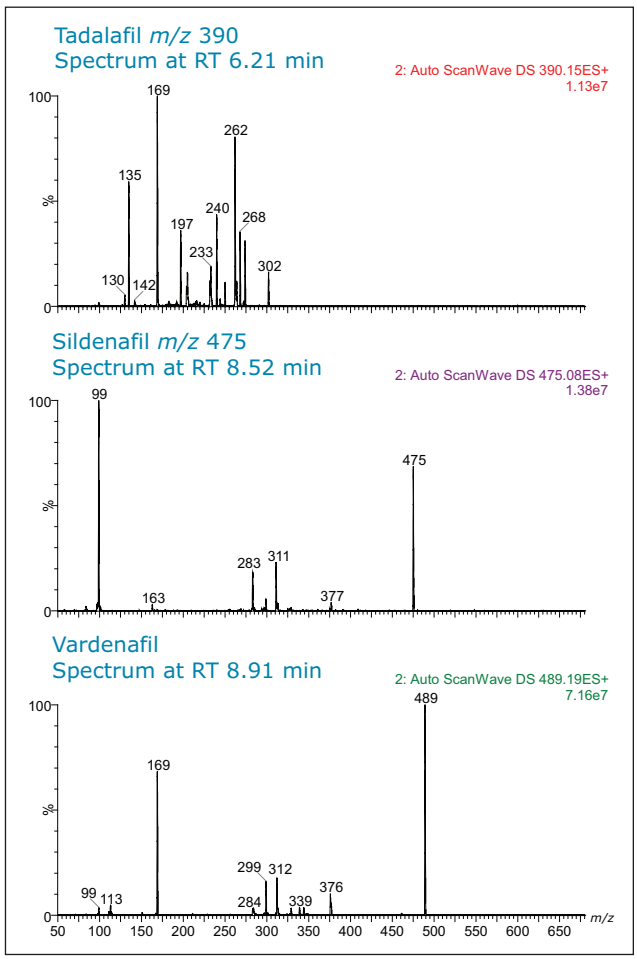

Figure 5. Spectra in ScanWave DS mode for tadalafil, sildenafil, and vardenafil, of the chromatographic peaks shown in Figure 3 (top).

Tadalafil adulteration

Sample 1 was found to be adulterated with tadalafil, as can be seen in Figures 6 and 7. The spectrum of a tadalafil standard at a collision energy of 35 eV (Figure 7 inset) exactly matches the spectrum taken at 6.18 minutes in the Sample 1 chromatogram. The survey scan was set up to collect two MS/MS functions, one at a collision energy of 25 eV and a second at 35 eV. This is advantageous in the analysis of complex samples, where the range of collision energies required to obtain useful structural information can vary.

The signature fragments of tadalafil, *m/z* 268, *m/z* 169, and *m/z* 135, can be seen in the spectra.

This sample indicated the presence of many natural ingredients, including *Dioscorea spinosina* (wolfberry fruit), *Glycyrrhiza glabra* (liquorice root), as well as others. Neither the patient information nor the packaging declared the presence of tadalafil in the product.

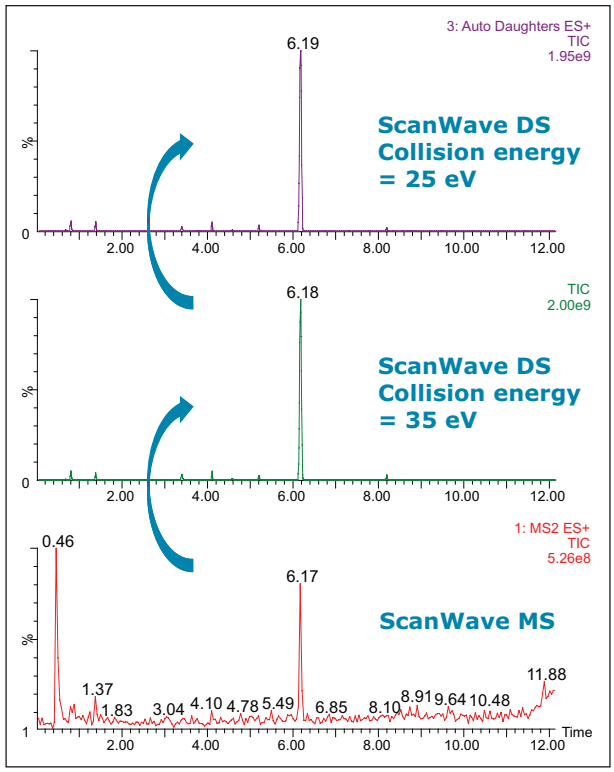

Figure 6. Survey ScanWave MS (lower trace) switching to ScanWave DS at collision energies of 25 and 35 eV for Sample 1.

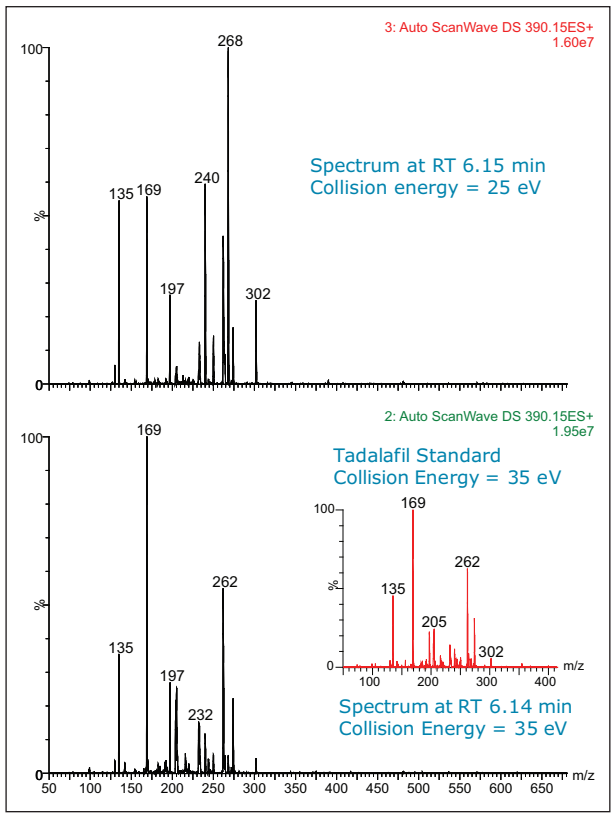

Figure 7. MS/MS spectra in ScanWave DS mode from the chromatographic peaks at RT 6.18 min at collision energies of 25 and 35 eV.

Tadalafil and sildenafil adulteration

Sample 3, in tablet form, was found to be adulterated with tadalafil and sildenafil. A survey function is shown in Figure 8 and selected spectra are shown in Figure 9. The spectra taken at RT 6.18 min and RT 8.5 min match with the spectra from a standard mix of tadalafil and sildenafil (see Figure 4). The survey scan was set up to collect two collision energy functions, one at 25 eV and a second at 35 eV. The expected diagnostic fragments were apparent in the sildenafil spectrum *m/z* 311, *m/z* 283, and *m/z* 99.

In addition to finding known compounds, it can be seen from the MS/MS fragmentation patterns in Figure 9 that there are other potentially related impurities in the sample. Many of them are above the ICH guideline's reporting threshold of 0.05% of the active pharmaceutical ingredient (API) peak (measured at 230 nm). In spectrum 1 shown in Figure 9, the precursor mass of *m/z* 503 gives rise to MS/MS fragments *m/z* 99, *m/z* 283, and *m/z* 311.

In spectra numbers 2, 4, 5, 7, and 8, one or more of these fragments are present. This information was obtained from one survey experiment without the need for extra confirmatory MS/MS analyses. This allows the analyst to acquire important structural information in a single run.

The patient information leaflet for this tablet sample said it contained 10 rare animal and plant extracts. It also stated on the packaging and in the patient information that it was acceptable for persons with heart disease and hypertension to take it. However, use of synthetic PDE5 inhibitors is contraindicated when a patient is currently taking nitrate medications (i.e., nitroglycerin) as it can cause low blood pressure – there can be a synergistic effect of these drugs in relaxing vascular smooth muscle and drastically lowering blood pressure.[18] Consequently, men in this group who suffer from ED may resort to seeking out this kind of herbal alternative – not knowing the remedy's actual risk to their heath.

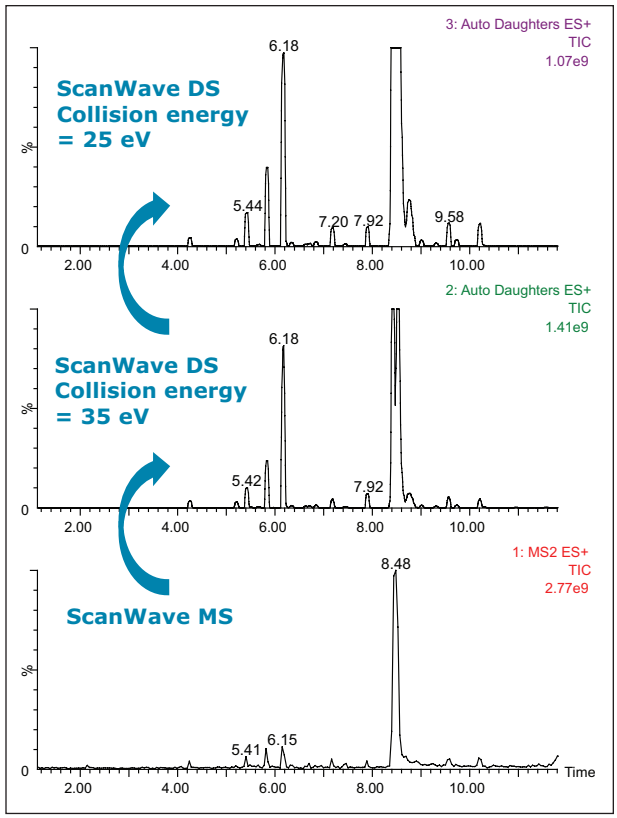

Figure 8. Survey ScanWave MS (lower trace) switching to ScanWave DS at a collision of 25 and 35 eV for Sample 3.

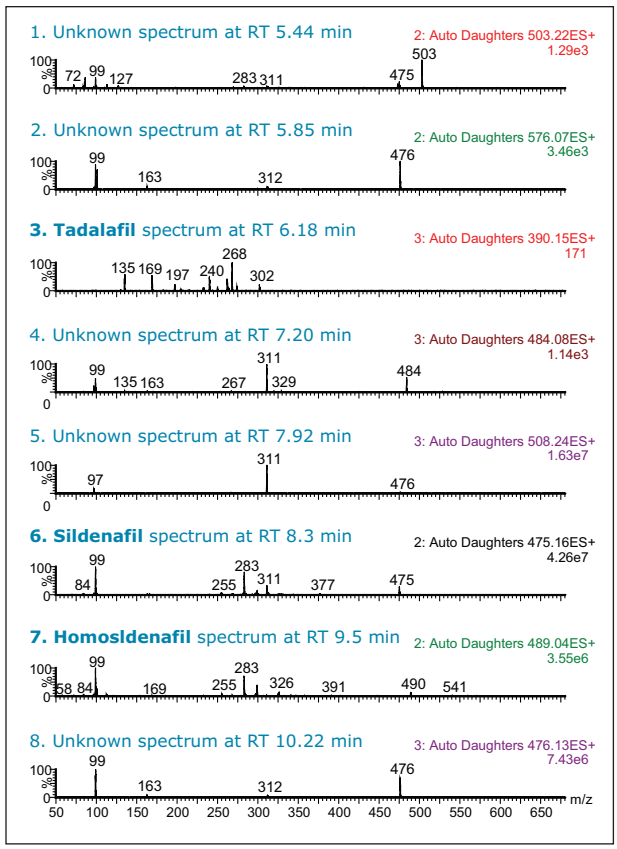

Figure 9. MS/MS spectra in ScanWave DS mode from selected chromatographic peaks shown in Figure 8.

Adulteration with analogues

Sample 5 was analyzed using a more targeted approach. A precursor scan of *m/z* 99 was used to trigger a ScanWave DS MS/MS function. Sildenafil, tadalafil, and vardenafil were not found in this sample. A chromatographic peak with a RT of 8.38 min had precursor mass of *m/z* 489. The retention time of this component did not match that of vardenafil, *m/z* 489 at RT 8.92 min, or homosildenafil, *m/z* 489 at RT 9.5 min.

However, when Sample 5 was subjected to MS/MS fragmentation, the characteristic fragments of sildenafil were seen: *m/z* 99, *m/z* 283, and *m/z* 311. There was also another fragment observed, *m/z* 113. The presence of this ion in certain analogues has been reported previously in literature.[13] The other chromatographic peaks, at retention times 10.70, 12.06, and 12.71 min, showed common fragments for the *m/z* 99 and *m/z* 113. These peaks are likely to arise from structural analogues of sildenafil.

This sample also declared on its packaging that it is all-natural, stating that it has helped support male performance for centuries. The supplement's ingredients were supposed to contain wild yam extract, Siberian Ginseng extract, jujube extract, and cayenne extract, as well as others.

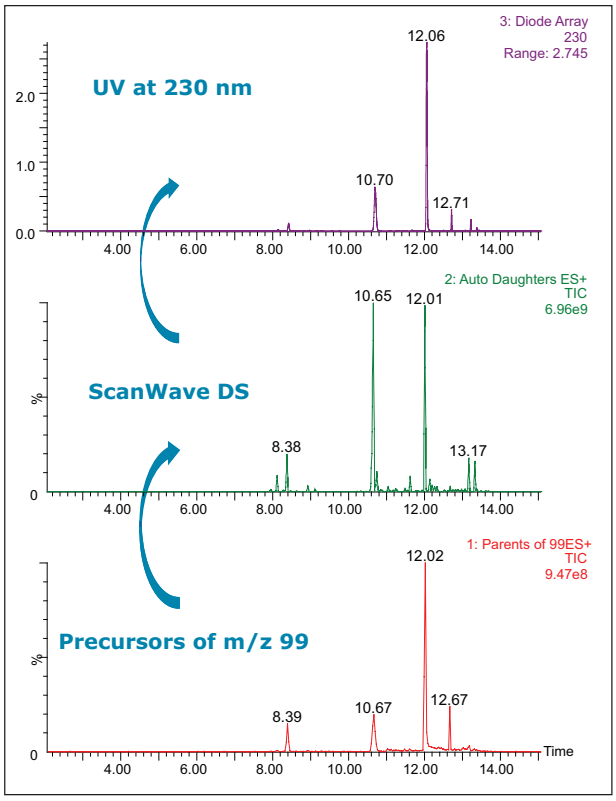

Figure 10. Survey precursors of m/z 99 are observed (lower trace) when switching to ScanWave DS for Sample 5. UV data was collected simultaneously.

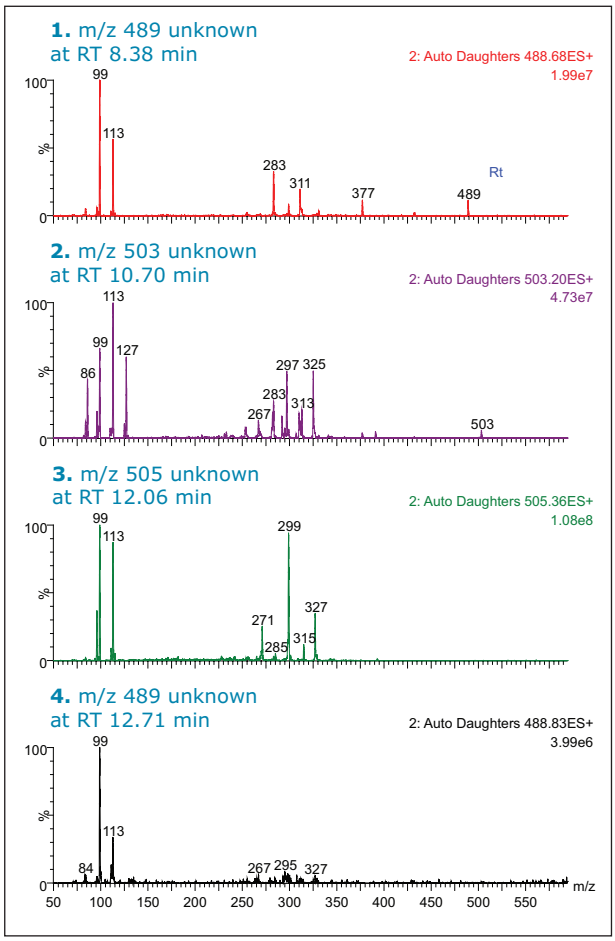

Figure 11. MS/MS Spectra in ScanWave DS mode from selected chromatographic peaks shown in Figure 9.

Quantitation of tadalafil and sildenafil in HDS samples

In an effort to understand the levels of adulteration to the samples, sildenafil and tadalafil were quantified. The drug substances for tadalafil and sildenafil citrate were purchased. Stock solutions of sildenafil and tadalafil were prepared at a concentration of 1.0 mg/mL in methanol. Working standard solutions were prepared at concentrations of 0.1 ng/mL to 500 ng/mL in a simulated matrix. This was performed using extraction of a mixture of some of the ingredients listed on the patient information of the HDS samples and subsequently spiking the resulting solution with the appropriate levels. Quantitation curves were injected in triplicate and were linear over the calibration range for both tadalafil and sildenafil (Figures 12 and 13). QC samples were within acceptable limits (< 15%).

The range of sildenafil measurements quantified in the HDS samples that were purchased via the Internet was 0.078 to 116.68 mg/dose. The range of tadalafil measurements was 13.47 to 52.14 mg/dose.

For samples 1 through 4, these concentrations would be at therapeutic levels.

- In Sample 2, the sildenafil was calculated to be 116.7 mg/dose.
- Sample 3 contained both sildenafil and tadalafil at levels of 88.7 and 26.7 mg/dose.
- Sample 5 could not be accurately quantified as it contained suspected analogues for which there was no available standard.

It should be noted that the analytical method used for quantitation of the samples had not been validated at the time when these results were reported.

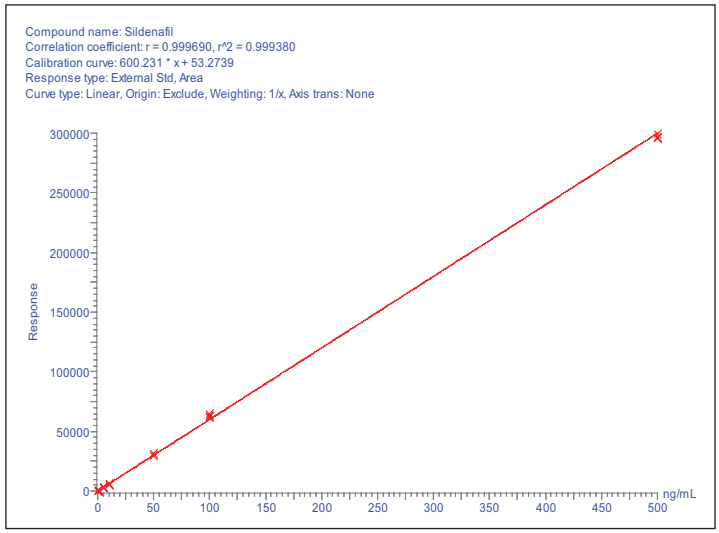

Figure 12. Quantitation curve for sildenafil 0.1 to 500 ng/mL.

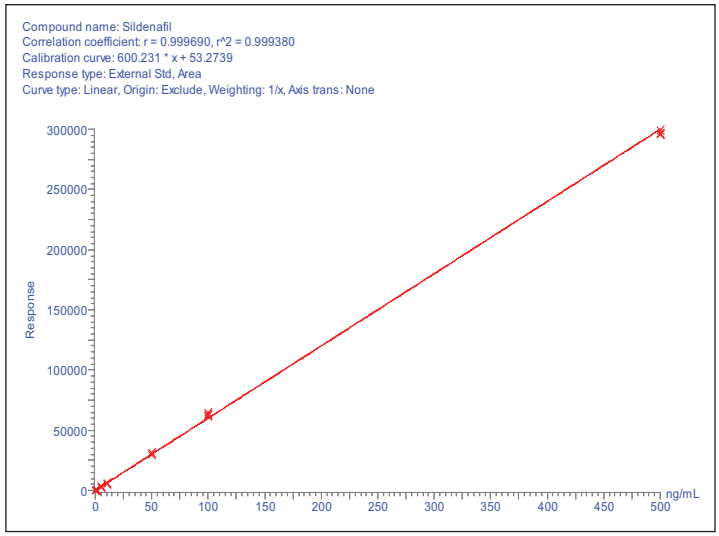

Figure 13. Quantitation curve for tadalafil , 0.1 to 500 ng/mL.

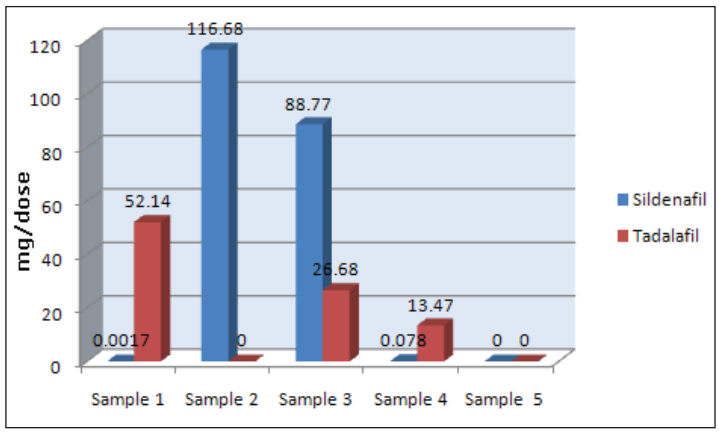

Figure 14. Summary of the calculated concentrations of sildenafil and radalafil for the HDS Samples 1 through 5.

CONCLUSIONS

Adulteration of herbal dietary supplements with synthetic pharmaceuticals is a growing problem. There have been reports in published literature that show many of the supposed natural alternatives to approved synthetic PDE5 inhibitors used to treat ED are actually adulterated with them. Many reports also indicate that this adulteration of herbal additives is a growing trend. Due to the threat to public health posed by unknowingly ingesting both the known PDE5 inhibitors and their analogues, it is vital that analysts have tools that allow them to characterize these complex samples adequately.

The Waters Xevo TQ Mass Spectrometer, with its unique collision cell design, facilitates the simultaneous acquisition of MS and MS/MS data in one LC/MS run. Its high scan speed of up to 10,000 amu/sec allows for these experiments to be performed with sufficient points across the peak to accurately define the narrow peaks produced by UPLC. This capability facilitates data-dependant experiments where real-time switching between MS and MS/MS allows more information to be acquired from a single injection. This reduces the need for separate experiments and accelerates the process of structural identification and unknown compound determination.

The value of the using the Waters Xevo TQ MS has been demonstrated for the analysis of adulterated herbal dietary supplements. Furthermore, use of the Xevo TQ MS for quantitation of the samples revealed that the doses of the sildenafil and tadalafil are sufficiently high to be therapeutic.

References

1. Koh HL, Woo SO. Chinese proprietary medicine in Singapore: regulatory control of toxic heavy metals and undeclared drugs. *Drug Saf.* 2000 Nov; 23(5): 351–62.
2. Huang WF, Wen KC, Hsiao ML. Adulteration by synthetic therapeutic substances of traditional Chinese medicines in Taiwan. *J Clin Pharmacol.* 1997 Apr; 37 (4): 344–50.
3. Bogusz MJ, Hassan H, Al-Enazi E, Ibrahim Z, Al-Tufail M. Application of LC-ESI-MS-MS for detection of synthetic adulterants in herbal remedies. *J Pharm Biomed Anal.* 2006 May 3; 41(2): 554–64. Epub 2006 Jan 19.
4. Lau AJ, Holmes MJ, Woo SO, Koh HL. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography-mass spectrometry-mass spectrometry. *J Pharm Biomed Anal.* 2003 Feb 26; 31(2): 401–6.
5. Gratz SR, Flurer CL, Wolnik KA. Analysis of undeclared synthetic phosphodiesterase-5 inhibitors in dietary supplements and herbal matrices by LC-ESI-MS and LC-UV. *J Pharm Biomed Anal.* 2004 Nov 15; 36(3): 525–33.
6. Liang Q, Qu J, Luo G, Wang Y. Rapid and reliable determination of illegal adulterant in herbal medicines and dietary supplements by LC/MS/MS. *J Pharm Biomed Anal.* 2006 Feb 13; 40(2): 305–11. Epub 2005 Sep 19.
7. Reepmeyer JC, Woodruff JT. Use of liquid chromatography-mass spectrometry and a hydrolytic technique for the detection and structure elucidation of a novel synthetic vardenafil designer drug added illegally to a "natural" herbal dietary supplement. *J Chromatogr A.* 2006 Aug 25; 1125(1): 67–75. Epub 2006 Jun 5.
8. Zou P, Oh SS, Hou P, Low MY, Koh HL. Simultaneous determination of synthetic phosphodiesterase-5 inhibitors found in a dietary supplement and pre-mixed bulk powders for dietary supplements using high-performance liquid chromatography with diode array detection and liquid chromatography-electrospray ionization tandem mass spectrometry. *J Chromatogr A.* 2006 Feb 3;1104 (1–2):113–22. Epub 2005 Dec 20.
9. Reepmeyer JC, Woodruff JT. Use of liquid chromatography-mass spectrometry and a chemical cleavage reaction for the structure elucidation of a new sildenafil analogue detected as an adulterant in an herbal dietary supplement. *J Pharm Biomed Anal.* 2007 Aug 15; 44(4): 887–93. Epub 2007 Apr 18.
10. Venhuis BJ, Blok-Tip L, de Kaste D. Designer drugs in herbal aphrodisiacs. *Forensic Sci Int.* 2008 May 20; 177(2–3): e25–7. Epub 2008 Feb 21.
11. Ge X, Low MY, Zou P, Lin L, Yin SO, Bloodworth BC, Koh HL. Structural elucidation of a PDE-5 inhibitor detected as an adulterant in a health supplement. *J Pharm Biomed Anal.* 2008 Dec 1; 48(4): 1070–5. Epub 2008 Aug 23.
12. Lam YH, Poon WT, Lai CK, Chan AY, Mak TW. Identification of a novel vardenafil analogue in herbal product. *J Pharm Biomed Anal.* 2008 Mar 13; 46(4): 804–7. Epub 2007 Dec 8.
13. Singh S, Prasad B, Savaliya AA, Shah RP, Gohil VM, Kaur A. *Trends in Anal. Chem.* 2009; (28): 1.
14. Reepmeyer JC, d'Avignon DA. Structure elucidation of thioketone analogues of sildenafil detected as adulterants in herbal aphrodisiacs. *J Pharm Biomed Anal.* 2009 Jan 15; 49(1):145–50. Epub 2008 Oct 21.
15. Zou P, Hou P, Oh SS, Chong YM, Bloodworth BC, Low MY, Koh HL. Isolation and identification of thiohomosildenafil and thiosildenafil in health supplements. *J Pharm Biomed Anal.* 2008 Jun 9; 47(2): 279–84. Epub 2008 Jan 18.
16. Venhuis BJ, Zomer G, de Kaste D. Structure elucidation of a novel synthetic thiono analogue of sildenafil detected in an alleged herbal aphrodisiac. *J Pharm Biomed Anal.* 2008 Mar 13;46(4): 814–7. Epub 2007 Dec 15.
17. Twohig M, Alden P, Fujimoto G, Kenny D, Plumb RS. Improving MS/MS Sensitivity using Xevo TQ MS with ScanWave. Waters Corporation. 2008; 720002828en.
18. 15 DeBusk RF, Kloner RA. Rationale for not combining nitrates and PDE5 inhibitors. *Journal of Family Practice.* 2005; Dec.

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Using a Scientific Data Management System to Manage Impurity Profiling Test Results and Data

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Waters Corporation, Milford MA, U.S.

APPLICATION BENEFITS

This application note describes the advantages of employing the data management and reporting capabilities of NuGenesis SDMS during an impurity profiling project.

WATERS SOLUTIONS

NuGenesis® SMDS

SDMS Vision Publisher

KEY WORDS

Impurity profiling, data management, compliant-ready reporting

INTRODUCTION

The objective of impurity profiling is to identify and quantitate impurities that are present in an API or drug product. Impurities may take the form of three broad classifications: (1) organic impurities, (2) inorganic impurities, and (3) residual solvent impurities. Organic impurities typically arise from the manufacturing process and may include unreacted starting materials, reaction intermediates, degradation products, and reaction by-products. Some of these impurities may even be genotoxic. Inorganic impurities include ligands and catalysts, heavy metals, inorganic salts, and filter aids. Residual solvents can be either organic or inorganic solvents used during manufacturing.

The U.S. FDA and other regulatory agencies require identification and quantitation of impurities above specific levels. Hence, conducting impurity profiling projects requires thorough documentation, robust data management, and the use of a variety of analytical techniques, e.g., LC/UV, LC/MS, and NMR, in order to provide prove that the impurities have been properly characterized.

With the variety of techniques used and the complexity of the data and reports generated, the scientists and management involved need a robust documentation system to systematically store and catalog the data and then combine the data and results into suitable reports. This laborious and potentially error-prone task is often performed manually due to the vastly different data formats the analytical instruments generate.

The Waters NuGenesis® Scientific Data Management System (SDMS) can automatically capture and catalog analytical data produced during an impurity profiling project into a centralized data repository (Figure 1). The system captures both raw analytical data and printed test reports from all laboratory instruments used during an impurity profiling project, e.g., LC/UV, LC/MS, NMR, and GC/MS. Capturing data within a centralized repository aids data review and approval, streamlines report creation, and promotes interdisciplinary collaboration.

In addition to data collection and archiving functionality, NuGenesis SDMS includes an analytical Electronic Laboratory Notebook (ELN) called SDMS Vision Publisher™. SDMS Vision Publisher gives scientists and management the ability to quickly and easily create reports in many formats.

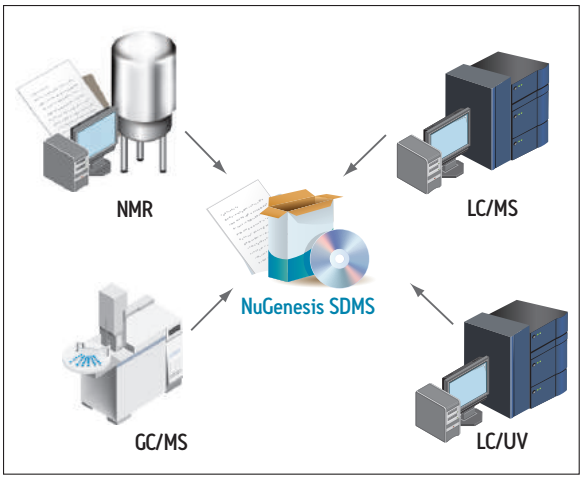

Figure 1. NuGenesis SDMS serves as the compliant-ready central analytical data repository for an impurity profiling project. The system can capture and catalog analytical raw data and printed test reports from a variety of analytical instruments during the course of any analytical project.

Managing impurity profiling data

A typical impurity profiling experiment may take place using four key steps (Figure 2). First, a sample is collected and prepared for analysis. Second, the sample is analyzed by using various analytical instruments such as LC/MS or NMR. Third, the raw data created by the instrument is processed using the relevant software application to provide information that will assist with identification and quantification. Fourth, a printed test result is created to summarize the instrumental results. Subsequently, this impurity profiling workflow can generate four different types of data:

- Sample preparation records
- Instrument data that includes both the physical measurement and the instrumental parameters
- A data file describing the data processing parameters and results
- Test results (electronic and or printed) that summarize instrumental parameters and findings, including examples of spectra or chromatograms.

In many analytical laboratories, data management tasks are typically the responsibility of laboratory personnel. With NuGenesis SDMS, electronic and printed raw data and reports can be automatically captured and cataloged in the SDMS database (Figure 2). This level of automation can significantly reduce the time and effort associated with data acquisition, processing, reporting, and archiving.

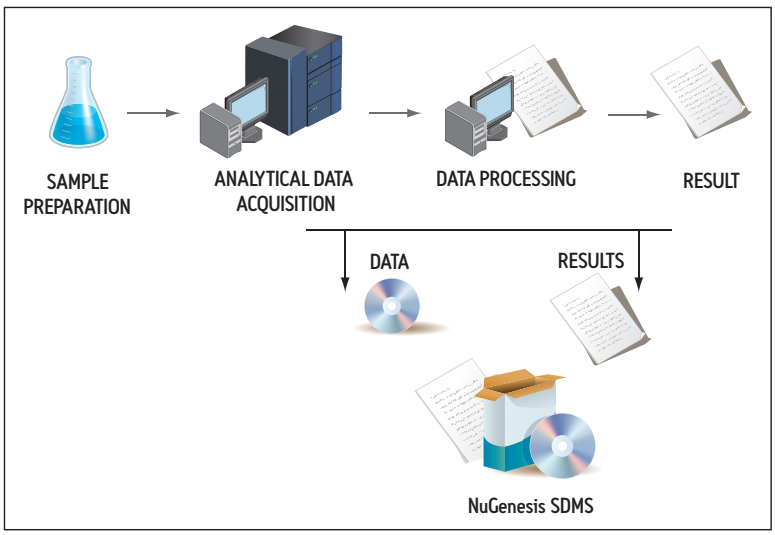

Figure 2. Typical impurity profiling workflow proceeds in four primary steps: (1) Sample collection and preparation, (2) analysis of sample by using an analytical technique, (3) processing of raw analytical data to return a result, and (4) the analytical test result report. NuGenesis SDMS automatically captures and catalogs all analytical data and reports to support an impurity profiling workflow.

Summarizing key findings

Capturing and cataloging diverse analytical data (such as that generated during an impurity profiling project) into NuGenesis SDMS enables centralized data storage and standardized data format. Then, SDMS Vision Publisher, acting as a portal into the SDMS data repository, streamlines impurity report creation by providing capabilities to combine diverse data and analytical reports into one seamless summary report. The documentation workflow from NuGenesis SDMS to SDMS Vision Publisher is shown in Figure 3.

For example, a typical summary report (which may consist of comments, observations as well as chromatograms, spectra, tables, etc.) is shown in Figure 4.

In addition, it is possible to directly incorporate a precursor ion scan (LC/MS/MS) test result from within the integrated NuGenesis SDMS data repository into the impurity profiling report. This is shown in Figure 5.

The integration of SDMS Vision Publisher with NuGenesis SDMS provides many benefits to the laboratory scientist and their management. For example, the SDMS Vision Publisher report is traceable back to the original results by following hyperlinks. Completed reports are finalized by electronic review and sign-off. In addition, by using SDMS Vision Publisher for report creation along with electronic review and sign-off, the time and effort required to create impurity profiling reports is dramatically reduced thereby enhancing the productivity of scientists and the analytical laboratory.

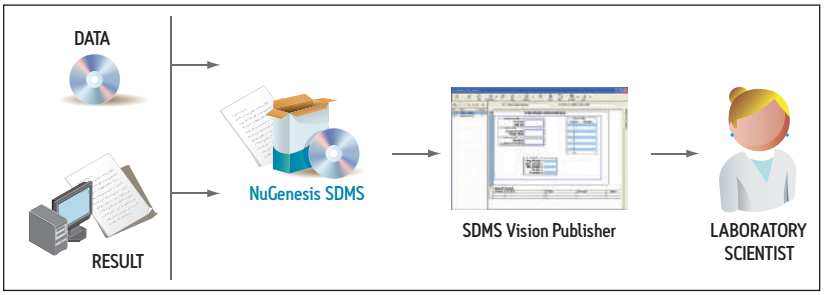

Figure 3. An impurity profiling analyst utilizes SDMS Vision Publisher to summarize key findings into a final research report. SDMS Vision Publisher serves as a portal into the NuGenesis SDMS central data repository to facilitate integration of key information from a variety of instrumental data and reports into a finalized impurity profiling report.

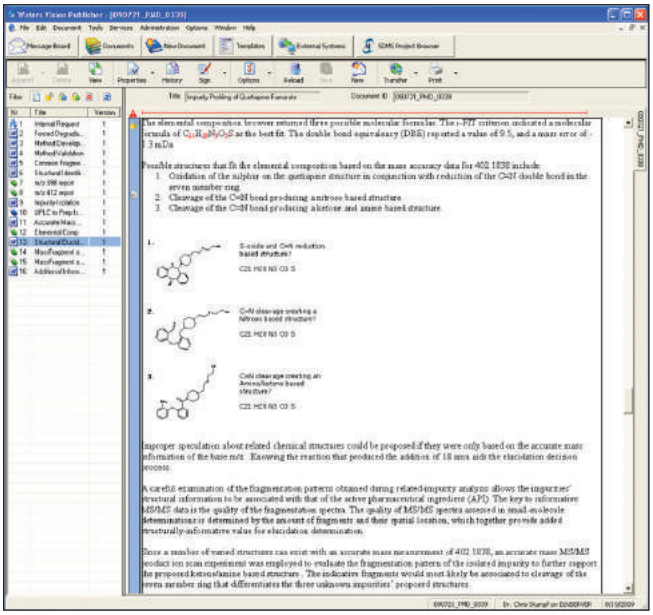

Figure 4. An example impurity profiling summary report created within SDMS Vision Publisher. The report consists of observations made by the scientist as well as printed results that were integrated via the NuGenesis SDMS analytical data repository.

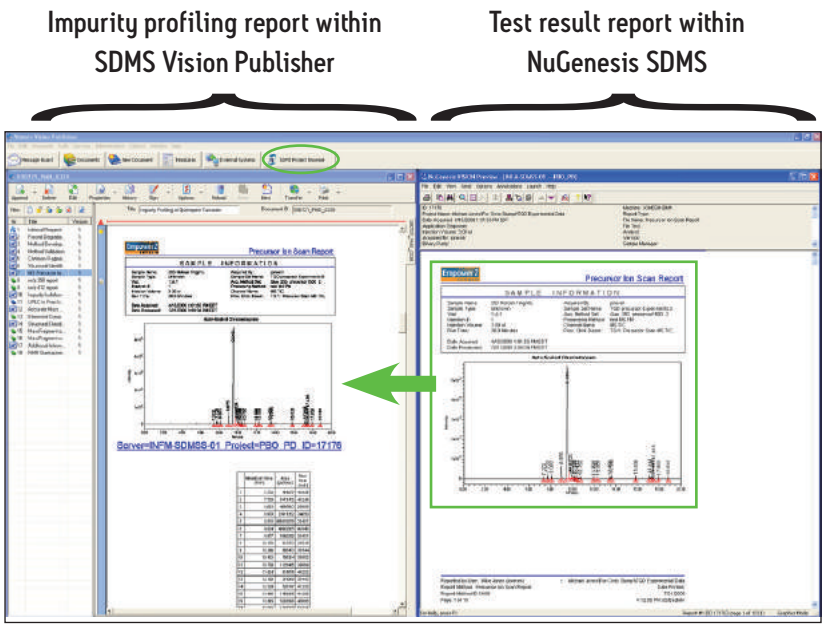

Figure 5. The integration of SDMS Vision Publisher with the NuGenesis SDMS data repository (green oval) streamlines transfer of important results (green rectangle) into an impurity profiling report. Data traceability is maintained within the impurity profiling report by incorporation of a hyperlink back to the original test result held within the NuGenesis SDMS data repository.

CONCLUSIONS

NuGenesis SDMS and SDMS Vision Publisher streamline data management and report authoring for all analytical laboratory experiments including impurity profiling.

Key benefits of this integrated Informatics solution include:

- Automatically capture and catalog data and test results from all analytical instruments, e.g. LC/UV, LC/MS, GC/MS, and NMR.
- Enhanced laboratory productivity for all personnel involved in analytical experiments and reporting, including a standardized and central data repository that streamlines review and signoff.
- Integrated analytical ELN facilitates content re-use and report authoring.
- The solution is 21 CFR Part 11 compliant-ready.

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Rapid Identification of Genotoxic Impurities in Tablets Using the ASAP Probe

Joanne Mather, Dominic Moore, Robert S. Plumb, and Paul Rainville
Waters Corporation, Milford, MA, U.S.

APPLICATION BENEFITS

Demonstrates a rapid, simple and powerful approach to genotoxic impurity identification at the Threshold of Toxicological Concern (TTC) using the Xevo TQD when used in a qualitative manner with the ASAP Probe and product ion confirmation (PIC).

WATERS SOLUTIONS

Xevo® TQD

ASAP Probe

KEY WORDS

IntelliStart, genotoxins, impurities

INTRODUCTION

Alkyl sulfonic acids, particularly methanesulfonic acid, benzenesulfonic acid, and p-toluenesulfonic acid, are a common class of alkylating agents used in the pharmaceutical industry as alkylating reagents, catalysts, and in purification steps in the chemical synthesis of an API. In addition, these sulfonic acids are often used as the final salt form of the drug due to improved chemical properties or bioavailability.

The presence of any residual alcohols from synthetic reaction or re-crystalization steps may result in the formation of alkyl esters of the sulfonic acids. Many of these mesylate, besylate, or tosylate esters are known to be genotoxic while others are potentially genotoxic, requiring monitoring in the drug substance and drug product.

Typical methods utilized in the past for the analysis of these akyl sulfonate esters have been based on GC/MS or HPLC/UV/MS, with derivatization typically using run times in the order of 20 to 30 minutes. We have previously demonstrated how good results can also be achieved using UPLC®/MS with run times of less than five minutes.

In this application note, we show how the presence of genotoxic impurities at Threshold of Toxicological Concern (TTC) can be quickly and easily detected using the Xevo TQD, a tandem quadrupole detector, with an Atmospheric Pressure Solids Analysis Probe (ASAP Probe).

EXPERIMENTAL

The Xevo TQD was tuned to each of the three impurity standard solutions using the on-board fluidics and IntelliStart™ allowing the instrumental conditions for the tablet analysis to be chosen quickly and easily.

MS conditions

MS system:	Xevo TQD

ASAP positive ion

Polarity:	API+
Corona:	0.50 µA
Corona:	1.5 kV
Cone:	30.00 V
Extractor:	3.00 V
Source temp.	150 °C
Probe temp.	450 °C
Desolvation gas flow:	400 L/Hr

ASAP negative ion

Polarity	API-
Corona:	0.80 µA
Corona:	1.5 kV
Cone:	30.00 V
Extractor:	3.00 V
Source temp.	150 °C
Probe temp.	450 °C
Desolvation gas flow:	400 L/Hr

RESULTS AND DISCUSSION

A single 10-mg amlodipine besylate tablet was crushed and solubilized in 5 ml of acetonitrile. The supernatant was removed and diluted 1:1 with water to give a 1 mg/mL solution of amlodipine besylate. Standard solutions of methyl- and ethyl- benzene sulfonates and ethyl-toluene sulfonate were prepared at 1 mg/mL in acetonitrile, and diluted to 15 µg/mL with water. These were spiked into the 1 mg/mL amlodipine solution using a 1:100 dilution, which equates to final impurities concentration of 1.5 µg (0.015%) per 10 mg tablet or the TTC when based on a single 10 mg per day dose of amlodipine besylate.

The ASAP probe was dipped into the spiked tablet solution, placed into the source and analyzed directly in multiple reaction monitoring (MRM) mode with product ion confirmation (PIC)[1] enabled. The Xevo TQD can be used to perform quantification of a sample with simultaneous characterization of the MRM peak as it elutes from the chromatographic system – or as shown in this case the ASAP MRM peak.

This eliminates the need for separate injections when qualitative confirmation of MRM peaks is required and reduces the total analysis time in these situations. When used routinely, PIC increases user confidence in qualitative results from complex matrixes, and thus reduces the need for re-analysis.

The presence of each of the impurities was confirmed in the spiked tablet solution, and the identities of the impurities confirmed using PIC.

Compound	Formula/Mass		Parent m/z	Cone Voltage	Daughters	Collision Energy	Ion Mode
methyl benzenesulfonate	C7H8O3S	1	172.92	34	91.08	20	ES+
		2	172.92	34	76.97	16	ES+
		3	172.92	34	65.00	32	ES+
		4	172.92	34	141.02	14	ES+

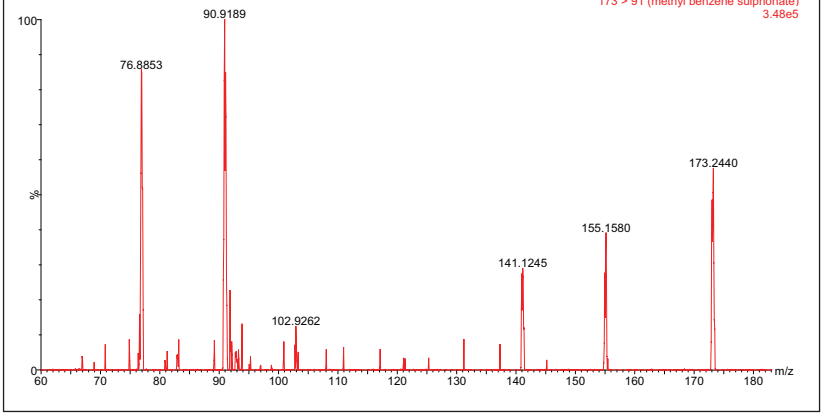

Figure 1. Methyl benzenesulfonate.

ethyl benzenesulfonate	C8H10O3S	1	204.07	6	76.95	34	ES+
		2	186.88	24	158.94	10	ES+
		3	186.88	24	76.96	24	ES+

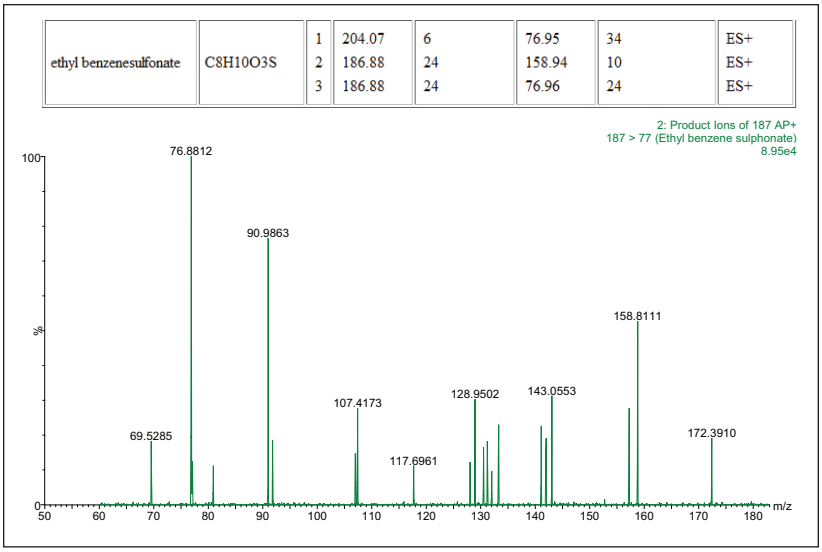

Figure 2.
Ethyl benezenesulfonate.

ethyl p-toluenesulfonate	C9H12O3S	1	201.15	20	173.05	8	ES+
		2	201.15	20	91.09	22	ES+
		3	218.21	12	91.10	30	ES+
		4	201.15	20	155.01	16	ES+

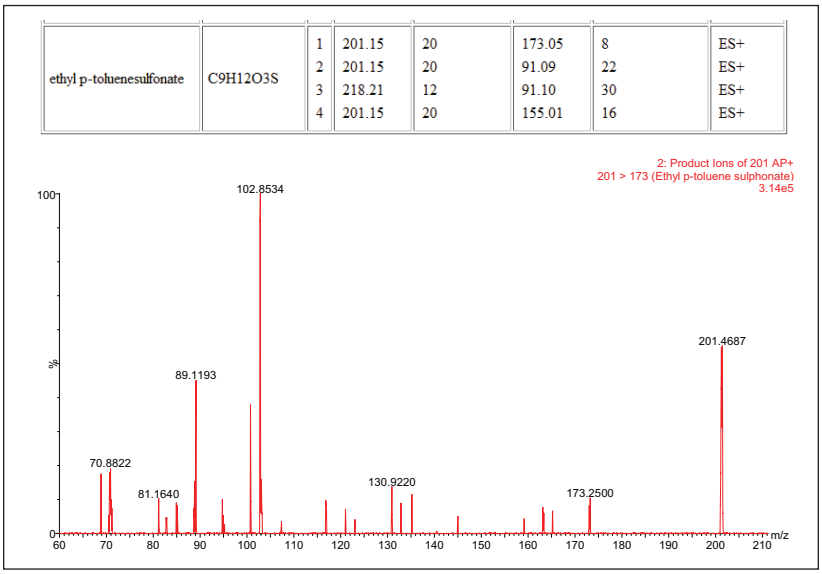

Figure 3. Ethyl
p-toluenesulfonate.

methanesulfonic acid	CH4O3S	1	94.89	40	79.94	16	ES-

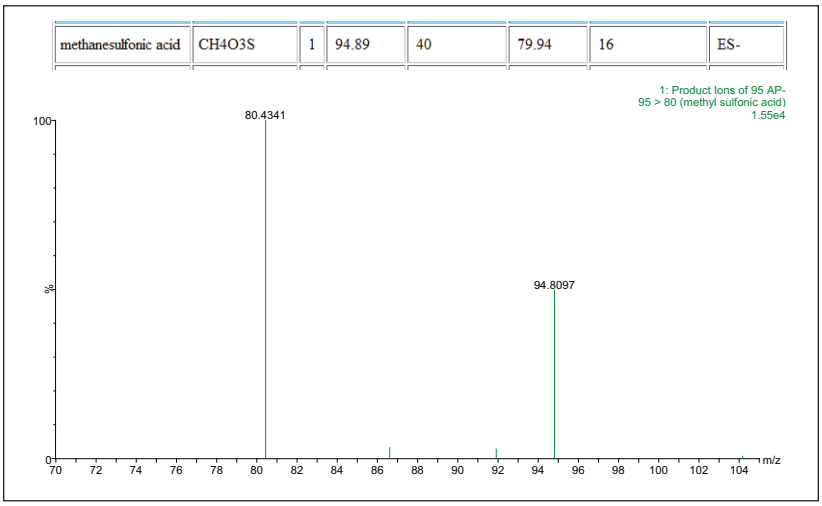

Figure 4.
Methanesulfonic acid.

CONCLUSIONS

Genotoxic impurities at the Threshold of Toxicological Concern (TTC) can easily be identified using the Xevo TQD when used in a qualitative manner with the ASAP probe and PIC. This allows for rapid check for the presence of genotoxic and other identified impurities and also allows confirmation of identity through the use of PIC. This rapid, simple and powerful approach allows productivity gains into a routine laboratory setting that has not been possible before.

Reference

1. Confirming Peak Identification in Bioanalytical Studies Utilizing Xevo TQ MS Product Ion Confirmation. Waters Application Note. 2009: 720002858en.

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Detection and Identification of Extractable Compounds from Polymers

GOAL

To detect and identify unknown polymer extractables not found using conventional GC/MS techniques. To apply the well-established QTof accurate mass measurement workflow to GC/MS analysis.

BACKGROUND

Containers specified for packaging pharmaceutical products are required to be tested for extractables to verify the absence of toxic impurities that could transfer to the drug. Often the monomer and polymer manufacturers do not provide all necessary compound information. Additional compounds may also be formed in the molding process. Therefore, there is need for identification of substances in the polymer that can potentially contaminate the drug product. Typically, this is accomplished by extracting the component with three different solvents and analyzing the extracts by LC/MS and GC/MS.* With EI on a single quadrupole GC/MS, sufficient sensitivity for library identification often cannot be accomplished for all prospective analytes.

Initial analyses of the nylon sample by single quadrupole GC/MS using conventional EI and CI were unable to provide data of sufficient intensity and quality to identify impurities. However, once this EI data revealed the presence of an impurity, it was important to establish its identity to ensure that this extractable would not impart undesirable qualities to the drug product through contact with the nylon.

APGC/QTof with MS^E allows elemental composition determination of compounds that could not otherwise be identified.

THE SOLUTION

For the analysis, sample preparation was performed using 2 g nylon resin microwave extracted 3h/70 °C in 10 mL isopropanol. The GC/MS system was a Waters Xevo® G2 QTof with an Atmospheric Pressure Gas Chromatography (APGC) source and 7890A GC.

APGC provides soft ionization resulting in a large peak for the molecular ion leading to improved sensitivity. In addition, the analysis can be performed with concurrent acquisition of both high and low collision energy data (MS^E). This facilitates structural elucidation by providing accurate mass data for both intact molecular ions as well as structurally significant fragment ions.

Figure 1 shows the EI TIC (A) compared with the two simultaneously acquired MS^E TICs from the APGC QTof experiment. The peak for the analyte at 15.75 min is readily observed in both APGC traces despite the fact that using conventional CI there was no

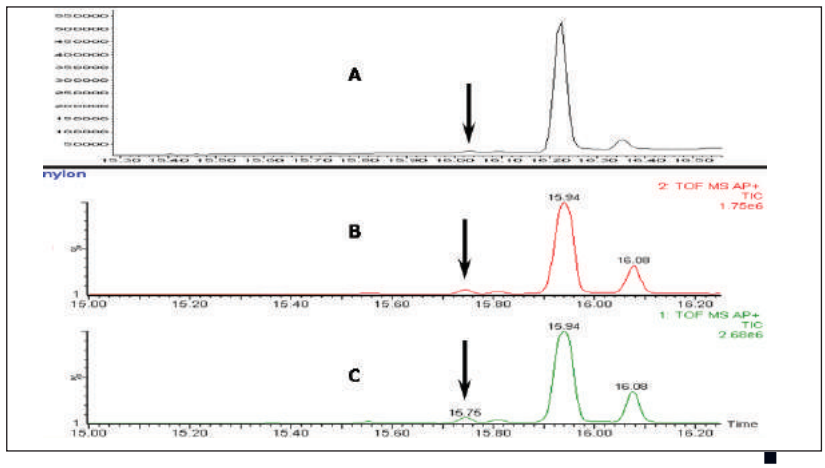

Figure 1. A = EI TIC, B = high energy/fragmentation APGC TIC, C = low energy/molecular ion APGC TIC.

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discernable peak. As a result of the sensitive detection of the analyte in both traces, high quality spectra for the intact molecular ion as well as a full range of fragment ions (Figure 2) is available for interpretation using accurate mass measurement and structural elucidation software.

In order to better qualify the sensitivity of the technique, the XIC for 222.2222 Da was plotted with a portion of the background magnified, as shown in Figure 3. This clearly demonstrates signal-to-noise in excess of 1000:1 for a compound undetected using convention vacuum source CI. Furthermore, upon plotting this XIC additional peaks of the same mass are observed. One of these, at 15.91 min, coelutes with the main extractable component of the nylon and would fail to be detected without the sensitivity and the high resolving power, at 22,500 FWHM, of the QTof. The stability and resolving power of the QTof together provide excellent mass accuracy (Figure 3), which allows determination of the elemental composition of the analyte not possible with previously acquired EI and CI data.

The comparison of the acquired data to the theoretical isotope pattern in Figure 3 helps show the dynamic range of the QTof as well as its ability to accurately measure and represent the naturally occurring isotope abundances. The proposed molecular formula and fragments support a structure that is a degradant of a proprietary processing aid identified by the resin manufacturer. The exact structure is not included here due to the proprietary nature of the formulation.

SUMMARY

The soft ionization of APGC provides an orthogonal technique to conventional EI and CI revealing previously undetectable compounds of interest and providing spectra with a controllable extent of fragmentation. This provides greater confidence in product purity for drugs that contact polymers during storage and delivery.

In this study, EI GC/MS on a single quadrupole was demonstrated to lack sufficient sensitivity to provide reliable library matches. Additionally, there is a high likelihood that polymer extractables will not be present in commercially available libraries making a Xevo G2 QTof with an APGC for accurate mass information a more fit-for-purpose solution in the determination of unknowns. As a result, APGC/QTof with MS^E allows elemental composition determination of compounds that could not otherwise be identified even with sufficiently intense EI spectrum.

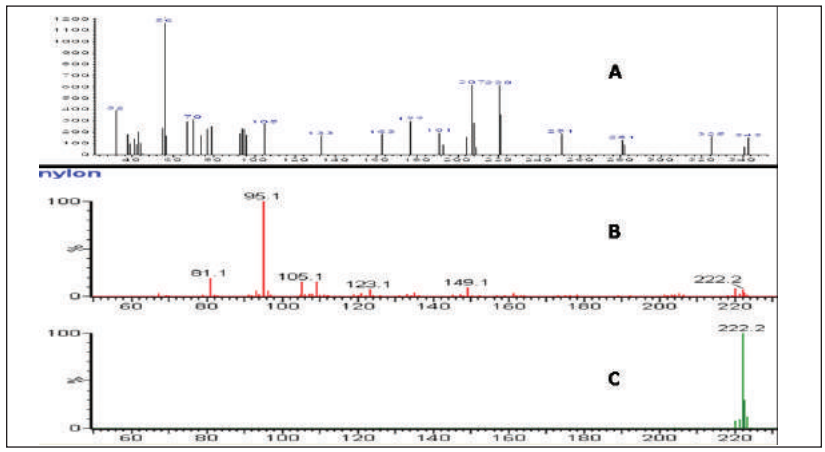

Figure 2. A = EI spectrum, B = high energy/fragmentation APGC spectrum, C = low energy/molecular ion APGC spectrum.

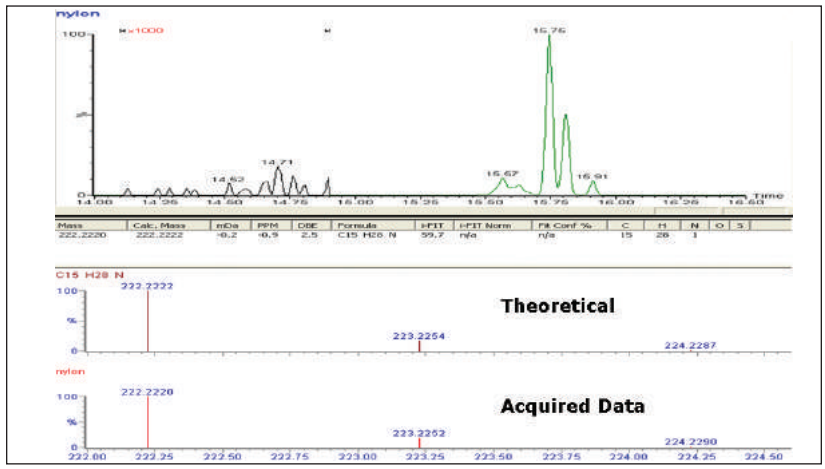

Figure 3. Upper, accurate mass XIC of 222.2222 Da. Lower, accurate mass spectrum from low energy MS^E data along with the theoretical isotope model for the calculated elemental formula.

* Safety Thresholds and Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products, Leachables and Extractables Working Group, Product Quality Research Institute (PQRI), 2006 (www.pqri.org).

Waters would like to acknowledge Baiba Cabovska and Arthur Bailey from MannKind Corporation (Danbury CT, U.S.) for their contribution to this work.

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Improving Performance and Throughput of the USP Organic Impurities Analysis of Tioconazole on an Alliance HPLC System

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APPLICATION BENEFITS

- Updating lengthy USP organic impurities methods using ***XP*** columns for faster analysis with reduced solvent usage, while remaining within USP Chapter <621> guidelines
- Reducing sample run time by up to 57%, increasing throughput
- Reducing solvent usage by at least 57%, decreasing operating costs

WATERS SOLUTIONS

- Alliance® HPLC System
- XSelect™ CSH™ C_{18} columns
- Empower® 3 Software
- e***X***tended Performance (***XP***) 2.5 µm columns
- TruView™ LCMS Certified Maximum Recovery Vials

KEY WORDS

USP methods, Alliance HPLC System, tioconazole, ACQUITY UPLC Columns Calculator, Waters® Reversed Phase Column Selectivity Chart, generic drugs, CSH

INTRODUCTION

Organic impurities in generic drugs are routinely analyzed by pharmaceutical manufacturers worldwide. Performing organic impurities analyses with older instrumentation and column technology can be time-consuming and costly, as these methods can be very lengthy and require large amounts of solvent. However, by using more modern chromatographic tools, organic impurities assays can become more efficient due to significant improvements in both instrumentation and column technology. e***X***tended ***P***erformance (***XP***) columns are 2.5 µm particle size columns designed for use on both HPLC and UPLC instrumentation. These columns are ideal for modernizing USP methods as they allow chromatographers to realize the benefit of smaller particle sizes, while operating according to the USP Chromatography Chapter <621> guidelines.[1]

Tioconazole is an imidazole antifungal compound used in the treatment of yeast infections. The method that was transferred is the organic impurities analysis of tioconazole.[2] Organic impurity methods are used to determine the presence and quantity of impurities in a sample. The USP method was scaled from the original column dimensions to ***XP*** columns on an Alliance HPLC System. The integrated fluidic design and efficient solvent management features of the Alliance HPLC System make it robust for reliable quality data generation. Updating the current USP method using ***XP*** columns on an HPLC instrument can reduce run times, thereby increasing sample throughput in a routine analytical laboratory.

EXPERIMENTAL

Alliance HPLC System conditions

Mobile phase A:	44:40:28 Acetonitrile/ methanol/water with 2 mL ammonium hydroxide
Separation mode:	Isocratic
Detector:	2998 photodiode array (PDA)
UV wavelength:	219 nm
Column (L1):	XSelect CSH C_{18} 4.6 x 250 mm, 5 µm; XSelect CSH ***XP*** C_{18} 4.6 x 150 mm, 2.5 µm; XSelect CSH ***XP*** C_{18} 4.6 x 100 mm, 2.5 µm
Column temp.:	25 °C
Needle wash:	95:5 ACN/water
Seal wash:	50:50 MeOH/water
Flow rate:	1.0 mL/min
Injection volume:	25 µL (250 mm column), 12 µL (150 mm column), 8 µL (100 mm column)
Data management:	Empower 3 Software

Sample description

The tioconazole sample was prepared in 100% methanol to the concentrations described in Table 1. The sample was then transferred to a TruView Maximum Recovery Vial for injection.

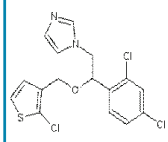
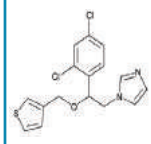
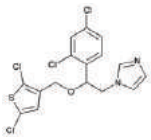
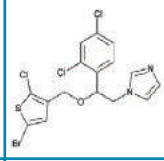
	Tioconazole	Related compound A	Related compound B	Related compound C
Structure				
Concentration (mg/mL)	6.6	0.2	0.2	0.2

Table 1. Structures and concentrations of tioconazole and related compounds.

RESULTS AND DISCUSSION

Tioconazole is produced generically and routinely analyzed worldwide by pharmaceutical manufacturers. In this application, the separation of tioconazole and tioconazole related compounds A, B, and C was demonstrated using the USP organic impurities method specified in the USP monograph on several different column dimensions. The Alliance HPLC System is used for its robust and reproducible performance in high throughput environments. The simplified fluidic path and integrated sample and solvent management result in pulse-free solvent flow, reduced system dispersion, and generate high quality reliable data. Tioconazole related compounds A, B, and C were used as low level impurity standards, as tioconazole impurities were not readily available. Organic impurity methods listed in the USP are used to analyze complex sample formulations. The well-resolved separation of multiple components in the samples often requires the use of longer column dimensions. The use of longer columns with larger particle sizes (≥3.5 µm) results in long run times and large amounts of solvent consumption. For example, the original USP organic impurities analysis of tioconazole requires a 4.6 x 250 mm, 5 µm column and 30 mL of solvent per sample per analysis with the separation taking thirty minutes to complete. However, using e***X***tended ***P***erformance (***XP***) 2.5 µm particle columns, run times may be reduced while meeting assay requirements. With shorter run times, throughput can increase with less solvent used per analysis, leading to overall cost savings. The current USP <621> Chromatography chapter provides allowable method changes that include ±70% change in column length, -50% change in particle size, and ±50% change in flow rate.[1] These guidelines were followed throughout the method transfers demonstrated here. A USP resolution of 1.5 between related compounds B and C was used as a requirement in this application to demonstrate that this critical pair can be consistently resolved as the method is transferred across different column dimensions.

Organic Impurities Analysis using *XP* Columns on HPLC Instrumentation

The organic impurities method for the analysis of tioconazole requires the use of an L1 USP designated column with the listed column for this separation a LiChrosorb RP-18.[2] Using the Waters Reversed Phase Column Selectivity Chart, the more modern XSelect CSH C_{18} stationary phase was chosen. The XSelect CSH C_{18} column was chosen because of its similarity to the listed column and its ability to provide full scalability of dimensions and particle sizes between HPLC and UPLC instrumentation. The USP method for this separation was first run using an Alliance HPLC System with an XSelect CSH C_{18} 4.6 x 250 mm, 5 µm column with a flow rate of 1.0 mL/min. The acceptance criteria for this separation were met, as shown in Table 2. The total run time for this separation was 30 minutes, which poses challenges in both time and financial management in high throughput environments where samples are continuously analyzed. Using the original USP method, an eight-hour work shift would result in only 16 samples being analyzed with 480 mL of solvent used. By using ***XP*** columns, up to 80 samples can be analyzed in the same eight-hour shift using only 240 mL of solvent, thus significantly increasing throughput and reducing operating costs.

The versatility of modernizing the compendial method using ***XP*** 2.5 µm columns across different column configurations on the Alliance HPLC System, while remaining within USP <621> guidelines, is shown in Figure 1.

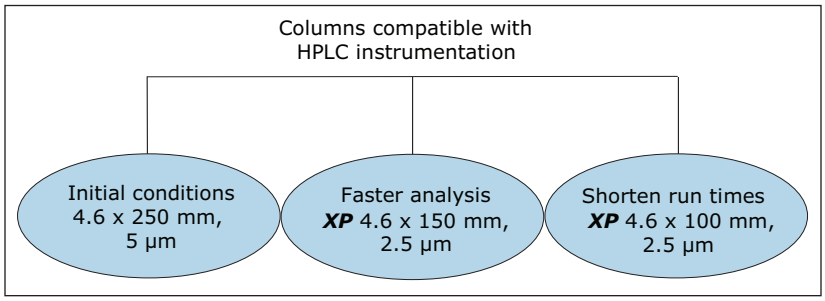


*Figure 1. Versatility of **XP** 2.5 µm particle columns on an HPLC system, while remaining within USP <621> guidelines.*

The compendial method was first transferred from the original 4.6 x 250 mm, 5 µm column to an ***XP*** 4.6 x 150 mm, 2.5 µm column to demonstrate that shorter run times can be achieved by using smaller particle sizes. Adopting smaller particle sizes can also lead to an increase in resolving power, measured by the column length to particle size ratio (L/dp). In this case, the L/dp increases from 50,000 (initial conditions) to 60,000 when moving to an ***XP*** 4.6 x 150 mm column. According to the ACQUITY UPLC Column Calculator, the properly scaled flow rate for this column is 2.0 mL/min.[3] However, that flow rate does not comply with USP <621> guidelines. A flow rate of 1.0 mL/min was used to remain within USP guidelines. The separation of tioconazole and its related compounds on the original column compared to the ***XP*** 4.6 x 150 mm column are shown in Figures 2A and 2B. The ***XP*** 4.6 x 150 mm column shows a 43% reduction in run time, along with a 5% increase in resolution, as shown in Table 2.

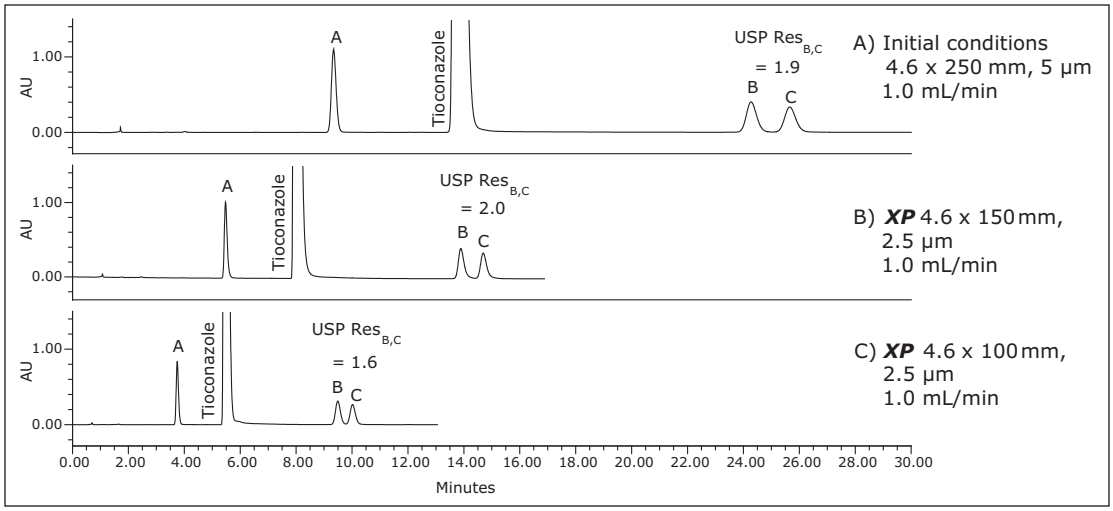


Figure 2. A comparison of the separation of tioconazole and tioconazole related compounds A, B, and C using various XSelect CSH C_{18} columns on an Alliance HPLC System, displaying a reduction in run time with comparable resolution.

Next, the separation was performed using a shorter ***XP*** 4.6 x 100 mm, 2.5 µm column to demonstrate a faster separation while maintaining acceptable resolution. The reduced run times especially benefit organic impurity methods as these methods generally have longer run times than other methods due to the added complexity of the separation. It is important to note that moving to a shorter column with lower resolving power (L/dp 40,000) may not always be an option. For example, cases exist where closely eluting excipients and impurities may require the resolving power of the original separation. Figure 2C shows the separation using the ***XP*** 4.6 x 100 mm, 2.5 µm column resulting in a 57% reduction in run time compared to the initial conditions while meeting all of the acceptance criteria, as shown in Table 2. In this case, the reduction in L/dp from 50,000 (initial conditions) to 40,000 resulted in a 15% drop in resolution between related compounds B and C; however, the resolution may still be adequate, depending on the complexity of the original separation.

	Acceptance criteria	4.6 x 250 mm, 5 µm (1 mL/min, Initial conditions)	***XP*** 4.6 x 150 mm, 2.5 µm (1 mL/min)	***XP*** 4.6 x 100 mm, 2.5 µm (1 mL/min)
USP resolution	NLT 1.5 between B and C	1.9	2.0	1.6
L/dp	N/A	50,000	60,000	40,000
Run time (min)	N/A	30	17	13

*Table 2. Suitability results for five replicate injections of tioconazole sample using the Alliance HPLC System with various dimensions of the XSelect CSH C_{18} column stationary phase including **XP** 2.5 µm columns.*

CONCLUSIONS

When performing the often lengthy and costly analysis of organic impurities, the use of e***X***tended ***P***erformance [***XP***] 2.5 µm columns on existing HPLC systems can significantly reduce run times and solvent usage by up to 57%, compared to the original compendial USP procedure. The availability of ***XP*** columns, capable of being run on both HPLC and UPLC instrumentation, allows USP methods to be updated while following the current USP <621> guidelines. In routine analytical laboratories, modernizing USP methods using columns with smaller particle sizes can result in significant time and operating cost savings.

References

1. USP General Chapter <621>, USP35-NF30 Page 258. The United States Pharmacopeial Convention, official from August 1, 2012.
2. USP Monograph. Tioconazole, USP35-NF30, 4875. The United States Pharmacopeial Convention, official from August 1, 2012.
3. Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721en. 2010 Sept.

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Using e*X*tended *P*erformance (*XP*) Columns to Modernize the USP Organic Impurities Analysis of Tioconazole

Kenneth D. Berthelette, Mia Summers, and Kenneth J. Fountain
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Updating lengthy USP organic impurities methods using ***XP*** columns for faster analysis with reduced solvent usage, while remaining within USP Chapter <621> guidelines.
- Reducing sample run time by up to 80%, thereby increasing throughput.
- Reducing solvent usage by up to 90%, resulting in lower operating costs.

WATERS SOLUTIONS

ACQUITY UPLC® H-Class System

Alliance® HPLC System

XSelect™ CSH™ C_{18} Columns

Empower™ 3 Software

e*X*tended *P*erformance (*XP*) 2.5 µm Columns

TruView™ LCMS Certified Maximum Recovery Vials

KEY WORDS

USP methods, tioconazole, ACQUITY UPLC Columns Calculator, Waters® Reversed-Phase Column Selectivity Chart, generic drugs

INTRODUCTION

Organic impurities in generic drugs are routinely analyzed by pharmaceutical manufacturers worldwide. Performing organic impurities analyses with older instrumentation and column technology can be a time-consuming and costly task, as these methods require large amounts of solvent over extended time periods. However, organic impurities assays can become more efficient by using significantly improved instrumentation and column technology. e*X*tended *P*erformance (*XP*) columns are 2.5-µm particle size columns designed for use on both HPLC and UPLC® instrumentation. These columns are ideal for modernizing USP methods as they allow chromatographers to realize the benefit of smaller particle sizes and low dispersion systems, while operating under the USP Chapter <621> Chromatography guidelines. The <621> chapter outlines allowable changes to the method.

Tioconazole is an imidazole antifungal compound used in the treatment of yeast infections. The method that was transferred was the organic impurities analysis of tioconazole.[2] Organic impurity methods are used to determine the presence and quantity of impurities in a sample. The USP method was scaled from the original column dimensions on an HPLC system to ***XP*** columns on both HPLC and UPLC instrumentation. Updating the current USP method using ***XP*** columns on an HPLC instrument can reduce run times, increasing sample throughput in a routine analysis laboratory, while using ***XP*** columns on a UPLC system can further reduce run time and solvent usage compared to HPLC, resulting in overall cost savings.

EXPERIMENTAL

Alliance 2695 HPLC Conditions

Mobile phase:	44:40:28 acetonitrile/ methanol/water with 2 mL ammonium hydroxide
Separation mode:	Isocratic
Detection:	UV at 219 nm
Column (L1):	XSelect CSH C_{18}, 4.6 x 250 mm, 5 µm, p/n 186005291;
	XSelect CSH C_{18} ***XP***, 4.6 x 150 mm, 2.5 µm, p/n 186006729;
	XSelect CSH C_{18} ***XP***, 4.6 x 100 mm, 2.5 µm, p/n 186006111
Column temp.:	25 °C
Needle wash:	95:5 ACN/water
Sample purge:	95:5 water/ACN
Seal wash:	50:50 MeOH/water
Flow rate:	Scaled with method
Injection volume:	Scaled with method

ACQUITY UPLC H-Class Conditions

Mobile phase:	44:40:28 acetonitrile/ methanol/water with 2 mL ammonium hydroxide
Separation mode:	Isocratic
Detection:	UV at 219 nm
Column (L1):	XSelect CSH C_{18} ***XP***, 4.6 x 150 mm, 2.5 µm, p/n 186006729;
	XSelect CSH C_{18} ***XP***, 4.6 x 100 mm, 2.5 µm, p/n 186006111;
	XSelect CSH C_{18} ***XP***, 2.1 x 150 mm, 2.5 µm, p/n 186006727

Sample Description

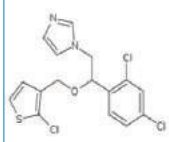
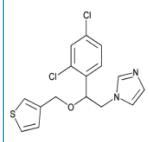
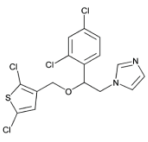
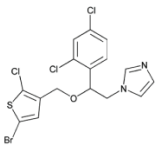
	Tioconazole	Related Compound A	Related Compound B	Related Compound C
Structure				
Concentration (mg/mL)	6.6	0.2	0.2	0.2

Table 1. Structures and concentrations of tioconazole and related compounds.

The Tioconazole sample was prepared in 100% methanol to the concentrations described in Table 1. The sample was transferred to a TruView Maximum Recovery Vial for injection, p/n 186005662CV.

RESULTS AND DISCUSSION

Tioconazole, produced generically, is routinely analyzed worldwide by pharmaceutical manufacturers. In this application, the separation of tioconazole and tioconazole related compounds A, B, and C is demonstrated using the USP organic impurities method specified in the USP monograph on several different column dimensions. Tioconazole related compounds A, B, and C were used as low level impurity standards, as tioconazole impurities were not readily available. Organic impurity methods listed in the USP are used to analyze complex sample formulations. The well-resolved separation of multiple components in the samples often requires the use of longer column dimensions. The use of longer columns with larger particle sizes (≥3.5 µm) results in long run times and the use of large amounts of solvent. For example, the original USP organic impurities analysis of tioconazole required a 4.6 x 250 mm, 5 µm column with the separation lasting thirty minutes, using 30 mL of solvent per sample per analysis. However, by using e***X***tended ***P***erformance (***XP***) 2.5 µm particle columns, run times may be reduced while still meeting assay requirements. With shorter run times, throughput can increase with less solvent used per analysis, leading to overall cost savings. The current USP Chapter <621> Chromatography guidelines provide allowable method changes that include ±70% change to column length, -50% change in particle size, and ±50% change in flow rate.[1] These guidelines were followed throughout the method transfers demonstrated here. A USP resolution of 1.5 between related compounds B and C was used as a requirement in this application to demonstrate that this critical pair can be consistently resolved as the method is transferred between different columns and systems.

Column temp.:	25 °C
Needle wash:	95:5 ACN/water
Sample purge:	95:5 water/ACN
Seal wash:	50:50 MeOH/water
Flow rate:	Scaled with method
Injection volume:	Scaled with method
Data management:	Empower 3 Software

Organic Impurities Analysis using *XP* Columns on HPLC Instrumentation

The organic impurities method for the analysis of tioconazole requires the use of an L1 designation column, and the listed column for this separation is a LiChrosorb RP-18.[2] Using the Waters Reversed-Phase Column Selectivity Chart, the more modern XSelect CSH C_{18} stationary phase was chosen. The XSelect CSH C_{18} Column was chosen because of its similarity to the listed column, and it offers full scalability of dimensions and particle sizes between HPLC and UPLC instrumentation. The USP method for this separation was first run using an Alliance HPLC System with an XSelect CSH C_{18}, 4.6 x 250 mm, 5 µm Column with a flow rate of 1.0 mL/min. The acceptance criteria for this separation were met, as shown in Table 2. The total run time for this separation was thirty minutes, which poses challenges in both time and financial management in high throughput environments where samples are continuously analyzed. Using the original USP method, an eight-hour work shift would result in only 16 samples being analyzed with 480 mL of solvent used. By using ***XP*** columns, up to 80 samples can be analyzed in the same eight-hour shift using only 240 mL of solvent, thus significantly increasing throughput and reducing operating costs.

The versatility of modernizing the compendial method using ***XP*** 2.5 µm columns across different systems, while remaining within USP Chapter <621> guidelines, is shown in Figure 1. ***XP*** columns are 2.5-µm particle HPLC and UPLC columns that are packed to a high efficiency and designed to withstand the higher pressures of a UHPLC system, allowing the ***XP*** columns to run across both HPLC and UPLC instrumentation.

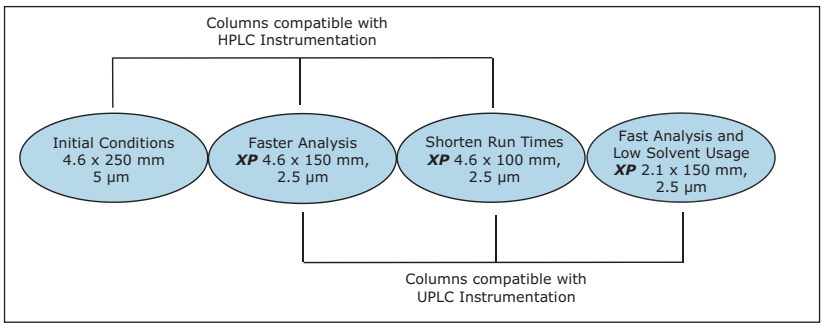


*Figure 1. Versatility of **XP** 2.5 µm particle columns across both HPLC and UPLC systems, while still remaining within USP Chapter <621> guidelines.*

The compendial method was first transferred from the original 4.6 x 250 mm, 5 µm column to an ***XP*** 4.6 x 150 mm, 2.5 µm column to demonstrate that shorter run times can be achieved by using smaller particle sizes. Using smaller particle sizes can also lead to an increase in resolving power, measured by the column length to particle size ratio (L/dp). In this case, the L/dp increases from 50,000 (initial conditions) to 60,000 when moving to an ***XP*** 4.6 x 150 mm column. According to the ACQUITY UPLC Column Calculator, the properly scaled flow rate for this column is 2.0 mL/min.[3] However, that flow rate is outside the USP Chapter <621> guidelines. A flow rate of 1.0 mL/min was used to remain within USP guidelines, and to accommodate back pressure limitations of the HPLC system. The separation of tioconazole and its related compounds on the original column are compared to the ***XP*** 4.6 x 150 mm column, as shown in Figures 2A-B. The ***XP*** 4.6 x 150 mm column shows a 43% reduction in run time, along with a 5% increase in resolution, as shown in Table 2.

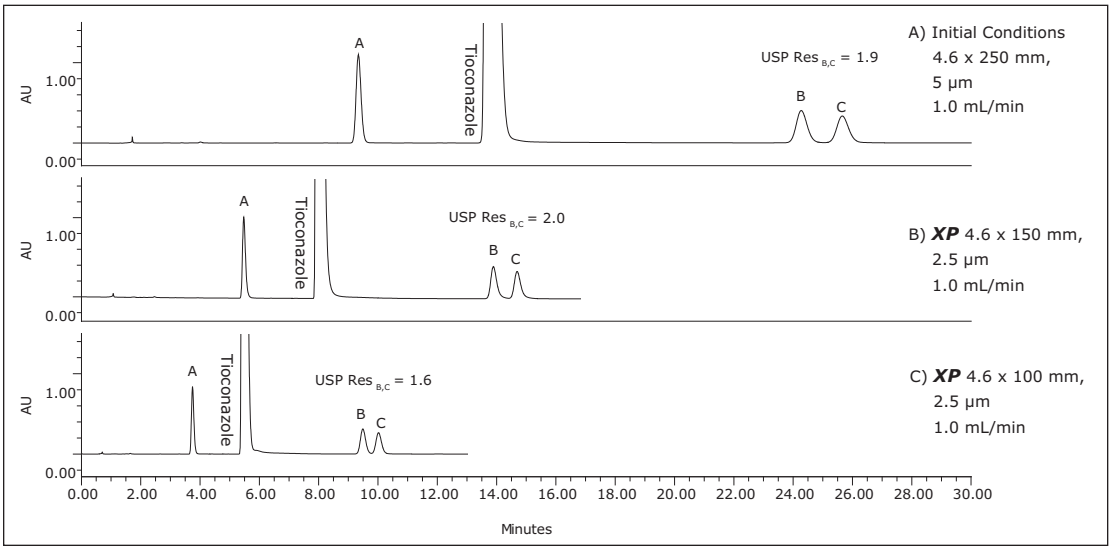


Figure 2. A comparison of the separation of tioconazole and tioconazole related compounds A, B, and C using various XSelect CSH C_{18} Columns on HPLC instrumentation, displaying a reduction in run time with comparable resolution.

Next, the separation was performed using a shorter ***XP*** 4.6 x 100 mm, 2.5 µm column to demonstrate a faster separation while maintaining acceptable resolution. The reduced run times especially benefit organic impurity methods as these methods generally have longer run times than other methods due to added complexity of the separation. It is important to note that moving to a shorter column with lower resolving power (L/dp 40,000) may not always be an option, for instance, in cases where closely eluting excipients and impurities may require the resolving power of the original separation. Figure 2C shows that the separation using the ***XP*** 4.6 x 100 mm, 2.5 µm column results in a 57% reduction in run time compared to the initial conditions and all of the acceptance criteria are still met, as shown in Table 2. In this case, the reduction in L/dp from 50,000 (initial conditions) to 40,000 resulted in a drop in resolution between related compounds B and C of 15%; however, the resolution may still be adequate, depending on the complexity of the original separation.

	Acceptance Criteria	4.6 x 250 mm, 5 µm [Initial Conditions]	*XP* 4.6 x 150 mm, 2.5 µm (1.0 mL/min)	*XP* 4.6 x 100 mm, 2.5 µm (1.0 mL/min)
USP Resolution	NLT 1.5 between B and C	1.9	2.0	1.6
L/dp	N/A	50,000	60,000	40,000
Run Time (min)	N/A	30	17	13

*Table 2. Suitability results for five replicate injections of tioconazole sample using an Alliance HPLC System with various dimensions of the XSelect CSH C_{18} Column stationary phase including **XP** 2.5-µm columns. L/dp is defined as the column length to particle size ratio.*

Organic Impurities Analysis using *XP* Columns on UPLC Instrumentation

As Figure 1 outlines, the method can be transferred from an Alliance HPLC System to an ACQUITY UPLC H-Class System by using both ***XP*** columns and the ACQUITY UPLC Column Calculator. Newer instrumentation, such as the ACQUITY UPLC H-Class System, can offer faster, more efficient separations due to high back pressure capabilities, faster equilibration between injections, and significantly lower system volume and dispersion. To compare the separation capabilities of the HPLC and UPLC systems, the organic impurities method using the ***XP*** 4.6 x 150 mm, 2.5 µm particle column, shown in Figure 2B, was re-run on an ACQUITY UPLC H-Class System, shown in Figure 3A. The change in instrumentation alone, from HPLC to UPLC, resulted in a 5% increase in resolution between peaks B and C, and a 12% reduction in run time, as shown in Tables 2 and 3. This increase in resolution is due to the low system volume and low dispersion of the UPLC system, as both of these attributes can improve peak shape.

To further demonstrate the benefits of UPLC instrumentation, the separation using the ***XP*** 4.6 x 100 mm column on a UPLC system was performed, as seen in Figure 3B. This separation resulted in an increase in resolution between peaks B and C from 1.6 using an HPLC system shown in Table 2 to 1.8 using a UPLC system shown in Table 3. Using the UPLC system with an ***XP*** 4.6 x 100 mm column, the separation has approximately the same resolution as the original method on the HPLC system but performed 57% faster than the original method.

	Acceptance Criteria	*XP* 4.6 x 150 mm, 2.5 µm (1.0 mL/min)	*XP* 4.6 x 100 mm, 2.5 µm (1.0 mL/min)	*XP* 2.1 x 150 mm, 2.5 µm (0.5 mL/min)
USP Resolution	NLT 1.5 between B and C	2.1	1.8	1.8
L/dp	N/A	60,000	40,000	60,000
Run Time (min)	N/A	15	13	6

*Table 3. Suitability results for five replicate injections of tioconazole sample using an ACQUITY UPLC System with various dimensions of the XSelect CSH C_{18} Column stationary phase including **XP** 2.5-µm columns. L/dp is defined as the column length to particle size ratio.*

Lastly, the compendial method was transferred to an ***XP*** 2.1 x 150 mm, 2.5 µm column. This column was tested to demonstrate that by reducing column interior diameter, run times can decrease further, while maintaining comparable resolution and using considerably less solvent. According to the ACQUITY UPLC Column Calculator, the proper flow rate for the method on this column is 0.42 mL/min. However, this flow rate is outside the USP <621> guidelines; therefore, a flow rate of 0.5 mL/min was used to maintain compliance. The resulting chromatogram, displayed in Figure 3C, shows an 80% reduction in run time compared to the original conditions while suitability requirements are still easily met, as shown in Table 3. In addition, by reducing only the interior diameter of the column, the analysis is 63% faster than the ***XP*** 4.6 x 150 mm column, as shown in Figure 3A. Finally, by using the ***XP*** 2.1 x 150 mm column, solvent usage was reduced by 90% compared to the original compendial method, resulting in significant cost savings. The resolution between peaks B and C of this separation dropped from 1.9 to 1.8 as the flow rate was adjusted to remain within USP Chapter <621> guidelines, but the resolution remained within assay specifications.

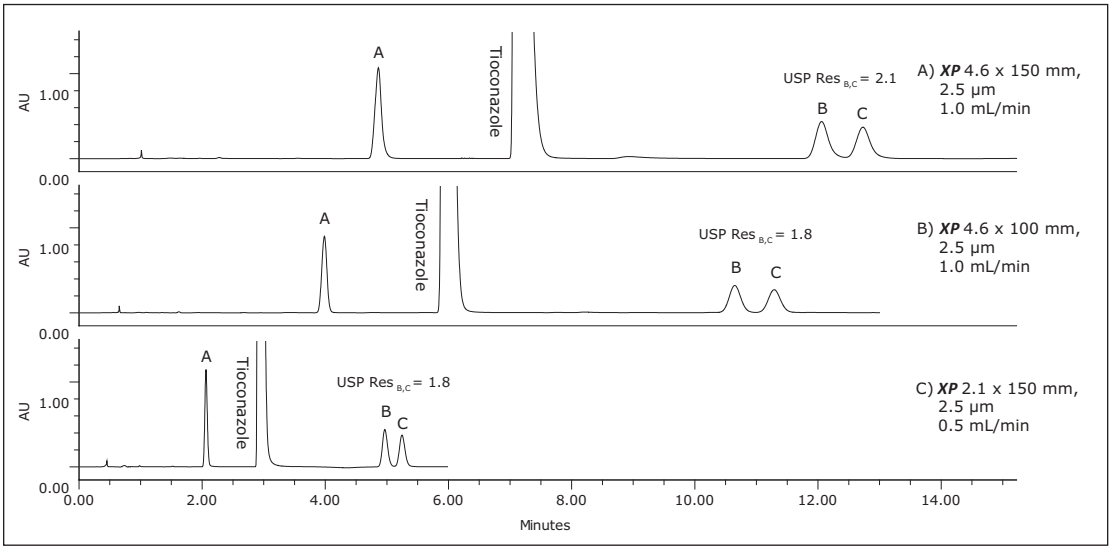


*Figure 3. Comparison of the separation of tioconazole and its related compounds using various **XP** columns on UPLC instrumentation, showing decreased run times by altering column dimensions.*

CONCLUSIONS

When performing the often lengthy and costly analysis of organic impurities, the use of e*X*tended *P*erformance [*XP*] 2.5 µm columns on existing HPLC systems can significantly reduce run times and solvent usage by up to 57%, compared to the original compendial USP procedure. By combining *XP* columns with UPLC instrumentation, run times can be reduced by up to 80%, while reducing solvent usage by 90%. The availability of *XP* columns, which are capable of being run on both HPLC and UPLC instrumentation, allows USP methods to be updated while following the current USP Chapter <621> guidelines. In routine analysis laboratories, modernizing USP methods using columns with smaller particle sizes can result in significant time and operating cost savings.

References

1. USP General Chapter <621>, USP35-NF30, 258. The United States Pharmacopeial Convention, official from August 1, 2012.
2. USP Monograph. Tioconazole, USP35-NF30, 4875. The United States Pharmacopeial Convention, official from August 1, 2012.
3. Jones MD, Alden P, Fountain KJ, Aubin A. Implementation of Methods Translation between Liquid Chromatography Instrumentation. Waters Application Note 720003721en. 2010 Sept.

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[TECHNOLOGY BRIEF]

Streamlining Analysis of Impurities in the Pharmaceutical Products using Empower 3 ICH Impurity Processing

Margaret Maziarz, Mark Wrona, Paul Rainville, Mia Summers, and Jade Byrd

GOAL

To demonstrate the use of Empower® 3 ICH Impurity Processing for streamlining the analysis of impurities of Ziprasidone HCl by defining allowable threshold limits and quickly identifying results above the limits.

BACKGROUND

Impurity profiling including identity and quantity in the drug substances or drug products are a requirement with which every manufacturer must comply. Impurities that develop from the active pharmaceutical ingredient (API) during the formulation and development process of drug product need to be assessed quickly and accurately. Presence of the impurities can compromise safety and efficacy of the end pharmaceutical product and must be effectively monitored. The International Committee for Harmonization (ICH) has published guidelines on impurities in drug substances[1] and drug products,[2] providing allowable threshold limits for impurities to monitor safety.

The Empower 3 ICH Impurity Processing function simplifies quantitative analysis by quickly identifying impurities above the ICH allowable limits defined by the user. Empower Software automatically compares the calculated amount of impurities against the limits and flags any failing results. This allows quick evaluation of the pharmaceutical products for safety during formulation or release testing.

Utilizing the Empower 3 ICH Impurity Processing enables users to define allowable threshold limits for impurities and quickly identify whether results pass these limits.

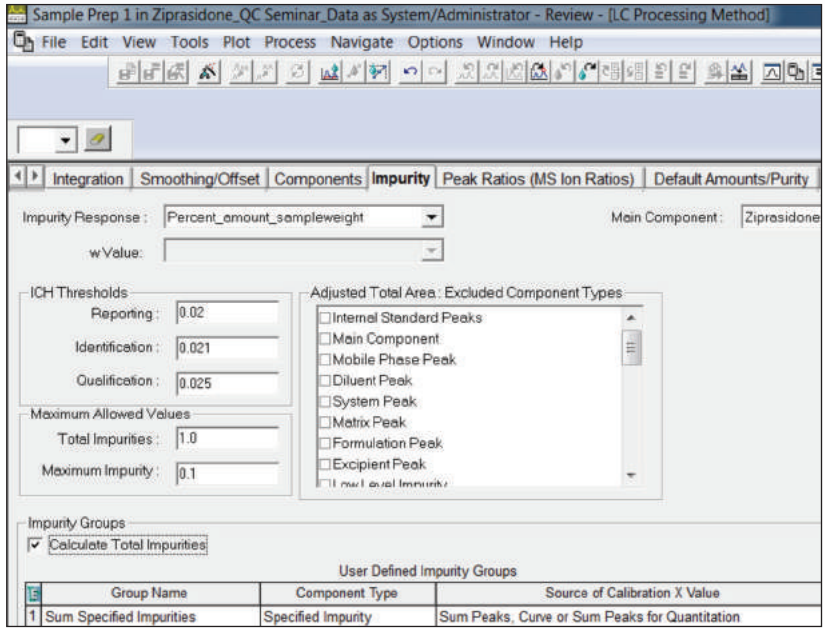

Figure 1. Empower 3 ICH Impurity Processing. Selection of field for impurity response, ICH thresholds, maximum allowed values, and group name to sum specified impurities.

This technology brief illustrates use of Empower 3 ICH Impurity Processing for streamlining quantitative analysis of impurities of Ziprasidone HCl in a capsule formulation.

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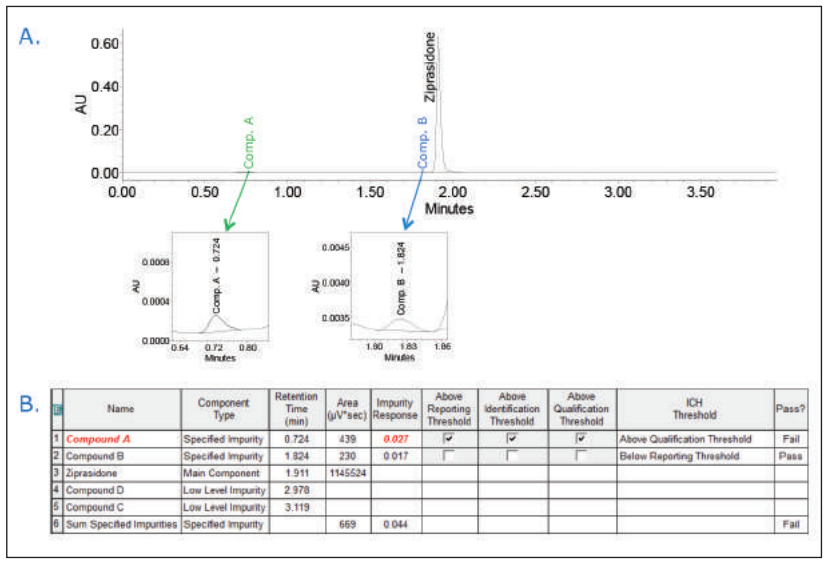
THE SOLUTION

The UPLC method for Ziprasidone HCl and its USP-specified related substances was previously developed in the Waters application note 720004639EN.[3] Empower 3 ICH Impurity Processing was used to streamline the quantitative analysis of related substances in the Ziprasidone HCl capsule formulation by defining the ICH threshold limits and identifying results that did not pass these limits. First, we specified the formula for calculating quantity of impurities or impurity response in the Empower processing method (Figure 1). Next, we defined the ICH thresholds for reporting, identification, and qualification of impurities. Then, we specified maximum allowed values for the total impurities and maximum impurity. In this example, we selected more stringent criteria compared to the ICH guidelines to demonstrate the workflow of identifying and flagging peaks that exceed impurity threshold limits. We used a reporting threshold of 0.02% of API. This is lower than the ICH guidelines for reporting impurities in new drug products of 0.1% based on maximum daily dosage of ≤1 g.[2] In addition, we can select tighter limits than the generic ICH thresholds for each specified impurity of the pharmaceutical product. Furthermore, we can group specific types of impurities to calculate their total.

Finally, we processed the chromatographic data to determine quantity of impurities. The data (Figure 2A) shows that two impurities, compounds A and B, were detected in the capsule sample, using Empower's proprietary peak detection algorithm ApexTrack.™ Empower Software quickly identified the out-of-specification (OOS) result for compound A (flagged in red), as this value was above the threshold limits defined in the processing method (Figure 2B). The results for analysis of impurities can be easily reported (Figure 3). The report template is customizable to tailor the reporting requirements for each user's needs and can facilitate the use of electronic signatures as shown in Figure 3.



	Name	Component Type	Retention Time (min)	Area (µV*sec)	Impurity Response	Above Reporting Threshold	Above Identification Threshold	Above Qualification Threshold	ICH Threshold	Pass?
1	Compound A	Specified Impurity	0.724	439	0.027	☑	☑	☑	Above Qualification Threshold	Fail
2	Compound B	Specified Impurity	1.824	230	0.017	☐	☐	☐	Below Reporting Threshold	Pass
3	Ziprasidone	Main Component	1.911	1145524						
4	Compound D	Low Level Impurity	2.978							
5	Compound C	Low Level Impurity	3.119							
6	Sum Specified Impurities	Specified Impurity		669	0.044					Fail

Figure 2. Analysis of impurities of Ziprasidone HCl in the capsule sample: A. Capsule sample with UV at 229 nm., B. Results for quantitative determination of impurities.

Empower 3 SOFTWARE

%Impurity_Report

SAMPLE INFORMATION

Sample Name:	Sample Prep 1		
Sample Type:	Unknown	Sample Set Name	Impurities_UPLC BEH
Vial:	1:A,6	Acq. Method Set:	UPLC_BEH
Injection #:	1	Processing Method	Impurities_ICH Imp Process_2
Injection Volume:	1.40 ul	Channel Name:	229nm
Run Time:	4.0 Minutes	Proc. Chnl. Descr.:	ACQUITY TUV ChA
Result Set Id 5934		Calibration Id 5954	

% Impurity

	SampleName	Name	RT (min)	Sample Weight (mg)	Dilution (mL)	Amount (mg)	Reporting Threshold	% Impurity	Pass?
1	Sample Prep 1	Compound A	0.724	1.301	10	0.000348	0.02	0.027	Fail
2	Sample Prep 1	Compound B	1.824	1.301	10	0.000221	0.02	0.017	Pass
3	Sample Prep 1	Ziprasidone	1.911	1.301	10				
Mean									

ICH Tresholds:
Reporting Threshold: 0.02
Identification Threshold: 0.021
Qualification Threshold: 0.025

Sign Off Full Name: Jane Smith (Lab_Manager)
Sign Off Reason: Sign Off Level 1, Reason: Approval

Figure 3. Empower report for analysis of impurities of Ziprasidone HCl in the capsule sample. Results approved and signed off by lab manager via electronic signature (OOS flags shown in red).

SUMMARY

Utilizing the Empower 3 ICH Impurity Processing enables users to define allowable threshold limits for impurities and quickly identify whether results are within these limits. The threshold limits defined by the user are clearly displayed by Empower during data review or in a report. This reduces the time and potential errors associated with manual verification and enhances the confidence that any results above the threshold limits are quickly identified.

Overall, Empower 3 ICH Impurity Processing is a powerful (yet simple) tool that can be utilized by any laboratory to monitor levels of impurities during formulation or release testing of the pharmaceutical products and streamline their quantitative analysis protocols.

References

1. ICH Q3A(R2), Impurities in New Drug Substances, International Conference on Harmonization, October 2006.
2. ICH Q3B(R2), Impurities in New Drug Products, International Conference on Harmonization, June 2006.
3. Maziarz M, Jones MD, Potts WB, Forini F. Transfer of Two UPS Compendial Methods for Impurities of Ziprasidone HCl to a Single UPLC Method. Waters Application Note 720004639EN, April 2013.

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India 91 080 49292200 03

Ireland 353 1 448 1500

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Korea 82 2 6300 9200

Mexico 52 55 52 00 1860

The Netherlands 31 76 508 7200

Norway 47 6 384 6050

Poland 48 22 101 5900

Portugal 351 21 893 61 77

Puerto Rico 1 787 747 8445

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