Extractables, Leachables, and Food Contact Materials

Application Notebook

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Extractables, Leachables, and Food Contact Materials Testing

The safety of pharmaceuticals, cosmetics, and foodstuffs may be compromised by chemical compounds in the various types of packaging and food contact materials (FCMs) that are in direct contact with the consumer product. These chemical compounds are typically categorized as:

- Extractables – compounds which are extracted from packaging or device components under controlled extraction conditions.
- Leachables – compounds which migrate from the packaging into the product during its normal shelf life.
- Non-intentionally added substances (NIAS) – degradation products from FCMs, impurities of starting materials, and contaminants from recycling processes.

Due to continuously increasing global regulations, the characterization of packaging and FCMs has become more critical than ever for the manufacturers that supply the pharmaceutical, food, and cosmetics industries.

To ensure regulatory compliance, avoid product recalls and protect their brands, these organizations must carefully control and monitor their products to eliminate the potential risks associated with extractable, leachable, and NIAS compounds.

Waters provides a wide range of technologies including Convergence Chromatography, Supercritical Fluid Extraction, Atmospheric Pressure GC, and Time-of-Flight Mass Spectrometry that enable accurate, rapid, and cost effective identification of extractable, leachable, and NIAS contaminants.

Table of Contents

EXTRACTABLES AND LEACHABLES

EXTRACTABLES AND LEACHABLES

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Probing for Packaging Migrants in a Pharmaceutical Impurities Assay Using UHPLC with UV and Mass Detection

Michael Jones
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- The ACQUITY™ Arc™ System is a quaternary-based, modern LC system for scientists working with established methods who are looking for the versatility and robustness required to bridge the gap between HPLC and UPLC™
- Run HPLC and UHPLC methods on one system
- Mass detection offers the ability to probe the identity of unexpected peaks and unknowns

WATERS SOLUTIONS

ACQUITY Arc System

ACQUITY UPLC PDA Detector

ACQUITY QDa™ Mass Detector

X-Bridge™ BEH C18 Column, 130 Å, 3.5 µm, 4.6 mm × 150 mm

X-Bridge BEH C18 Column, 130 Å, 2.5 µm, 2.1 × 100 mm

Empower™ 3 Chromatography Data Software (CDS)

KEYWORDS

Active pharmaceutical ingredient, API, impurities, extractables, leachables, mass detection, UHPLC

INTRODUCTION

The synthesis of pharmaceutical products frequently involves the formation of intermediates and byproducts. Low levels of some of these may be present in the drug product as impurities either through formation in the manufacturing process or via degradation during storage. Regulators such as the U.S. FDA and other international healthcare agencies require drug product manufacturers to control and remove these impurities to the extent possible. In addition to impurities and degradants related to the API of a drug product, polymeric packaging materials may impart chemical impurities to the final formulation during storage.

These chemical compounds contributed by packaging are typically categorized as:

Extractables – Compounds that are extracted from packaging or device components under controlled extraction conditions.

Leachables – Compounds that migrate from the packaging into the product during its normal shelf life.

While the analysis of extractables is quite straightforward, the presence of active pharmaceutical ingredients and pharmaceutical impurities can make the analysis of leachables much more complicated. Finished drug formulations will also contain various fillers, stabilizers, and excipients. These can contribute a multitude of unidentified peaks to observed chromatograms and make complete resolution of actives challenging. Mass detection enables a chromatographer in a QC method development setting to quickly and effectively suggest a number of possibilities for these unknown peaks, identify possible co-elutions or peak impurities and overall, increase confidence in results without resorting to costly and time-consuming central MS laboratory analysis of unknowns.

This application note describes the utility of Waters™ ACQUITY Arc System coupled with PDA and the ACQUITY QDa Mass Detector for the analysis of betamethasone valerate (BMV) scalp application for impurities, according to USP-NF 35 monograph methods. The flexibility of the ACQUITY Arc System is also highlighted with a redeveloped method based on USP-NF 35 that allows for the analysis of known, expected pharmaceutical impurities as well as compounds known to leach from high density polyethylene (HDPE) packaging.

EXPERIMENTAL

Replication of the USP method for Betamethasone Valerate (BMV) impurities

The ACQUITY Arc System was utilized to analyze betamethasone valerate (BMV) 0.1% w/w scalp application according to the USP-NF 35 impurities method described below to determine the presence of API based impurities with PDA detection at 240 nm.

Sample preparation

Samples and standards were prepared according to USP-NF 35. Three samples of BMV scalp application, stored under different conditions were analyzed by the method. The first sample (New Sample) was purchased from an online pharmacy and tested immediately for the presence of impurities. The second sample (Aged Sample) had previously been sourced and stored at ambient conditions for 6 months. The third sample (Forced Degradation Sample) had been stored at elevated temperature, relative humidity, and exposed to UV radiation to replicate conditions found in forced degradation studies.

HPLC conditions

HPLC system: ACQUITY Arc

Detection: PDA, 240 nm at 4.8 nm;

ACQUITY QDa:
SIR [M+formate-H]-
for API and impurities

Column: XBridge BEH C_{18}, 130Å,
3.5 µm, 4.6 mm × 150 mm,
p/n: 186003034

Injection volume: 100 µL

Flow rate: 1.00 mL/min

Mobile phase A: 20 mmol ammonium formate (aqueous)

Mobile phase B: Acetonitrile

Gradient:

Time (min)	MP A (%)	MP B (%)
0.0	63	37
7.0	63	37
15.0	30	70
19.0	30	70
19.1	10	90
21.0	10	90
21.1	63	37
25.0	63	37

Data management: Empower 3 CDS

See Figure 7B for representative HPLC and UHPLC standard chromatograms.

UHPLC conditions

UHPLC system: ACQUITY Arc

Detection: PDA: 240 nm at 4.8 nm;

ACQUITY QDa:
SIR [M+formate-H]$^-$
for API and impurities

Column: XBridge BEH C_{18}, 130Å,
2.5 µm, 4.6 mm × 50 mm,
p/n: 186006029

Injection volume: 16 µL

Flow rate: 0.4 mL/min

Mobile phase A: 20 mmol ammonium formate (aqueous)

Mobile phase B: Acetonitrile

Gradient:

Time (min)	MP A (%)	MP B (%)
0.0	63	37
1.38	63	37
5.19	30	70
7.09	30	70
7.14	10	90
8.05	10	90
8.10	0	37
14.5	0	37
14.6	63	37
16.0	63	37

Data management: Empower 3 CDS

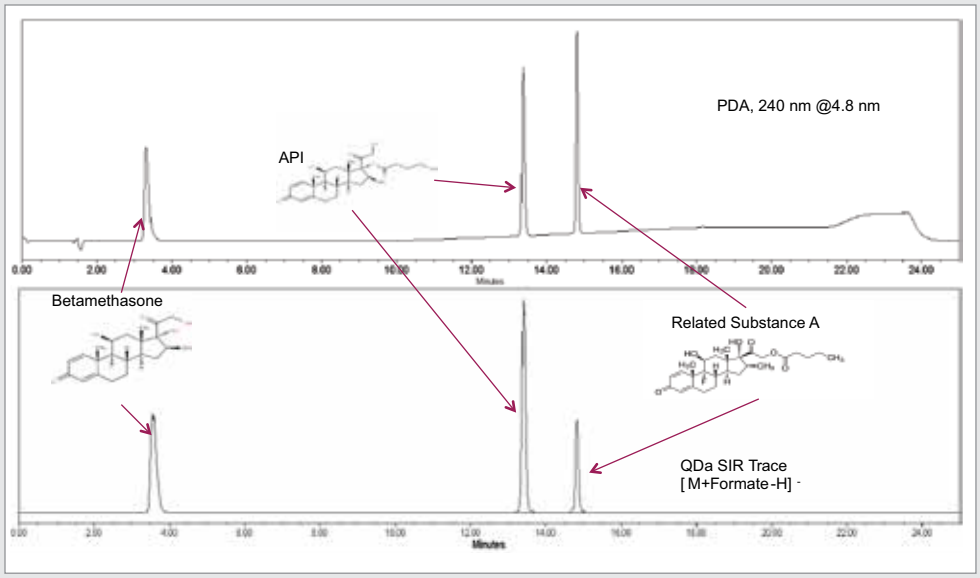


Figure 1. PDA chromatogram (top) and ACQUITY QDa SIR chromatogram (bottom) of solvent standards for betamethasone, betamethasone valerate, and Related Substance A.

RESULTS AND DISCUSSION

COMPARISON OF NEW, AGED, AND FORCED DEGRADATION SAMPLES FOR THE PRESENCE OF PHARMACEUTICAL IMPURITIES

All of the samples showed the presence of Related Substance A at levels below the reporting limit of 1.0% for the New Sample, and above the reporting limit for Aged Sample, and the sample held at forced degradation conditions. Analysis by mass detection on the ACQUITY QDa lowers the limits of quantitation and detection for API impurities compared to UV detection.

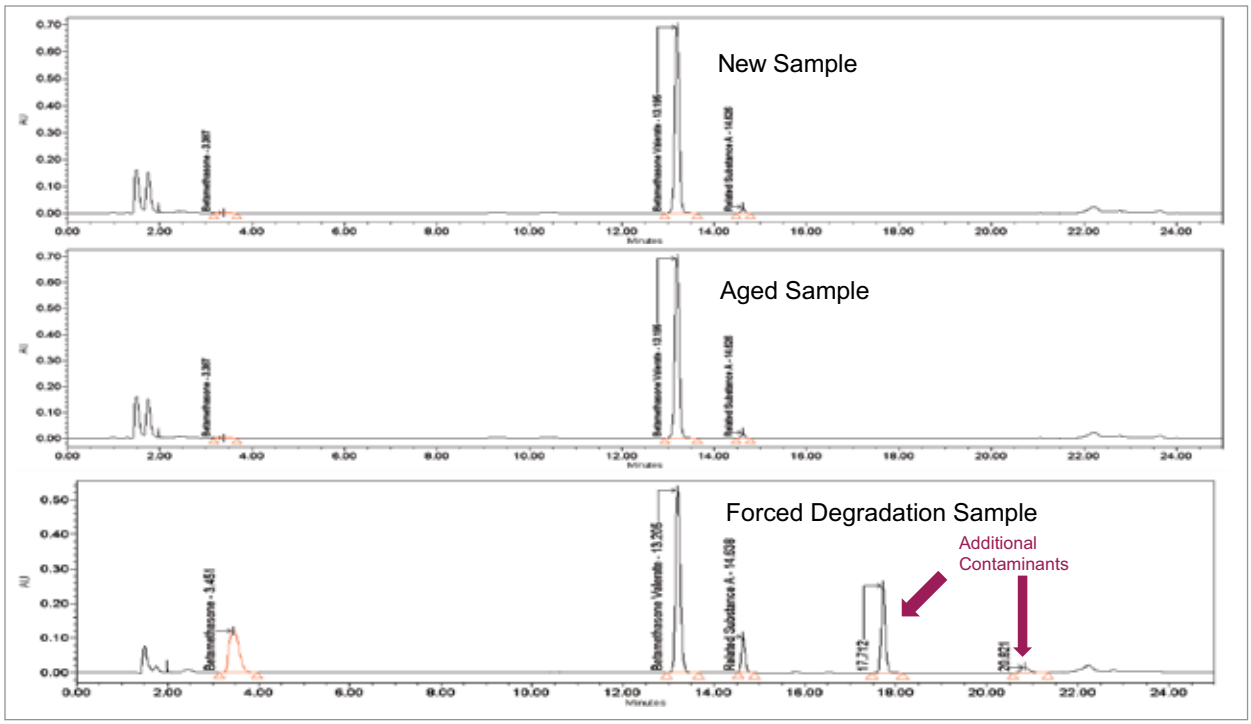


Figure 2. PDA chromatograms of the New Sample BMV Scalp application (top), Aged Sample (middle), and Forced Degradation Sample (bottom).

New sample

Peak Results

	Name	RT	Area	Height	Amount	Units	% Impurity
1	Betamethasone	3.440					
2	Betamethasone Valerate	13.291	4666875	226824	0.03330	ug/mL>	
3	Related Substance A	14.692	65544	6200	0.00030	ug/mL>	1.0
4		21.166	81869	7639			

Aged Sample

Peak Results

	Name	RT	Area	Height	Amount	Units	% Impurity
1	Betamethasone	3.440					
2	Betamethasone Valerate	13.269	4971263	283740	0.03577	ug/mL>	
3	Related Substance A	14.674	131883	13230	0.00032	ug/mL>	1.9
4		21.147	80001	7229			

Forced Degradation Sample

Peak Results

	Name	RT	Area	Height	Amount	Units	% Impurity
1	Betamethasone	3.479	1814046	103818	0.01053	ug/mL>	43.7
2	Betamethasone Valerate	13.246	3769222	472488	0.03407	ug/mL>	
3	Related Substance A	14.687	557883	100910	0.00352	ug/mL>	10.9
4		17.260	5485	1005			

Figure 3. Empower 3 Peak Results tables for the New Sample, Aged Sample, and Forced Degradation Sample.

Analysis of the sample held at forced degradation conditions by PDA at 240 nm suggested the presence of two additional impurities in the formulation, which eluted at 17.89 min and 21.12 min.

The Mass Analysis window in Empower 3, shown in Figure 4, offers significant benefits when probing spectral and chromatographic data on unexpected peaks found during routine analysis. Interrogating this spectral data showed that the two potential contaminants found in the forced degradation sample were likely to be unrelated to the API or any known process impurities. The UV spectra extracted from the PDA chromatogram at the retention times of the two unknown peaks were markedly different to those of the API and known impurities. The spectra of the two unknowns showed characteristic UV maxima (274–278 nm) for substituted benzene containing compounds. Extracting the UV spectrum at 278 nm allowed for interrogation of m/z data collected by the ACQUITY QDa Mass Detector at the retention times of interest. API and Related Substance A (Peaks 2 and 3) gave strong m/z signals of 521.2 in negative ionization mode, corresponding to $[M+Formate-H]^-$. Betamethasone gave a corresponding m/z signal for the $[M+Formate-H]^-$ adduct at 437.19. The first unknown peak, Peak 4 (Figure 4), eluted at 17.7 min, and ionized strongly in positive mode giving m/z signals at 279.2, 205.15, and 149.05. Peak 5 ionized strongly in negative mode with an m/z value of 219.15.

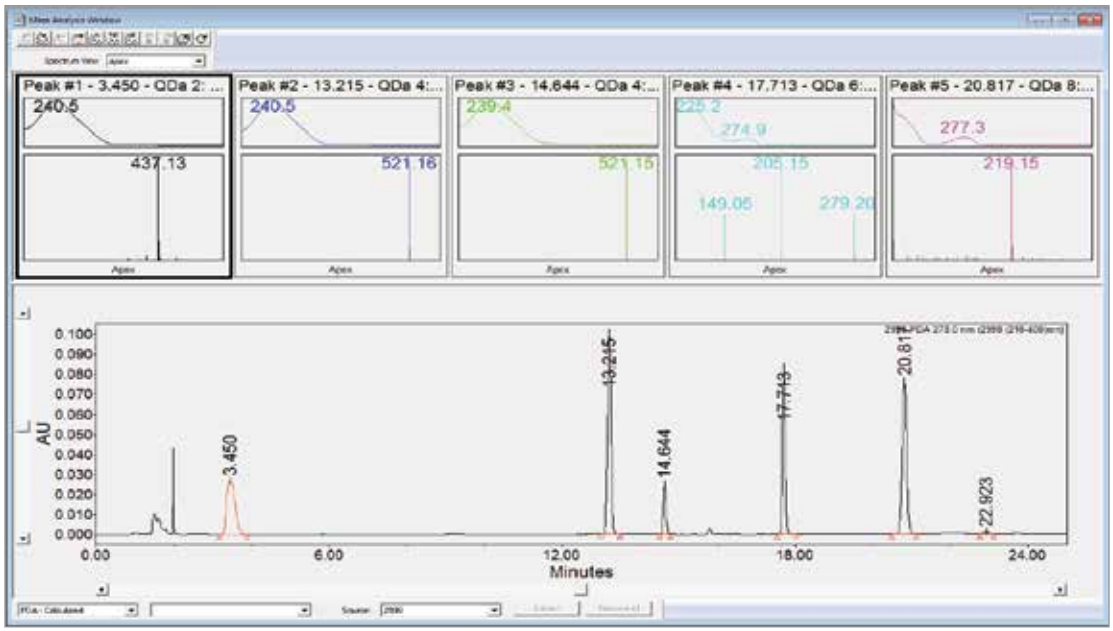


Figure 4. Empower 3 Software Mass Analysis window for the Forced Degradation sample showing UV spectra and MS scan data for peaks 1–5 in the chromatogram.

These data, coupled with the increased hydrophobicity of the contaminants and UV spectra suggested impurities with different structural motifs to the API and its degradants. The polymeric material used for the formulation packaging was found to be high density polyethylene (HDPE). Literature research[1] into potential polymer additives containing phenyl moieties and matching the observed m/z values showed two commonly used polymer additives, dibutyl phthalate and butylated hydroxyl toluene (BHT), and one additive currently banned for use in the EU due to its adverse safety profile as a potential endocrine disruptor, nonylphenol. In order to increase evidence for the presence of dibutyl phthalate, BHT, or nonylphenol in the Forced Degradation samples, solvent and matrix spiked standards of the three potential leachates were analyzed by the HPLC method. Solvent standard UV chromatograms are shown in Figure 5.

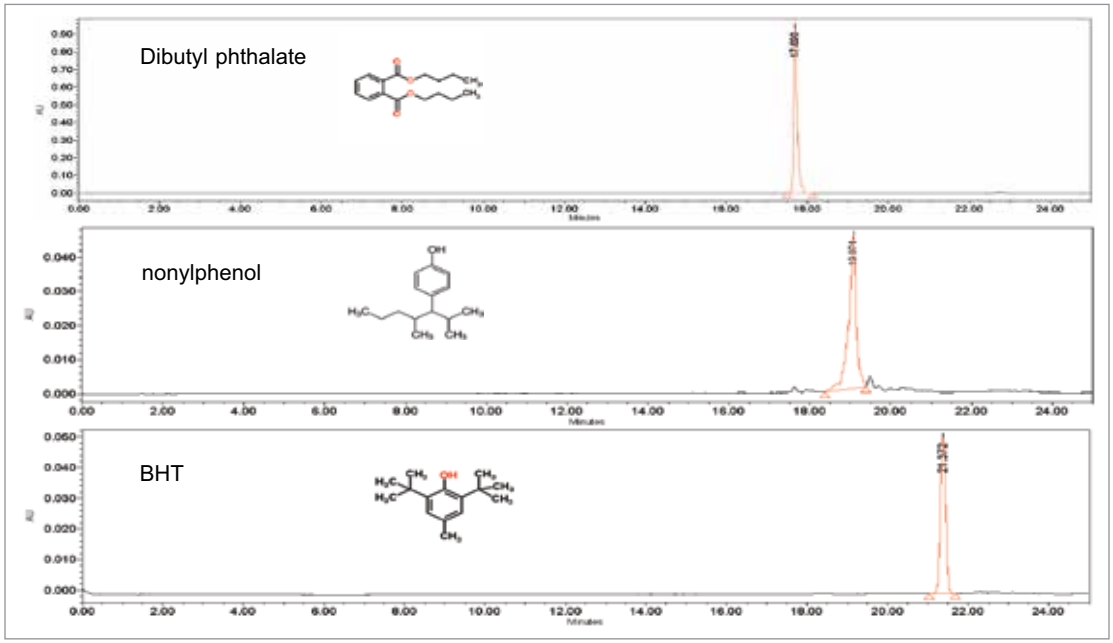


Figure 5. UV Chromatograms of solvent standards of dibutyl phthalate (top), nonylphenol (middle), and butylated hydroxyl toluene (BHT) (bottom).

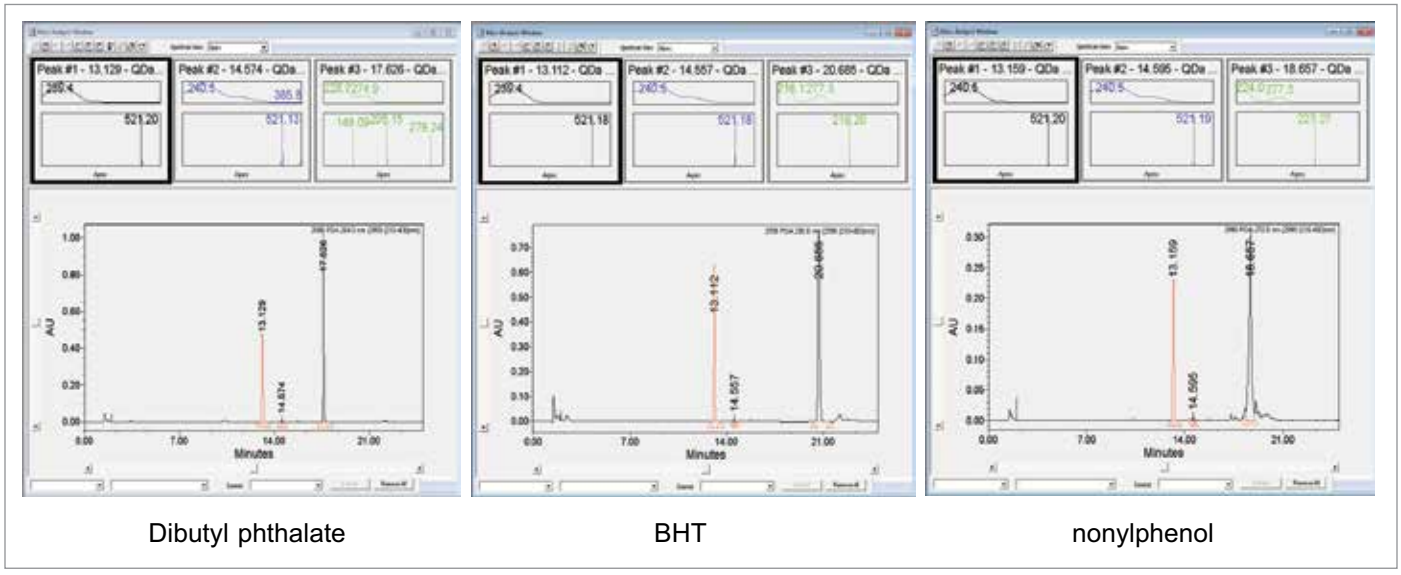


Figure 6. Empower Mass Analysis window summaries of matrix samples spiked with dibutyl phthalate (left), BHT (middle), and nonylphenol (right).

Empower's Mass Analysis windows for the matrix spiked samples are shown in Figure 7. Retention times, UV spectra, and MS signals corresponded with the spiked samples of dibutyl phthalate and BHT, offering strong evidence that harsh forced degradation conditions cause polymer additives to migrate from the packaging into the formulation. In the case of nonylphenol spiked matrix samples the Mass Analysis window in Empower showed important UV spectral differences in terms of UV maxima, while the ACQUITY QDa Mass Detector provided a strong m/z signal at 227.27 under the experimental conditions. This m/z signal would correspond to a molecular species of $[M+Formate-C_3H_3]^-$ Taken together, this data offers strong evidence for the absence of the banned polymer additive nonylphenol in the Forced Degradation sample.

The ACQUITY Arc's dual flow path technology allows for the utilization of both traditional HPLC and UHPLC stationary phases. Conversion of gradient methods is now easily achieved with the use of automated converters. The original USP method was input to the ACQUITY Column Calculator to convert the gradient to UHPLC dimensions (Figure 7).

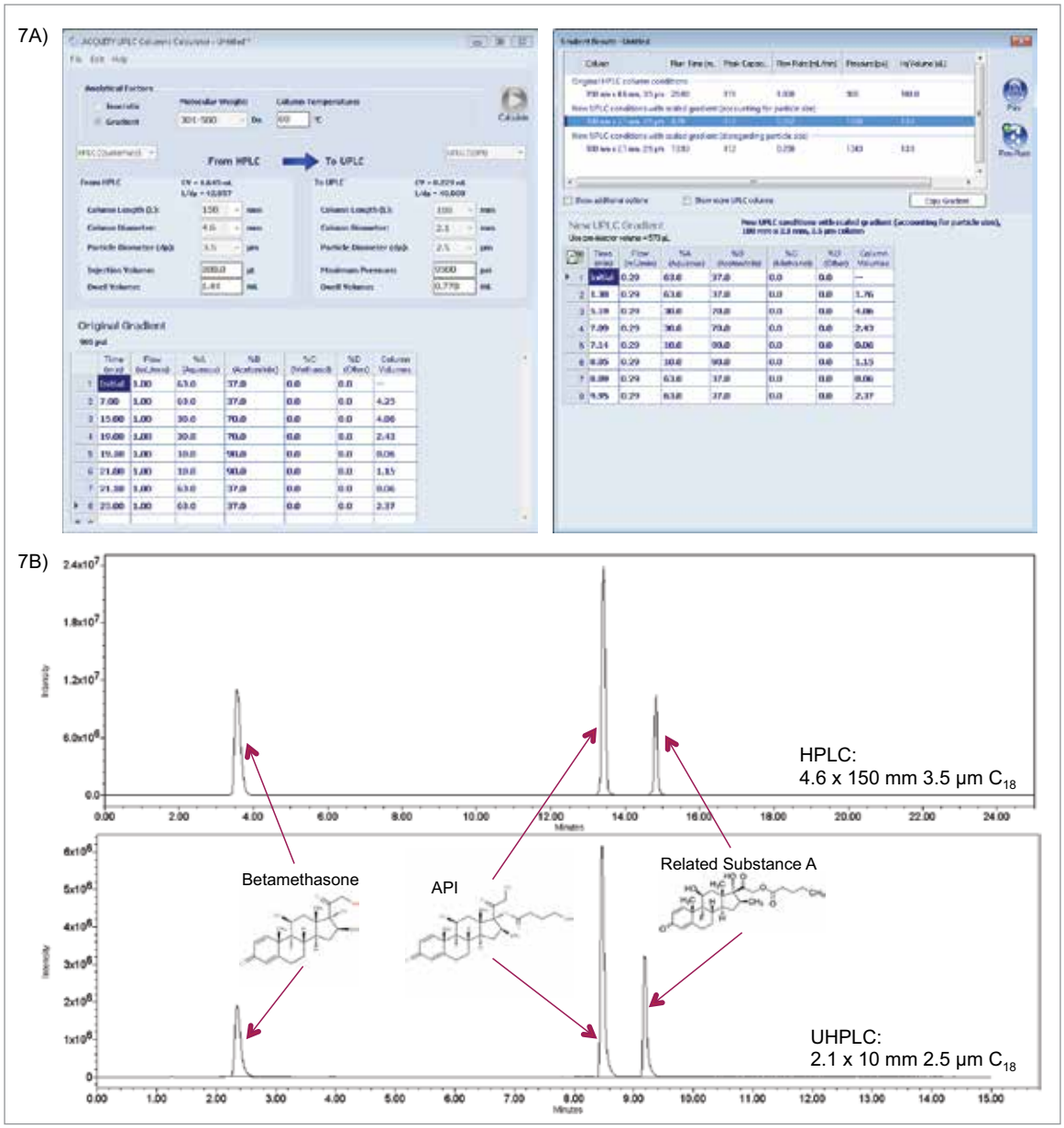


Figure 7A. Automated gradient scaling with the ACQUITY Column Calculator; 7B. Comparison of HPLC and UHPLC chromatograms.

With the reduced run times associated with moving from HPLC to UHPLC scale separations it is now practical to probe for the presence of additional low polarity polymer additives in the Forced Degradation sample through the addition of a 100% organic hold to the end of the UHPLC gradient profile.

Initially, the UV spectrum of the forced degradation sample was compared to that of the New Formulation sample to probe for the appearance of new UV active impurities. Studies were concentrated on any impurities eluting under 100% B conditions. Figure 8 shows features of the UV chromatograms common to both the New Formulation and the Forced Degradation samples.

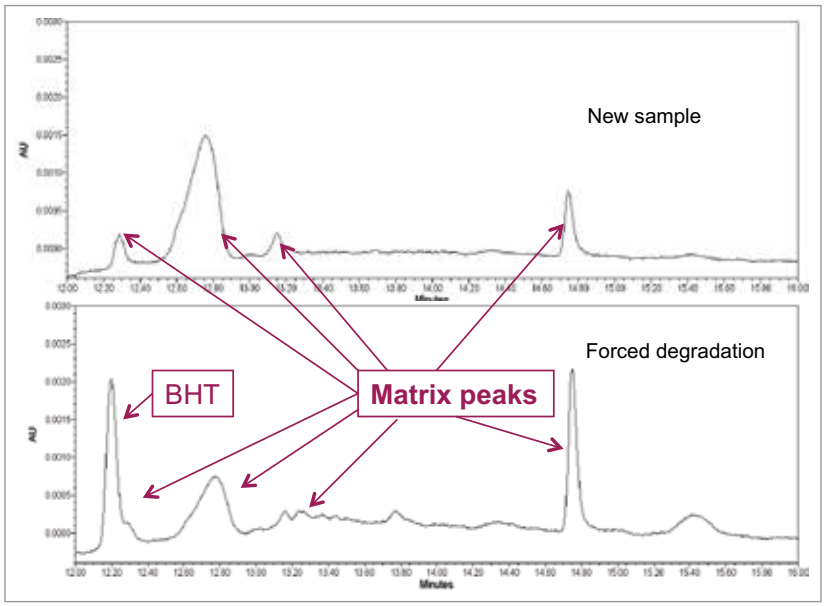


Figure 8. UV Chromatograms of the New Formulation sample (top) and Forced Degradation sample (bottom) showing matrix features common to both samples.

In the absence of any additional UV active peaks in the Forced Degradation sample compared to the New Formulation, the benefits of mass detection provided by the ACQUITY QDa Detector are highlighted when an MS scan experiment is performed on the same portion of the chromatographic run.

In addition to operating in single ion recording (SIR) mode, the ACQUITY QDa can be programmed to operate in MS scan mode in both positive and negative polarities for all or part of the chromatographic run time.

In this instance, the ACQUITY QDa was programmed to record MS scan data between 100 and 600 amu during the 100% B portion of the chromatographic run.

Figure 9 shows the total ion chromatogram (TIC) for each MS scan experiment when comparing freshly prepared New Formulation Forced Degradation samples.

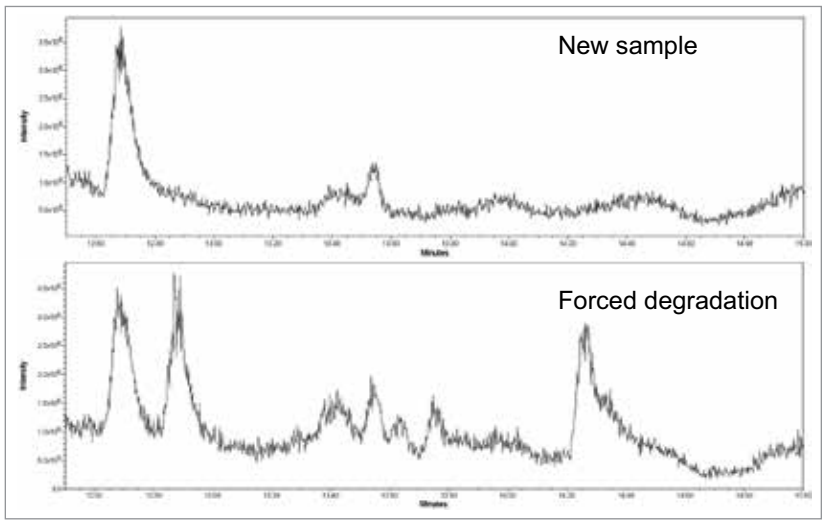


Figure 9. Comparison of the TIC (positive scan mode) for the New Formulation and Forced Degradation samples.

Figure 10 summarizes the interrogation of the mass spectral data across the samples. In regions that show matching components of both the New Formulation and Forced Degradation samples (A and B) suggest that matrix peaks detected by both PDA and ACQUITY QDa were due to a polyethylene glycol species present in the formulation. This is evidenced by repeating MS units increasing by m/z 46 in those peaks. When comparing the TIC chromatograms of new and degraded formulations show two regions where there are marked differences, suggesting the presence of further additional compounds leached from the packaging under degradation conditions. The first potential (C) leachate eluted at 12.9 min and interrogation of the MS scan data reveals an m/z value of 282.3. A second potential leachate (D) eluted between 14.2 and 14.4 min and corresponds to an m/z value of 338.3. Common polymer additives used in HDPE formulations that would correspond to $[M+H]^+$ values 282.3 and 338.3 are oleamide and erucamide respectively.

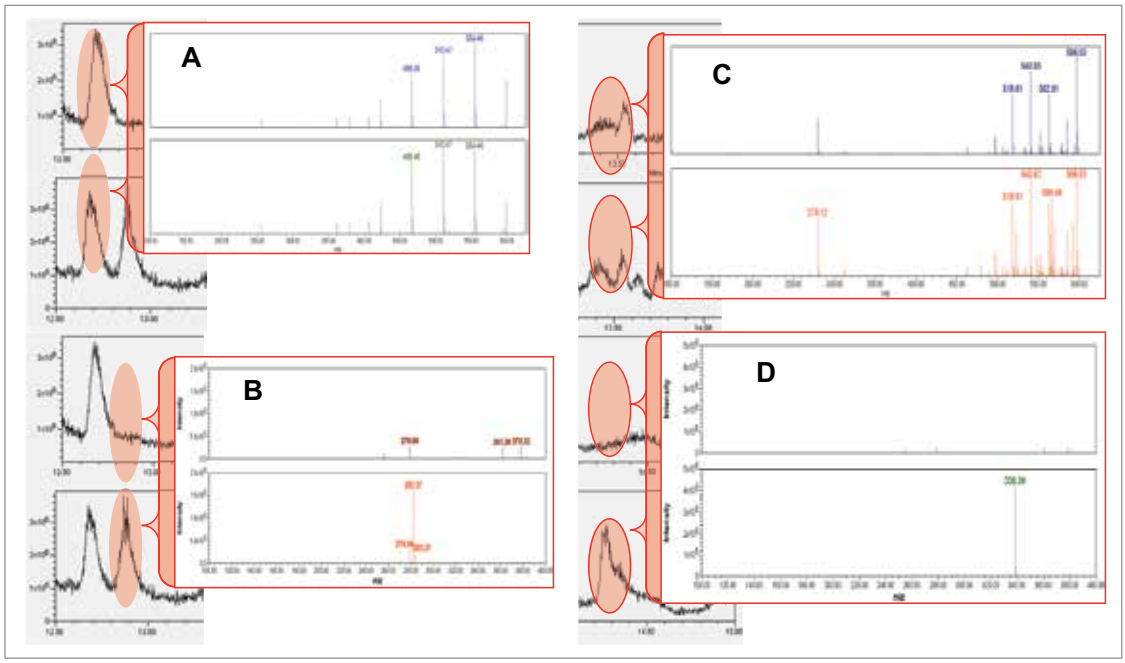


Figure 10. Probing the mass spectral data for differences between the New Formulation and Forced Degradation samples.

Analytical standards of oleamide and erucamide were analyzed by the method (Figure 11) to determine if chromatographic and mass spectral data were comparable to the contaminants found in the degraded samples.

It is clear from Figure 11 that retention times and $[M+H]^+$ values for the oleamide and erucamide standards matched the unknown peaks in the Forced Degradation sample providing strong evidence that these compounds have migrated from the packaging under forced degradation conditions. As is the case for previously putatively identified compounds dibutylphthalate and BHT, high resolution mass spectrometry (HRMS) data would be required before confident identification could be achieved.

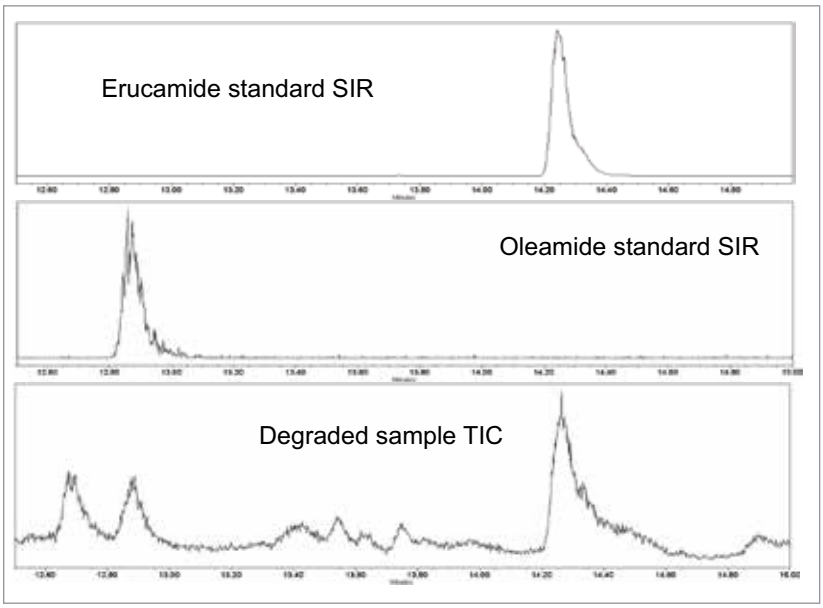


Figure 11. Comparison of erucamide and oleamide standards with degraded sample TIC.

Having tentatively identified the presence of four known polymer additives in the Forced Degradation sample, new formulation samples were prepared with decreasing concentrations of those additives to determine limits of detection and quantitation in the formulation by the UHPLC method in its current iteration. Those limits are summarized in Tables 1 and 2.

Table 1. Detection limits, HPLC method.

Cmpd	UV LOD (ppm)	UV LOQ (ppm)	QDa LOD (ppm)	Qda LOQ (ppm)
Erucamide	N/A	N/A	ND	ND
Nonylphenol	0.05	0.1	0.5	1
BHT	0.1	0.3	0.05	0.2
Butylphthalate	0.05	0.1	0.2	0.5

Table 2. Detection limits, UHPLC method.

Cmpd	UV LOD (ppm)	UV LOQ (ppm)	QDa LOD (ppm)	Qda LOQ (ppm)
Erucamide	N/A	N/A	ND	ND
Oleamide	N/A	N/A	ND	ND
Nonylphenol	0.2	0.5	0.05	0.2
BHT	0.4	1.0	0.05	0.2
Butylphthalate	0.05	0.1	0.01	0.03

N/A = not applicable, ND = not determined.

CONCLUSIONS

The ACQUITY Arc System was used to successfully replicate an HPLC-based assay and related substance testing of betamethasone valerate 0.1% w/w ointment according to USP 35 monograph methods. The system achieved prescribed system suitability requirements according to the described methods and it was able to characterize differences in the levels of actives, degradants, and process impurities between freshly sourced formulations and samples stored under ambient conditions for long periods (>6 months).

The addition of mass detection to the method via hyphenation with the ACQUITY QDa Mass Detector provided lower limits of detection for degradants and related substances than those achieved with PDA detection alone. The addition of mass detection also allowed for the putative identification of two impurities observed upon subjecting the formulations to forced degradation conditions.

Scaling the analytical method to 2.5 µm particle sizes allowed for further investigation of the components in the Forced Degradation samples eluting under 100% organic conditions and putative identifications were again made possible through mass detection.

Reference

1. Jenke D. *Compatibility of Pharmaceutical Products & Contact Materials*. J Wiley & Sons, Inc., Hoboken, NJ. 2009.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Non-Targeted Screening of Extractables and Leachables in E-Cigarettes Using UPLC and GC Coupled to QTof-MS

Naren Meruva, Baiba Cabovska, Dimple Shah, Kari Organtini, Gareth Cleland
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Comprehensive characterization of extractables and leachables using UPLC™ and GC which can be configured to a single QTof-MS
- Accurate mass screening using MSE data acquisition combined with scientific libraries streamlines identification of potential extractables
- Sample comparison workflows and structure elucidation toolkits for characterization of unknown compounds
- Metabolite ID workflow can be used to evaluate possible degradation or transformation products of formulation components

WATERS SOLUTIONS

ACQUITY™ UPLC HSS T3 Column

ACQUITY UPLC I-Class System

Atmospheric Pressure Gas Chromatography (APGC)

Xevo™ G2-XS QTof Mass Spectrometer

UNIFI™ Scientific Information System

KEYWORDS

Extractables, leachables, e-cigarette, e-liquid, tobacco, UPLC, GC, QTof-MS, APGC

INTRODUCTION

Characterization of extractables and leachables is essential for ensuring the safety, quality, and efficacy of inhalation tobacco products such as e-cigarettes. The initial step for characterizing extractables from e-cigarettes involves targeted screening where you analyze the extract and quantify against known impurity standards. This is a well-established process that can be performed using analytical techniques such as GC-MS, LC-MS/MS and ICP-MS. However the finished products (e-liquids, refill cartridges, and e-cigarette aerosol) may have impurities present from the starting materials and other packaging and device components that need to be further evaluated by non-targeted screening analysis.

E-cigarette regulations are still evolving due to a lack of scientific information and lack of product quality and safety standards. Both the US FDA regulation and the revised EU Tobacco Products Directive (TPD2; 2014/40/EU) subject e-cigarette manufacturers to product and ingredient disclosures and good manufacturing practices to ensure e-cigarette products are appropriate for the protection of the public health.[1,2] In the UK, the MHRA (Medicines and Healthcare Products Regulatory Agency) regulates e-cigarettes as nicotine delivery devices and requires manufacturers to provide complete quality information for licensing e-cigarette devices including the composition of the e-cigarette device, the plastic, polymer, and metal components used, the quality of the nicotine and excipients, data from extractables and leachables studies, and product stability data during use, and shelf-life.[3]

In this study, the various components of an e-cigarette device (end caps, mouth piece, gauze, heating element, and flavor formulation) were extracted individually and subjected to non-targeted high resolution screening using UPLC and GC which can be configured to the same QTof-MS. Accurate mass data for precursor and fragment ions was acquired using alternating high and low collision energy states (MS^E) across the full analytical mass range. Data from the sample component extracts was compared to the reagent blank to determine differences and identify potential extractables. In this application note, we describe a workflow on how non-targeted screening for extractables and leachables testing can be performed in e-cigarettes. The workflow demonstrated here is also applicable to non-targeted screening for extractables and leachables in packaging for food, cosmetics, and pharmaceuticals.

EXPERIMENTAL

The various components of a closed system e-cigarette cartridge (outer and inner end caps, mouth piece, gauze with flavor formulation, paper wrap, and metal shell) were extracted separately using isopropanol solvent for 30 minutes and subjected to non-targeted high resolution screening using UPLC and GC coupled to QTof-MS. As part of the batch QC analysis, Waters™ Extractables and Leachables Screening Standard [p/n: 186008063], that includes 18 common polymer additives, was used to evaluate and benchmark the high resolution UPLC-QTof-MS system. The Extractables and Leachables Screening Standard covers a mass range of up to 1176 Da, supporting both positive and negative ionization modes.

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Column:	ACQUITY UPLC BEH C_{18}, 130Å, 1.7 µm, 2.1 × 100 mm
Column temp.:	45 °C
Sample temp.:	4 °C
Mobile phase A:	10 mM ammonium acetate (pH 5.0) in water
Mobile phase B:	10 mM ammonium acetate (pH 5.0) in MeOH
Flow rate:	0.45 ml/min
Needle wash:	50:50 water:methanol (v/v)
Syringe purge:	10:90 methanol:water (v/v)
Total run time:	17 min
Injection volume:	10 µL

Gradient:

Time (min)	%A	%B
0.00	98	2
0.25	98	2
12.25	1	99
13.00	1	99
13.01	98	2
17.00	98	2

MS (ESI) conditions

MS system:	Xevo G2-XS QTof
Capillary voltage:	0.8 kV
Sampling cone:	20.0
Source temp.:	120 °C
Source offset:	80
Carrier gas:	Nitrogen
Cone gas flow:	50 L/Hr
Desolvation gas flow:	1000 L/Hr
Acquisition range:	50–1200 *m/z*
Scan time:	0.25 sec
Lockmass:	Leucine enkephalin (556.2771 *m/z*)

GC conditions

GC system:	A7890 (with APGC Interface)
Column:	DB-5MS 0.25 µm, 30 m × 0.25 mm
Desolvation temp.:	550 °C
Flow rate:	1.2 mL/min
Initial temp.:	35 °C (1.6 min)
Ramp:	25 °C/min
Final temp.:	320 °C (7 min)
Run time:	20 min
Inlet mode:	Splitless
Inlet type:	Multimode
Temp.:	280 °C
Injection volume:	1 µL
Make-up gas:	Nitrogen
Make-up gas flow:	250 mL/min
Transfer line temp.:	310 °C

MS (API) conditions

QTof System:	Xevo G2-XS QTof MS (with APGC interface)
Corona current:	3.0 µA
Sampling cone:	20.0
Source temp.:	120 °C
Source offset:	80
Cone gas flow:	175 L/Hr
Auxiliary gas flow:	50 L/Hr
Acquisition range:	50–1200 *m/z*
Scan time:	0.25 sec
Lockmass:	Siloxane bleed (281.0517 *m/z*)

Data acquisition and processing

Accurate mass data from both the GC and UPLC–QTof-MS analysis of the e-cigarette component extracts were acquired and processed using the UNIFI Scientific Infomation System.

RESULTS AND DISCUSSION

The Xevo G2-XS QTof-MS couples to either UPLC or GC to provide a full system solution for chemical profiling. Accurate mass data from both the GC and UPLC-QTof-MS analysis of e-cigarette component extracts were acquired and processed using the extractables and leachables workflow in the UNIFI Scientific Information System. Precursor and fragment ions were acquired simultaneously using alternating low- and high-collision energy states (MS^E) across the full analytical mass range. Potential candidate markers were screened against a library of known extractables and leachables compounds in UNIFI, and automatically interrogated using multiple matching criteria including accurate mass for precursor and fragment ions, adducts, and isotopic fit.

The GC-QTof-MS profiles of e-cigarette component extracts are shown in Figure 1. Potential extractables were short-listed based on the following criteria: detector response >1000, mass error ± 5 ppm and the number of expected fragments detected >0. The established UNIFI workflow utilizes accurate mass precursor and fragment ion data, and applied criteria to simplify data review and facilitate the decision-making process. It allows analysts to evaluate complex data in a more efficient way and enables rapid identification of known and unknown compounds.

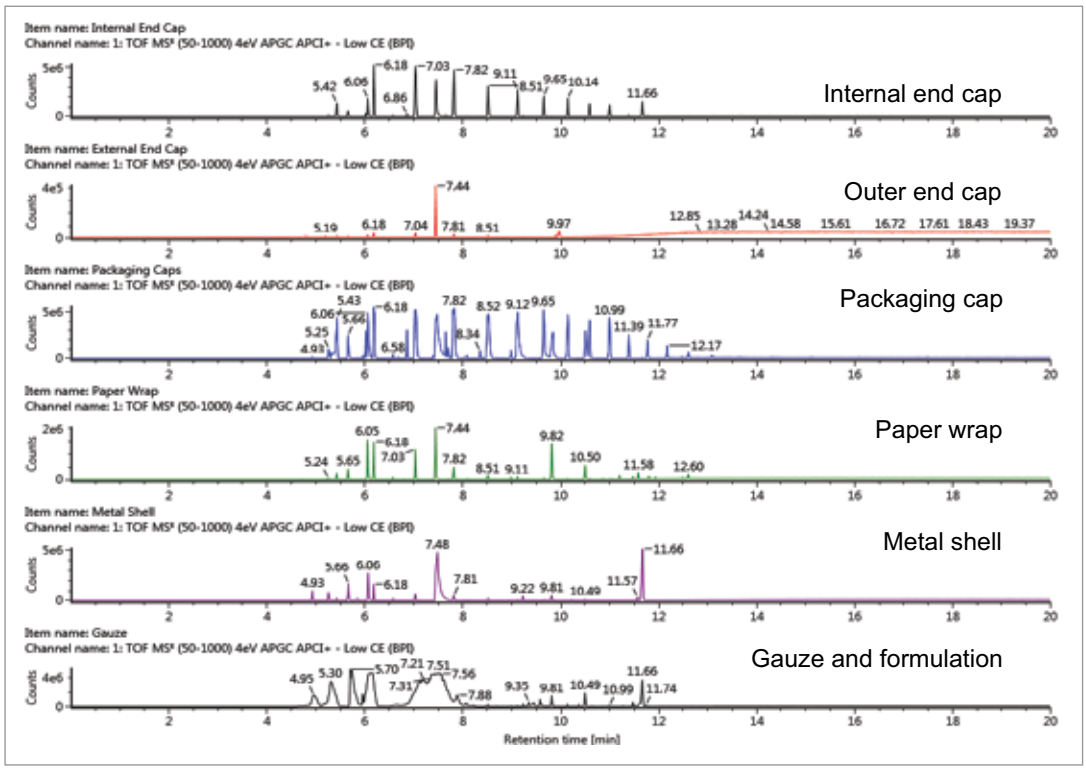


Figure 1. GC-QTof-MS profiles of e-cigarette component extracts.

Figure 2 exhibits the identification of dibutyl phthalate (DBP), a common plasticizer, in the internal end cap, metal shell, and gauze extracts using GC-QTof-MS analysis. The DBP peak had a high detector response (>11,000) in the component extracts compared to the solvent blank, one identified fragment ion, and a low measured mass error (<2.5 ppm). The migration of DBP across the internal end cap, metal shell, and gauze is possible as these components come in contact with each other in the e-cigarette cartomizer assembly.

Figure 2. Identification of dibutyl phthalate (DBP) in the internal end cap, metal shell, and gauze using GC-QTof-MS.

Figure 3 shows the identification of HMBTAD, a light stabilizer in the internal end cap, metal shell, and gauze extracts using UPLC-QTof-MS analysis. The HMBTAD peak had a high detector response (>42,000), low mass error (<1.5 ppm) and was not identified in solvent blanks. The relative levels of HMBTAD are higher in the gauze containing the flavor formulation, potentially to increase the product shelf-life stability.

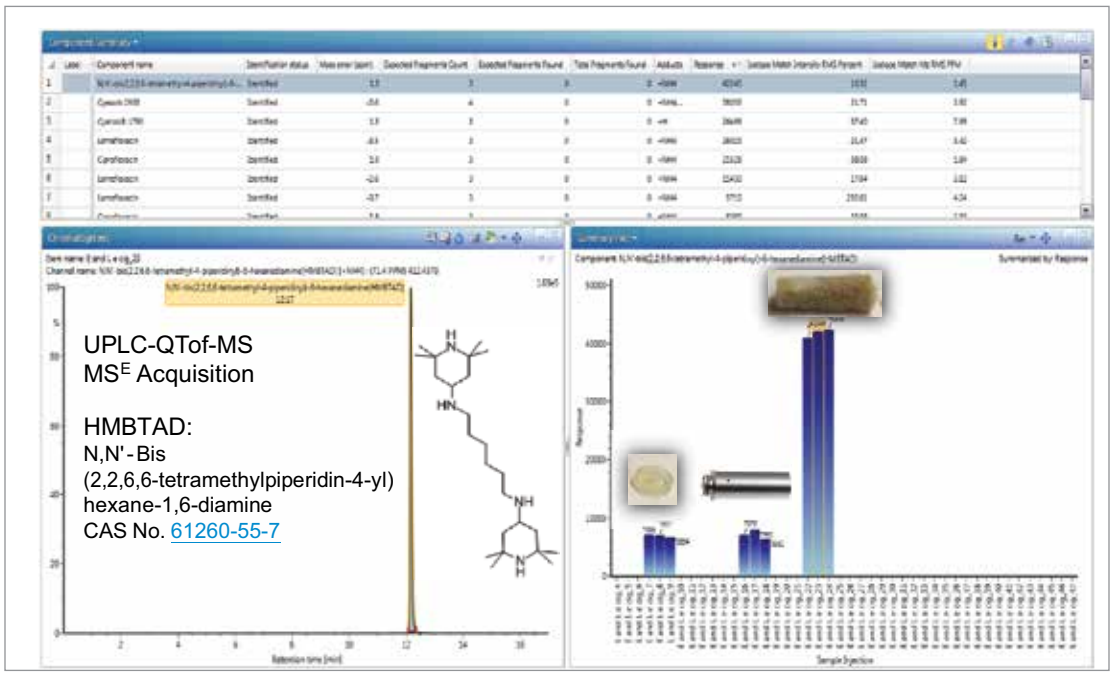


Figure 3. Identification of HMBTAD in inner end cap, metal shell, and gauze using UPLC-QTof-MS.

Table 1 lists the potential extractables detected in various e-cigarette component extracts analyzed by GC-QTof-MS and UPLC-QTof-MS. These compound identifications are based on the targeted match between the experimental data and the UNIFI Scientific Library for the accurate mass precursor and fragment ions, low mass error (± 5 ppm) and relatively high detector response (>1000).

Table 1. Tentative identifications of potential extractables using UPLC-GC-QTof-MS analysis.

Analysis	Extractables ID	Function	Internal end cap	Outer end cap	Packaging cap	Paper wrap	Metal shell	Gauze
GC-QTof-MS	Dibutyl phthalate (DBP)	Plasticizer	✓				✓	✓
	Octadecanoic acid	Surfactant/ softening agent			✓	✓		✓
	Dioutyl sebacate	Plasticizer			✓	✓		
	4-methyl benzophenone (4-MBP)	Stabilizing agent			✓	✓	✓	✓
	Sorbic acid	Food preservative					✓	✓
	N,N-Dimethyl-p-phenylenediamine	Polymer additive				✓	✓	
UPLC-QTof-MS	HMBTAD	Light stabilizer	✓				✓	✓
	Disperse red 11	Dye			✓			
	Uvinul 120	Anti-oxidant			✓			
	Irgafos 168	Light stabilizer			✓			

CONCLUSIONS

Comprehensive characterization of extractables and leachables requires evaluation using multiple chromatographic techniques (UPLC and GC), multiple modes of ionization, and an integrated informatics workflow (UNIFI). Accurate mass screening using MS^E data acquisition, combined with scientific libraries can be used to automatically identify target components.

UNIFI's sample comparison and elucidation toolsets are useful for quickly identifying known targets and characterizing unknown compounds. A metabolite identification workflow can be used to evaluate possible degradation or transformation products of formulation components in e-cigarette products. This study demonstrates an integrated workflow for targeted and non-targeted screening using UPLC and GC on a single MS platform with UNIFI informatics for extractable and leachable screening in e-cigarettes, food, cosmetics, and pharmaceutical packaging applications.

References

1. FDA Deeming Regulation (May 2016) – FDA's New Regulations for E-Cigarettes, Cigars, and All Other Tobacco Products. https://www.fda.gov/tobaccoproducts/labeling/rulesregulationsguidance/ucm394909.htm.
2. EU Tobacco Products Directive (TPD2; 2014/40/EU) https://ec.europa.eu/health/sites/health/files/tobacco/docs/dir_201440_en.pdf.
3. Medicines and Healthcare Products Regulatory Agency (2016). E-cigarettes: Regulations for Consumer Products. Relevant guidance documents available via https://www.gov.uk/guidance/e-cigarettes-regulations-for-consumer-products.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Application of UPC² in Extractables Analysis

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

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

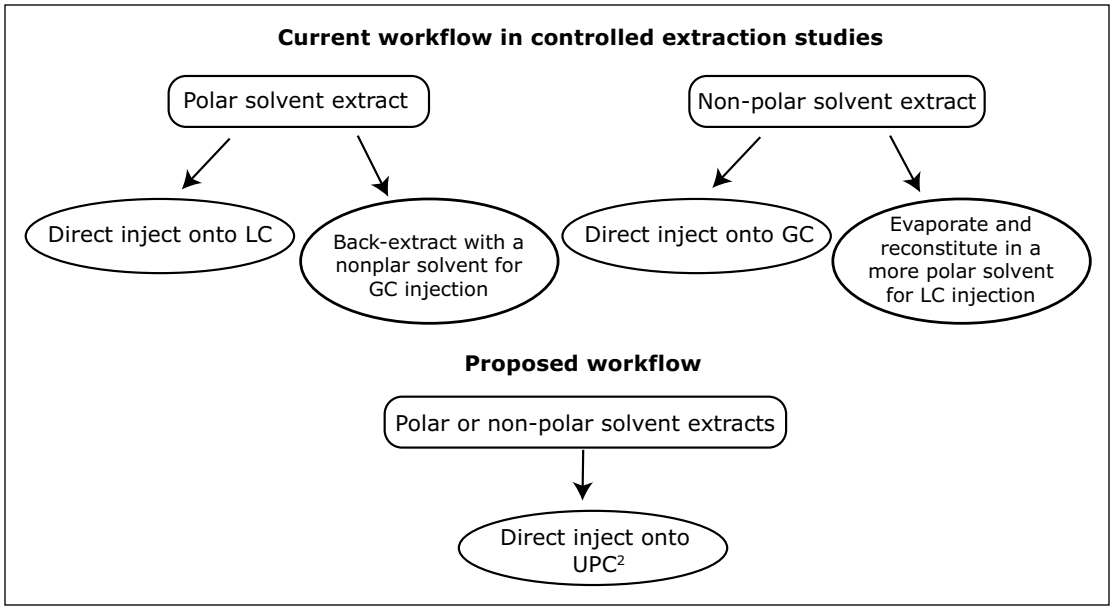
Column:	HP-5MS 30 m x 0.32 mm, 1.0 µm film
Carrier gas:	He at 2 mL/min
Temperature program:	35 °C for 5 min, 20 °C/min to 320 °C, hold 20.75 min
Injection port:	300 °C
Injection type:	1 µL splitless, 1 min purge
Makeup gas:	N_2 at 400 mL/min
Transfer line:	350 °C
Scan range:	100 to 1500 *m/z*
Run time:	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 3 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.

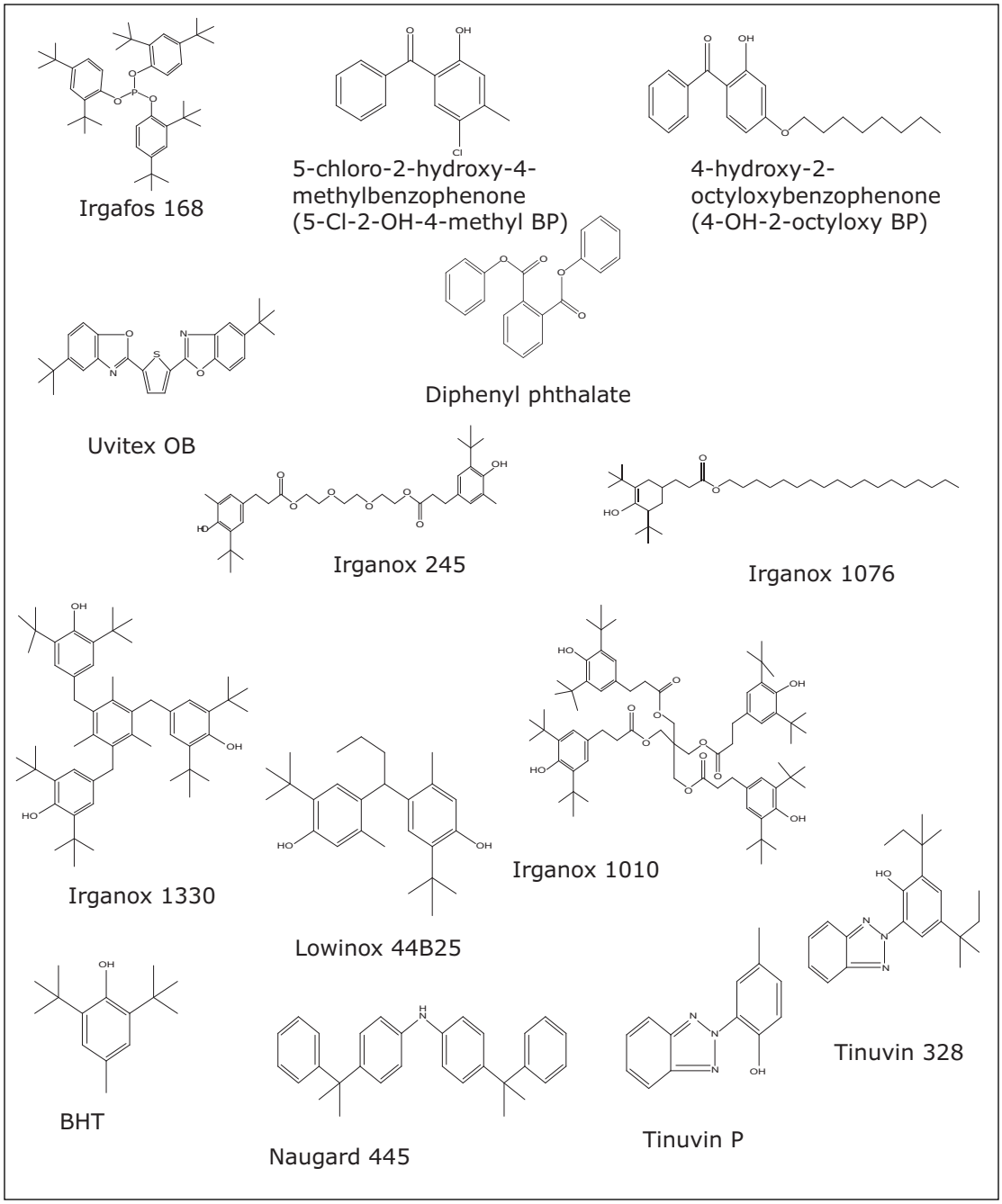


Figure 1. Polymer additives and their structures.

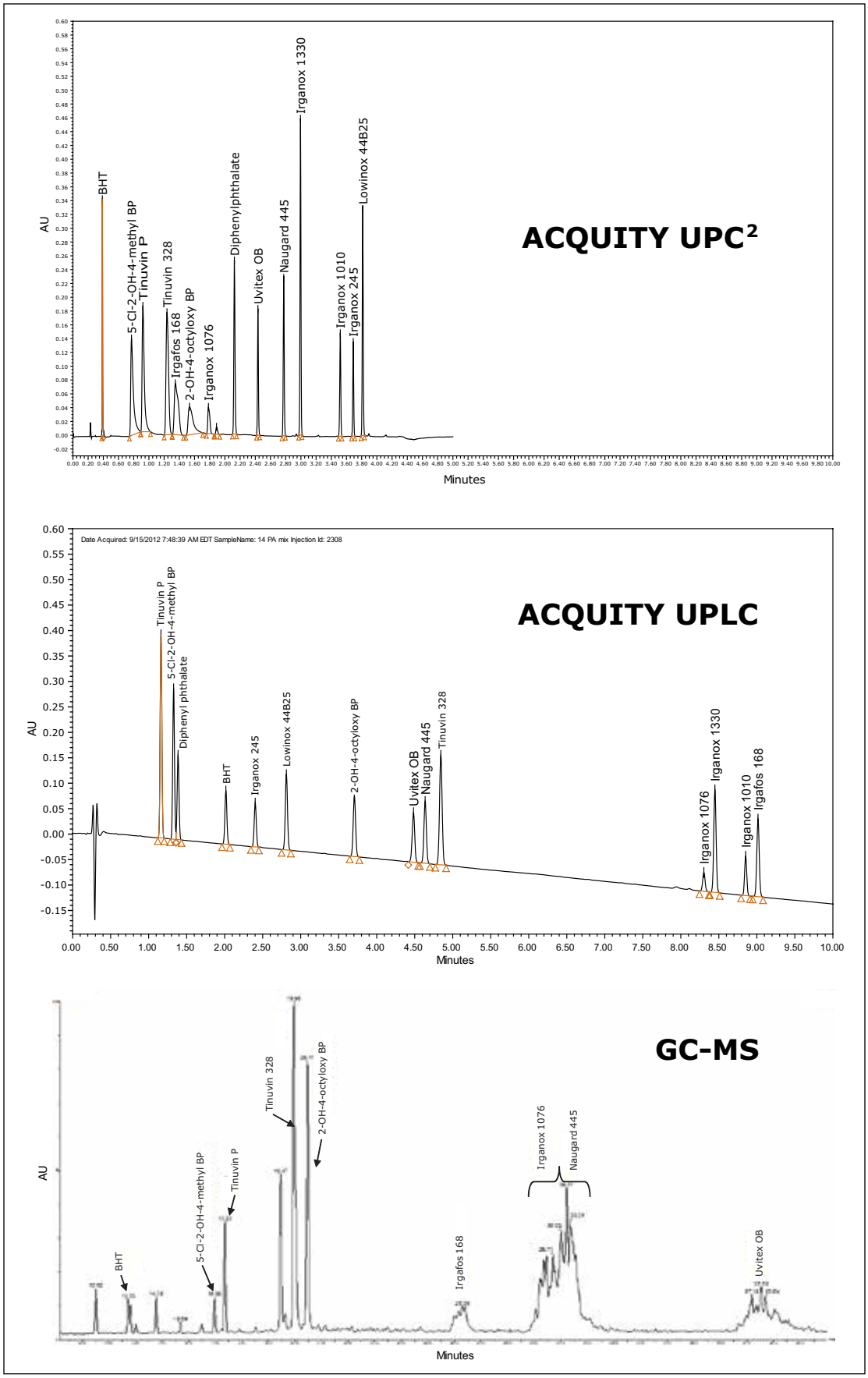


Figure 2. Chromatograms for standards separation.

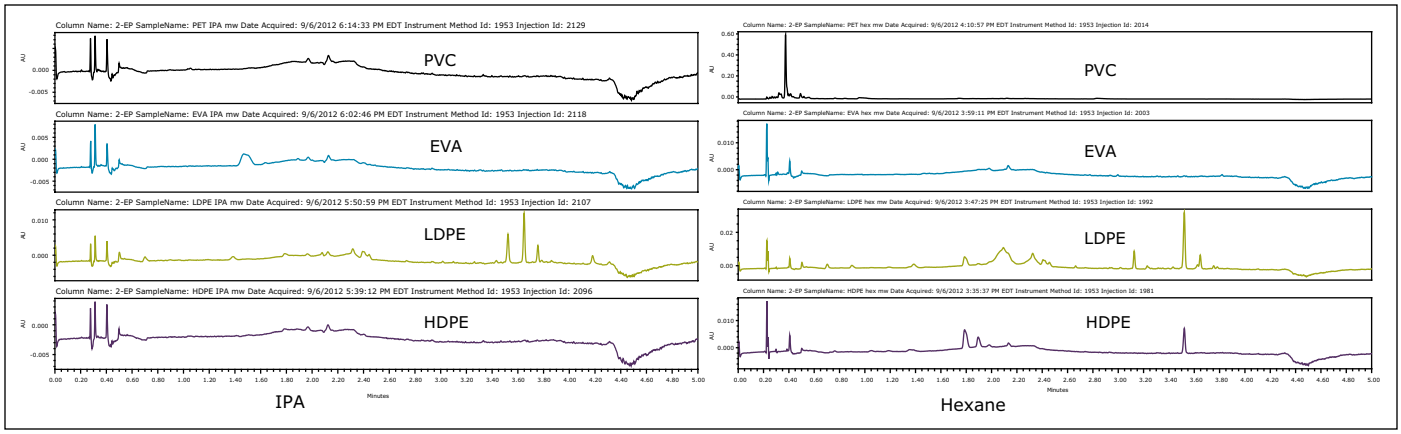


Figure 3. ACQUITY UPC² System chromatograms for IPA and hexane sample extracts.

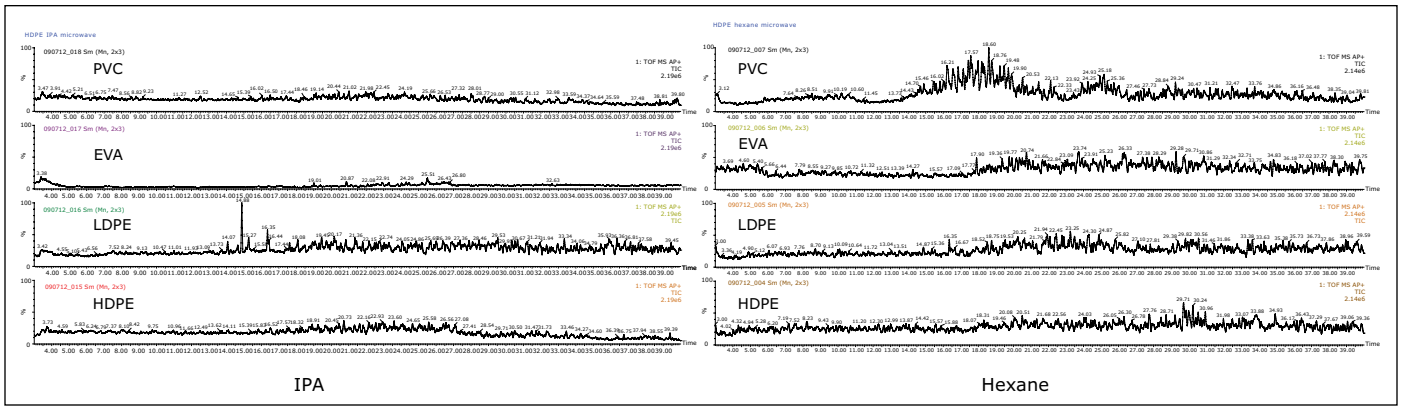


Figure 4. GC-MS chromatograms for IPA and hexane extracts.

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.

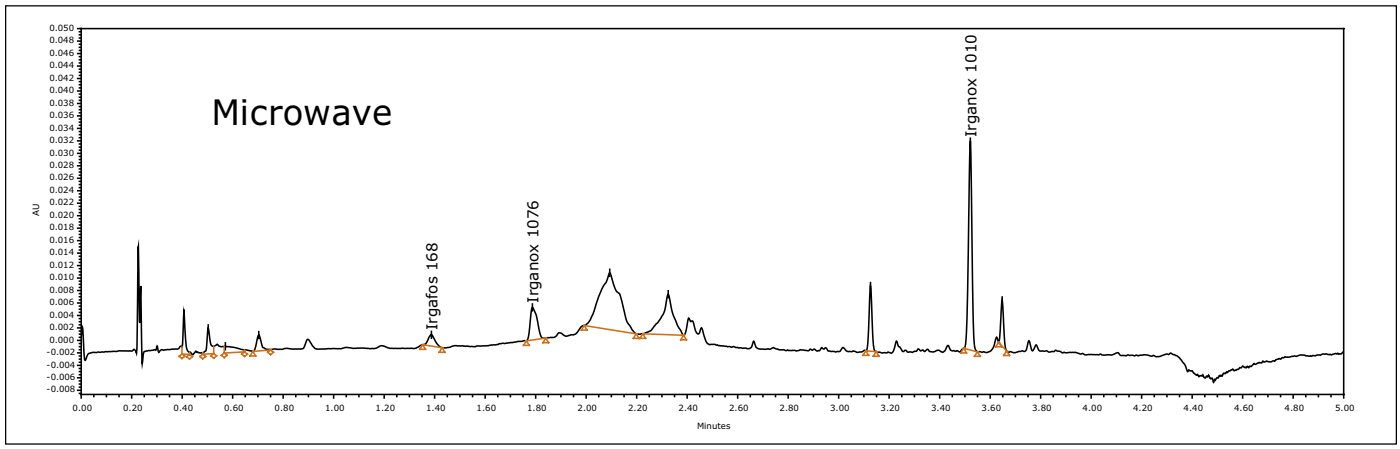


Figure 5. Identified extractables in LDPE hexane extract using ACQUITY UPC².

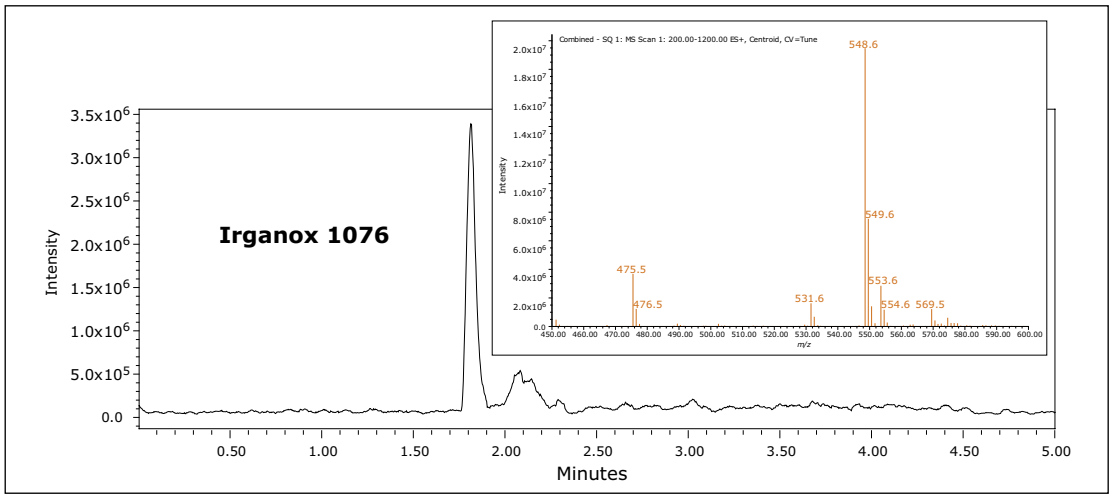


Figure 6. Irganox 1076 in LDPE hexane extract by UPC².

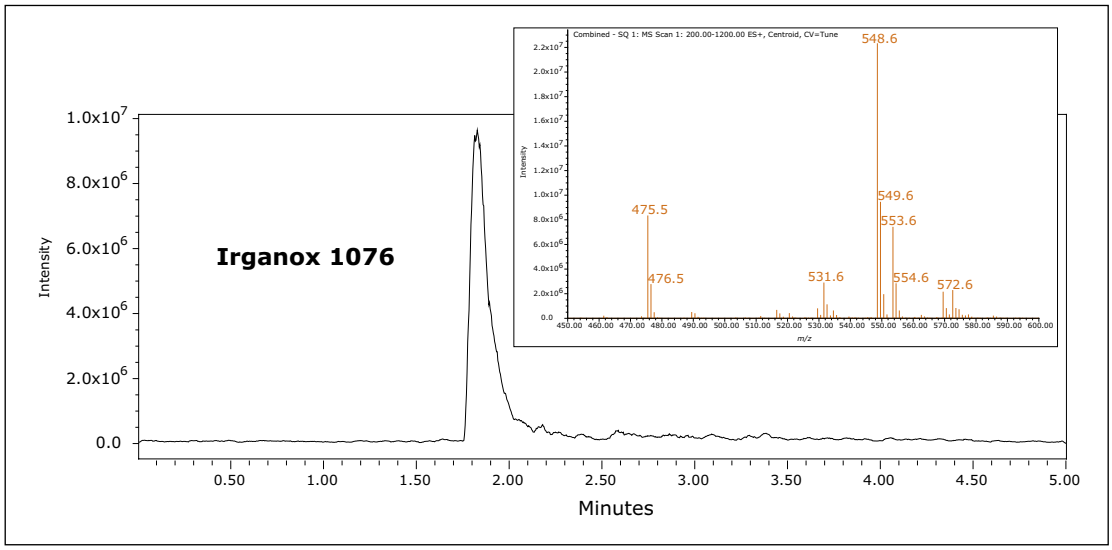


Figure 7. Irganox 1076 standard by UPC².

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.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Streamlining Current Approaches for Extractable Analysis Utilizing Waters MV-10 ASFE and ACQUITY UPC² 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
- UPC²® enhances extractables analysis by streamlining the workflow

WATERS SOLUTIONS

ACQUITY UPC² System configured with PDA and SQD Detection

MV-10 ASFE™ System

Empower® 3 Software

KEY WORDS

Extractables, SFE, UPC², 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 UPC²® 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 (UPC²) 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 hours. 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.

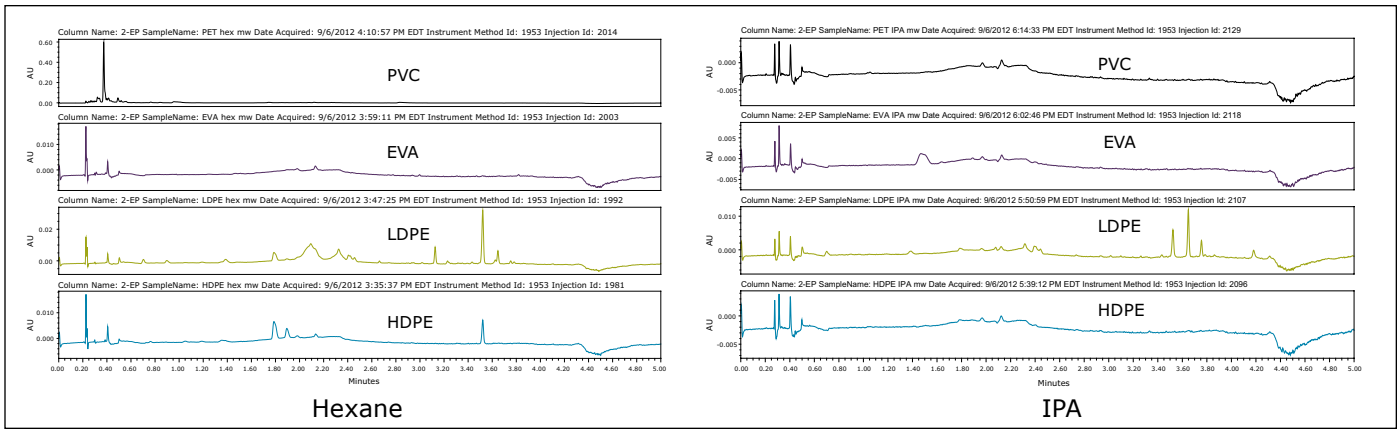


Figure 1. Hexane and IPA extracts using the microwave extraction technique.

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.

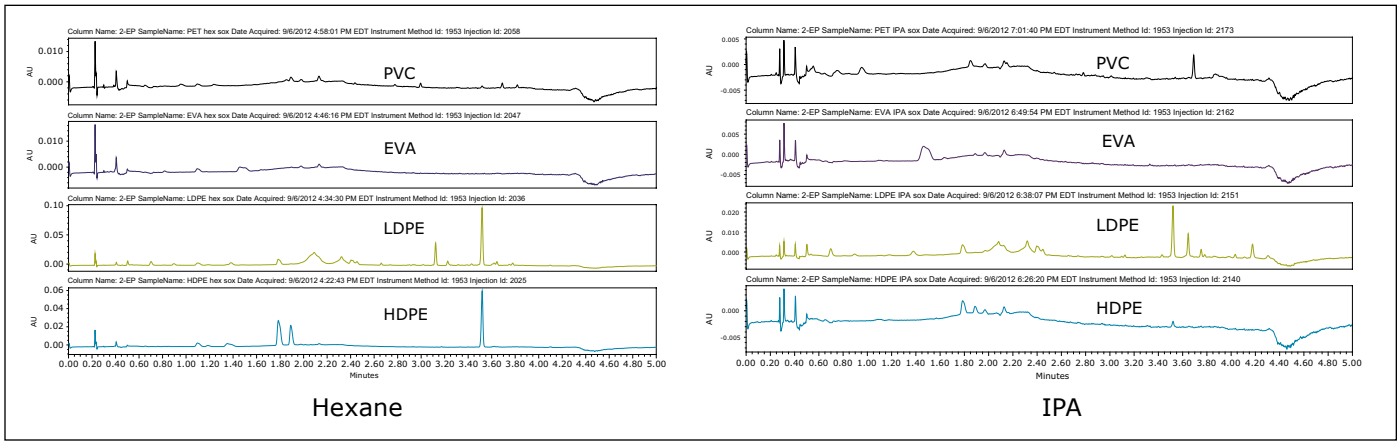


Figure 2. Hexane and IPA extracts using the Soxhlet extraction method.

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.

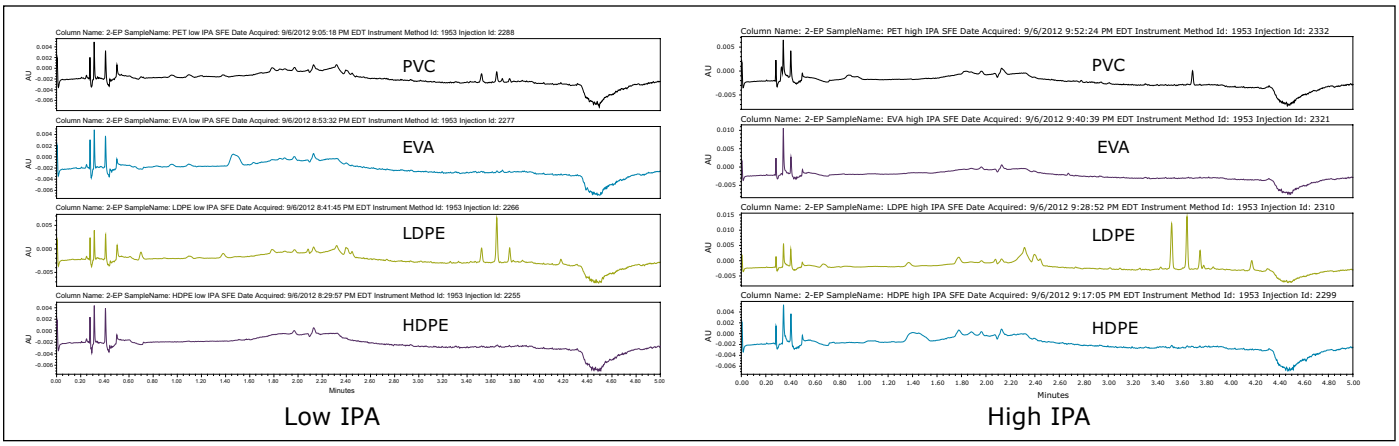


Figure 3. SFE extracts with low and high volumes of IPA co-solvent.

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.

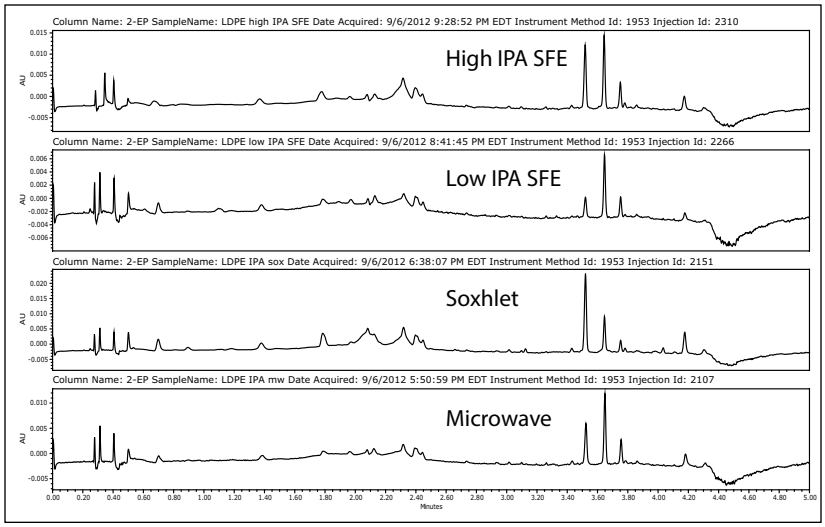


Figure 4. IPA extracts for LDPE.

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

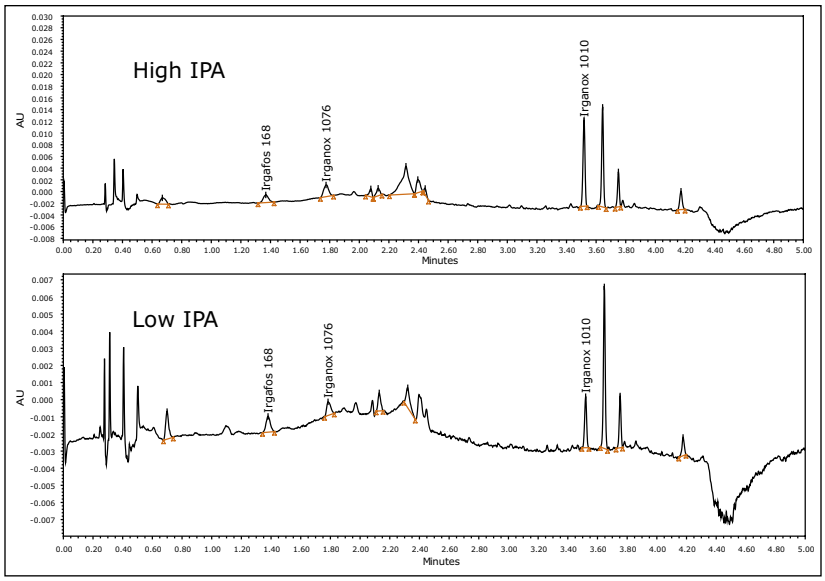


Figure 5. Identified extractables in LDPE, SFE extracts.

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 no. 720004490en. 2012 November.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

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

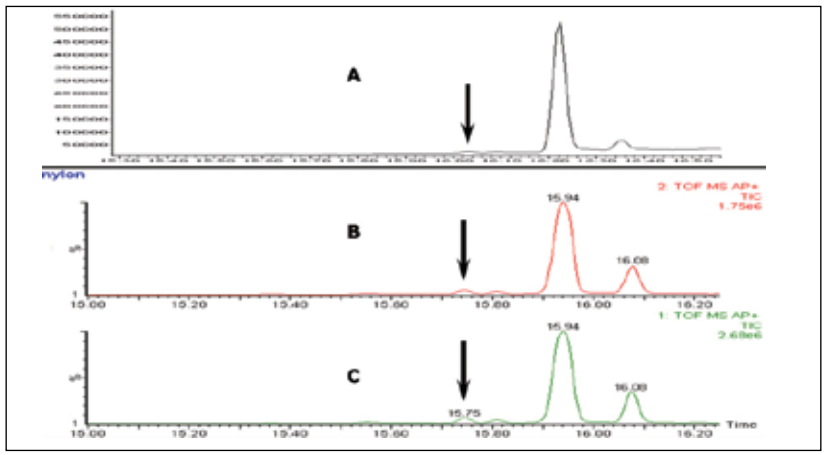


Figure 1. A = EI TIC, B = high energy/fragmentation APGC TIC, C = low energy/molecular ion APGC TIC.

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

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.

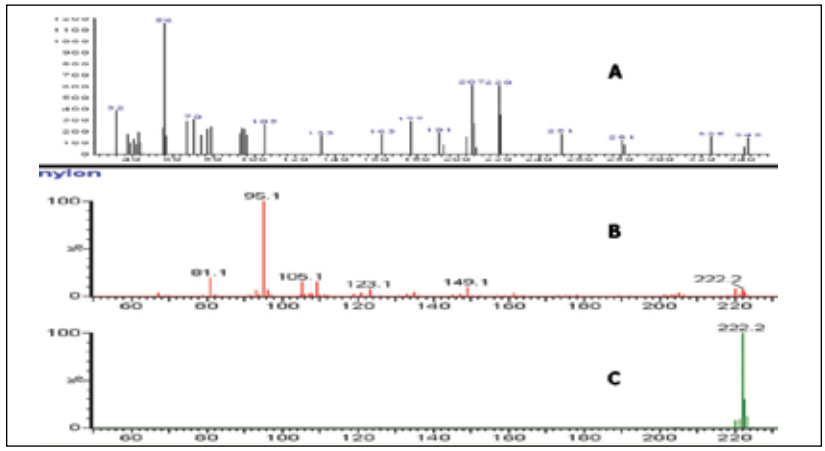


Figure 2. A = EI spectrum, B = high energy/fragmentation APGC spectrum, C = low energy/molecular ion APGC spectrum.

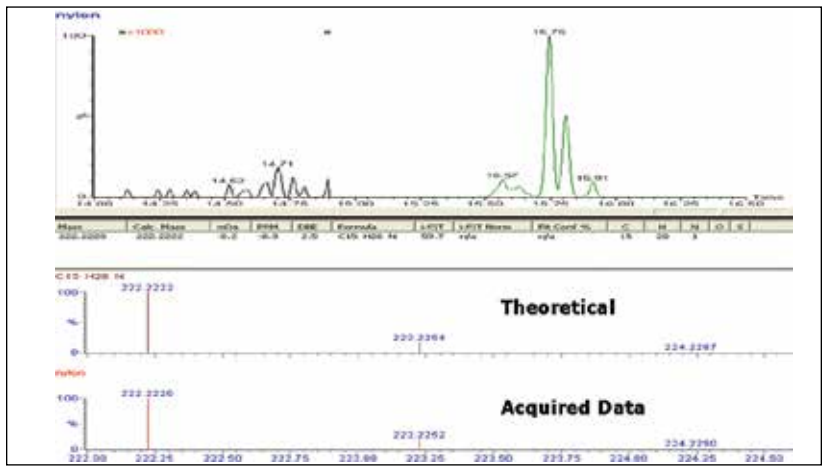


Figure 3. Upper, accurate mass XIC of 222.2222 Da. Lower, accurate mass spectrum from low energy MS^E data along with the theoretical isotope model for the calculated elemental formula.

* 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.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

[APPLICATION NOTE]

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Identifying Leachables and Extractables from Packaging Materials

Baiba Čabovska,[1] Douglas M. Stevens,[1] A. John Cunningham,[2] Arthur E. Bailey[2]
[1]Waters Corporation, Milford, MA, USA
[2]Mannkind Corporation, Danbury, CT, USA

APPLICATION BENEFITS

- Facilitates the daunting task of identifying unknown compounds in any field that deals with structural elucidation, such as Pharmaceutical, Chemical, 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

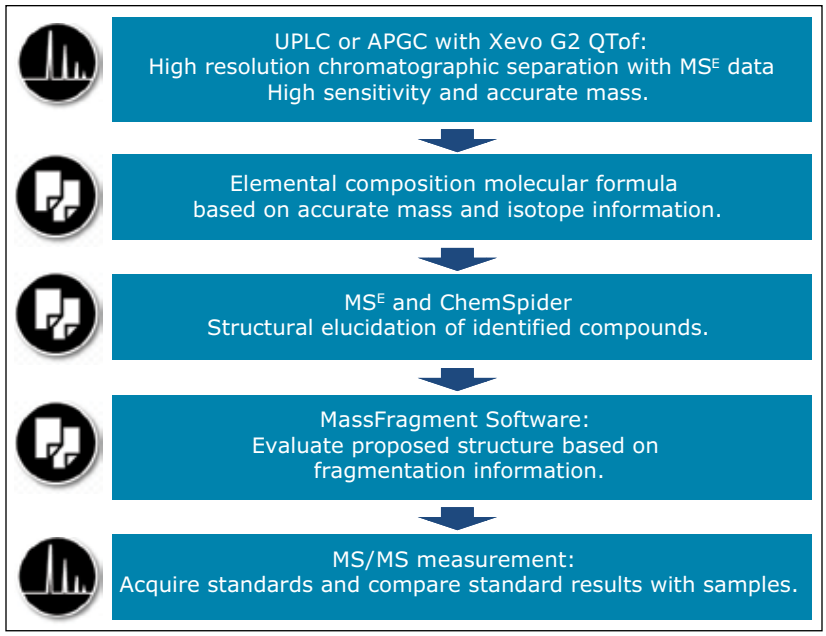
Samples were prepared by microwave extraction. The samples of polypropylene and nylon (2 g) were extracted in 10 mL of isopropanol for 3 h at 70°C. After the extraction the supernatant was transferred to the GC vials.

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.

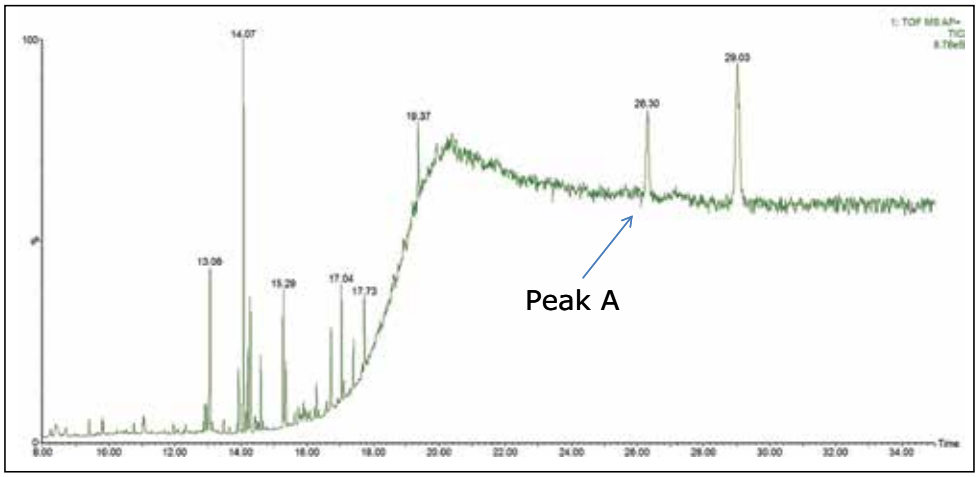


Figure 1. Polypropylene TIC.

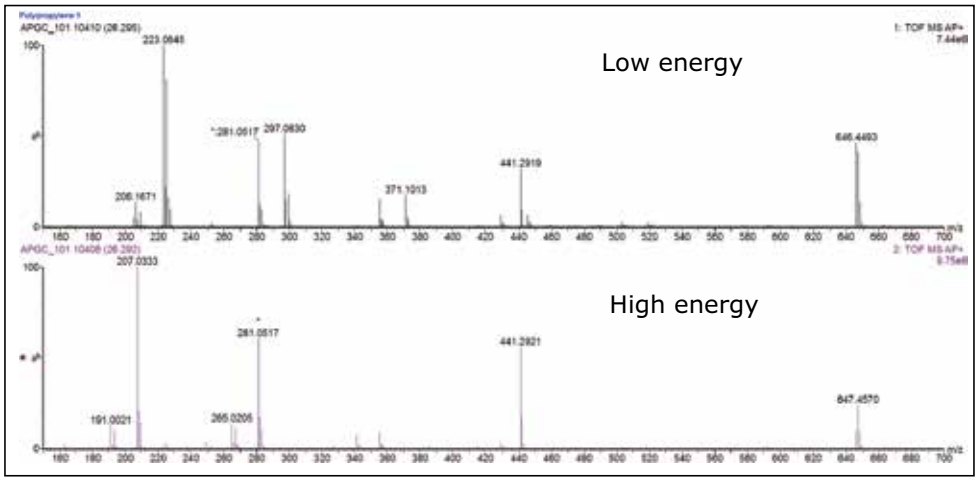


Figure 2. High and low energy spectra for Peak A.

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.

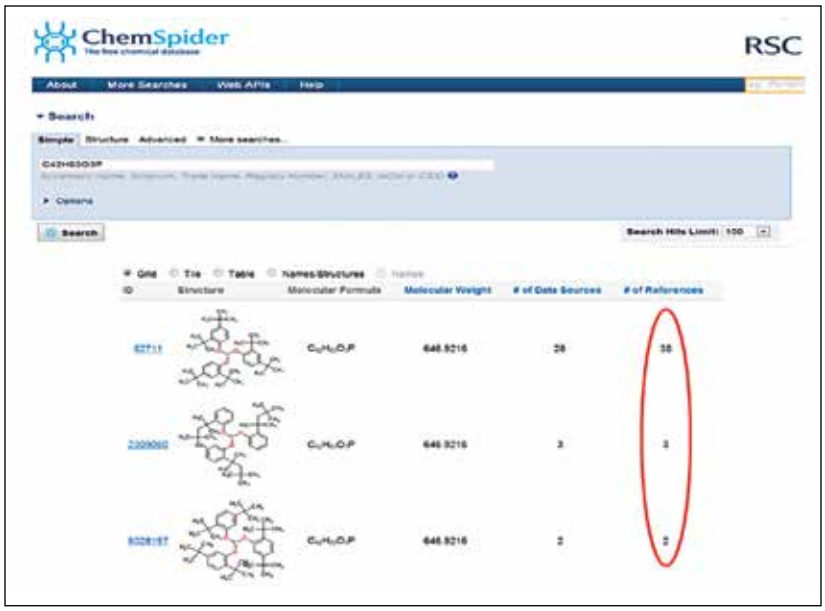


Figure 4. ChemSpider search for $C_{42}H_{63}O_3P$, first match is Irgafos 168. The search hits are ordered by number of references and data sources.

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.

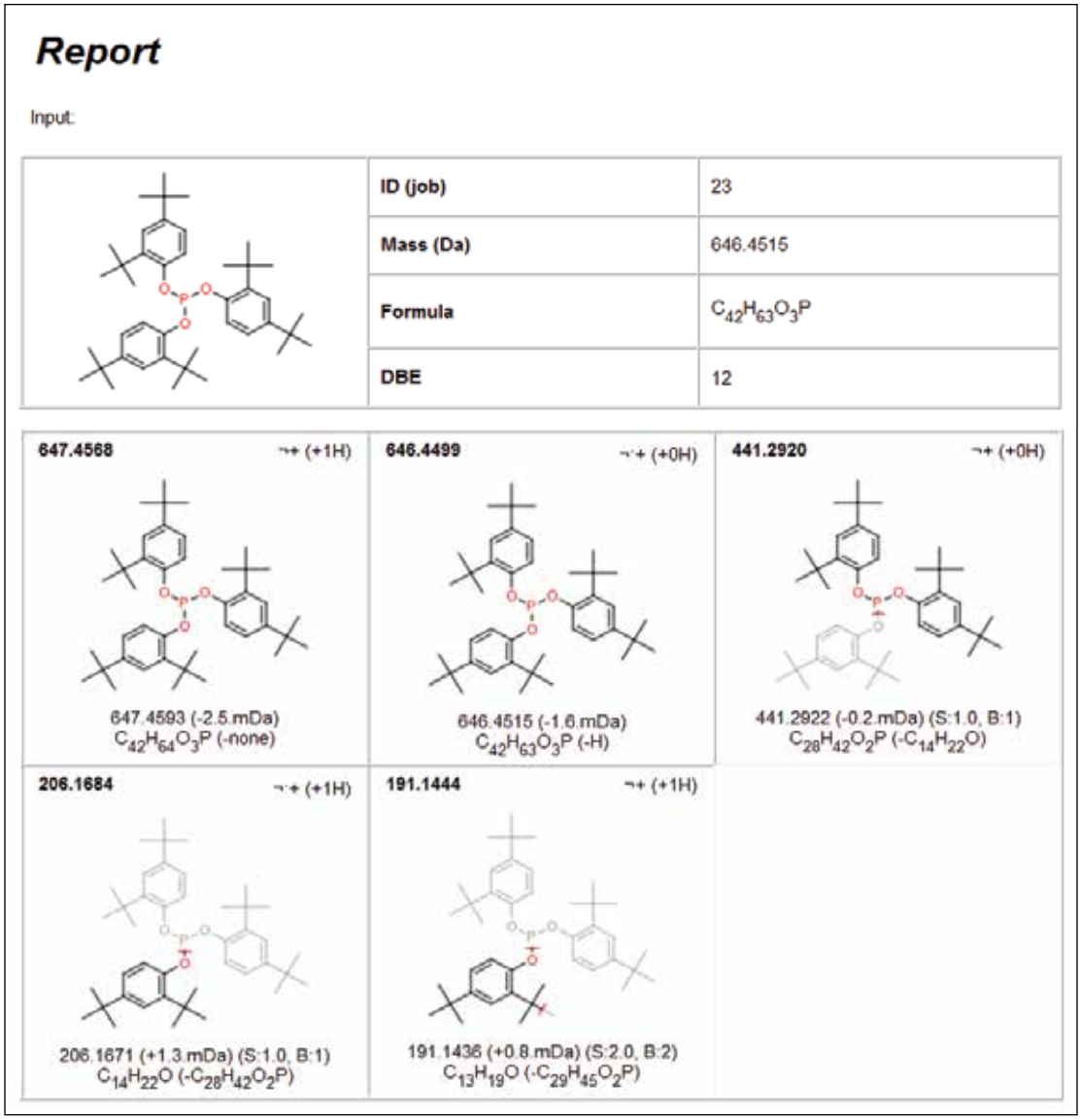


Figure 5. MassFragment Software report for confirmation of Irgafos 168.

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.

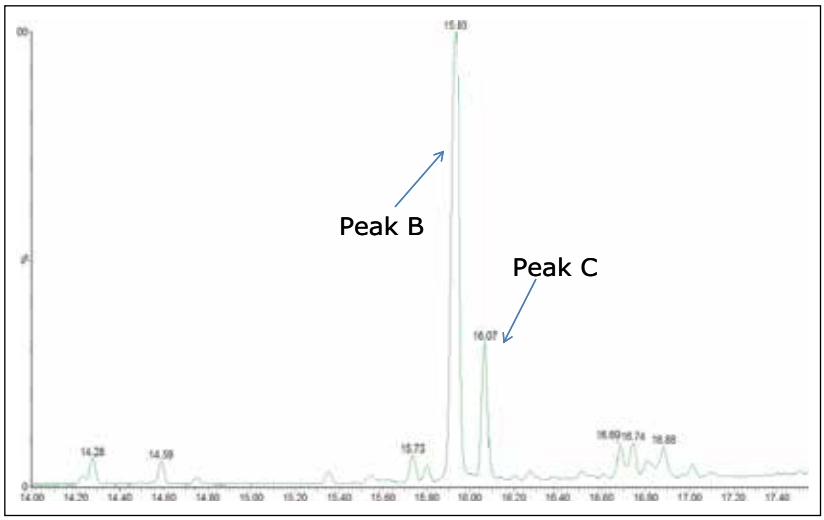


Figure 6. TIC for nylon extract.

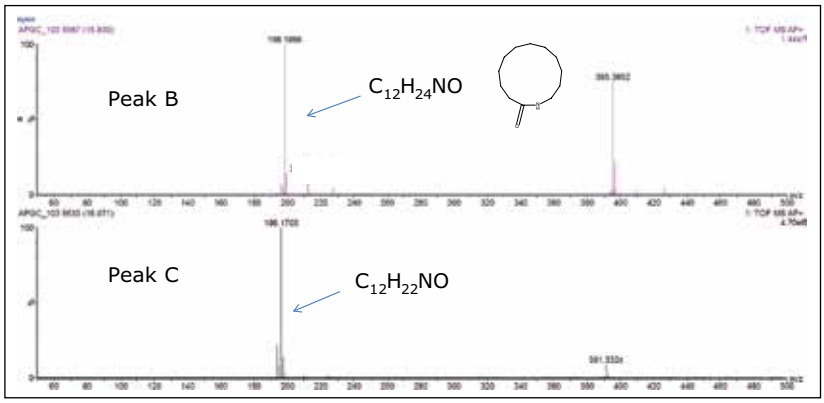


Figure 7. Spectra and molecular formula $[M+H]^+$ for Peaks B and C.

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.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Screening Workflow for Extractables Testing Using the UNIFI Scientific Information System

Baiba Čabovska
Waters Corporation, Milford, MA, USA

TECHNOLOGY BENEFITS

- Simple MS methodology using high-resolution mass spectrometry (HRMS) that can be adopted for cosmetics, 3D printing media, food, and pharmaceutical packaging extractable applications.
- Streamlines the structural elucidation process for packaging extracts by utilizing MS^E data of accurate mass precursor and fragment ion information on a single software platform
- A rapid and automated way to evaluate information for an unknown component (*m/z*) by ranking the possible elemental compositions, and searching databases for likely structures ranked based on fragmentation matching.

WATERS SOLUTIONS

UNIFI® Scientific Information System

Ion Mobility Mass Spectrometry

Quadrupole Time-of-Flight Mass Spectrometry

KEYWORDS

extractables, screening, elucidation, multivariate statistics, scientific library, MS^E, $HDMS^E$

INTRODUCTION

Characterization of packaging, food contact materials, medical devices, and many other consumables used in various industries is becoming more and more important due to ever-increasing global regulations. The initial step in characterizing extractables from packaging includes targeted screening, i.e. testing the extracts for known compounds. This is a well-established process and can be performed in various ways by using analytical techniques ranging from GC-FID-MS to LC-UV-MS. However, the final packaging may have impurities present from starting materials and additional degradants such as those formed during the molding process. The structural elucidation of unknowns is typically a very complex and time-consuming process that requires the analyst to have a high level of expertise. Waters® UNIFI Scientific Information System provides a simple workflow that includes scientific library creation, multivariate statistical analysis, elucidation, and reporting. This single platform Informatics solution enables analysts to evaluate complex data in a more efficient way through simplifying data review and facilitating the decision-making process.

DISCUSSION

As shown in Figure 1, the workflow starts with a non-targeted, data independent analysis (MS^E or $HDMS^{®E}$) acquired on a quadrupole time-of-flight mass spectrometer (QToF) or on an ion mobility QTof mass spectrometer (IMS-QTof). The QTof MS is operated in the alternate scanning MS^E mode (where the E represents elevated collision energy), as this technique provides two MS scan functions for data acquisition in one analytical run. The first scan function acquires MS data using low collision energy and collects information on the precursor ions in the sample. For the second scan function the collision energy is ramped from low to high energy which allows for the collection of fragment ions over a wide *m/z* range. With IMS-QTof, an additional dimension of separation is achieved by the inclusion of ion mobility, thus achieving High Definition Mass Spectrometry$^{E®}$ ($HDMS^E$). These types of data acquisitions allow simultaneous collection of precursor and fragment ion information, which is crucial when doing elucidation for unknown compounds. In extractables testing complete information about sample extract is rarely available. Therefore after the targeted screening, the elucidation steps in non-targeted screening are essential.

The sample separation prior to MS analysis can be performed by liquid chromatography using UPLC®, by gas chromatography using APGC, as well as by convergence chromatography using UPC.$^{2®}$

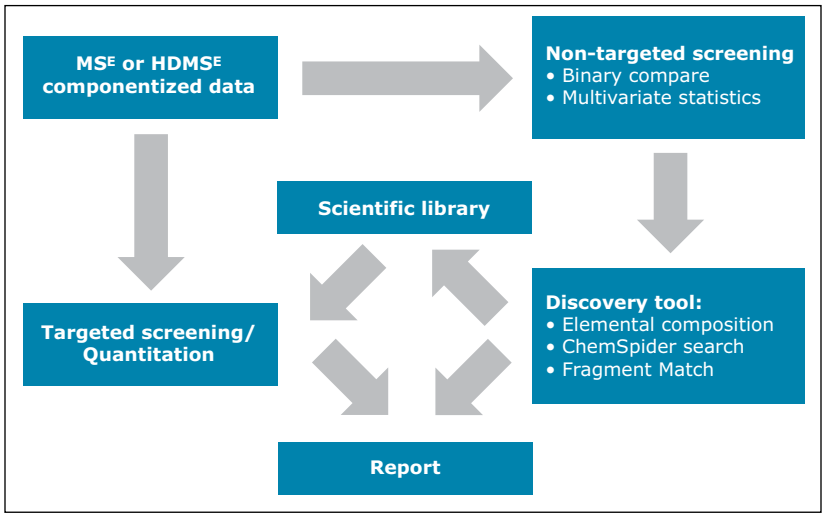


Figure 1. Screening workflow in UNIFI.

Prior to starting data analysis, the user can create a scientific library based on knowledge of expected compounds in the sample extract, i.e. if the starting compounds in the formulation of a plastic material are known, or a literature search has provided list of compounds that are typically encountered in similar types of packaging. Additionally, regulations provide lists of compounds that are either allowed or prohibited in certain types of packaging, (e.g. food contact materials).

The scientific library (Figure 2.) can contain as much information as is available. The most common information typically included is the compound name, its molecular formula, structure, item tag, and fragmentation information. For ion mobility data, the information needed in screening would be the collisional cross section value (CCS).[1] More extensive information about each compound in the library can reduce the number of false positives during targeted screening analysis. Examples of additional information that could be added to a UNIFI scientific library include MS spectra and other relevant documents (e.g. MSDS, articles, SOPs).

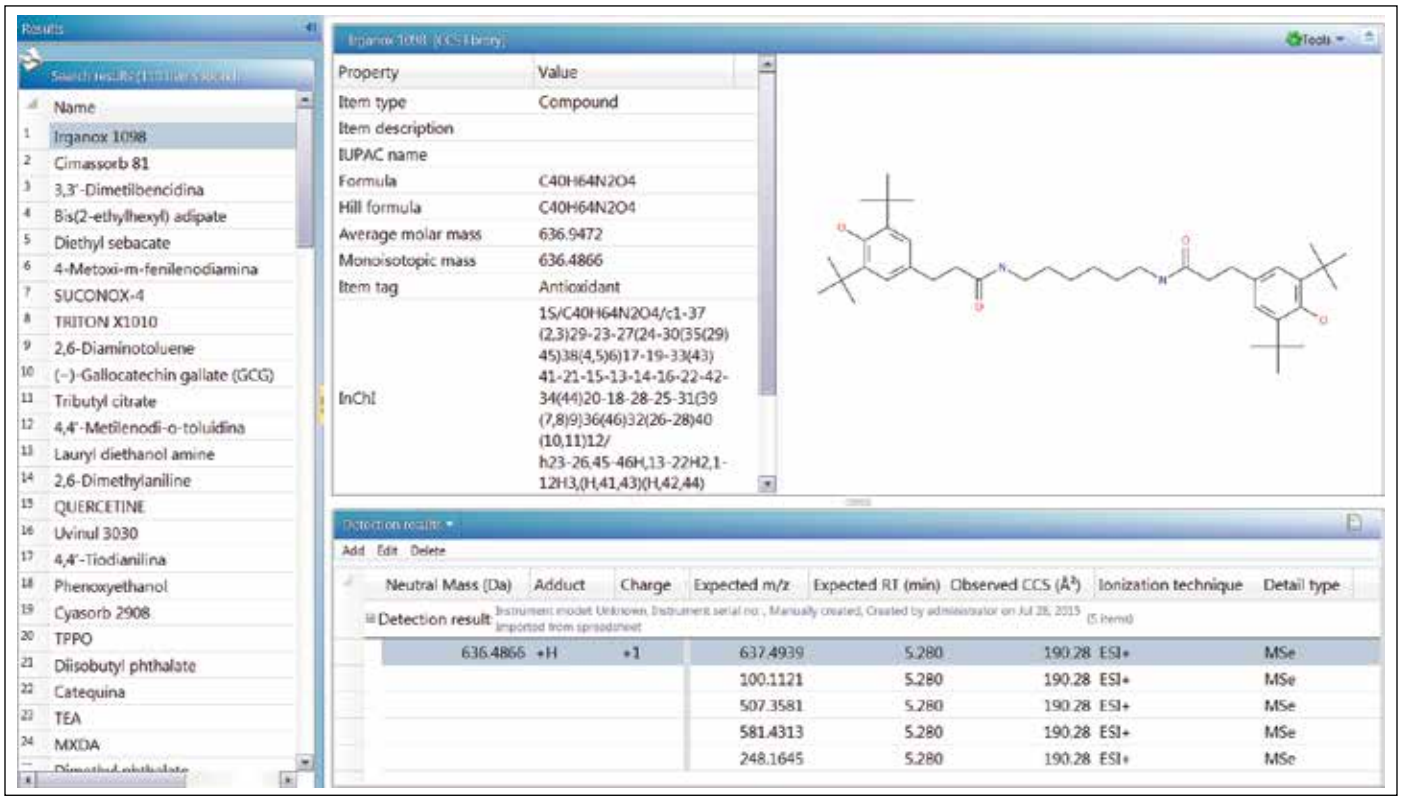


Figure 2. UNIFI scientific library screen shot.

Once the data has been acquired, UNIFI uses a target list created by the user from the library to process the raw data and search for compounds which match acceptance criteria. It is also possible to create target lists manually, if required. The user can set up processing criteria such as retention time and mass accuracy tolerances. Subsequently, it is possible to review the proposed identifications based on the number of expected fragments versus the number of expected fragments found, expected and observed CCS values for IMS data with CCS delta (%), isotope intensity matches in ppm or %, among other parameters.

UNIFI allows each user to have a customized workflow which displays information in the preferred dashboard for review (Figure 3). It is possible to review the spectrum for precursor and fragments. Extracted ion chromatograms (XIC) can be displayed for all precursors as well as for fragments. Summary plots can be used to verify the presence or absence, or changes in intensity of a target in other injections.

If the appropriate standards have been analyzed during the sample run and a calibration curve is available, it is possible to quantify the identified targets in the analysis.

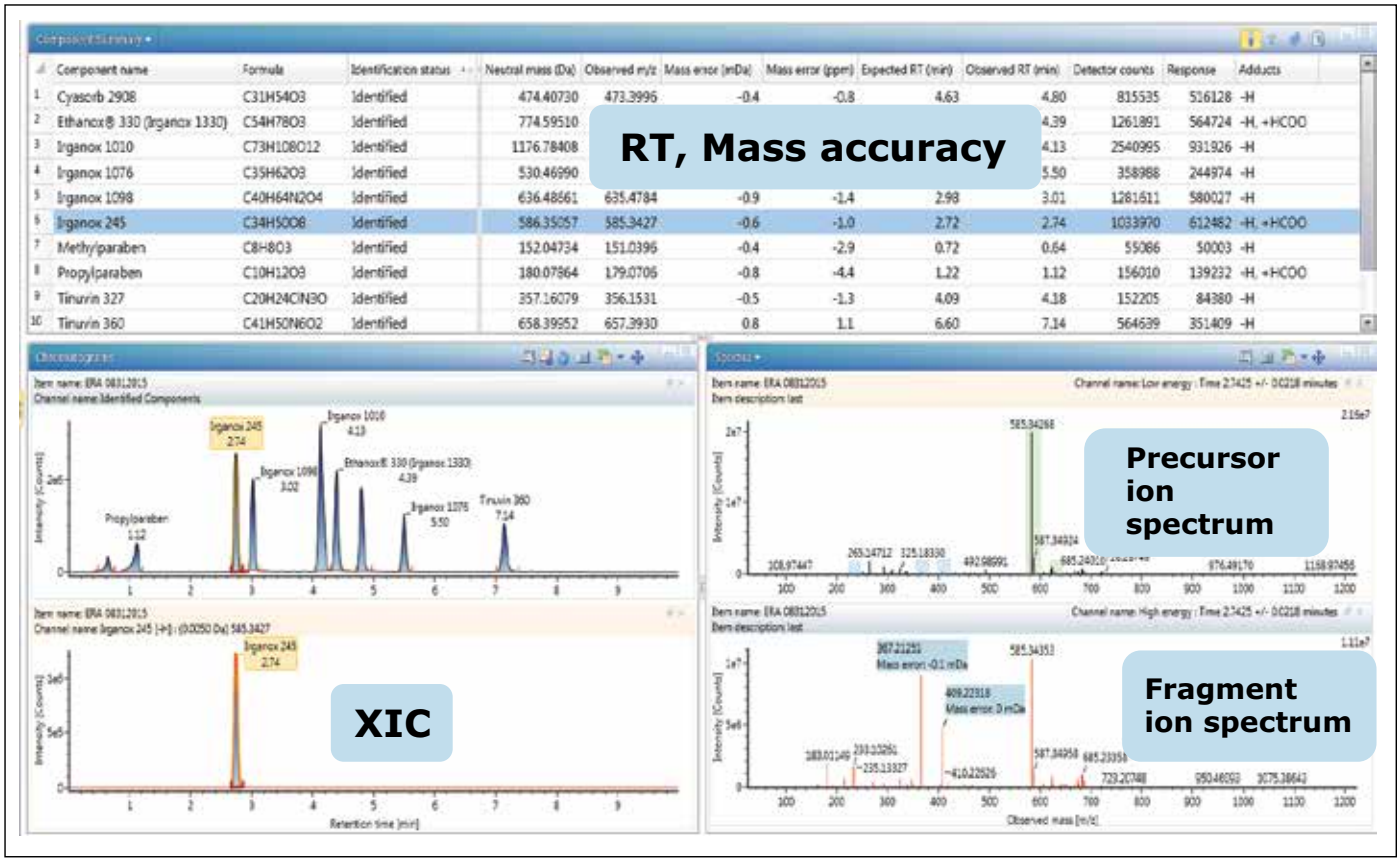


Figure 3. Example data review window.

After reviewing the identified targets for false positives and removing them from the identified list, the next question to be answered is "What else is in my sample?" or "What are the differences between a blank extract and sample extract or between these two samples?". UNIFI has two tools for comparison and statistical analysis. The first one, Binary Compare, allows the user to compare two injections. One injection must be labeled as a reference sample, in this case, an extraction blank.

Masses in the reference spectra and the unknown spectra are considered to be the same component if they are within the specified mass and retention time tolerances. The comparison can be presented graphically as a mirror image of base peak intensity chromatograms (BPI), total ion count chromatograms (TIC), or as a table of candidate masses (Figure 4). Also the spectra of the compound in the reference sample can be displayed in comparison to the unknown sample. The column labeled "Match Type" shows whether the candidate is present only in the unknown sample or in the reference sample, or both. The corresponding match types would be Unknown Unique, Reference Unique, or Common. Typically, compounds that are unique to the sample and absent in the reference sample would be of most interest in an analysis.

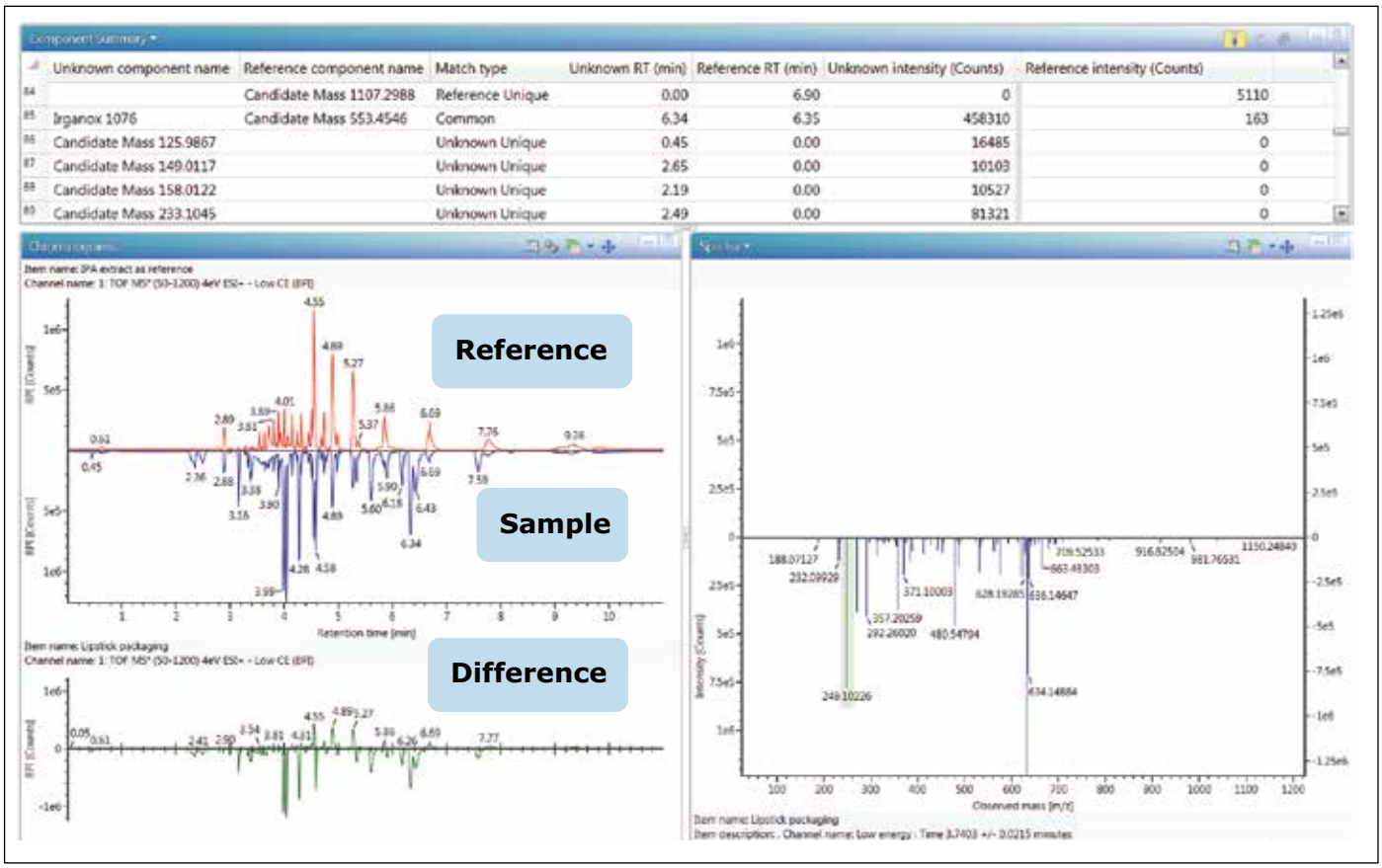


Figure 4. Binary Compare plot, table, and spectra.

If there is a need to compare more than two samples or groups of samples, UNIFI provides Principal Component Analysis (PCA) and other models for data reduction and evaluation by an integrated workflow with statistical software package- EZInfo. PCA is a statistical tool which allows the reduction of a large set of multivariate data into uncorrelated variables called principal components. The differences among the groups of samples are emphasized by Projection to Latent Structures Discriminant Analysis (PLS-DA) model (Figure 5), where a sample group is specified. PLS-DA models the quantitative relationships between the variables X (predictors) and Y (responses) for all of the sample groups. Subsequently, Orthogonal Projection to Latent Structures Discriminant analysis (OPLS-DA) plot demonstrates the differences between two groups.[2] The data points (markers) in the loadings plot and S-plot are called Accurate Mass/Retention Time pairs (AMRTs). Individual markers that contribute to the biggest differences between the samples can be selected from either the loadings plot or the S-plot and transferred back to the discovery tool for elucidation. When transferring the selected markers, labels can be added to make the data easier to sort and to keep track of markers for different sample groups. When an individual marker is selected from the marker matrix table, a TrendPlot is displayed, allowing the analyst to quickly evaluate its presence in the other samples or injections (Figure 6).

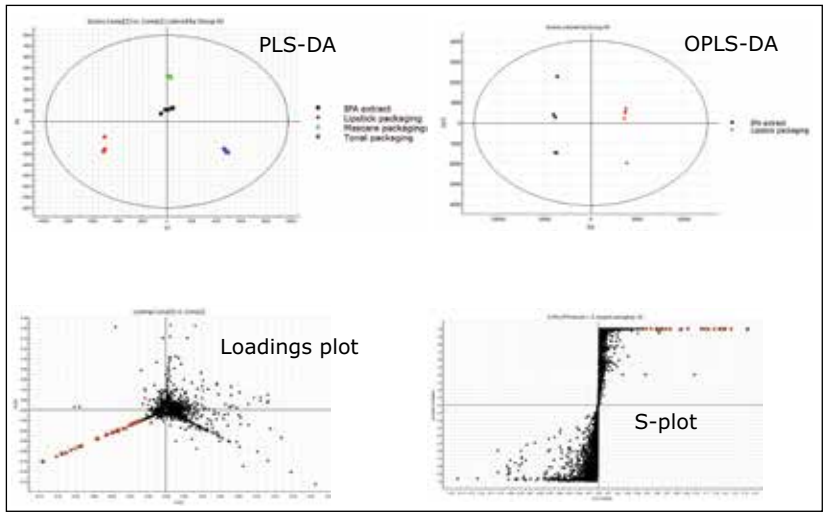


Figure 5. Example of statistical plots provided with UNIFI's multivariate analysis tools.

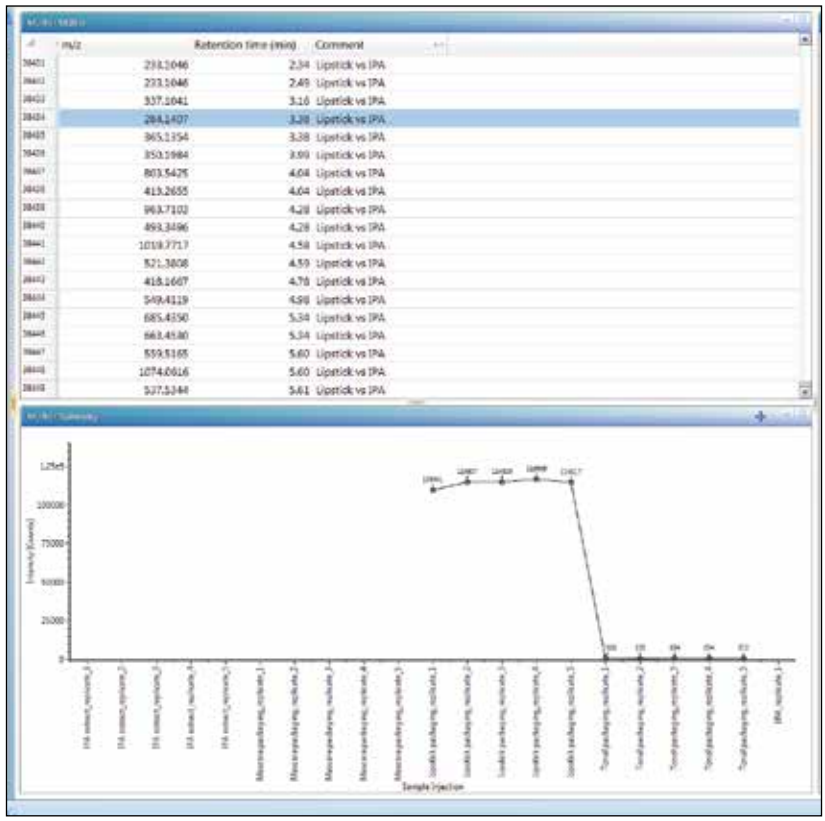


Figure 6. Markers from statistical analysis and a trendplot.

Once the markers are selected either from Binary Compare or from MVA analysis, UNIFI's Discovery tool can be used to find the possible identity of the ion. The Discovery tool automatically combines all of the analytical information contained in the data: accurate mass, isotope pattern, fragmentation in the high collision energy channel – with the structural database search. The Results table shows the possible molecular formulas for the ion, corresponding structures from a ChemSpider search, and a number of fragments that can be matched to each structure based on fragmentation data. Information returned from the search (Figure 7) also includes the number of citations and synonyms used for each structure. Many polymer additives have common names like Irganoxes and Tinuvins, which helps in further narrowing down the possible compound choices.

When the decision for the compound identity is made, the chosen structure and name can be assigned to the candidate mass ion. Assignment will change the identification status of the candidate to "identified". All of the elucidated compounds can be added to UNIFI's scientific library to be used in subsequent targeted screening analysis.

One of the final steps of the analysis is to create a report. A report template can be embedded in the UNIFI Analysis method which can be used for similar types of analysis (Figure 8). The report can be customized to include all the relevant information such as analysis method, processing parameters, chromatograms, spectra, and identified compound summary tables, among others.

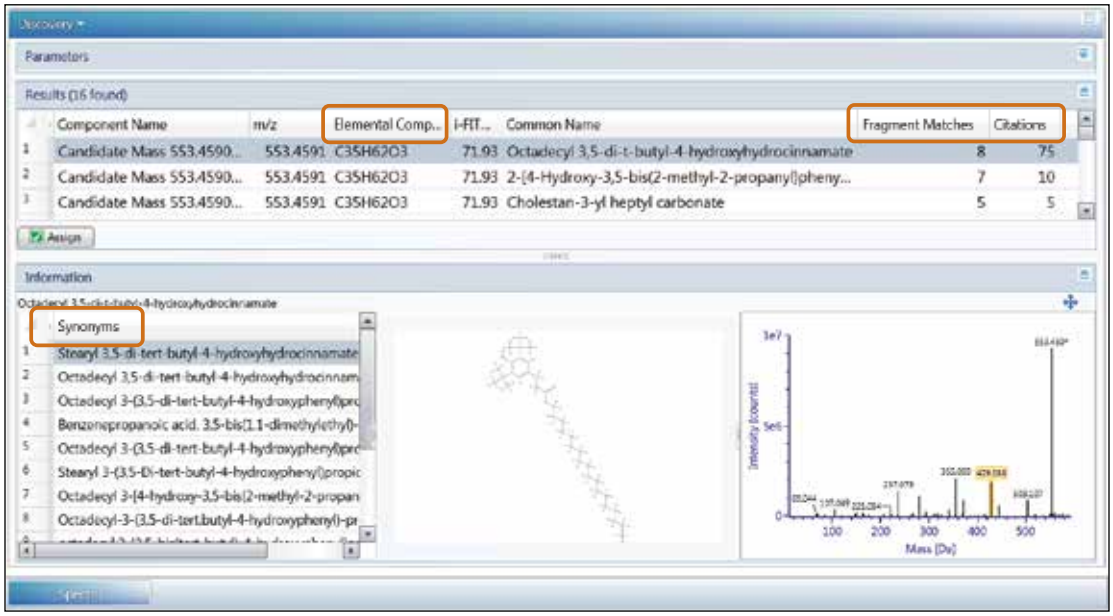


Figure 7. UNIFI Summary table for Discovery toolset.

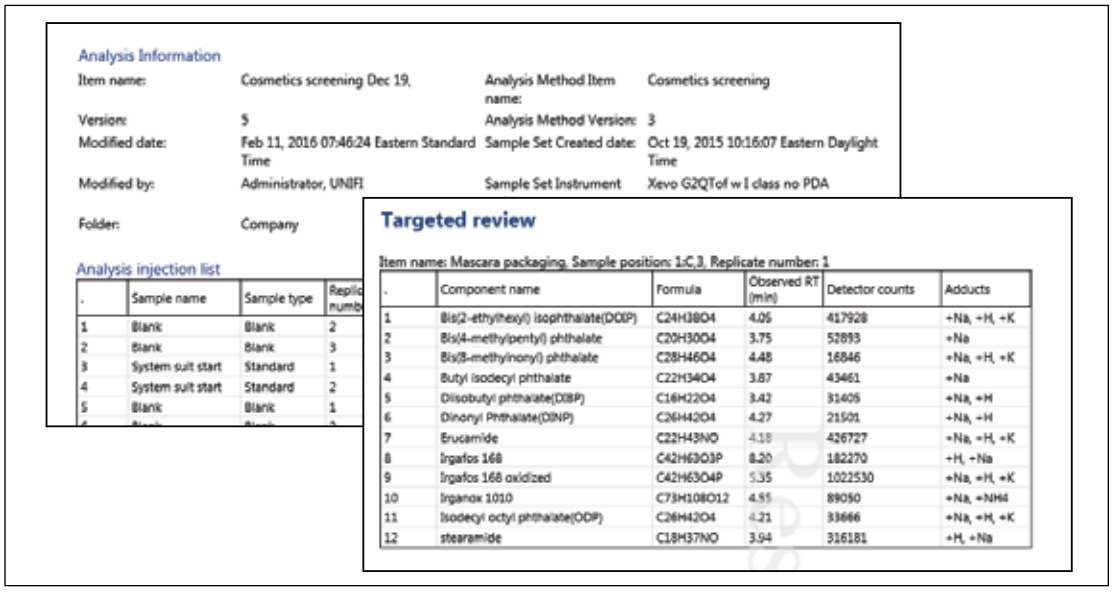


Figure 8. Report examples for analysis information and target summary.

CONCLUSIONS

The UNIFI Scientific Information System provides analysts with a well-established workflow for extractable screening analysis. The UNIFI workflow starts with the scientific library for targeted screening, followed by statistical analysis for the determination of markers or relevant compounds. The Discovery tool automatically utilizes information-rich raw data for elemental compositions, followed by a structural database search and fragmentation assignments. This integrated workflow reduces the amount of time required for extractables screening analysis with structural elucidation.

References

1. M McCullagh, V Hanot, and S Goscinny. Use of Ion Mobility Spectral Cleanup and Collision Cross Section Values to Increase Confidence and Efficiency in Pesticide Residues Screening Strategies. Waters application note no. 720005080en. June, 2014.
2. B Cabovska. Non-targeted screening analysis of packaging extracts using the UNIFI Scientific Information System. Waters application note no. 720005326en. March, 2015.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Non-Targeted Screening Analysis of Packaging Extracts Using the UNIFI Scientific Information System

Baiba Čabovska
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Simple LC-MS methodology leverages high-resolution mass spectrometry that can be adopted for cosmetics, food, and pharmaceutical packaging extractable applications.
- Streamlines the structural elucidation process for packaging extracts by utilizing MS^E data of accurate mass precursor and fragment ion information on a single software platform.
- Rapidly evaluate information for an unknown component (*m/z*) by ranking the possible elemental compositions and performing database searches for likely structures ranked based on fragmentation matching.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

UNIFI® Scientific Information System

Xevo® G2-XS QTof Mass Spectrometer

CORTECS® C_{18} Column

KEY WORDS

Extractables, leachables, packaging, cosmetics, screening, elucidation, accurate mass, QTof, non-targeted analysis, informatics

INTRODUCTION

Characterization of packaging in various industries has become more important due to ever-increasing global regulations. The first regulations for plastics used in food packaging and contact materials were established in 1982 in Europe,[1] which have been expanded in recent years.[2] In the pharmaceutical field the need for extractables testing was recognized in the 1990s.[3] Manufacturers are required to evaluate packaging for the possible migration of additives and ingredients into the final product because of the potential impact extractables and leachables can have on patients' health.[4,5] Extractables in the pharmaceutical industry are defined as compounds that can be extracted from packaging materials or devices under controlled experimental conditions. Leachables, a subset of extractables, are compounds that actually migrate into the final product during expected shelf or contact time. The latest addition to the industries that require testing of packaging is the cosmetics industry. The most recent regulations for the cosmetics industry in Europe (EU Regulation 1223/2009) Annex 1 states that "impurities, traces, information about the packaging material must be determined".[6] For the cosmetics industry the impact from leachables would depend on the route of application. For example, it would be less critical for cosmetic products that are applied to the skin such as body creams than it would for products that can be ingested or absorbed through the eyes, such as lipstick or mascara.

The initial step for characterizing extractables from packaging involves targeted screening, i.e., testing the extracts for known compounds. This is a well-established process that can be performed using various analytical techniques ranging from GC-FID-MS to LC-UV/MS. However, the final packaging may have impurities present from the starting materials and additional degradants such as those formed during the molding process. The first step in ensuring that these compounds do not pose any toxicological risks to the consumer is to identify the extractables, or at least their structural class. The structural elucidation of unknowns is typically a very complex and time-consuming process that requires the analyst to have a higher level of expertise. Waters® UNIFI Scientific Information System utilizes accurate mass and fragment information to simplify data review and facilitate the decision-making process. It allows analysts to evaluate complex data in a more efficient way and quickly make decisions about the possible identity of an unknown compound.

EXPERIMENTAL

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Separation mode:	Gradient
Column:	CORTECS UPLC C_{18} 90Å, 1.6 µm, 2.1 mm x 100 mm
Column temp.:	40 °C
Injection volume:	5 µL
Flow rate:	0.5 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	60% B held for 30s, increased to 99% over 2.5 min, held at 99% for 5 min, then re-equilibrated back to 60%

MS conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI +
Capillary voltage:	3.0 kV
Desolvation temp.:	450 °C
Source temp.:	150 °C
Cone voltage:	25 V
Collision ramp:	10 to 40 eV
MS scan range:	50 to 1200 *m/z*

Data acquisition and processing

UNIFI Software was used for acquisition and data processing.

Sample preparation

Mascara packaging made of polypropylene, lipstick packaging and tonal cream packaging made of polyethylene were chosen as samples. The cosmetics products were removed from the packaging, which was subsequently cut into 1x1 cm pieces. Sample extracts were prepared in isopropanol (IPA) by extracting ~2 g in 5 mL of IPA by sonication in glass scintillation vials for 6 hours.

RESULTS AND DISCUSSION

Typically, screening experiments for packaging extracts are performed using generic gradient LC-MS methods. As it is not known what kind of chromatographic profile the extract might have, the screening methods are not optimized for each individual packaging material at this initial stage in R&D. If the chromatogram only has one or two peaks, it is easy for analysts to decide where to start their investigation. However, if the extract has a multiple chromatographic peaks that are not completely resolved, or if several groups of samples must be compared, the analyst needs to determine which compounds are unique to the extract and are not present in the extraction blank (Figure 1). Furthermore, less intensively ionized compounds or trace-level compounds of toxicological concern may not be visible in the total ion current (TIC) chromatogram, or even in the base peak intensity (BPI) chromatogram.

Binary compare

In cases where only two samples must be compared, for example a blank extract (reference) and a sample (unknown), UNIFI Software's binary comparison feature allows the analyst to directly compare the chromatographic and spectral results of an analyte sample with those of a reference sample. Masses (*m/z*) in the reference and unknown spectra are considered to be the same component if they are within the user-specified mass, retention time, and intensity difference tolerance. The comparison can be presented graphically as a mirror image of BPI or TIC chromatograms, or as a table of Candidate Masses (Figure 2). The candidates are accurate mass and retention time pairs which have common peak features in the raw data. They are grouped according to retention time alignment and isotope spacing.

UNIFI shows a comparison between the mass spectrum of the compound in the unknown sample with the reference sample, and displays any differences. Figure 2 shows the comparison between an IPA blank extract "Reference sample" and lipstick packaging extract "Unknown sample" with the column "Match type" highlighting if the candidate is present in only the unknown sample, the reference sample, or both - the corresponding match types would be Unknown Unique, Reference Unique or Common. In this case, the most interesting candidates for further evaluation would be those that are not present in the extraction blank- Unknown Unique.

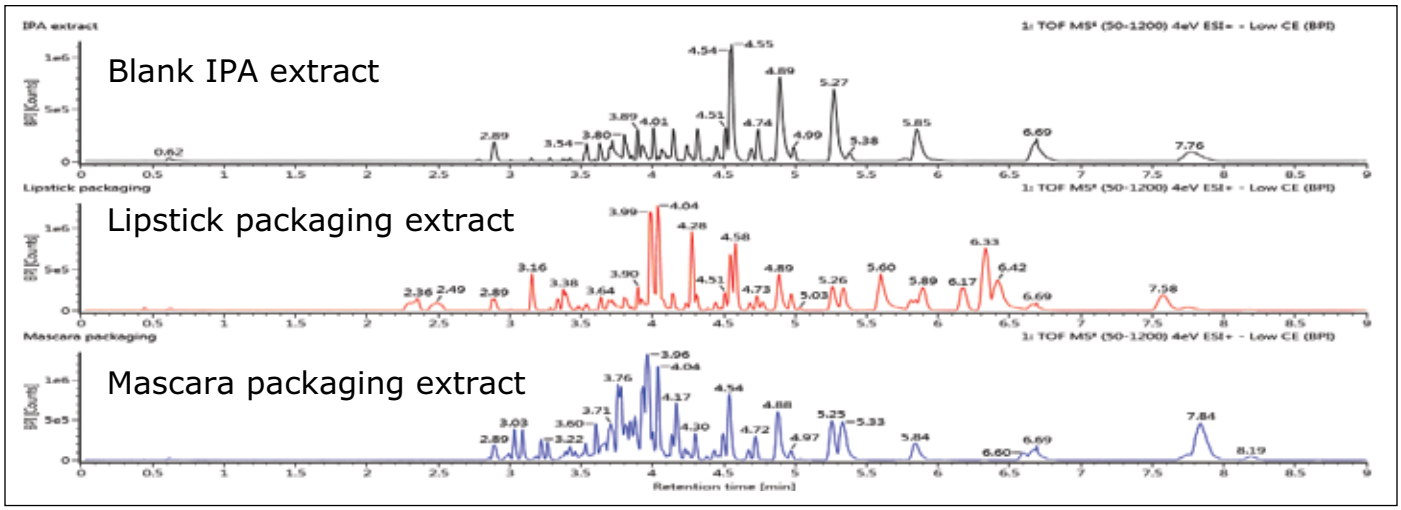


Figure 1. Mass chromatograms for packaging extracts and a blank extract.

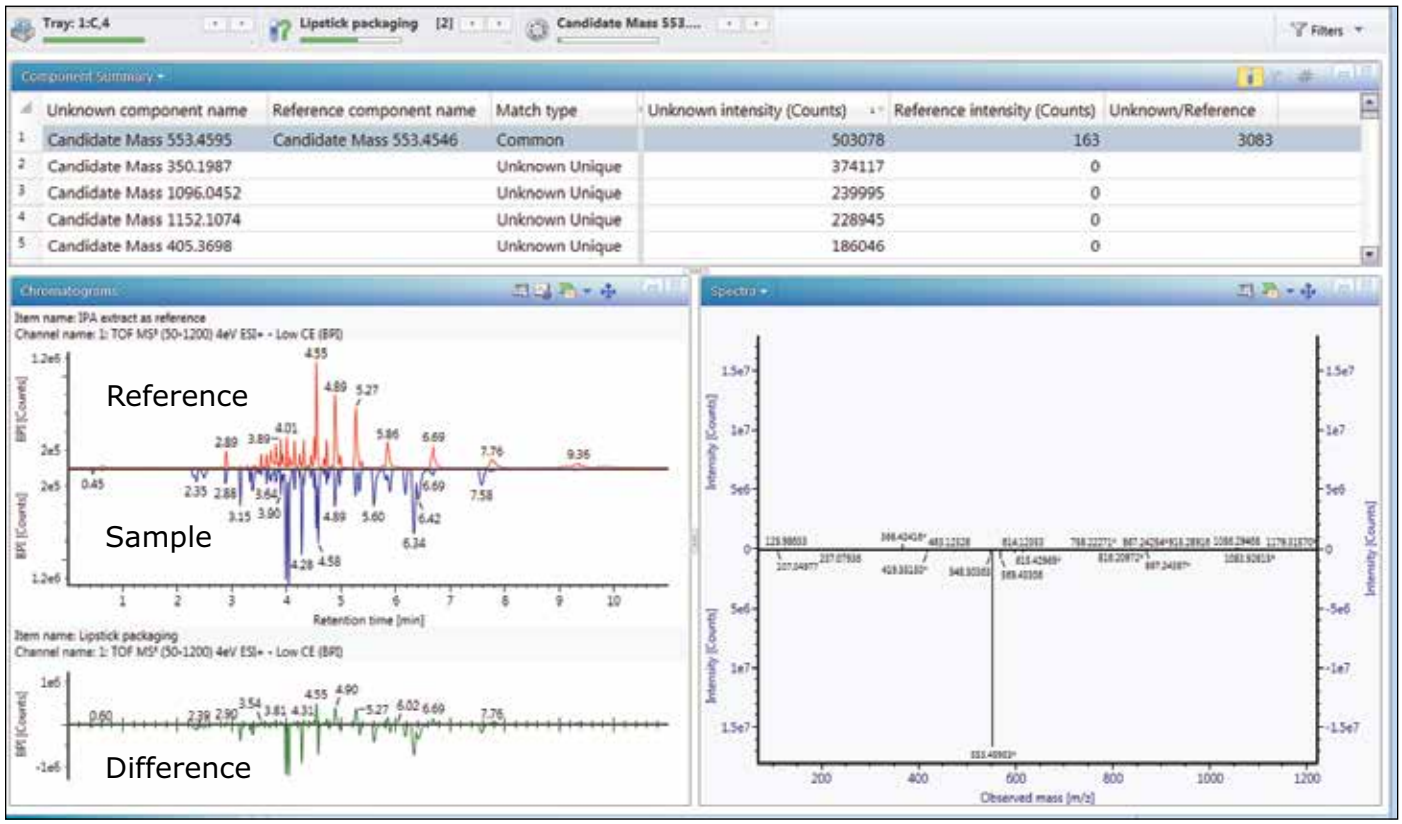


Figure 2. Binary compare results window for the IPA reference sample extract and lipstick packaging sample. The red trace shows the BPI chromatogram of the reference sample (IPA blank extract); the blue trace shows the BPI chromatogram of the lipstick packaging extract; and the green trace shows the difference between the samples.

Due to increases in instrument sensitivity and the ubiquitous presence of many extractables in LC-MS solvents, extraction vessels, plastic pipette tips, *etc.*, it is often difficult to obtain a clean blank. It is useful to evaluate the compounds where the candidate intensity in the unknown sample is much higher than in the reference sample. The column labeled Unknown/Reference (Figure 2) shows a ratio for common components, allowing users to quickly identify common extractables that may be persistent, but have a fold change that is significant. For candidate mass *m/z* 553.4595 the response ratio is over 3000 which indicates potential presence of the candidate in the extraction blank or a carryover.

High resolution mass spectrometry provides very comprehensive, high-quality information, but interpreting the data sets manually can be challenging. Therefore data processing software is of utmost importance for managing and reviewing data in an more efficient way. UNIFI Software allows users to set up their workflow in order to facilitate visualization of their data in the most productive way, and only display data that is relevant – all with a single click. The processed data can then be filtered using criteria defined by the user. In this case, to make the information in the table easier to manage the data was filtered based on specifications that showed Unknown Unique candidate masses with an intensity over 10,000 counts and Common candidate masses with a response ratio of Unknown/Reference of at least 300.

Once the data has been organized in a way that is most appropriate for the analyst, the next step is to proceed to elucidation of the candidates of interest (most intense for example) by utilizing the accurate mass information and high-collision energy fragment information.

Multivariate analysis (MVA)

Binary compare is useful for comparing two samples, but when multiple samples or sample groups need to be compared, the use of multivariate statistical analysis tools such as principal component analysis (PCA) facilitate the identification of differences between samples or groups. UNIFI can generate marker matrices based upon user-defined criteria which can then be automatically transferred to EZInfo 3.0.3 for MVA. PCA is a statistical tool that reduces a large set of multivariate data into uncorrelated variables called principal components. If additional discrimination among the investigated sample groups is required, the differences can be emphasized by using a Projection to Latent Structures Discriminant Analysis (PLS-DA) model (Figure 3). PLS-DA creates models of the quantitative relationships between the variables X (predictors) and Y (responses) for all sample groups. However, in these plots, each sample is presented by a single point, which does not allow individual markers contributing to the differences between the groups to be observed.

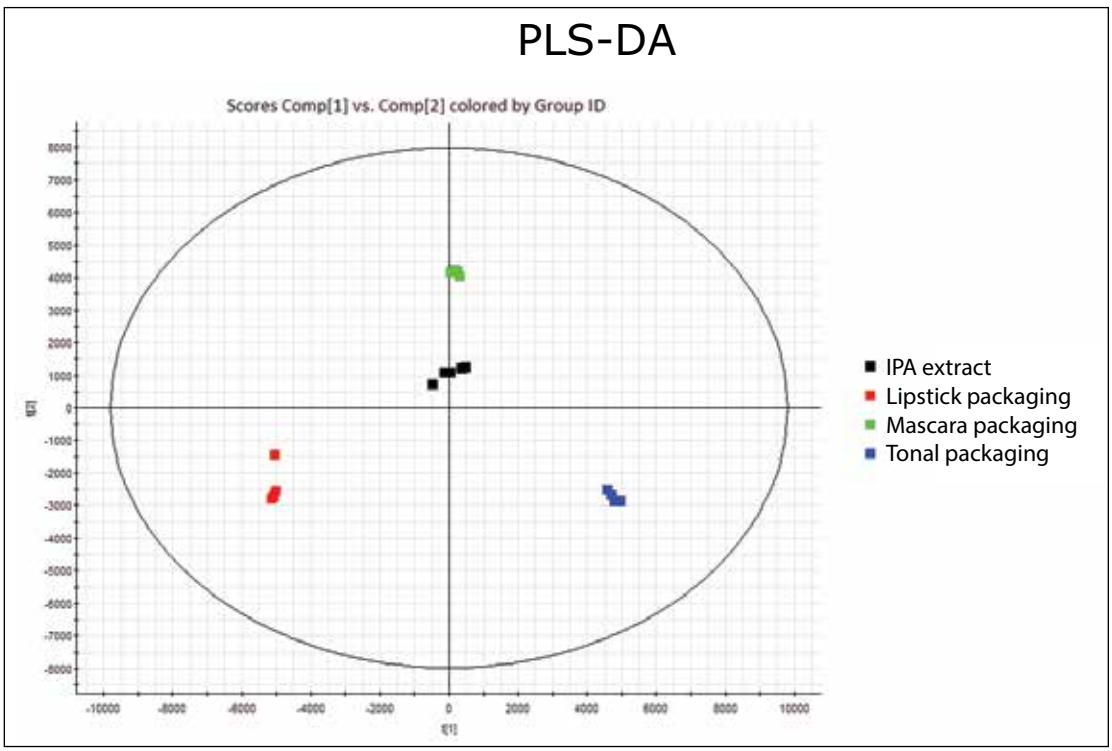


Figure 3. PLS-DA model for all of the packaging sample groups.

In order to investigate group differences down to individual markers, a loadings plot can be used. The loadings plot displays how the X variables correlate to each other, with points further away from the center being the most dissimilar between the sample groups (Figure 4). The data points in these plots are called Accurate Mass/Retention Time (AMRT) pairs. The quadrants in the loadings plot correspond to the PLS-DA model, thus the AMRTs in the lower left quadrant represent the unique markers in the lipstick packaging. Markers selected in red contribute most to the difference between the lipstick packaging and all the other packaging samples.

The differences between the groups can come from analytes that are not present in one of the groups, or from analytes with the greatest change in intensity (concentration) between the groups.

The individual markers that represented the biggest differences between the lipstick packaging and the rest of the group were selected (highlighted in red in Figure 4) and transferred back into UNIFI's Discovery tool for elucidation. When transferring selected markers from the loadings plot, labels can be added to make the data easier to sort and keep track of markers from different sample groups (Figure 5). When an individual marker is selected from the Marker Matrix table, a trend plot is displayed which allows users to quickly evaluate its presence in the other samples or injections.

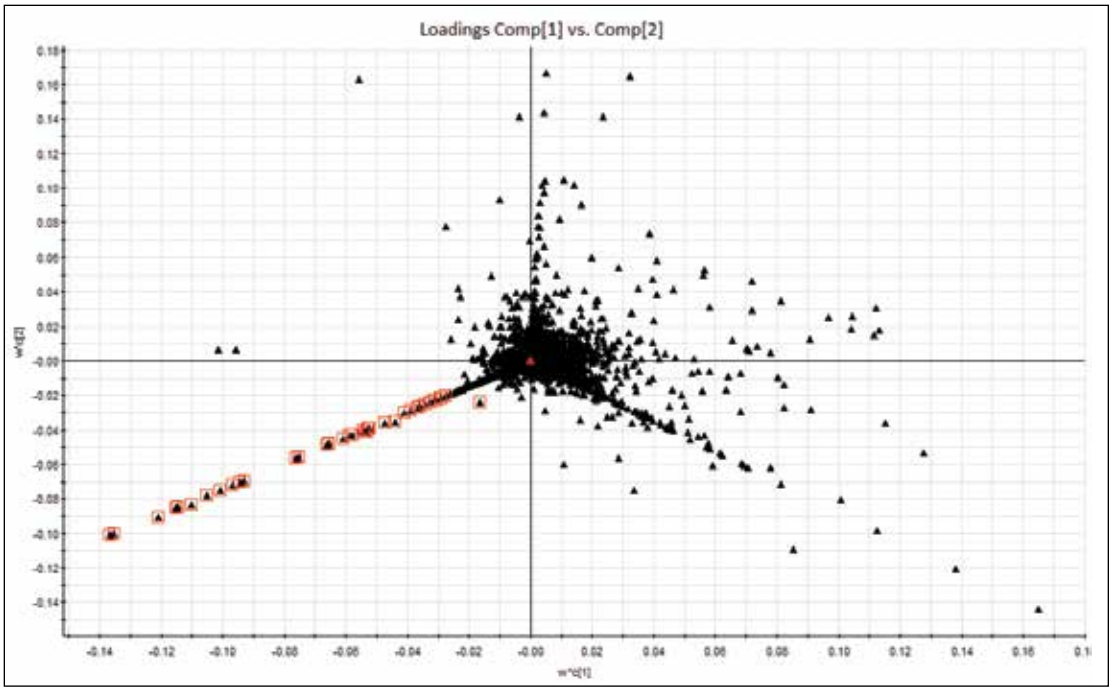


Figure 4. Loadings plot for all of the packaging samples.

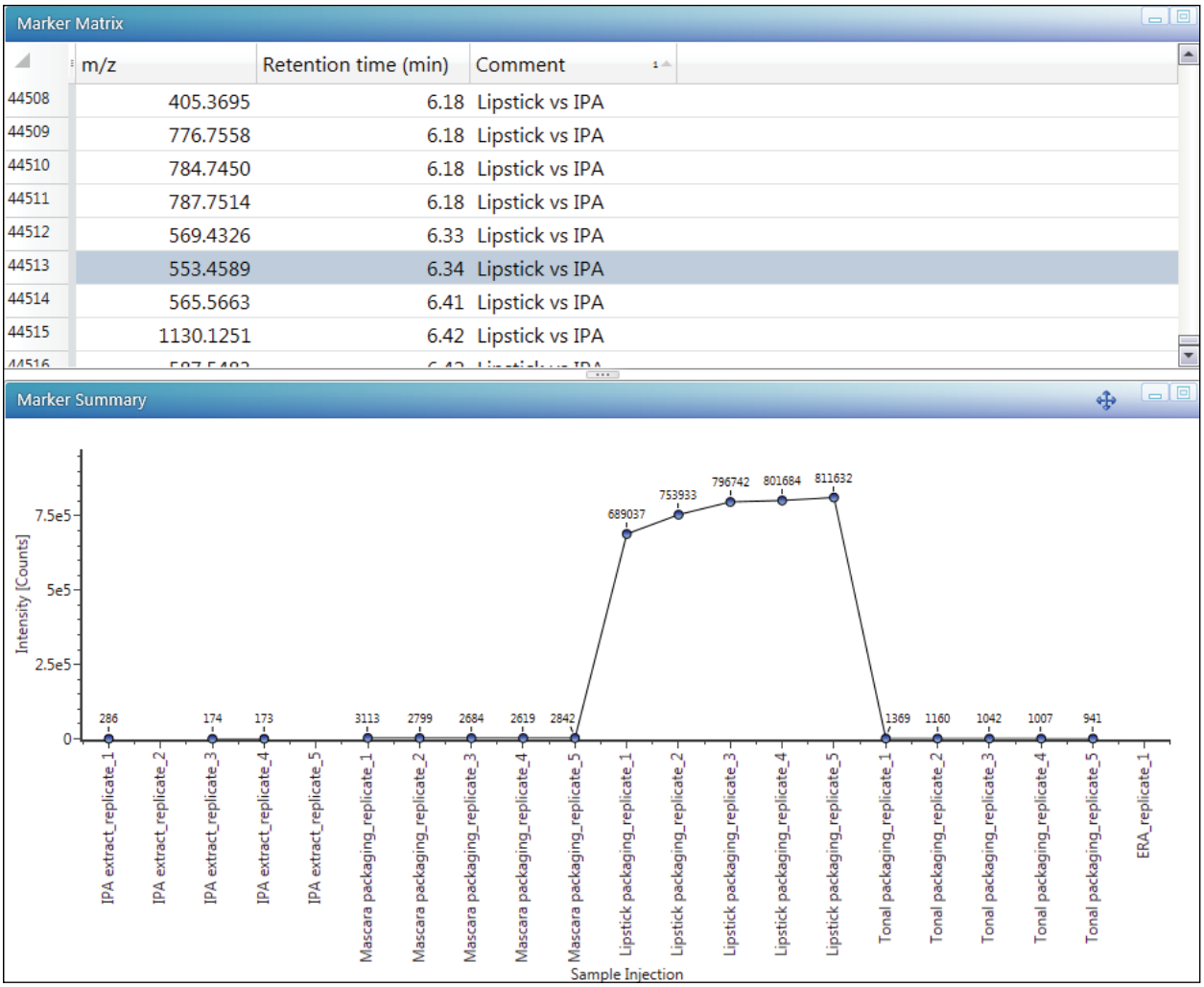


Figure 5. Marker Matrix with labeled markers and a trend plot for a marker 553.4589 at RT 6.34 min.

Discovery tool

Regardless of whether a marker or candidate of interest was obtained by binary compare or multivariate analysis, the next step in the workflow is structural elucidation. The Discovery tools within UNIFI's Elucidation toolset include automated elemental composition, database searching through ChemSpider or UNIFI's configurable Scientific Library, as well as fragment matching of high-collision energy data (Figure 6) of individual or batches of candidates. The best matches are displayed based upon the number of identified high energy fragments, citations from ChemSpider, and mass accuracy. The elemental composition algorithm uses accurate mass and isotope information to calculate the possible compositions for each marker. Using the Discovery tool settings, analysts can specify an acceptable level of isotope match (i-FIT™), elements to be included in the elemental composition search, which libraries to select from ChemSpider (all or specific ones), and minimum number of citations in ChemSpider, among other things.

The final results for the candidate mass *m/z* 360.3236 in the mascara packaging are displayed in a table that lists the elemental compositions within specified limits, possible structures with citations from the ChemSpider database, and how many fragments can be matched to the high collision energy data for each structure (Figure 7).

Many polymer additives form adducts during LC-MS (Na+ being the most common). The adduct ion can be more intense than the protonated species, or the protonated ion can be absent entirely. In this case, the initial evaluation of the mass using +H ion, did not provide a reasonable molecular formula (no i-FIT above 50% and no structure from ChemSpider). Therefore Na+ was selected as an adduct and the Discovery tool process was repeated. As shown in Figure 7, the molecular formula C22H43NO has a 100% i-FIT, meaning that the isotope ratio for the *m/z* is consistent with the proposed composition. ChemSpider returned a lot of possible structural hits for this formula. When sorted by the number of citations, it can be seen that the top choice also has one of the highest number of possible fragment matches in the high energy data. Additionally, common names are returned from the ChemSpider search that can help analysts determine the correct structure. Many polymer additives have common names such as Irganox's or Tinuvin's which are much easier to recognize than just a chemical name. The most cited chemical with the elemental composition C22H43NO has several common names indicating a polymer additive e.g. Armoslip E. Researching the identity of the chemical further, it turned out to be erucamide – a fatty acid derivative that is commonly used as a slip agent in packaging materials.

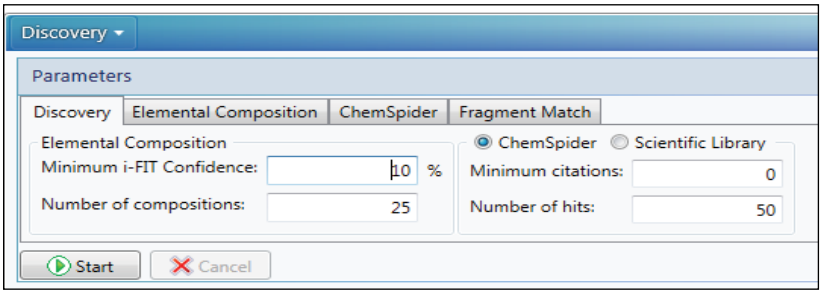


Figure 6. Interface for UNIFI's Discovery tool.

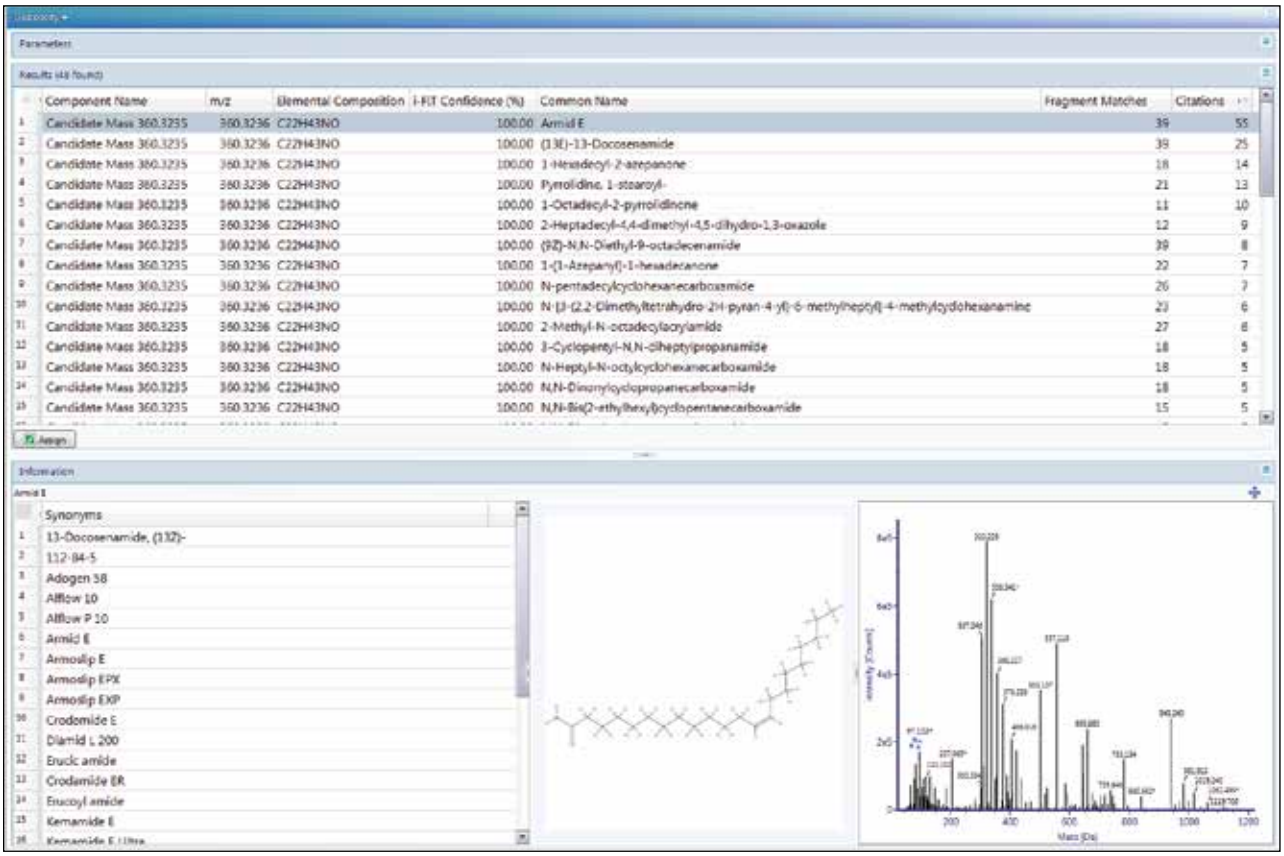


Figure 7. Results from UNIFI's Discovery tool for m/z 360.3236 at RT 4.18 in the mascara packaging.

CONCLUSIONS

Characterizing component spectra in non-optimized LC-MS analysis can be complex, therefore it is advantageous to use automated software tools to quickly evaluate possible structures for candidate masses. The described LC-MS and Informatics workflow, which employs high-resolution mass spectrometry, can be adopted for cosmetics, food, and pharmaceutical packaging extractable applications. Utilization of MS^E data containing accurate mass precursor and fragment ion information on a single software platform streamlines the identification and review process.

An Informatics-based structural elucidation discovery tool provides a rapid process to evaluate information for an unknown *m/z* by ranking the possible elemental compositions and subsequently searching databases for possible structures that are prioritized based on fragmentation matching. The UNIFI Software workflow makes it easy to rank markers of importance and facilitates component identification.

References

1. Council Directive 82/711/EEC of 18 October 1982 laying down the basic rules necessary for testing migration of the constituents of plastic materials and articles intended to come into contact with foodstuffs, http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:31982L0711
2. *Official Journal of the European Union.* 2011, Regulation 10/2011/EU.
3. D L Norwood, L M Nagao, C L M Stults. *PDA Journal of Pharmaceutical Science and Technology,* 67 (5): 413–429. 2013.
4. M P Balogh. Testing the Critical Interface: Leachables and Extractables. *LCGC*, June 1, 2011.
5. F Mofatt. Extractables and leachables in pharma-Serious Issue. http://www.solvias.com/sites/default/files/solvias_whitepaper_web.pdf
6. The European Parliament and the Council of the European Union. Regulations (EC) No 1223/2009 of the European Parliament and of the Council.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

FOOD CONTACT MATERIALS

FOOD CONTACT MATERIALS

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Identification of Non-Intentionally Added Substances (NIAS) in Food Contact Materials Using APGC-Xevo G2-XS QTof and UNIFI Software

Nicola Dreolin and Peter Hancock
Waters Corporation, Wilmslow, UK

APPLICATION BENEFITS

- Reliable GC-MS method for screening and structural elucidation of non-intentionally added substances (NIAS) in food packaging materials
- Atmospheric Pressure Gas Chromatography (APGC) is a soft ionization technique that produces lower levels of fragmentation than EI, enabling improved detection of challenging molecular ions and the avoidance of possible erroneous identification
- UNIFI® Software provides customized workflows to streamline and simplify elucidation of unknown compounds from food packaging

WATERS SOLUTIONS

Atmospheric Pressure Gas Chromatography (APGC)

Xevo® G2-XS QTof Mass Spectrometer

UNIFI Scientific Information System

KEYWORDS

High resolution mass spectrometry, HRMS, food contact materials, leachables, non-targeted analysis, GC-MS, migration, componentization, elucidation, electron ionization, EI, MS^E

INTRODUCTION

Food comes into contact with many materials and articles during its production, processing, storage, preparation, and serving before its eventual consumption. Such materials and articles are called food contact materials (FCMs). Recently, concern about the wholesomeness and safety of food products has increased dramatically. Most of the concern usually focuses on food additives, monomers, oligomers, and non-intentionally added substances (NIAS). A non-intentionally added substance is defined in the European Union (EU) Regulation No 10/2011 as "an impurity in the substances used or a reaction intermediate formed during the production process or a decomposition or reaction product."[1,2] FCMs can, therefore, be considered materials containing a complex mixture of substances of known or unknown identity/origin. Depending on their physico-chemical properties and chemical composition, FCMs may transfer some constituents, both Intentionally Added Substances (IAS) and NIAS to foodstuffs. This mass transfer phenomenon is called migration, and may lead to high exposure to certain chemicals, which might cause a risk for human health.[3] Therefore, migration must be evaluated and controlled. Furthermore, where migration brings about an unacceptable change in the composition of food or brings about deterioration in the organoleptic properties of the food, it must be avoided.[4]

Before performing a migration study, a screening analysis of the packaging material is required to identify the chemicals that are present in the material and those that are more likely to migrate. This initial step usually involves a strong extraction of the material with an organic solvent or a mixture of solvents. The extract is then injected via LC-MS and/or GC-MS for non-targeted screening analysis of non-volatiles, and volatiles/semi-volatiles, respectively. With respect to semi-volatiles and volatiles analyses, a GC coupled to a quadrupole mass spectrometer equipped with electron ionization using 70 eV in the ion source is typically employed, since it allows the analyst to use scientific libraries, such as NIST, for comparing acquired spectra with those in the library. However, the identification process becomes almost impossible when the compound of interest is not listed in the library, or when the sensitivity of the quadrupole MS is not sufficient for reliable mass confirmation. Waters® Atmospheric Pressure Gas Chromatography (APGC) and Xevo G2-XS quadrupole time-of-flight (QTof) Mass Spectrometer, along with the UNIFI Scientific Information System provides an advantageous solution to overcome this hurdle.

APGC is a soft ionization technique which enables molecular ions to be observed.[5] Furthermore, the use of high resolution mass spectrometry (HRMS) and its proprietary MSE mode[6] allows analysts to simultaneously acquire data containing the accurate mass of precursor and fragment ions. Finally, UNIFI's Discovery tool utilizes accurate mass and fragment information to facilitate the decision-making process towards the eventual identification of unknown compounds. To illustrate the benefits of APGC-QTof against electron ionization (EI)-single quadrupole MS, a polymer extracted sample was injected into both systems using the same chromatographic conditions in order to perform a comparative study of the chromatographic traces.

EXPERIMENTAL

Sample preparation

The sample, consisting of novel starch-based biopolymer pellets (0.5 g), was extracted three times with 2.5 mL of methanol in an ultrasonic bath for 1 hour at 40 °C. The total extraction solution (7.5 mL) was concentrated to 1 mL under a gentle nitrogen flow at room temperature before injection.

GC conditions

GC system:	Agilent 7890A
Autosampler:	7683B
Column:	DB-5MS, 30 m x 0.25 mm I.D. x 0.25 µm film thickness
Injection type:	1 µL pulsed splitless
Pulse time:	1.2 min
Pulsed pressure:	32 psi
Inlet temp.:	250 °C
Carrier gas:	He at 1 mL/min
Oven temp. program:	50 °C held for 2 min, ramp 50 to 300 °C 10 °C/min, 300 °C held for 10 min

MS conditions

MS system:	Xevo G2-XS QTof, sensitivity mode
Scan range:	50 to 650 *m/z*
Corona current:	2.2 µA
Sample cone:	30 V
Source temp.:	150 °C
Cone gas flow:	140 L/h
Auxiliary gas flow:	225 L/h
Make-up gas:	N_2 300 mL/min at 300 °C
Collision ramp for MS^E:	20 to 30 eV
Lock mass:	Persistent column bleed peak, 207.0324 *m/z*
EI solvent delay:	4 min
Data management:	UNIFI Scientific Information System

RESULTS AND DISCUSSION

Data were acquired using dry conditions, where nitrogen charge transfer occurs and gives rise to the (radical cation) molecular ion $M^{+\cdot}$ information.

First, Total Ion Current (TIC) chromatograms acquired with EI (using an Agilent 6890N gas chromatograph with a MS 5975B detector) and APGC were compared. It is notable that APGC showed a higher number of peaks (Figure 1). This is due to the higher sensitivity of the QTof versus the single quadrupole, and to the intrinsic characteristics of the two different types of ionization techniques.

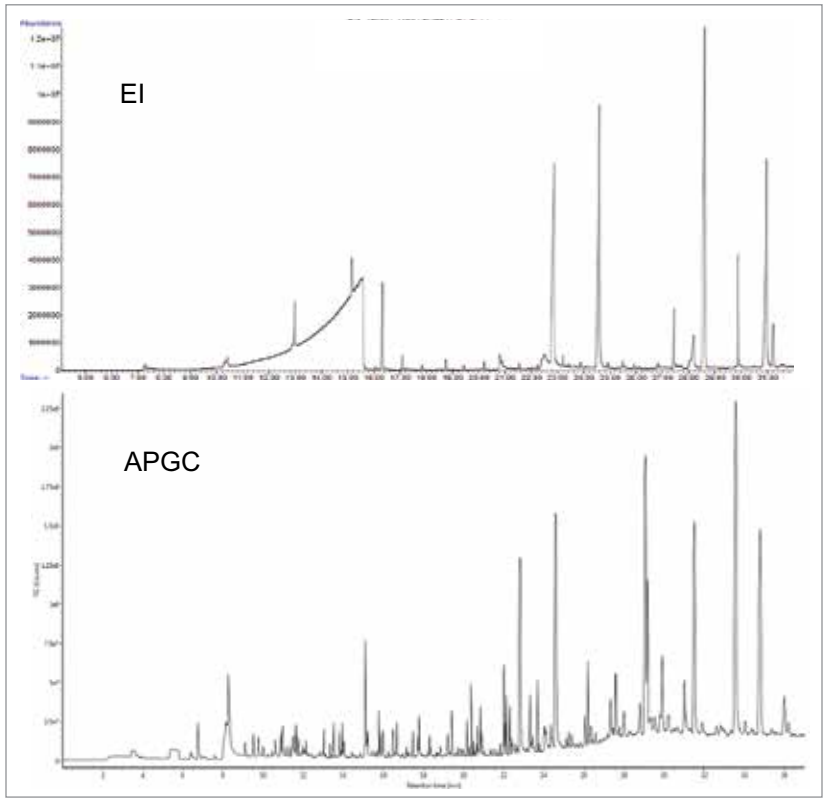


Figure 1. TIC chromatograms of the polymer extract acquired with EI (top), and with APGC at low collision energy (bottom).

BINARY COMPARISON

It is important to determine whether a peak comes from the tested material or from external contamination. Therefore, the analysis of a sample must always be accompanied by the analysis of its blank extract. UNIFI Software's Binary Compare feature allows direct comparison of the analysis results of an unknown sample with those of a reference (blank) sample, and to display the results in a mirror-image plot (Figure 2).

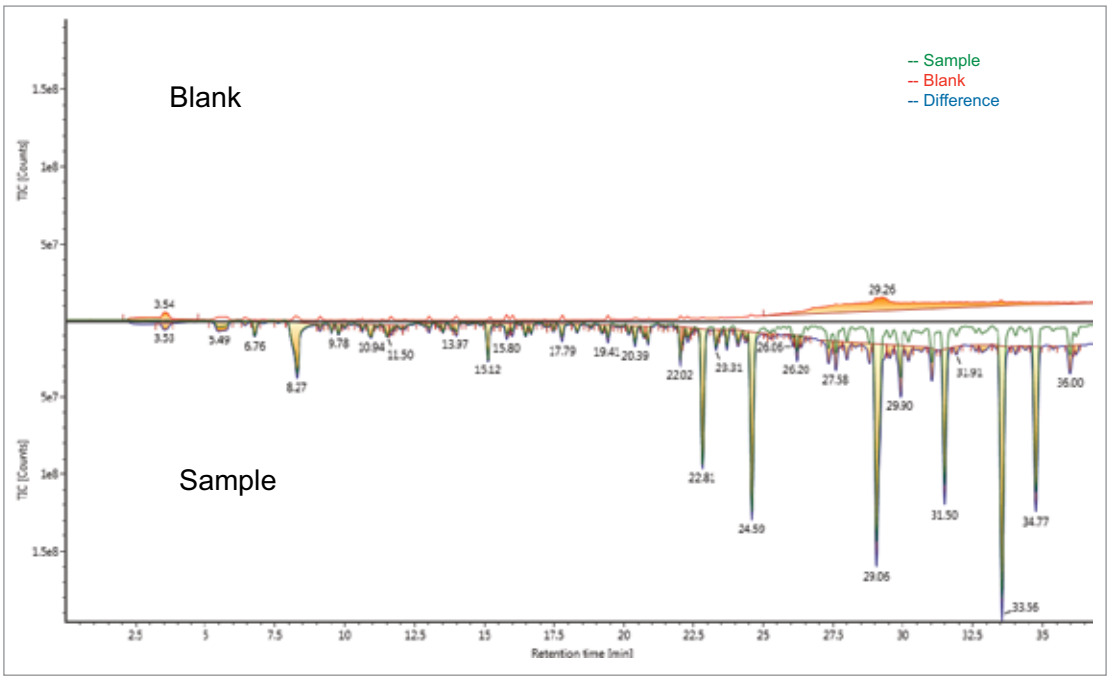


Figure 2. UNIFI's Binary Compare window shows the unknown sample and blank chromatographic profiles.

In addition, after specifying the mass tolerance, retention time tolerance, and intensity threshold of the unknown and reference samples in the comparison settings, UNIFI returns a Component Summary, where it is easy to identify the ions that are present in the unknown sample only, sorted by the intensity of response (Figure 3).

Component Summary

	Unknown component name	Unknown RT (min)	Unknown m/z	Match type	Unknown intensity (Counts)	Unknown/Reference	Reference m/z
1	Candidate Mass 480.4893	34.78	480.4893	Unknown Unique	4817260		0.0000
2	Candidate Mass 421.1843	33.57	421.1843	Common	3552189	104.3914	421.1836
3	Candidate Mass 452.4577	31.50	452.4577	Common	3513449	430.0651	452.4577
4	Candidate Mass 401.2153	29.07	401.2153	Common	3393889	66.9177	401.2153
5	Candidate Mass 481.4937	34.78	481.4937	Unknown Unique	3079423		0.0000
6	Candidate Mass 450.1754	33.57	450.1754	Common	2885954	160.5685	450.1748
7	Candidate Mass 430.2064	29.06	430.2064	Common	2880768	112.7112	430.2060
8	Candidate Mass 420.1770	33.57	420.1770	Common	2702121	97.1917	420.1765
9	Candidate Mass 400.2085	29.06	400.2085	Common	2615383	60.3286	400.2082
10	Candidate Mass 435.1651	33.58	435.1651	Common	2391867	137.5356	435.1639
11	Candidate Mass 453.4619	31.50	453.4619	Unknown Unique	2183887		0.0000
12	Candidate Mass 256.2635	22.85	256.2635	Common	2115949	83.8246	256.2634
13	Candidate Mass 285.2981	24.60	285.2981	Common	2100727	208.3426	285.2978

Figure 3. Excerpt of Component Summary table.

UNIFI's Binary Compare function is particularly useful when the blank samples present a high level of contamination, as well as when some of the peaks are not perfectly resolved. Furthermore, some components were not visible in the TIC chromatogram due to the trace-level nature of some NIAS from the packaging materials. In these circumstances, UNIFI Software helps the user to determine the unique compounds in the sample extract despite their low intensity, which would be labelled as "unknown unique".

CONFIRMING IDENTIFICATION

The first step is testing the applicability of APGC for the confirmation of compounds that are associated to a candidate in the NIST library with a high *match* value. By way of example, the peak at retention time 16.3 min was identified by EI as 1,6-Dioxacyclododecane-7,12-dione (molecular formula $C_{10}H_{16}O_4$, monoisotopic molecular mass 200.1049 amu, CAS number 777-95-7) with a match of 917 (Figure 4A).

The same peak was processed via APGC, and its spectrum showed a base peak at m/z 201.1120, which is attributed to the $[M+H]^+$ ion (Figure 4B).

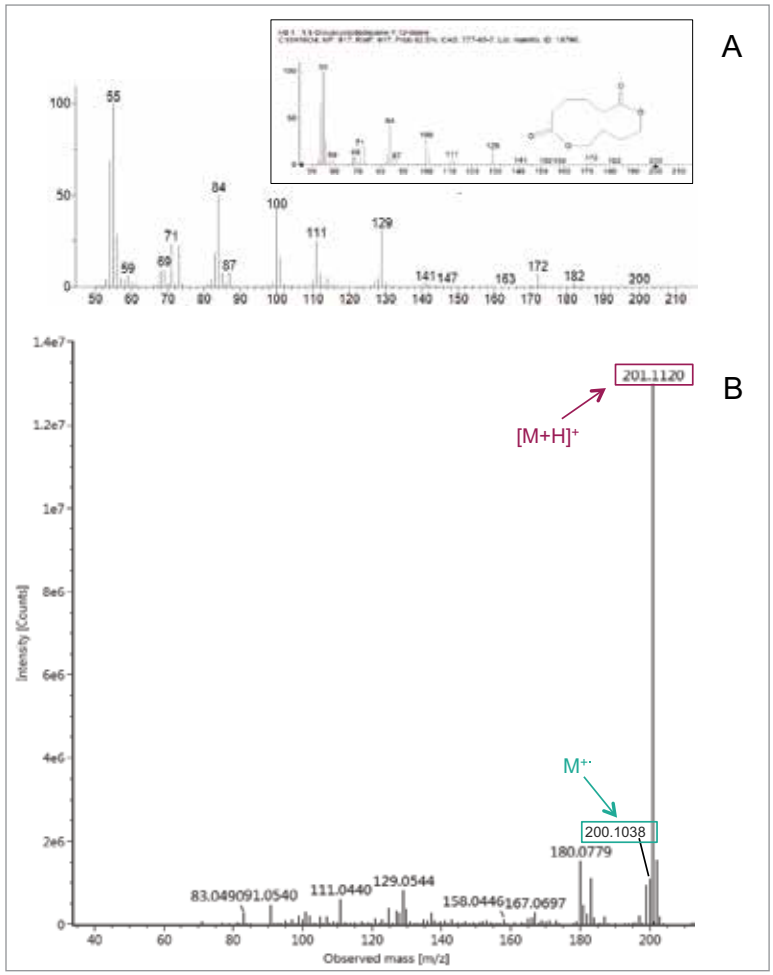


Figure 4. Comparison between the unknown and the reference for peak Rt = 16.2 min, showing (a) EI spectra, and (b) APGC low collision energy spectrum of the same chromatographic peak.

Using UNIFI's Mass Calculator feature, it is possible to obtain the exact mass of the adduct candidate molecular formula proposed by the EI library $[C_{10}H_{16}O_4+H]^+$. Hence, the mDa and ppm errors can be calculated. In the current example, the candidate molecular formula presents -0.14 mDa error and -0.7 ppm error. In APGC, the molecular ion $M^{+\cdot}$ at m/z 200.1038 is also present; in this case, the errors are -0.48 mDa and -2.4 ppm. Even though the presented APGC spectrum was obtained under dry conditions, protonation prevails over charge transfer because the structure of the investigated molecule favors accepting a proton, since even under dry conditions, the complete elimination of moisture in the ion source cannot be reached. The results demonstrate that the molecular formula of the candidate could be confirmed by the accurate mass of the molecular ion and the protonated adduct.

While linear adipates are usually employed as plasticizers in many plastic materials, 1,6-Dioxacyclododecane-7,12-dione is a cyclic adipate that was previously also found as a NIAS in biodegradable polyesters,[7] printing inks,[8] and polyurethane plastics.[9]

This example highlights the usefulness of APGC coupled with high resolution mass spectrometry when confirmation of the molecular formula is needed.

CORRECTING AN INCORRECT IDENTIFICATION

At the retention time 17.2 min in EI there was a very low intensity and broad peak that NIST attributed to 3,4-altrosan or beta-D-glucopyranose, 1,6-anhydro-, with a *match* value of 787. Both compounds have a molecular weight of 162 amu. However, by analyzing the same peak in APGC, a base ion peak at m/z 232.1817 appeared.

UNIFI Software allows users to create a customized workflow through the introduction of filters in order to get better visualization of data, and to save time by focusing on the most relevant components. For example, it is possible to select a specific Rt window to be analyzed and an ion intensity threshold. Applying this filter (Rt window 17.16–17.27 min and response >5000 counts) for peak Rt 17.2 min in APGC, UNIFI returns the component list that fits those settings. In this example, we displayed the processed and non-processed high collision energy spectra of the same component, shown in Figure 5. The processed spectrum appears "cleaner" because it focuses only on the component under investigation, without ions coming from other compounds that could partially coelute with the compound of interest.

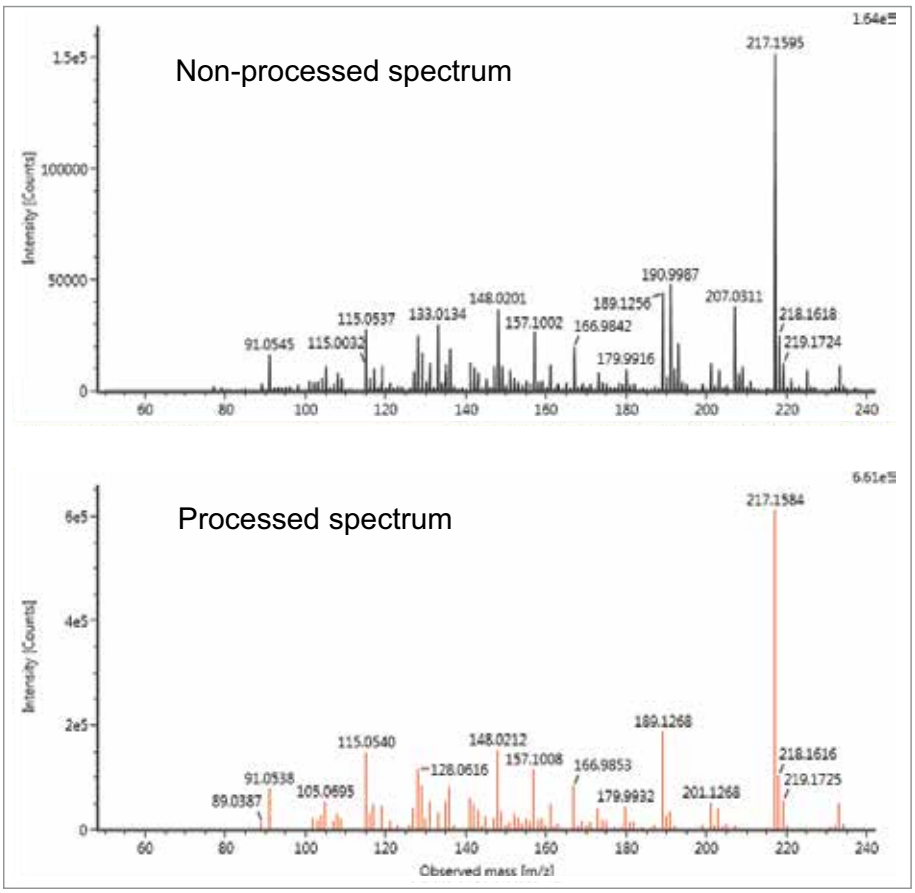


Figure 5. APGC high collision energy spectra of peak Rt 17.2 min. Non-processed spectrum (top) and processed spectrum based on component m/z 232.1817 (bottom).

UNIFI's filters, views, and workflow steps allow users to review data in a more timely, consistent, and accurate way. The componentization feature in UNIFI allows interrogation of entire datasets without having to interact with the raw data. Componentization also facilitates the selection of candidate components, which may represent unexpected substances within a sample; this is possible with UNIFI's 3D peak detection algorithm.[10]

When screening complex samples, the UNIFI Elucidation toolset can be used to investigate and potentially identify candidate components. The Elucidation toolset includes an elemental composition calculator that determines a number of possible formulas for an accurate mass peak. Elemental Composition uses an algorithm, i-FIT,™ to score each formula by the likelihood that the theoretical isotope pattern of the formula matches a cluster of peaks in the spectrum. To restrict the number of possible formulas, the i-FIT model can take into account fragment ion mass spectral peaks, the number of atoms of elements specified, valence state, the number of double bonds in a formula, the type of isotope pattern, and a series of chemical rules.

By applying the Elemental Composition tool to mass 232.1817 UNIFI proposed the molecular formula $C_{16}H_{24}O$ ($M^{+\cdot}$) with the lowest mDa error and the highest i-FIT confidence (%), as shown in Figure 6.

After searching ChemSpider, PubChem, and SciFinder, the suggested molecular formula was attributed to 1,2,3,4-tetrahydro-1-methoxy-1,6-dimethyl-4-(1-methylethyl) naphthalene (CAS number 60698-94-4). The Elemental Composition tool was also used to check the molecular formula of the most abundant fragments in the processed high collision energy spectrum, and to deduce their structures. In Figure 7 the proposed fragmentation pathway is shown, which confirmed the candidate structure of the molecular ion.

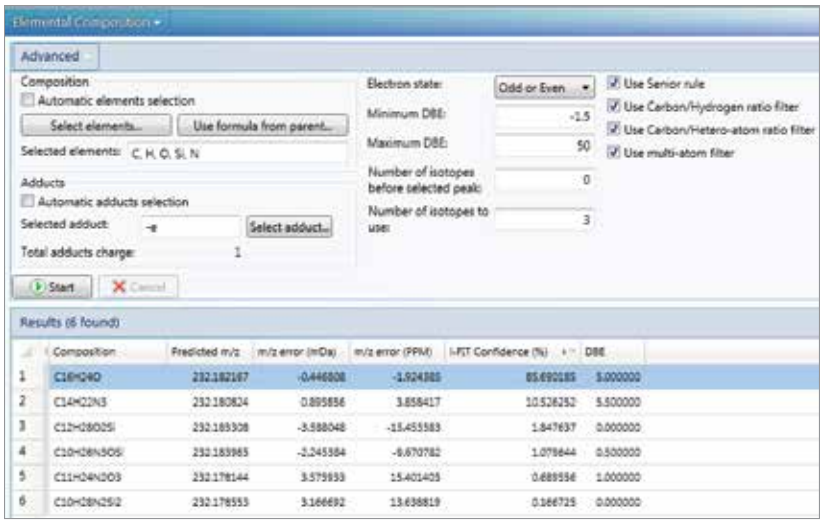


Figure 6. Results from UNIFI Software's Elemental Composition tool for the ion m/z 232.1817.

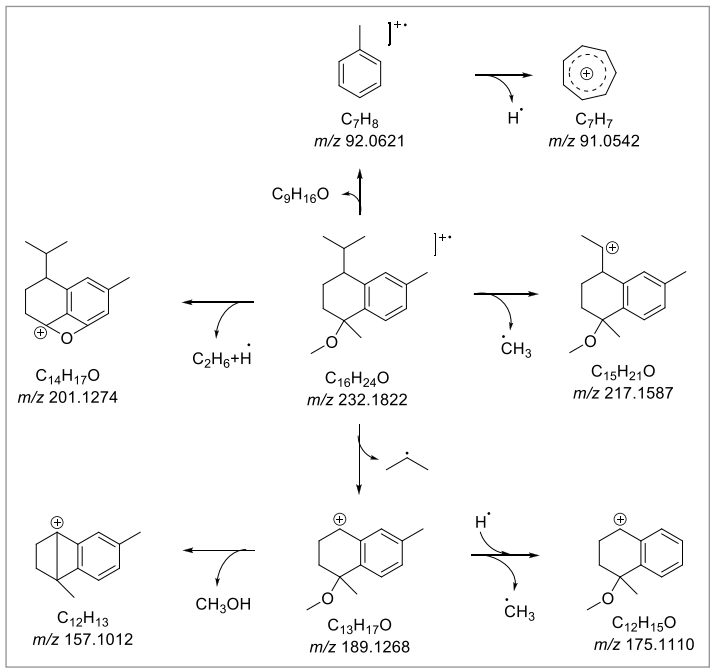


Figure 7. Proposed fragmentation pathway of the molecular ion $M^{+\cdot}$. Fragment ions are defined by their molecular formula and exact mass-to-charge ratio.

1,2,3,4-tetrahydro-1-methoxy-1,6-dimethyl-4-(1-methylethyl) naphthalene was also found in essential oil extracts of several plants, such as hops, pine and Japanese spicebush,[11-13] as well as in propoli extracts[14] as a component of the volatile profile.

Here, we were able to correct the EI identifications of components that presented a low match value or that were not listed in the libraries using APGC and UNIFI.

IDENTIFYING PREVIOUSLY NON-DETECTABLE PEAKS

Since the APGC-QTof MS system delivers enhanced sensitivity compared to EI-MS, APGC spectra lead to a significantly higher number of detected peaks. Consequently, it is possible to extend the identification process to a wider range of compounds. By way of example, the compound represented by the peak at Rt 27.3 min in the APGC spectrum was not present in the EI spectrum (Figure 8).

In this step, the Discovery tool in UNIFI was employed on the base ion peak m/z 410.3169.

In Figure 9 it can be noted that UNIFI attributed the component of interest to a predicted list of chemicals, recognized to be likely by an automatic search in ChemSpider. The table shows a list of possible compounds sorted by Predicted Intensity, i-FIT Confidence, Fragment Match, or number of citations.

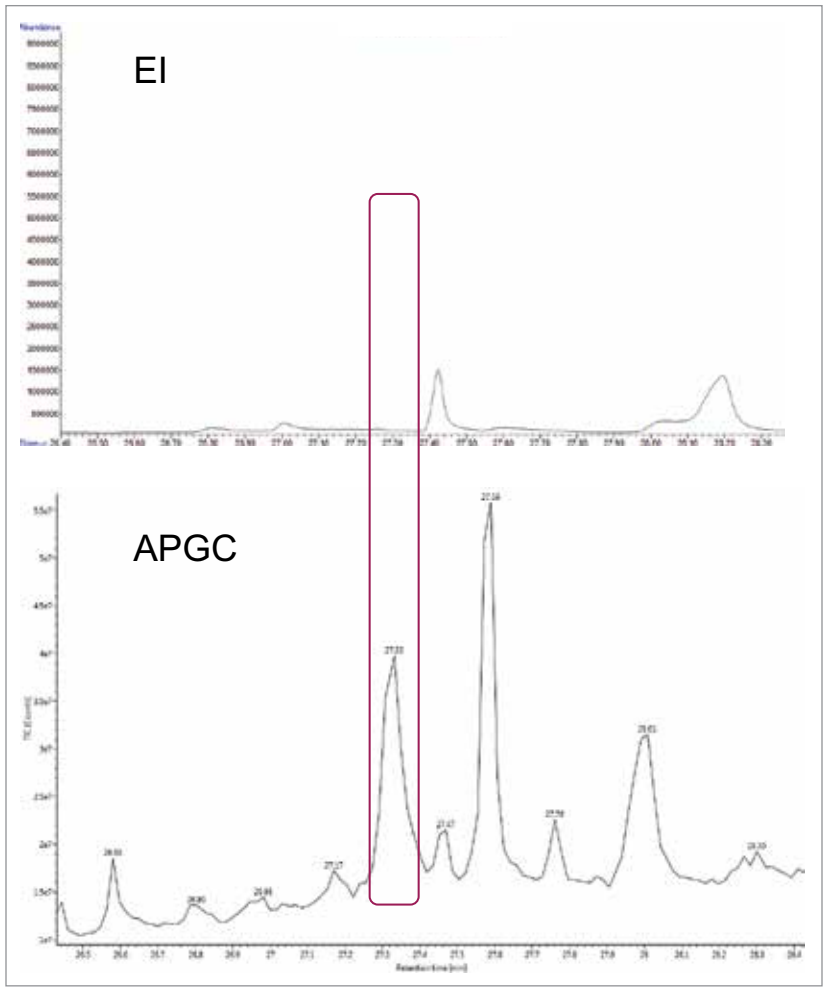


Figure 8. Comparison between the EI and APGC chromatograms within the range 26.4–28.4 min, highlighting the peak at 27.3 min in APGC, not detected with EI.

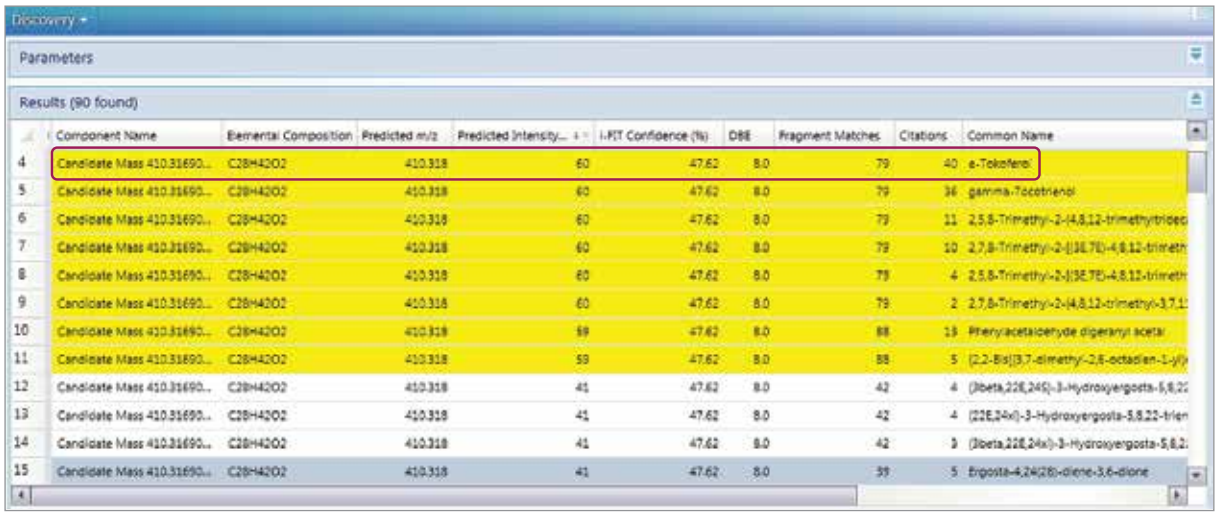


Figure 9. Results from UNIFI's Discovery tool for component m/z 410.3169 at Rt 27.33 min.

The candidates highlighted in yellow present a Predicted Intensity >50%. After analyzing the most important fragment ions, applying the common organic chemistry rules, and checking their molecular formula and mDa errors, the unknown compound was identified as e-tokoferol, more commonly called beta-tocotrienol, IUPAC name: [*R*-(*E,E*)]-3,4-dihydro-2,5,8-trimethyl-2-(4,8,12-trimethyl-3,7,11-tridecatrienyl)-2H-1-benzopyran-6-ol (CAS number 490-23-3). In Figure 10, the Discovery information output is illustrated. On the left side of the figure there is a list of synonyms for the candidate, while on the right side, the software shows the chemical structure and the high collision energy mass spectrum, where the most important fragments are pointed out.
It is possible to check out the molecule's cleavage points by clicking the fragment marker on the ion peak; the fragment m/z 191.1062 was chosen as an example.

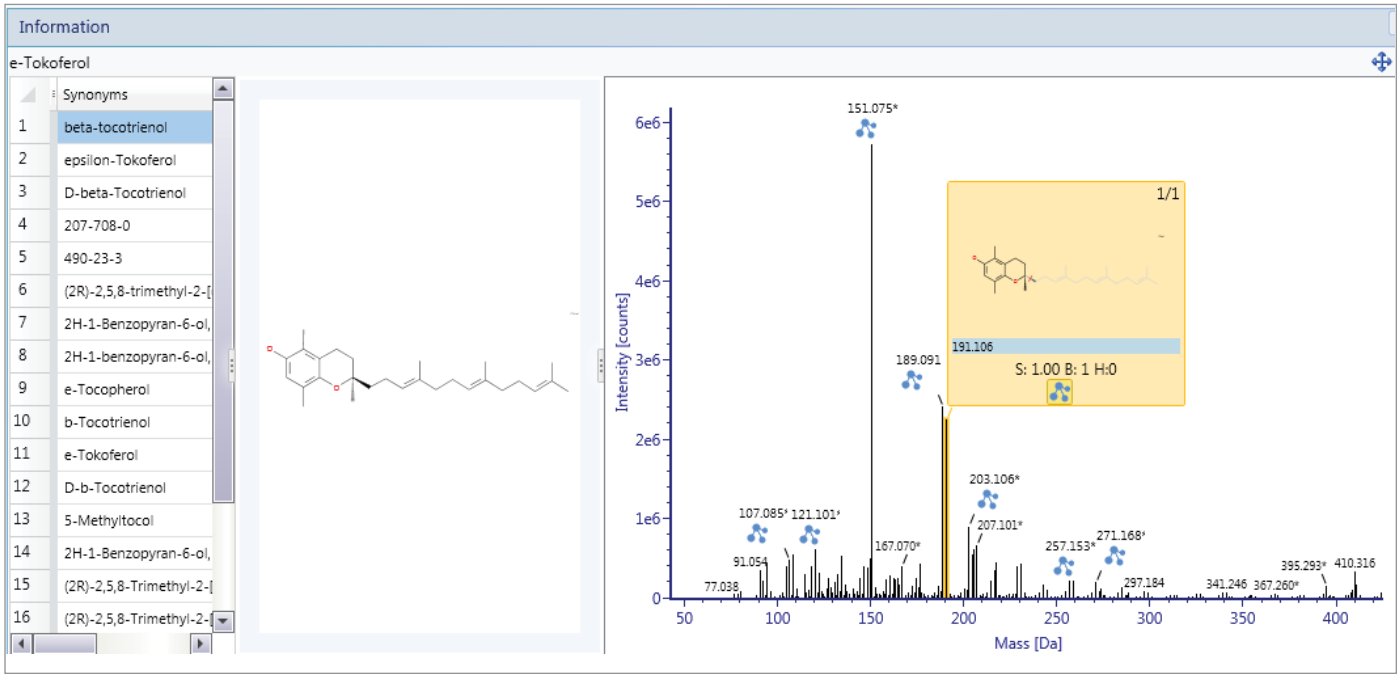


Figure 10. UNIFI's Discovery tool information output of beta-tocotrienol. Highlighted is one of the major fragments (m/z 191.1062).

Tocotrienols are members of the Vitamin E family, characterized by an unsaturated isoprenoid side chain (farnesyl isoprenoid tail) with three double bonds; their presence in the polymer could be due to their employment as antioxidant additives. In addition, tocotrienols are bioactive compounds normally present in many fatty foodstuff (such as vegetable oils), that have been used in many nutritional and pharmaceutical applications.[15]

UNIFI's Discovery tool saves analyst's time in the elucidation process and provides comprehensive high-quality information by sorting the possible candidates, based on several parameters set by the user. However, it should be noted that to reach a confidence level closer to 100% in the identification of an unknown compound, the candidate compound must be confirmed with a standard by verifying retention time, accurate mass, and common fragments.

CONCLUSIONS

Identifying unknown compounds in food contact materials is usually a challenging process. The UNIFI Scientific Information System simplifies the process by providing customizable workflows and achieving data containing accurate mass precursor and fragment ions information acquired by the MSE functionality.

EI-MS and APGC-QTof MS systems have been proven to be complementary when the compounds of interest are described in commercially available libraries, whereas APGC-QTof MS is particularly advantageous when the elucidation is required for volatile and semi-volatile components not listed in the libraries, or for those at trace or ultra-trace levels. APGC-Xevo G2-XS QTof with UNIFI can determine possible erroneous identifications and also facilitate component identification for peaks that are not detected using an EI quadrupole MS system.

Finally, UNIFI componentization eases the burden of data interpretation for the analyst, reducing potential false-positive assignments, and allowing results to be presented clearly and concisely.

References

1. The European Commission. Regulation EU No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food. *Official Journal of the European Union*, 2011.
2. S Koster, M H Bani-Estivals, M Bonuomo, E Bradley, M C Chagnon, M L Garcia, F Godts, T Gude, R Helling, P Paseiro-Losaba, G Pieper, M Rennen, T Simat, L Spack. *Guidance on Best Practices on the risk assessment of non intentionally added substances (NIAS) in food contact materials and articles*. International Life Sciences Institute, 2015.
3. O W Lau, S K Wong. Contamination in food from packaging material. *Journal of Chromatography A,* 2000.
4. The European Commission. Regulation EU No 1935/2004 of the European Parliament and of the Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. *Official Journal of the European Union*, 2004.
5. APGC. Waters White Paper, no 720004771en. August, 2013.
6. An overview of the principles of MSE, the engine that drives MS performance. Waters White Paper, no. 720004036en. October, 2011.
7. E Canellas, P Vera, C Nerin. UPLC-ESI-Q-TOF-MSE and GC-MS identification and quantification of non-intentionally added substances coming from biodegradable food packaging. *Analytical and Bioanalytical Chemistry*, 2015.
8. I Clemente, M Aznar, C Nerin, O Bosetti. Migration from printing inks in multilayer food packaging materials by GC-MS analysis and pattern recognition with chemometrics. *Food Additives and Contaminants*, 2016.
9. J S Felix, F Isella, O Bosetti, C Nerin. Analytical tools for identification of non-intentionally added substances (NIAS) coming from polyurethane adhesives in multilayer packaging materials and their migration into food stimulants. *Analytical and Bioanalytical Chemistry*, 2012.
10. Componentization following 3D-peak detection in the UNIFI Scientific Information System. Waters White Paper no. 720005480en. September, 2015.
11. D D Yan, Y F Wong, L Tedone, R A Shellie, P J Marriott, S P Whittock, A Koutoulis. Chemotyping of new hop (*Humulus lupulus L.*) genotypes using comprehensive two-dimensional gas chromatography with quadrupole accurate mass time-of-flight mass spectrometry. *Journal of Chromatography A,* 2017.
12. J J Kim, I Chung, E H Kim, K S Song, A Ahmad. Chemical composition of the essential oil and petroleum ether extract of Korean Pinus densiflora leaves. *Asian Journal of Chemistry*, 2012.
13. Z Liu, H Chen. GC-MS analysis of essential oils from leaves of Lindera obtusiloba. *Chinese Journal of Experimental Traditional Medical Formulae*. 2011.
14. W Bei, M Haile, Z Jiewen, L Lin. GC-MS fingerprints and clustering analysis of supercritical CO2 extracts of propolis from China. *Journal of Chinese Institute of Food Science and Technology.* 2011.
15. P Y Tan, T B Tan, H W Chang, B T Tey, E S Chan, O M Lai, B S Baharin, I A Nehdi, C P Tan. Effects of environmental stresses and in vitro digestion on the release of tocotrienols encapsulated within chitosan-alginate microcapsules. *Journal of Agricultural and Food Chemistry*, 2017.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Quantifying Primary Aromatic Amines in Polyamide Kitchenware Using the ACQUITY UPLC I-Class System and Xevo TQ-S micro

Steven Haenen and Marijn Van Hulle
Waters Corporation, Brussels, Belgium

APPLICATION BENEFITS

- Single method for analysis of 23 PAAs
- No need for ion-pairing reagents, or the removal of acetic acid from the sample extract prior to analysis
- Sensitive detection at levels well below the EU guidelines with Xevo® TQ-S micro Triple Quadrupole Mass Spectrometry

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

Xevo TQ-S micro

ACQUITY UPLC HSS T3 Column

MassLynx® MS Software

TargetLynx™ XS Application Manager

KEYWORDS

PAAs, primary aromatic amines, kitchenware, utensils, migration, food contact materials, FCMs

INTRODUCTION

Primary Aromatic Amines (PAAs) are a class of compounds of which the simplest form is aniline (Figure 1). PAAs are substances that are used, for example, in the production of certain colorants, so-called azo pigments, notably in the color range yellow – orange – red. Whereas a large number of PAAs are safe for human health, some PAAs are known human carcinogens. For kitchenware, paper napkins, baker's bags with colorful print and other printed items that come in contact with food, some PAAs may pose a health risk, if they are transferred to the food.

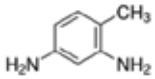
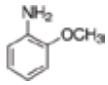
Compound	Mass	Structure
Aniline	93	
o-Toluidine	107	
2,4-Diaminotoluene	122	
o-Anisidine	123	

Figure 1. Chemical structures of some PAAs.

Because of the potential health risks, specific migration limits (SMLs) are put in place.[1] According to the regulation on plastics EU 10/2011: 'Plastic materials and articles shall not release primary aromatic amines, excluding those appearing in Table 1 of Annex I, in a detectable quantity into food or food simulant. The detection limit is 0.01 mg of substance per kg of food or food simulant. The detection limit applies to the sum of primary aromatic amines released'.

The provisions in Regulation 10/2011 state that for primary aromatic amine migration from polyamide kitchenware, only one migration test will be carried out, if this first extract is compliant with the summed SML (SML(T)) of 0.01 mg/kg . However, if this first simulant extract exceeds the permitted SML(T), two subsequent migration studies are required.[2] This PAAs migration testing is conducted with simulant B, 3% (w/v) acetic acid, as it has been demonstrated that this simulant represents the worst case for the migration of PAAs from polyamide kitchenware.[3]

PAAs are small, basic compounds, which are ionized with low pH. As a result of their basic properties and the 3% acetic acidic sample solvent, some PAAs don't focus well on the head of the column, resulting in poor peak shape and/or loss of retention. In order to improve chromatographic retention ion-pairing reagents are often used.[2] Unfortunately these reagents have a negative impact on the electrospray sensitivity and are to be avoided where possible.

In this application note we describe a LC-MS/MS method for the analysis of 23 common PAAs in kitchenware after migration using Waters® ACQUITY UPLC I-Class System coupled to a Xevo TQ-S micro Mass Spectrometer. The described method does not use an ion-pair reagent to improve chromatographic retention.

EXPERIMENTAL

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Sample manager:	Flow-Through Needle
Column:	ACQUITY UPLC HSS T3, 1.8 µm, 2.1 x 100 mm
Mobile phase A:	Water
Mobile phase B:	Methanol
Column temp.:	45 °C
Sample temp.:	10 °C
Flow rate:	0.4 mL/min
Run time:	15 min
Injection volume:	20 µL
Gradient:	

0 min	5% B
10 min	100% B
12 min	5% B
12.01 min	5% B
15 min	5% B

MS conditions

MS system:	Xevo TQ-S micro
Ionization mode:	ESI +
Capillary voltage:	2 kV
Desolvation temp.:	600 °C
Desolvation gas flow:	1200 L/hr
Source temp.:	150 °C
Acquisition:	Multiple Reaction Monitoring (MRM)

MS methods and data acquisition

Two MRM transitions were used, unless otherwise stated. The dwell times were chosen automatically using the built-in points-per-peak calculator in the MS method. The data were acquired using MassLynx v. 4.1 Software, and processed using TargetLynx XS Application Manager. Table 1 summarizes all MRM transitions. Figure 2 shows the retention time windows of the MRM method.

Compound	Transitions	Cone voltage (V)	Collision energy (eV)
Aniline	93.8>77.0	40	15
o-Toluidine	107.8>91.0 107.8>93.0	40 40	15 15
2,4-Diaminotoluene	122.8>106.2 122.8>108.3	40 40	15 18
o-Anisidine	123.9>65.0 123.9>109.0	40 40	20 15
4-Chloroaniline	127.8>93.1 129.8>93.1	40 40	18 18
3-Chloro-o-toluidine	140.8>77.1 140.8>95.1	40 40	10 10
2,4,5-Trimethyl aniline	135.9>91.0 135.9>121.0	40 40	20 15
2-Methoxy-5-methylaniline	137.8>78.1 137.8>123.1	40 40	25 15
4-Chloro-2-methylaniline	141.8>107.0 141.8>125.0	40 40	15 18
2-Amino naphthalene	143.8>117.1 143.8>127.0	40 40	20 20
2-Methyl-5-nitroaniline	152.8>107.0 152.8>121.0	40 40	15 10
4-Aminobiphenyl	169.9>92.0 169.9>152.1	40 40	20 25
2-Aminobiphenyl	169.9>92.0 169.9>152.1	40 40	20 25
Benzidine	184.9>167.1 184.9>168.1	40 40	25 18
4-Phenyl azoaniline	197.95>77.0 197.95>105.0	40 40	18 12
4,4'-Diamino diphenylmethane	199.0>77.1 199.0>106.0	40 40	22 22
4,4'-Oxydianiline	200.95>108.0 200.95>184.1	40 40	20 20
3,3'-Dimethyl benzidine	213.0>180.0 213.0>196.0	40 40	30 30
4,4'-Thiodianiline	216.95>124.0	40	20
o-Amino azotoluene	226.0>91.0	40	20
3,3'-Dimethyl-4,4'-diaminodiphenylmethane	227.0>120.2	40	20
3,3'-Dimethoxy benzidine	245.0>213.1 245.0>230.1	40 40	18 18
3,3'-Dichloro benzidine	252.9>182.1 252.9>217.0	40 40	25 20
4,4'-Methylene bis (2-chloroaniline)	266.9>140.1 266.9>231.1	40 40	25 22

Table 1. Overview of MRM transitions for all 23 PAAs.

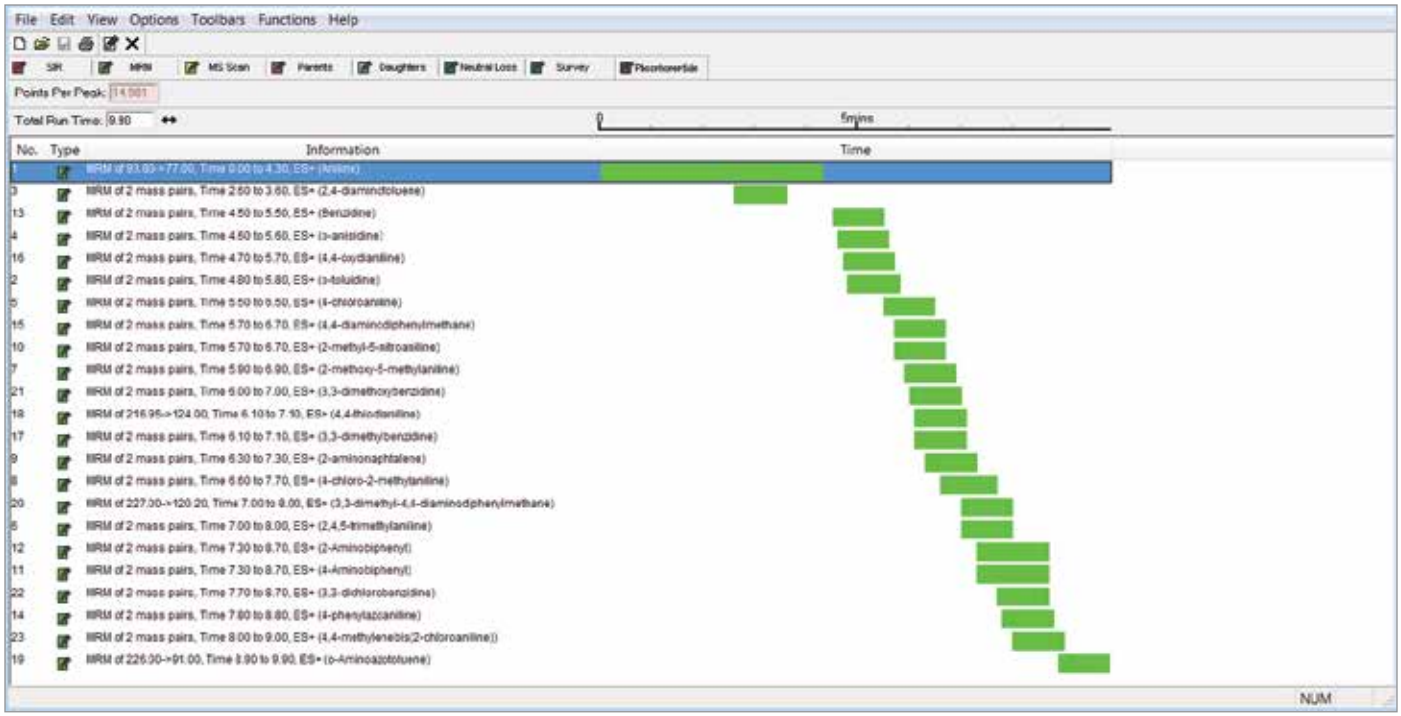


Figure 2. Retention time windows for the PAAs acquisition method.

Standards

A mixed standard solution containing all PAAs at a concentration of 100 µg/mL was used. The working standards were further diluted with the 3% acetic acid food stimulant solution. For the solvent calibration a dilution series starting at 100 ng/mL down to a level of 0.78 ng/mL was made.

Sample preparation

Nine polyamide kitchenware utensils were extracted with a 3% acetic acid solution according to the procedure described in the EU 10/2011 guidelines.[1]

RESULTS AND DISCUSSION

UPLC METHOD DEVELOPMENT

Because of the basic properties of PAAs, and the fact that acetic acid is used as a migration stimulant, some PAAs don't focus well on the head of the column, resulting in poor peak shape and/or loss of retention. Aniline elutes early and is therefore prone to this effect. As a result, some literature references cite the use of ion-pair reagents.[2] Adding ammmonium hydroxide to the 3% acetic acid samples prior to injection, the pH of the sample is increased and the polar and weakly basic PAAs such as aniline will be in their neutral form. A volume of 10 µL of a 25% NH_4OH solution was added to 1 mL of sample. This approach resulted in more robust results and is therefore preferred over the use of ion-pair reagent. Figure 3 shows a chromatogram of aniline with an unchanged pH (top) and adjusted pH (bottom). The neutralization of the pH drastically improves the peak shape of aniline, without the need for ion-pairing reagent.

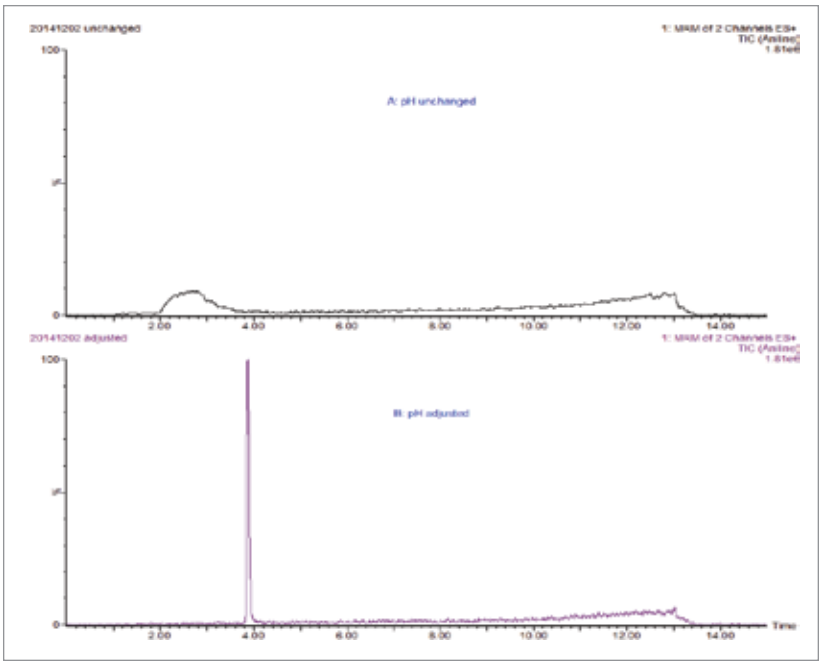


Figure 3. Chromatogram of aniline in 3% acetic acid food stimulant without (top) and with (bottom) pH adjustment.

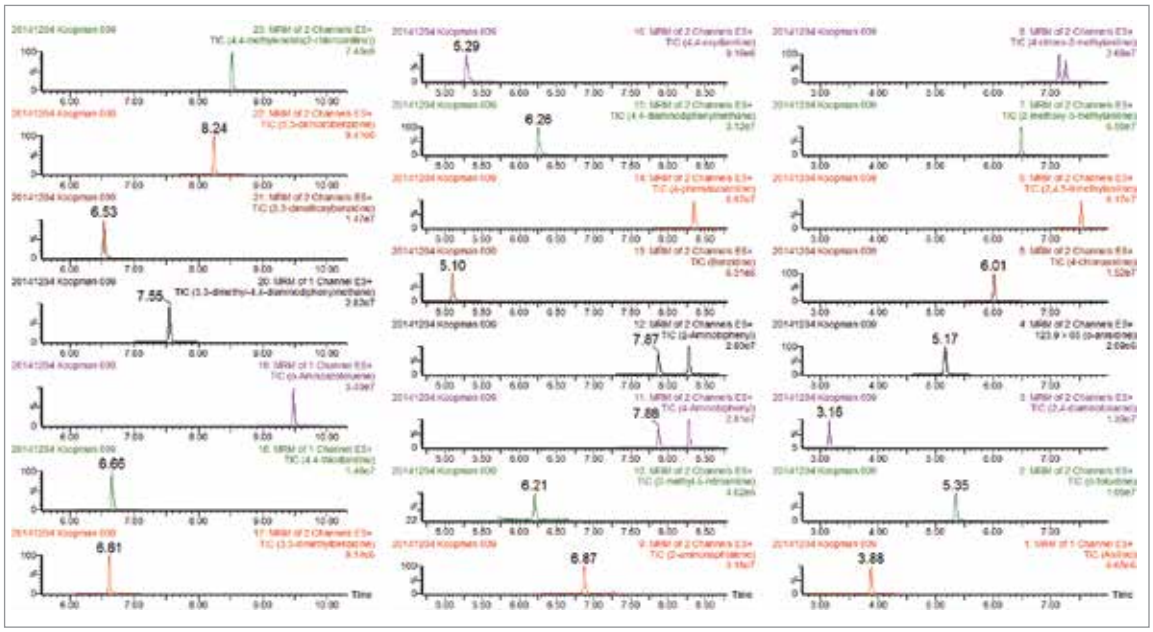


Figure 4. Chromatograms of all 23 PAAs.

LINEARITY

Calibration curves were prepared from 0.78 ng/mL to 100 ng/mL for all compounds. An example is given for aniline (Figure 5). For each calibration curve, a linear regression and a 1/X weighting was applied. All compounds show good linearity across the range of concentrations as well as excellent % residual values.

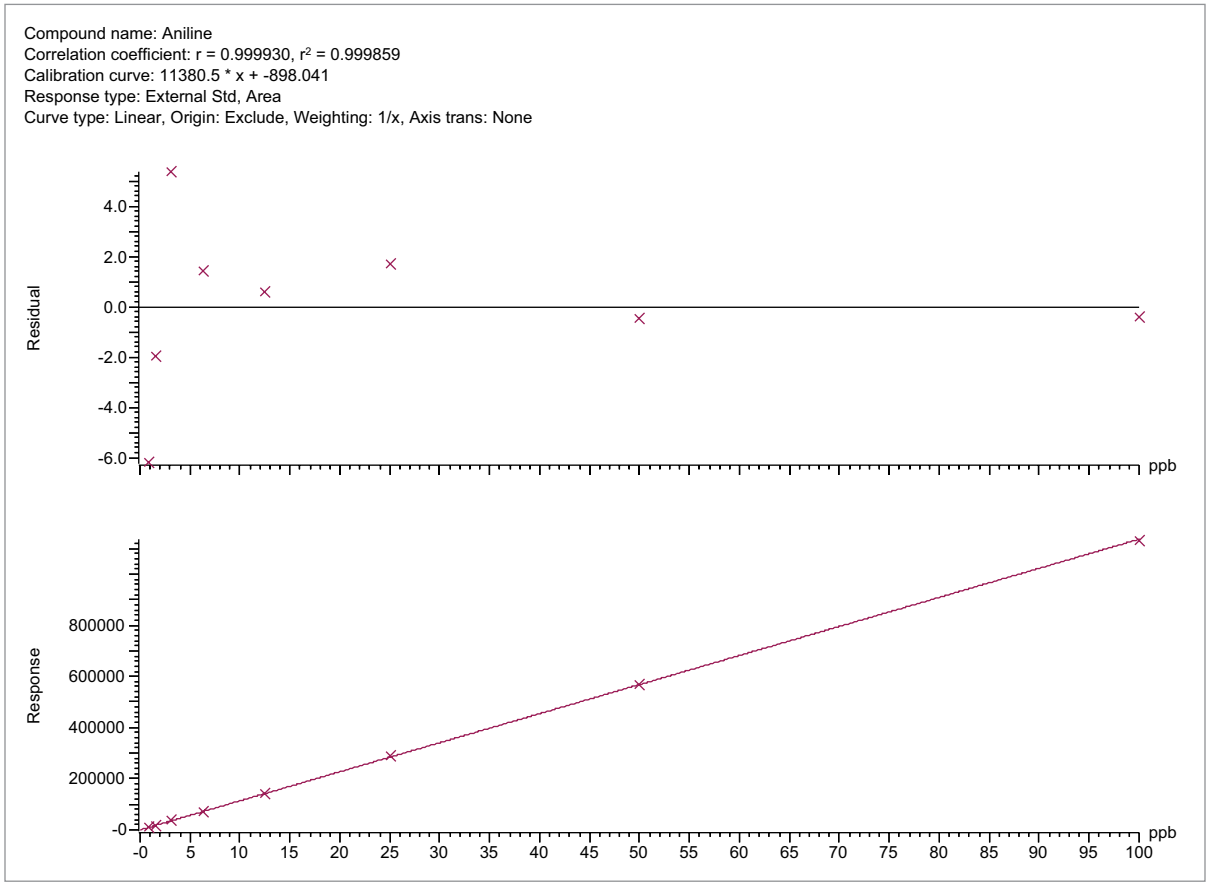


Figure 5. Calibration curve (bottom) and residuals plot (top) for aniline in the range 0.78 to 100 ng/mL.

Acidified mobile phases aid in the protonation of compounds and therefore improve the sensitivity in positive ion electrospray. As no acid was added to the mobile phases, we investigated whether a post-column addition (PCA) with formic acid would be beneficial. Using the Xevo TQ-S micro's built-in IntelliStart™ fluidics, a solution of 2% formic acid was infused at a constant flow rate of 20 µL/min into the UPLC® flow exiting the column. As such the formic acid solution was diluted 20-fold with the mobile phase, resulting in a final concentration of 0.1% of formic acid going into the ESI source. Figure 6 shows how this PCA was configured in the acquisition method, while Figure 7 shows the chromatograms for a selection of PAAs with (top trace) and without (bottom trace) this post-column addition. For better interpretation, the intensity axes have been linked. As can be seen from the chromatograms, the sensitivity is significantly improved when formic acid is added to the eluent.

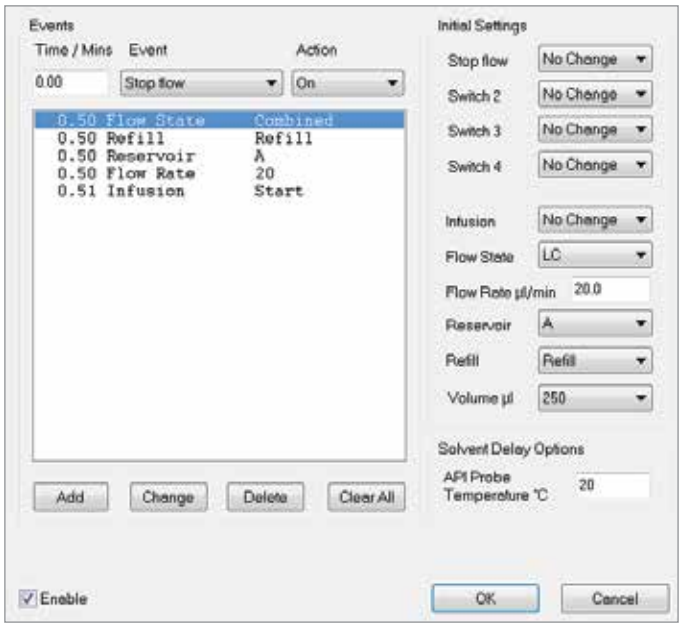


Figure 6. Post-column addition in the MS acquisition method.

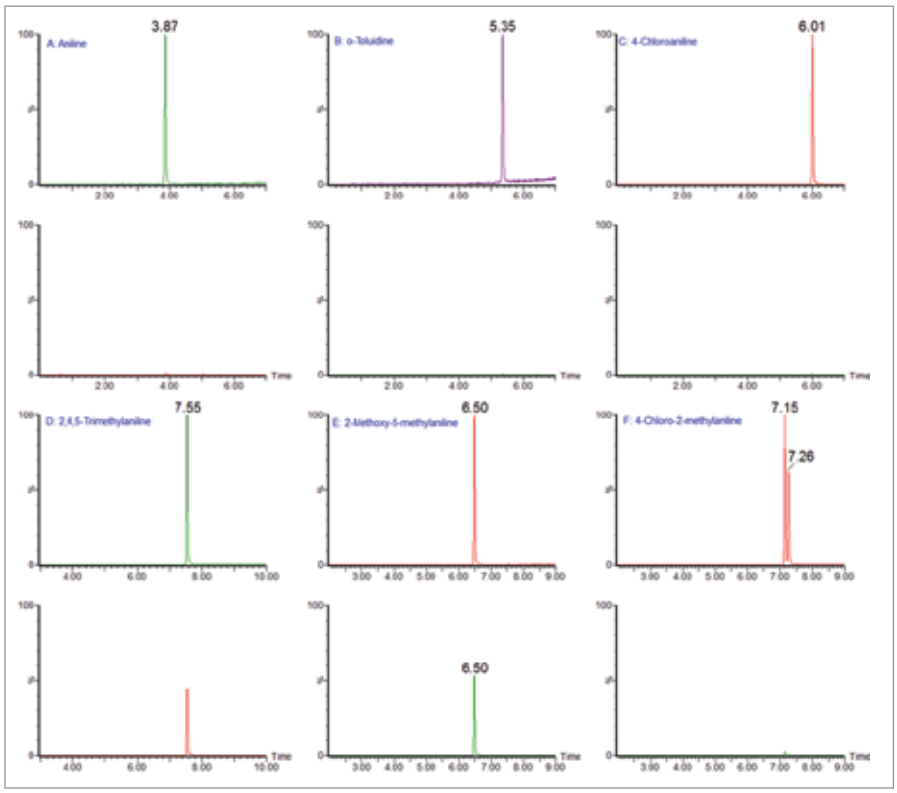


Figure 7. Increase in sensitivity with the use of a formic acid post-column addition (top), and without (bottom), illustrated for:
A. aniline,
B. o-Toluidine,
C. 4-Chloroaniline,
D. 2,4,5-Trimethylaniline,
E. 2-Methoxy-5-methylaniline, and
F. 4-Chloro-2-methylanaline.

Table 2 summarizes the quantitation limits (LOQ) for all compounds using this PCA approach. The LOQ is defined as the concentration giving rise to a signal-to-noise (S/N) value of 10:1. For the calculation of S/N, raw data was used and the peak-to-peak algorithm was applied. An extrapolation was made in most cases, as the reported S/N values were still significantly high, even at the lowest reported standard level of 0.78 ng/mL. Calculated LOQs below 20 pg/mL are not mentioned specifically but are cut off at this level. The reported LOQ concentrations range between 20 pg/mL and 300 pg/mL.

MATRIX EFFECTS

Internal standards were not used in this method. Therefore it was investigated whether the food simulant extract leads to ion suppression. One of the samples was spiked to a final concentration of 10 ppb and this sample was compared with a standard dissolved in the same food stimulant solution. All spike recoveries were within 90% to 107%, indicating that matrix effects were low to non-existing for the 23 compounds under investigation.

Compound	S/N ratio	LOQ (ng/mL)
Aniline	377	0.02
o-Toluidine	768	<0.02
2,4-Diaminotoluene	52	0.15
o-Anisidine	89	0.09
4-Chloroaniline	323	0.03
2,4,5-Trimethyl aniline	693	<0.02
2-Methoxy-5-methylaniline	1444	<0.02
4-Chloro-2-methylaniline	3503	<0.02
2-Amino naphthalene	1858	<0.02
2-Methyl-5-nitroaniline	27	0.29
4-Aminobiphenyl	226	0.04
2-Aminobiphenyl	272	0.03
Benzidine	559	<0.02
4-Phenyl azoaniline	1931	<0.02
4,4'-Diamino diphenylmethane	1353	<0.02
4,4'-Oxydianiline	312	0.03
3,3'-Dimethyl benzidine	165	0.05
4,4'-Thiodianiline	2582	<0.02
o-Amino azotoluene	1746	<0.02
3,3'-Dimethyl-4,4'-diaminodiphenylmethane	1818	<0.02
3,3'-Dimethoxy benzidine	528	<0.02
3,3'-Dichloro benzidine	926	<0.02
4,4'-Methylene bis (2-chloroaniline)	1522	<0.02

Table 2. Calculated S/N values at 0.78 ng/mL and estimated LOQ values for all 23 PAAs investigated.

KITCHENWARE SAMPLES

Using the external calibration curves, nine kitchenware samples were quantified. Except for aniline and 4,4'-diamino diphenylmethane found in all nine samples at levels between 0.4 to 1.1 ppb and 0.04 to 0.11 ppb, respectively, no other PAAs were detected. Figure 8 shows the chromatograms of aniline in the sample containing 0.4 ppb and of 4,4'-diamino diphenylmethane in the sample containing 0.04 ppb. As can be seen sensitivity was excellent at these sub ppb level.

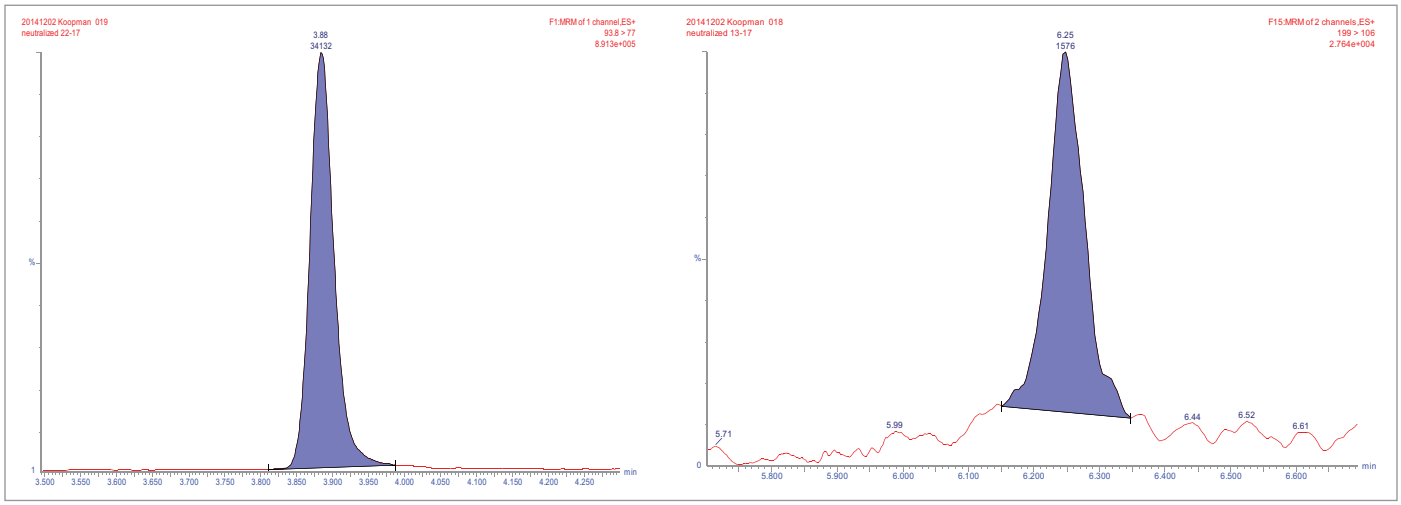


Figure 8. Chromatograms of aniline in kitchenware samples present at 0.4 ppb (left), and of 4,4'-Diamino diphenylmethane in the sample containing 0.04 ppb (right).

CONCLUSIONS

We have demonstrated a sensitive method for 23 PAAs with very easy sample preparation. The addition of ammonium hydroxide as neutralizing agent, and a post-column addition of formic acid into the Xevo TQ-S micro via IntelliStart's built-in fluidics – resulted in a very sensitive assay which could reach sub ppb levels. Linearity was observed over a large range and up to 100 ppb. The samples were all below detection limits except for aniline which was detected at 0.4 to 1.1 ppb, and 4,4'-diamino diphenylmethane which was detected at 0.04 to 0.11 ppb. The total PAAs content for all samples was below the SML(T) of 0.01 mg/kg as stipulated in the regulations EU 10/2011.

References

1. Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food.
2. LB-NA-24815-EN-N, Technical guidelines on testing the migration of primary aromatic amines from polyamide kitchenware and of formaldehyde from melamine kitchenware.
3. Analysis of primary aromatic amines (PAA) in black nylon kitchenware 2014. Selected samples from the Norwegian Market.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Chemical Analysis of Food Packaging Migrants and Other Chemical Contaminants in Infant Formula Using a TOF-Based Approach

Melvin Gay,[1] Antonietta Gledhill[2]
[1]Waters Pacific Pte Ltd, Singapore, [2]Waters Corporation, Manchester, UK

APPLICATION BENEFITS

- Unequivocal identification of potentially harmful food packaging migrants in infant formula containers.
- Simultaneous MS^E data acquisitions of both low energy precursor (MS) and high energy fragment ions (MS^E) in a single injection, for compound identification and confirmation.
- Structural elucidation and compound identification through the use of MarkerLynx™ MS, ChemSpider, and other software tools.
- MS/MS function of Xevo® G2 QTof provides compound confirmation, when used together with the commercially available standard.

WATERS SOLUTIONS

ACQUITY UPLC® System

Xevo G2 QTof

MarkerLynx XS Application Manager

KEY WORDS

Food packaging, TOF screening, Chemometrics, infant formula, benzoguanamine

GOAL

To identify possible food packaging migrants in infant formula containers.

INTRODUCTION

Packaging has become an indispensible element of food manufacturing processes. Packaging not only better protects consumers from microorganisms, biological, and chemical changes in food, thus providing longer shelf life, but it also makes foods easier to transport.

Recently, food packaging issues have gained widespread importance in food safety, due to the possible migration of chemicals from food contact materials into the food. Instances, such as the leaching of bisphenol-A (BPA) and BPA diglycidyl ether (BADGE) from plastic films to aqueous food simulants,[1,2] have caused serious health and legal issues. This incident led to more strict legislation by the European Union[3] and the U.S. Food and Drug Administration[4] that restricts packaging migration into foods, and better ensures consumer safety.

The internal surfaces of cans used to pack infant formula are often coated with layers of an epoxy liner that forms a barrier between the food and the metal of the can. However, the inert properties of this coating have raised important safety concerns, and thus the possible migration of contaminants from this surface is being actively investigated.

A wide variety of coating materials are used in food packaging, depending on the type of packaging and the food that is contained within the package. In many cases, the particular coating materials used to protect foods are not known to the analyst, thus posing a challenge in identifying potential chemicals that can migrate from the packaging into foodstuffs.

In this application note, an approach is described using TOF screening and a chemometric workflow to compare the similarities and differences between packaging materials, and to identify food packaging migrants in infant formula containers.

EXPERIMENTAL

LC conditions

LC system:	ACQUITY UPLC
Runtime:	10 min
Column:	ACQUITY BEH C_{18} 1.7 µm, 2.1 x 100 mm
Column temp.:	40 °C
Mobile phase A:	Water with 0.1% formic acid
Mobile phase B:	Methanol with 0.1% formic acid
Flow rate:	0.45 mL/min
Injection volume:	5.0 µL, PLUNO injection

UPLC gradients are detailed in Table 1

	Time (min)	Flow rate (mL/min)	%A	%B	Curve
1	Initial	0.45	90	10	0
2	0.25	0.45	90	10	6
3	7.75	0.45	0	100	6
4	8.50	0.45	0	100	6
5	8.51	0.45	90	10	6
6	10.00	0.45	90	10	6

Table 1. ACQUITY UPLC gradient for a 10-min screening run.

MS conditions

MS system:	Xevo G2 QTof
Ionization mode:	ESI +
Scan time:	0.2 s
Capillary voltage:	2.4 kV
Sampling cone:	30.0 V
Extraction cone:	4.0 V
Source temp.:	150 °C
Desolvation temp.:	500 °C
Desolvation gas:	1000 L/hr
Cone gas:	20 L/hr
Mass range:	50 to 1000 *m/z*

continued on next page

Sample preparation

The infant formula was purchased from a local supermarket. The contents were emptied and the container was washed and dried with nitrogen gas.

The tin was heated to 110 °C for 5 min to promote packaging migrants onto the surface of the tin, and 100 mL of methanol/water (50:50) was added into the tin. An aliquot of 2 mL (Day 0) was removed and stored in a -80 °C freezer. The remaining solvent in the tin was incubated at 40 °C. An aliquot of 2 mL was collected at the following time points, Days 1, 2, 3, 4, 5, 6, 7, and 8; and stored at -80 °C until analysis.

The samples were analyzed according to the parameters listed using the UPLC® gradient in Table 1.

RESULTS AND DISCUSSION

Currently, there is a limited amount of literature reporting the type of components migrating from infant formula containers into the formula, so an investigative approach was taken.

The investigative workflow used for these series of experiments is shown in Figure 1. This type of approach can also be applied to other food-related experiments where comparisons need to be made between a control sample and a test sample. In this case, the control sample was the packaging at T = 0, and the test samples were T = 1, 2, 3, 4, 5, 6, 7, and 8 days.

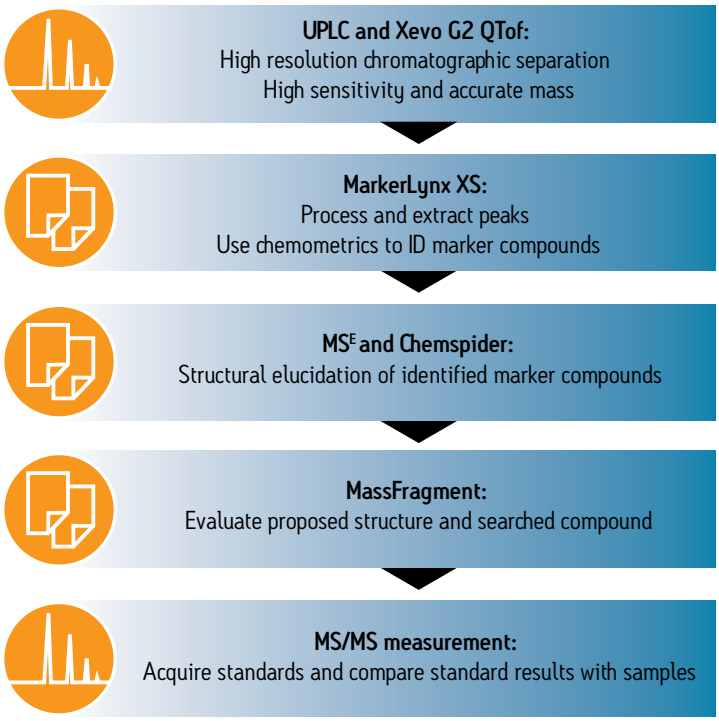


Figure 1. TOF screening workflow for packaging migration analysis.

MS^E conditions

Low energy:	6 eV
High energy ramp:	20 to 35 eV

MS/MS conditions

Set mass:	188.08 m/z
Scan time:	1.0 s
Collision energy:	25.0 eV
Mass range:	50 to 500 *m/z*

LockSpray™ conditions

Compound:	Leucine enkephalin
Masses:	*m/z* 556.2771 (MS^E); *m/z* 556.2771; and *m/z* 278.1141 (MS/MS)
Flow rate:	25 µL/min
Capillary voltage:	2.7 kV
Collision energy:	21.0 eV

With this challenge in mind, the ACQUITY UPLC System and Xevo G2 QTof were selected for this investigation. The increased resolution of the ACQUITY UPLC System, combined with exact mass performance, MS^E, and the MS/MS functionality of the Xevo G2 QTof, made this an excellent screening platform for this analysis.

After acquisition, the data were processed using MarkerLynx XS Application Manager, a chemometrics-based software package. The information was first investigated by using the Principle Component Analysis (PCA) approach to look at the differences between the packaging over the eight days of sampling. The samples can be easily compared, as shown in Figure 2.

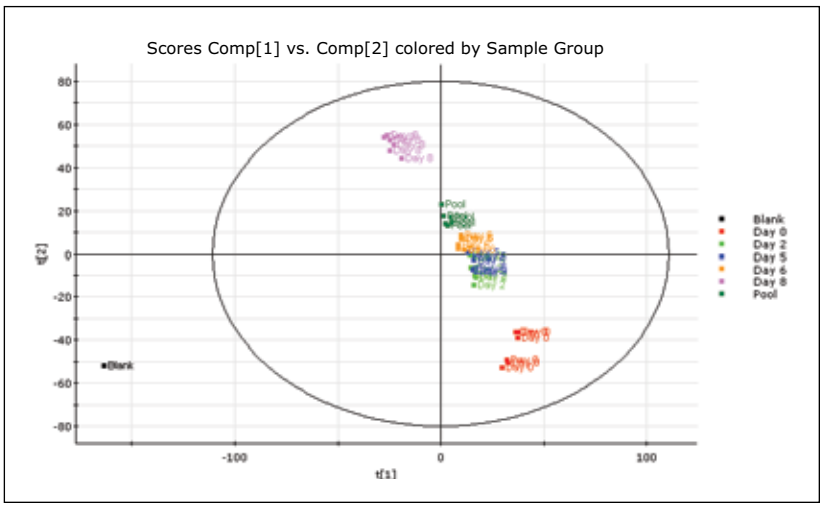


Figure 2. PCA model of the samples that underwent different incubation times.

Tight groupings were observed on the days that included repeat injections (good intra-group repeatability), and large differences were observed between Day 0 and Day 8. Further investigation using the Orthogonal Partial Least Squares (OPLS) model (which is used for comparing two groups) was employed to directly compare Day 0 to Day 8. The S-Plot derived from the OPLS model illustrating the comparison between the two groups (Day 0 and Day 8) is shown in Figure 3.

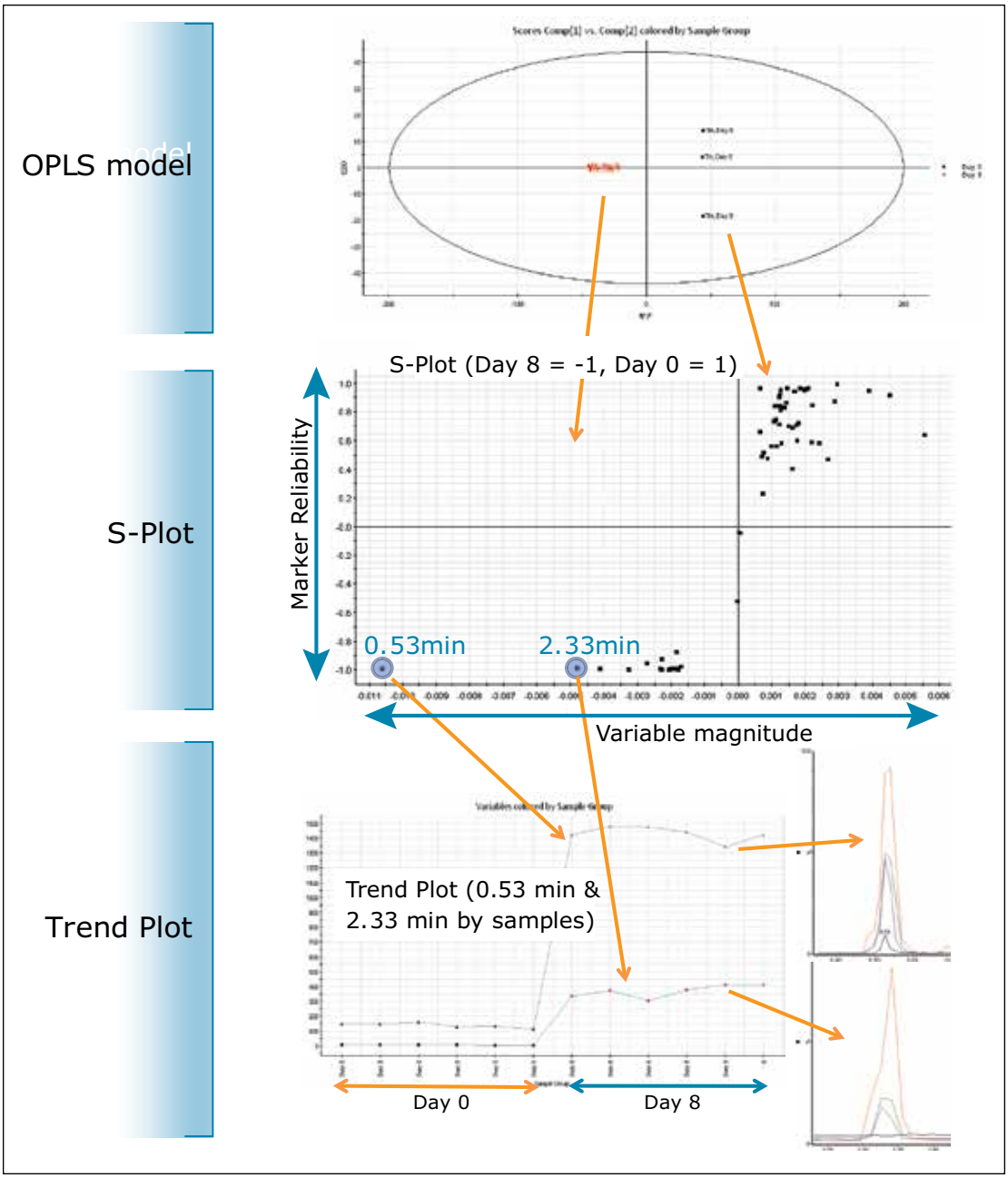


Figure 3.
From the OPLS model, the S-Plot was derived showing the increase of components at retention time 0.53 and 2.33 min. The BPI chromatogram showing gradual increase in the concentration of the two components between Day 0, 2, 6, and 8.

Further visualization using a trend plot shown in Figure 3 revealed that the concentrations of the two compounds, with retention times of 0.53 min and 2.33 min, increased on Day 8, compared to Day 0. The BPI chromatograms (Days 0, 2, 6, and 8) show a gradual increase in concentration of the two compounds, as shown in Figure 3. These unknowns were further investigated to elucidate the structure and identity of the compounds.

Structural elucidation is derived by utilizing the MS^E data, which are routinely acquired within an acquisition run. MS^E is an acquisition technique that provides a simple, unbiased, and parallel route to deliver exact mass, low energy precursor (MS) and high energy fragment ion (MS^E) information from every detectable component, without the need for multiple injections.

Using the MS^E data with exact mass measurement, the elemental composition of the unknown components were identified using ChemSpider (http://www.chemspider.com). The proposed structure was then evaluated using MassFragment™ Software, as shown in Figure 4. Combining information from MS^E, ChemSpider, and MassFragment Software, the compound with a retention time of 2.33 min was identified as benzoguanamine (2,4-diamino-6-phenyl-1,3,5-triazine), with a chemical formula of $C_9H_9N_5$. Benzoguanamine, which belongs to the same family as melamine, is often cross linked with saturated polyester resin, and is commonly used in can coating.

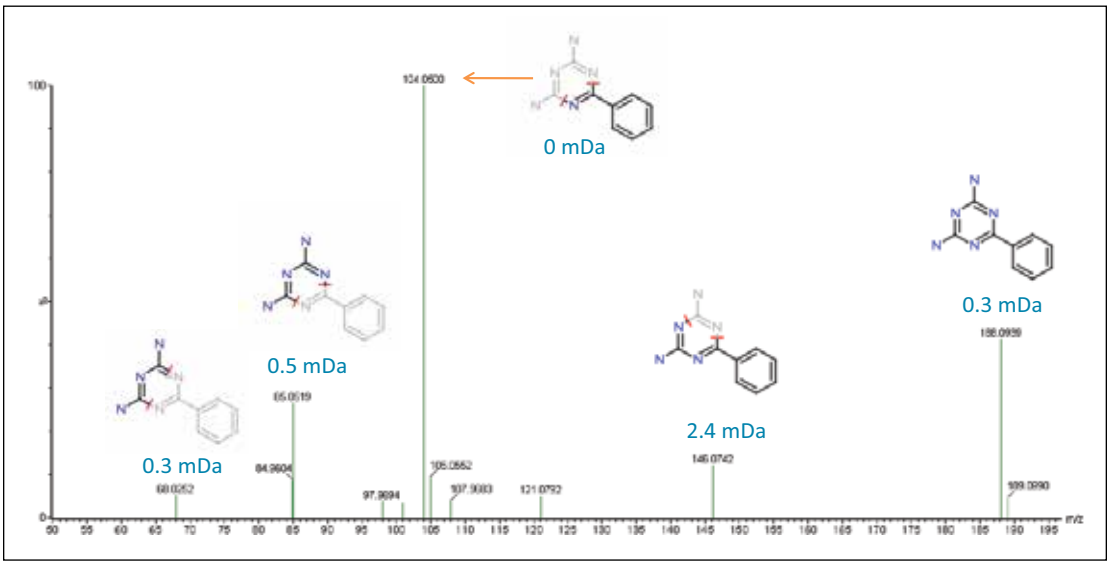


Figure 4.
Possible structures assigned to fragments (with mass error) from the component at 2.33 min attained from the S-Plot.

Further confirmatory analysis was performed using a commercially available benzoguanamine standard. MS/MS using Xevo G2 QTof was performed on both the standard and the Day 8 sample. The precursor mass (188.08 m/z) which corresponded to benzoguanamine produced identical fragment ion spectra in both the standard and the sample, shown in Figure 5, thus confirming the identity of the peak at 2.33 min.

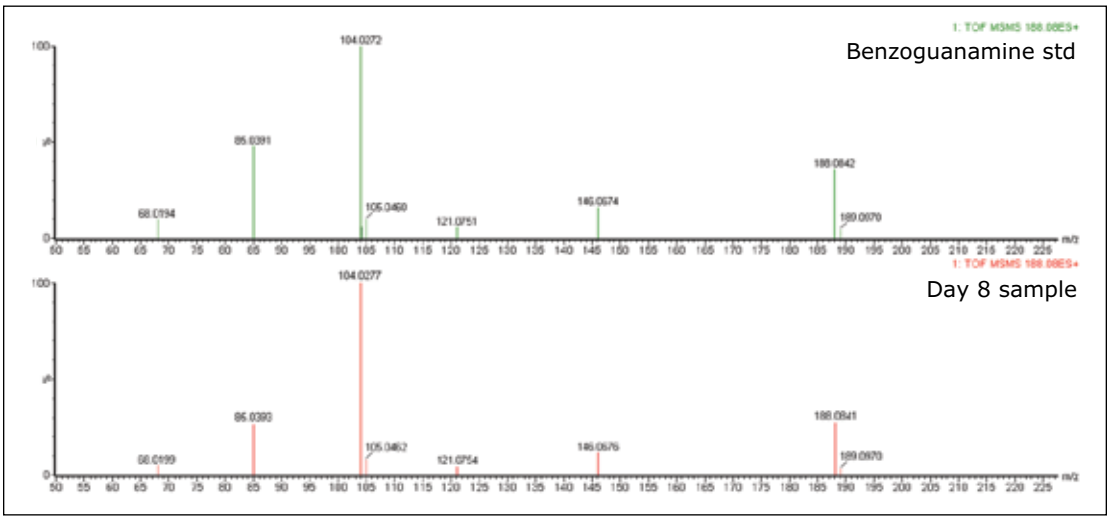


Figure 5.
MS/MS of Benzoguanamine standard (green) and Day 8 sample (red). Precursor ion selected was 188.08 m/z.

CONCLUSIONS

The experimental combination of ACQUITY UPLC, Xevo G2 QTof, and several data analysis software tools like MarkerLynx XS and MassFragment made possible the structural elucidation and identification of benzoguanamine from infant formula containers.

- The MS^E functionality of Xevo G2 QTof enabled the acquisition of both low energy precursor (MS) and high energy fragment ions (MS^E) in a single rapid screening run, for unequivocal compound identification.
- PCA and OPLS models were easily generated using MarkerLynx XS Software to identify differences between the different days.
- MS^E fragment ion data together with exact mass measurement provided added confidence and accuracy for structural elucidation.
- MassFragment Software aided structural elucidation by proposing and assigning fragmented structures to exact mass spectral data.
- MS/MS functionality of the Xevo G2 QTof, together with the use of commercial available standards, confirmed the identity of the compound as benzoguanamine.
- The same retention times achieved from both the sample and standard on the ACQUITY UPLC System provided added confidence in the identification of benzoguanamine.

References

1. Petersen H, Biereichel A, Burseg K, Simat TJ, Steinhart H. Food Addit Contam Part A Chem Anal Control Expo Risk Assess. Bisphenol A diglycidyl ether (BADGE) migrating from packaging material 'disappears' in food: reaction with food components. 25:911-920, 2008.
2. Cao XL, Dufresne G, Belisle S, Clement G, Falicki M, Beraldin F, Rulibikiye A. J Agric Food Chem. Levels of bisphenol A in canned liquid infant formula products in Canada and dietary intake estimates. 56:7919-7924, 2008.
3. Regulation (EC) No 1935/2004 of the European Parliament and Council of 27 October 2004 on materials and articles intended to come into contact with food and repealing Directives 80/590/EEC and 89/109/EEC. Official J Eur Union, L338 of 13.11.2004:4-17.
4. U.S. Food and Drug Administration (FDA). 21CFR175.300: Resinous and polymeric coatings (2002).

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

High Throughput Screening of Food Contact Materials

Malcolm Driffield[1], Antony Lloyd[1], Greg Noonan[2], and James Morphet[3]
[1]The Food and Environment Research Agency, Sand Hutton, York, UK, [2]Food & Drug Administration, 5100 Paint Branch Parkway, College Park, MD 20740 USA
[3]Waters Corporation, Manchester, UK

APPLICATION BENEFITS

The use of the ASAP probe can substantially reduce the time of analysis, producing qualitative results and identification of potential migrants with increased confidence when used in conjunction with high resolution MS detection techniques, such as time-of-flight (ToF) MS. The use of ToF-MS also allows full scan screening of the samples so potential migrants other than those specifically analyzed for may also be detected.

WATERS SOLUTIONS

Xevo® G2 QTof

ACQUITY UPLC® System

Atmospheric Pressure Solids Analysis Probe (ASAP)

INTRODUCTION

Most food and drink is packaged in some way. It is also highly likely that it comes into contact with other materials during harvesting, production, transport, storage, and cooking. A food contact material (FCM) is any material or article intended to be placed in contact with foodstuffs.[1] Food packaging materials are the most notable example, but also included are cutlery, dishes and plates, containers, parts of food processing equipment, etc.

When food comes into contact with a FCM there is the potential for migration of any of the chemicals from the material into the foodstuff. Depending on the chemical substance(s) involved, this can compromise the safety and/or the quality of the food, and so most countries have legislation in place to keep any chemical migration within acceptable limits. In Europe the EU Framework Regulation (EC) No. 1935/2004[2] provides general requirements for FCMs. Article 3 states that they should not endanger human health, bring about an unacceptable change in composition, or deteriorate any organoleptic characteristics.

Further to this framework regulation is more specific legislation. One example is the migration of primary aromatic amines (PAAs) which are regulated through the Plastics Directive 2002/72/EC[3], as amended, which states that:

- Plastic materials and articles shall not release primary aromatic amines in a detectable quantity (DL = 0.01 mg/kg of food or food simulant). The migration of the primary aromatic amines appearing in the lists in Annex II and III is excluded from this restriction.

Over the last couple of years there have been numerous notifications relating to the migration of PAAs from nylon kitchen utensils via the Rapid Alert System for Food and Feed[4] (RASFF). As concerns to human health grow regarding these FCMs, quicker and easier methods need to be developed to screen for compounds in the current legislation. This application note will detail the analysis of nylon kitchen utensils for PAAs and will show how the latest advances in mass spectrometer probe design help to achieve this goal.

EXPERIMENTAL

LC-MS conditions

LC-MS system:	ACQUITY UPLC with Xevo G2 QTof (used in Tof mode)
Ionization mode:	ASAP +
Corona current:	1.0 µA
Sample cone:	30 V
Source temperature:	120 °C
Desolvation gas:	Nitrogen, 800 L/Hr, 500 °C
Cone gas:	Nitrogen, 5 L/Hr

LockSpray™ conditions

Lock mass compound:	Leucine enkephalin, *m/z* 556.2771
Flow rate:	10 µL/min
Capillary voltage:	3 V
Collision energy:	6 eV

The samples tested were two black nylon kitchen utensils, a typical example is shown in Figure 1.

Figure 1. Example of a typical black nylon kitchen utensil.

Variables such as cone voltage, desolvation gas (nitrogen) temperature and corona pin current were optimized using solvent standards. Once the optimum settings were achieved the screening of the sample took a matter of minutes. The ASAP probe was used in the usual way; a new glass capillary was used for each sample removing sample carryover giving results that were more reliable by minimizing false positives.

The glass capillary was inserted into the source chamber at an elevated temperature for approximately one minute. This cleaned any contamination from the tip. The probe was then removed, cooled and the glass tip wiped backwards and forwards across the surface for 10 seconds. The mass spectrometer was set to an optimum desolvation gas temperature and the probe reinserted into the Xevo G2 QToF and the signal created recorded. This manual screening process was performed as quickly as 3 minutes per sample.

RESULTS AND DISCUSSION

Keeping a check on the migration of all the starting substances that may be used to make FCMs is a massive undertaking. This involves the chemical analysis of either the material itself or testing for migration of chemicals into foods or into model foods that are called food simulants. For this mass spectrometric methods and especially gas chromatography with mass spectrometric detection (GC-MS) and liquid chromatography with mass spectrometric detection (LC-MS) are widely used.

The use of the ASAP probe can substantially reduce the time of analysis, producing qualitative results and identification of potential migrants with increased confidence when used in conjunction with high resolution MS detection techniques, such as time-of-flight (ToF) MS. The use of ToF-MS also allows full scan screening of the samples so potential migrants other than those specifically analyzed for may also be detected.

Two different sampling techniques were tested to see which would achieve the better results. The ASAP probe was wiped across the surface of the kitchen utensils and then inserted into the MS. A fine powder was also prepared from the sample using sandpaper and the probe rubbed in this powder before insertion in to the MS. The strongest signal was seen for the powder approach, and the results for the two samples are shown in Figure 2.

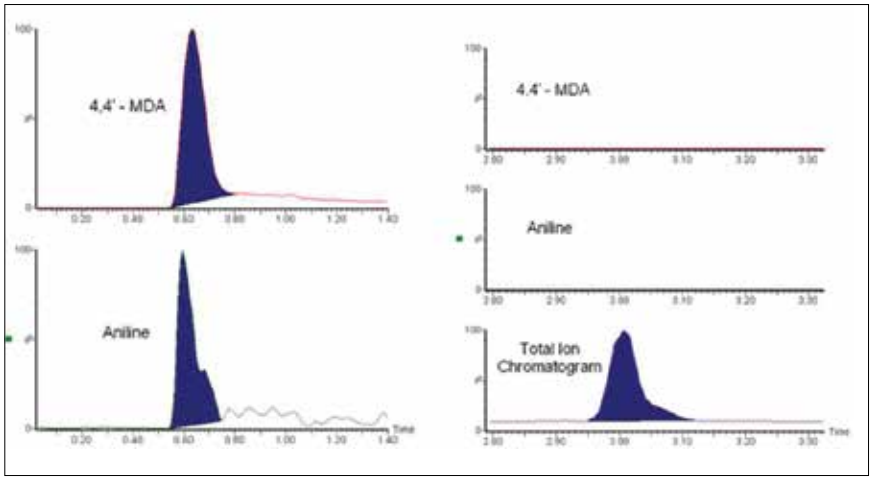


Figure 2. Extracted ion traces for aniline and 4,4'-metyhlenedianiline (4,4'-MDA) from the ASAP-ToF MS analysis of sample A (left) and sample B (right).

Sample A was found to contain levels of aniline and 4,4'-MDA ($[M+H]^+$ adduct seen in both cases). PAAs were not detected in sample B. The total ion chromatogram gives the location of the peak on the trace, showing that the compounds are not present. These were the only compounds to give a positive result for these samples.

A high degree of confidence was achieved with the identification of these compounds. All of the spectra across the 4,4'-MDA peak were assessed with respect to mass accuracy of the system. Figure 3 shows the spectrum acquired at the apex of the peak (spectrum 11), the total mass accuracy across the peak is shown in Table 1.

Having identified sample A as a potential positive, it clearly merits being subjected to migration testing using food simulants to see if it complies or not with migration limits for the PAAs identified.

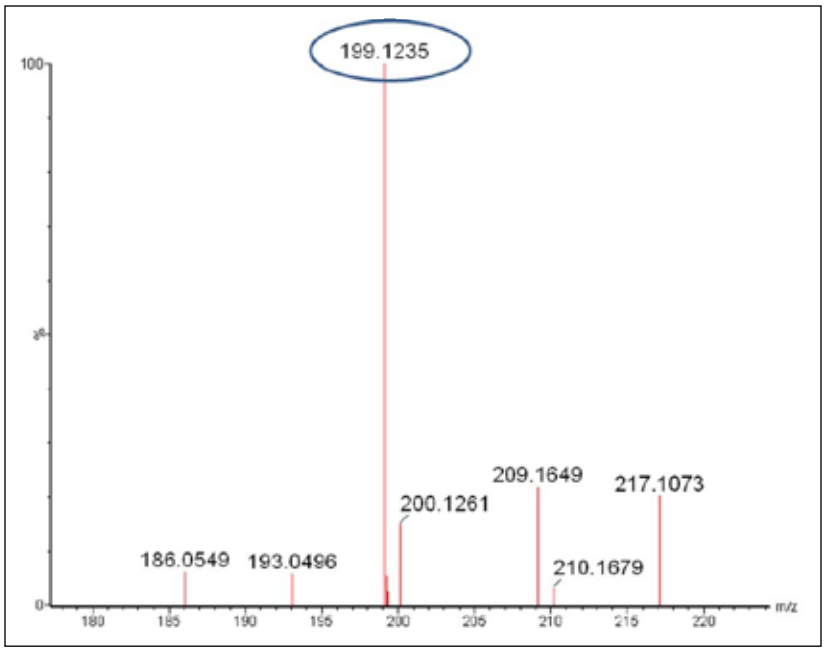


Figure 3. Spectra of 4,4'-metyhlenedianiline, m/z 199.1235.

Spectrum number	Exact mass	mDa error	Spectrum number	Exact mass	mDa error
1	199.1231	0.4	12	199.1238	0.3
2	199.1236	0.1	13	199.1237	0.2
3	199.1236	0.1	14	199.1237	0.2
4	199.1235	0.0	15	199.1236	0.1
5	199.1235	0.0	16	199.1236	0.1
6	199.1236	0.1	17	199.1236	0.1
7	199.1235	0.0	18	199.1235	0.0
8	199.1237	0.2	19	199.1238	0.3
9	199.1236	0.1	20	199.1236	0.1
10	199.1237	0.2	21	199.1238	0.3
11	199.1235	0.0	22	199.1235	0.0
				Mean mDa error	0.1
				Mean PPM error	0.7

Table 1. The mean mass accuracy of the 22 data points is 0.7 ppm for the 4,4'-MDA $[M+H]^+$ ion, m/z 199.1235.

This data was acquired using a Xevo G2 QToF in ToF mode. Further analysis of the data after it has been acquired is possible. In this example, the aim of the experiment was to look for PAAs, but examination of the ToF data revealed other potential migrants that were identified. Post acquisition interrogation of this sort would not be possible if a quadrupole MS system was used for the analysis that only acquired the data in SIR or MRM modes.

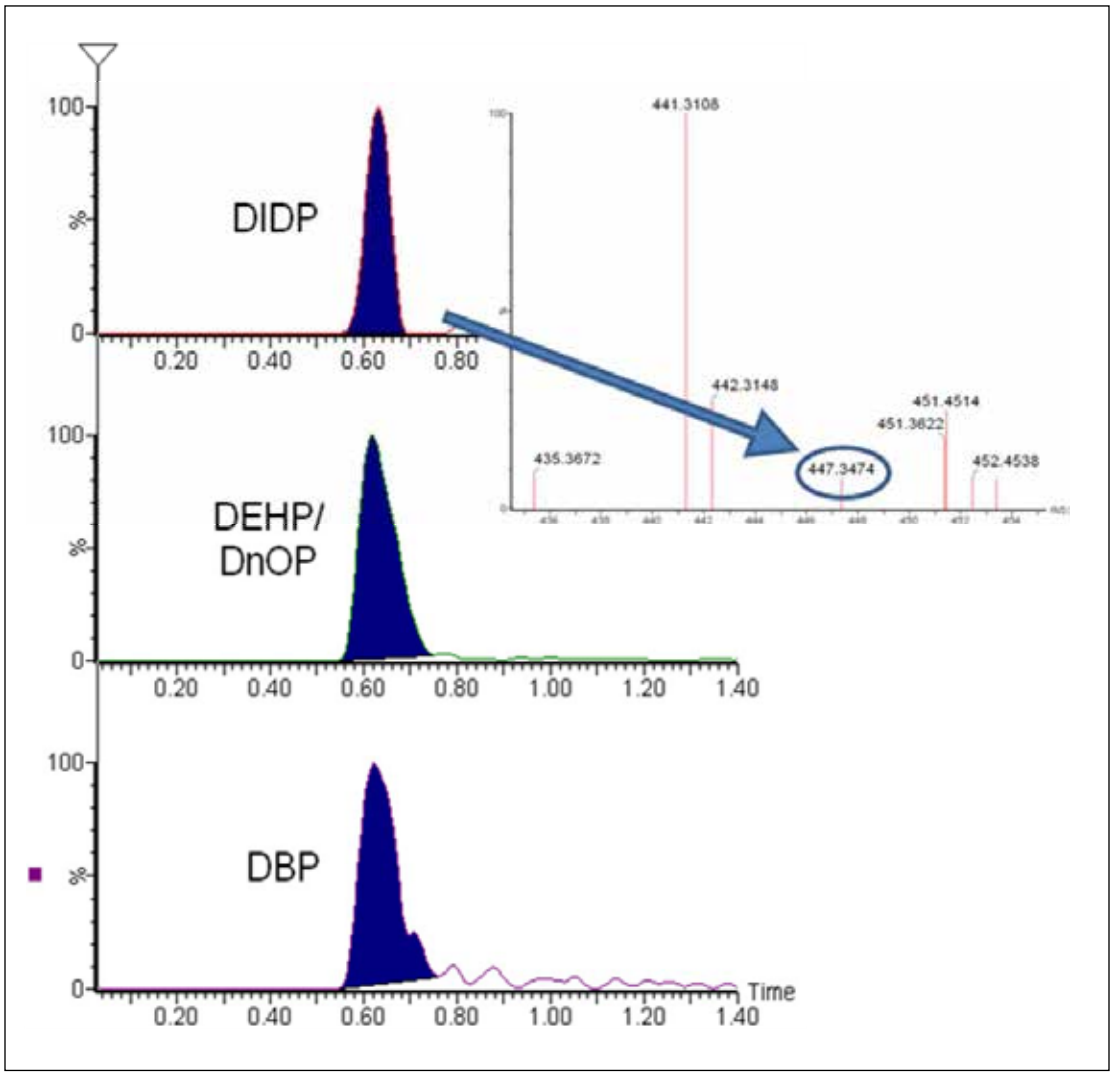


Figure 4. Further analysis of Sample A reveals that Di-n-butyl phthalate (DBP), Di-(2-ethylhexyl) phthalate (DEHP), Di-n-octylphthalate (DnOP), and/or Di-isodecyl phthalate (DIDP) are also present. The mass accuracy of the Xevo G2 QToF does not show any error, even when many compounds are being ionized at the same time.

The presence of some common phthalates in sample A is shown in Figure 4. A chromatographic separation is needed to allow quantification of the isobaric DEHP and DnOP. As phthalates are ubiquitous in the environment the presence of phthalates may be due to contamination of the nylon sample. Further abrasion and testing would prove the origin.

CONCLUSIONS

- Using the Xevo G2 QTof, in ToF mode, with an ASAP probe is a fast and easy method to screen for potential migrants from food contact materials.
- Sample preparation times for this approach can be less than 3 min per sample, allowing increased throughput and revenues to be maximized.
- Xevo G2 QTof allows for interrogation of data for compounds that were not on the original screening list when the analysis occurred.
- Xevo G2 QTof raises the level of confidence in results with excellent mass accuracy.

References

1. http://www.foodcontactmaterials.com/
2. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:338:0004:0017:en:pdf
3. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0072:20091109:en:pdf
4. http://ec.europa.eu/food/food/ rapidalert/index_en.htm

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

The Identification and Structural Elucidation of Potential Migrants from Paper and Board Food Packaging Using UPLC/Q-Tof MS with MS^E and MassFragment

Malcolm Driffield,[1] Antony Lloyd,[1] Emma Bradley,[1] Dominic Roberts[2]
[1]The Food and Environment Research Agency, York, UK
[2]Waters Corporation, Manchester, UK

APPLICATION BENEFITS

- MS^E data acquisition allows for the simultaneous collection of both low energy precursor ion and higher energy fragment ion data from a single injection for greater confidence in compound identification, and provides comprehensive historical data review.
- ChromaLynx™ XS Software provides rapid detection, identification, and confirmation of all components in complex mixtures. It allows the user to determine chemical formulae from accurate mass information, searching a user-prepared database of compounds.
- MassFragment™ is an intelligent software tool that automates structural assignment to fragment ion spectra making data processing significantly easier, and confirmation without standards possible.

WATERS SOLUTIONS

ACQUITY UPLC® System

ACQUITY UPLC HSS T3 Column

SYNAPT® G2 HDMS® System

ChromaLynx XS Software

MassFragment Software

KEY WORDS

TOF screening, database searching, structural elucidation, paper, board, food packaging, phthalate

INTRODUCTION

Recycling paper and board has clear benefits to the environment, relieving pressures on forestry resources and reducing the amount of waste disposal. Currently, there is limited control over the types of paper and board entering the recycling stream. End use of the recycled paper and board ranges from less demanding applications, such as newspapers and magazines, to cardboard boxes and cartons, and more demanding applications, such as food packaging.

In recent years, there have been issues reported in scientific literature and in the media relating to the use of recycled paper and board in food packaging. Contaminants associated with recycled paper and board have been detected in food. Mineral hydrocarbons have been found from inks used to print newspapers and magazines,[1-2] as well as phthalates, such as diisobutyl phthalate from adhesives in catalogues and brochures,[3] and photoinitiators and other components from printing on the external surface of the paper and board.[4] All of these chemical types have been shown to persist after passing through the recycling process.

This study is part of a larger project investigating suitable sources of paper and board for use in recycled food packaging.[5] Four different paper sources (plain white printer paper, newspapers and magazines, corrugated cardboard, and food packaging) have been examined and potential contaminants identified. UltraPerformance LC® with high resolution mass spectrometric detection (UPLC®/HR-MS) has been shown to be a useful tool to aid with identification of unknown compounds in the area of food contact materials and beyond.[6] The accurate mass, isotope patterns, and fragmentation information (if present) allow predictions of elemental composition which can be compared to a database of potential structures, if one is available, adding confidence to the identification process. The instrument used must be sufficiently sensitive and accurate to ensure that compounds are confidently identified.

The use of the ACQUITY UPLC System combined with the SYNAPT G2 HDMS and associated software to detect chromatographic peaks, determine accurate mass, and produce elemental composition is described here. A comparison with a user-prepared database containing over 6000 food contact material ingredients and contaminants is described, and, as an example, one of the proposed chemical structures is confirmed using fragmentation information acquired by MS^E without the need for authentic standards.

EXPERIMENTAL

Sample description

A selection of foodstuffs in paper and board packaging was purchased from a local supermarket. The food was removed from the packaging, cut into small pieces, and mixed well. The samples included breakfast cereals, pasta, frozen fish, cakes, and other baked products.

A portion of the mixed sample (5 g), d_{10}-benzophenone (100 µL of 1 mg/mL) to act as internal standard and ethanol (20 mL) was added to a vial, capped, and shaken overnight. A portion of the ethanol was removed and directly analyzed.

UPLC conditions

System:	ACQUITY UPLC
Column:	ACQUITY UPLC HSS T3 (Part No. 176001133) 1.8 µm, 150 x 2.1 mm
Column temp.:	45 °C
Flow rate:	0.45 mL/min
Injection volume:	1 µL
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid

Gradient:

Time (min)	%A	%B
0.0	90	10
15.0	0	100
18.0	0	100
18.1	90	10
20.0	90	10

MS conditions

MS system:	SYNAPT G2 HDMS
Acquisition mode:	Resolution mode, MS^E
Ionization mode:	Electrospray positive
Mass range measured:	50 to 1200 Da
Cone voltage:	25 V
Capillary voltage:	1.0 kV
Desolvation temp.:	500 °C
Source temp.:	120 °C
Collision energy:	Function 1 CE = 6 eV, Function 2 CE = 15 - 35 eV
Collision gas:	Argon
Lock mass:	Leucine enkephalin, *m/z* 566.2771
Data management:	ChromaLynx XS and MassFragment software

RESULTS AND DISCUSSION

The base peak ion chromatogram (BPI) for the ethanol extract of the pooled food packaging sample is shown in Figure 1.

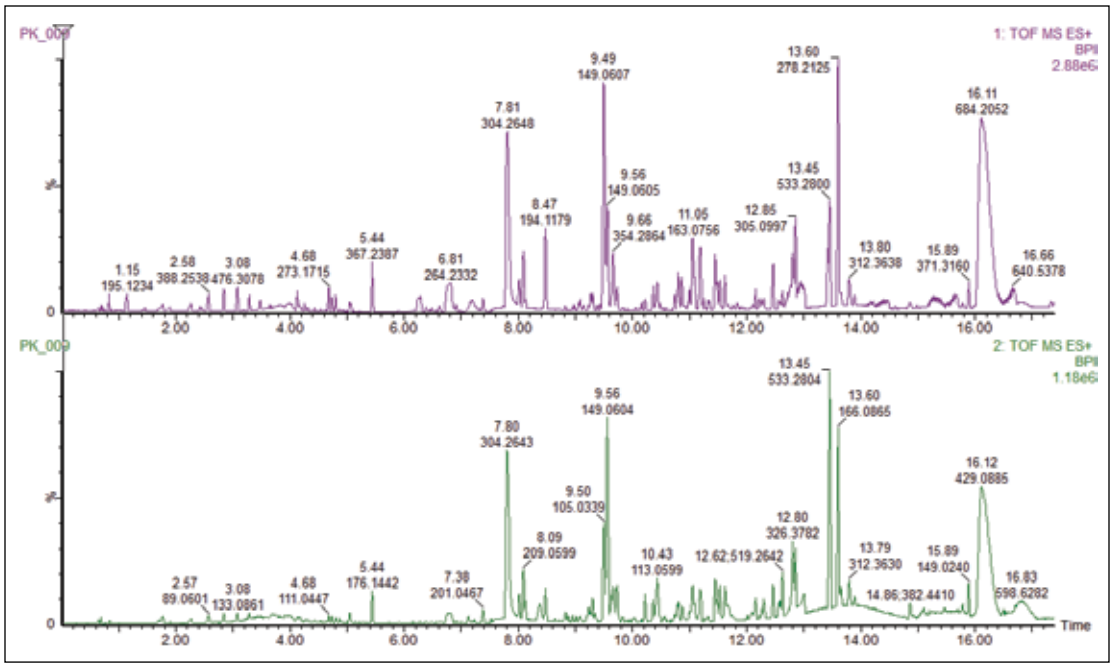


Figure 1. Base ion chromatogram (low energy positive electrospray ionisation mode) of paper and board food packaging ethanol extract.

ChromaLynx XS Software deconvolutes chromatograms, detects all chromatographic components present, and produces refined spectra for each identified component. These were processed in the 'targeted mode' producing a list of individual peaks that were then compared to a database containing potential structures. The software extracted 1380 individual components, many more than were evident from the TIC, highlighting the power of the software to detect components present at very low concentrations. ChromaLynx XS extracts the exact mass chromatograms of the targeted compounds and confirms their presence or absence.

The user-prepared database contains over 6000 known ingredients, potential contaminants, and reaction and breakdown products in food contact materials. The list contains the compound name and chemical formula that the software will search and report positive hits. Retention time and fragment ion information can also be included in the database, if authentic standards are available and have been analyzed. Figure 2 shows an example of the ChromaLynx XS output including: (A) the TIC, (B) the target list, (C) in particular the extracted ion chromatogram, and (D) associated mass spectrum for the peak at 13.6 minutes, as an example of the completed identification process. Of the 6000 compounds screened in this sample, a total of 45 were identified based on accurate mass. In the absence of analytical standards, these identifications can be supported using the simultaneously acquired fragment information.

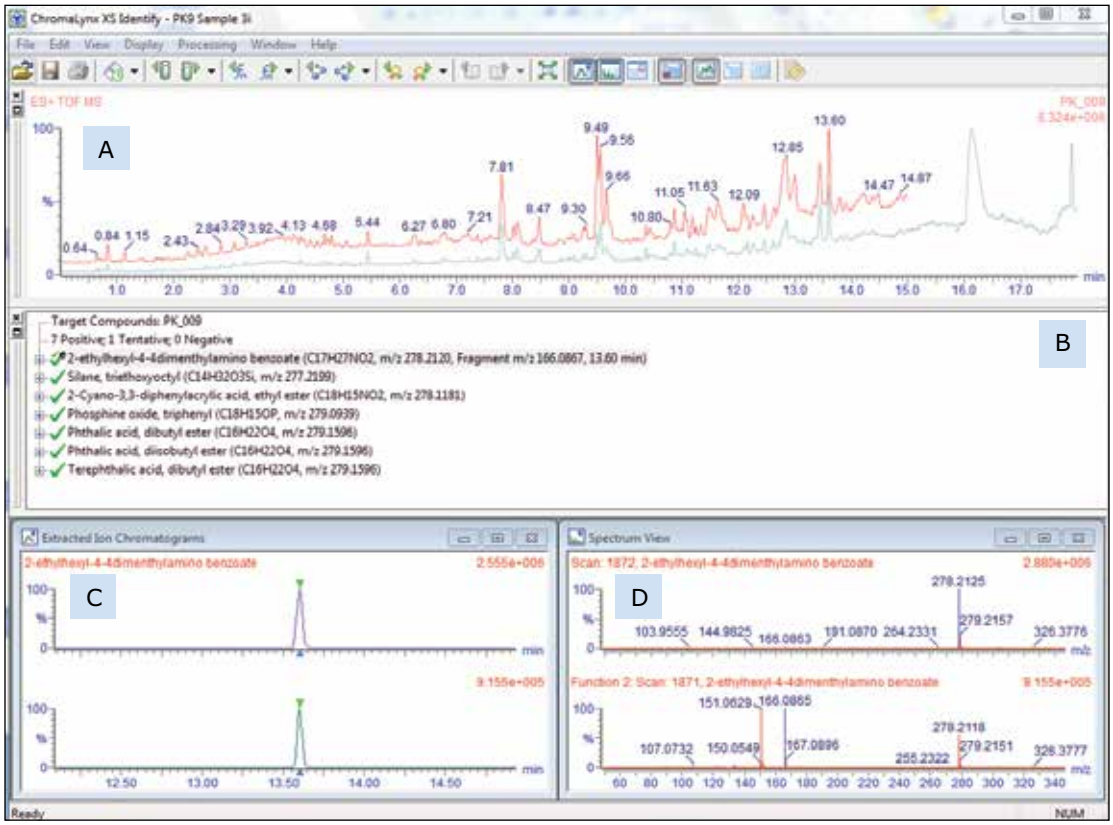


Figure 2. ChromaLynx XS output for the peak at 13.6 minutes corresponding to a database hit for 2-ethylhexyl-4-(dimethylamino) benzoate. This shows A) total ion chromatogram, B) target list, C) extracted ion chromatogram (m/z 278.2122) for the peak at 13.6 minutes, and D) mass spectrum (low energy) of the peak at 13.6 minutes.

Figure 3 shows the molecular species, $[M+H]^+$ at *m/z* 278.2122 which produced a chemical formula of $C_{17}H_{27}NO_2$. This gave a database hit of 2-ethylhexyl-4-(dimethylamino)benzoate, which is used as an amine co-initiator in UV-cured inks applied to paper and board substrates. The formula for the $[M+H]^+$ has a theoretical accurate mass of *m/z* 278.2120, differing by only 0.7 ppm from that measured. No authentic standard of 2-ethylhexyl-4-(dimethylamino)benzoate was analyzed at the same time as the food packaging sample to confirm identification. The SYNAPT G2 HDMS, however, was run in MS^E acquisition mode. This allows for the simultaneous collection of both low energy precursor ion ($[M+H]^+$ in this example) and higher energy fragment ion data from a single injection for greater confidence in compound identification.

Figure 3 shows the low and high energy mass spectra with the molecular adduct reducing in intensity at the higher energy, and fragment ions being formed.

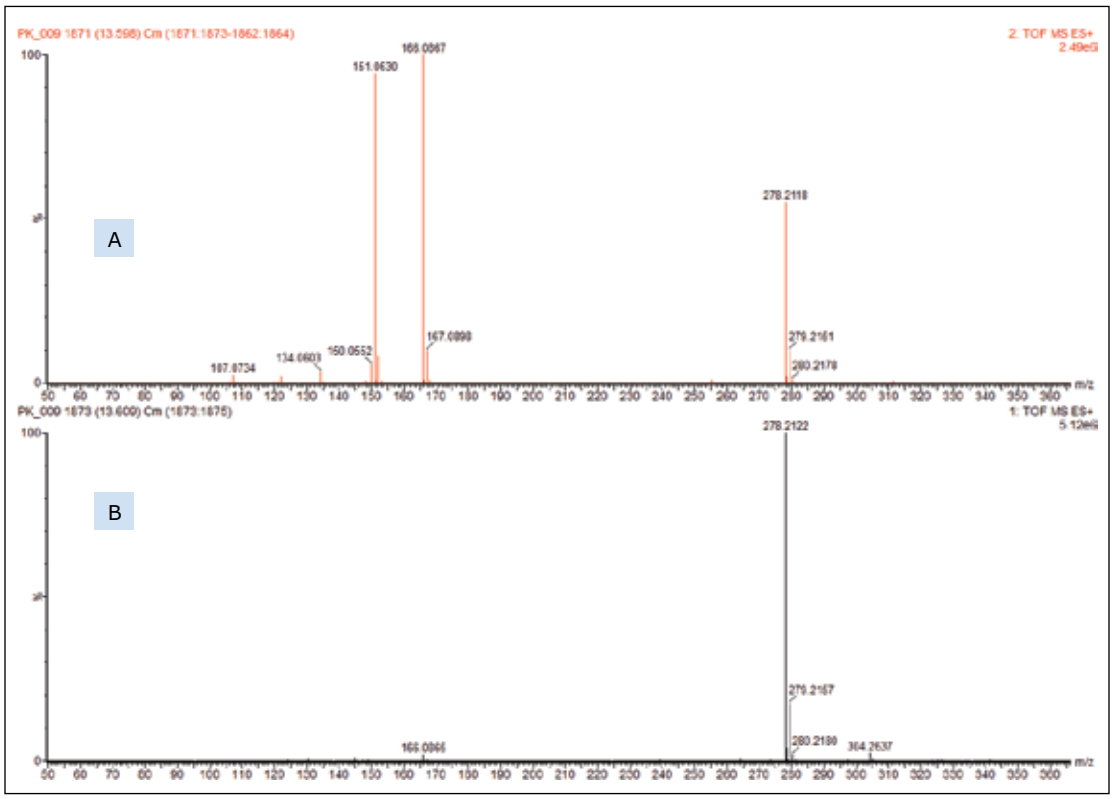


Figure 3. Mass spectra for the peak at 13.6 minutes. A) MS^E high energy showing fragment ions, B) MS^E low energy showing molecular adduct, $[M+H]^+$.

Like the molecular species, the accurate mass of the fragment ions can be used to determine potential elemental compositions. These were used in the MassFragment Software to determine likely structures based on the chemical structure of the proposed compound, for example 2-ethylhexyl-4-(dimethylamino)benzoate. The software utilizes systematic bond disconnections and a scoring system dependent on the types of bonds disconnected and the likelihood that this would happen. Inputting information into the program is simple. A .mol file can either be downloaded from ChemSpider online database or be prepared from most common chemical drawing packages, then imported along with the MS^E mass spectrum which provides the fragment ions.

The parameters can be adapted depending on the specific needs of the user. The mass window allowance is particularly important with the smaller the range used, the more confidence given to the structural assignment. In this example, a value of +/- 1 mDa was applied. Figure 4 gives the results generated by the software for the peak at 13.6 minutes, proposed to be 2-ethylhexyl-4-(dimethylamino)benzoate.

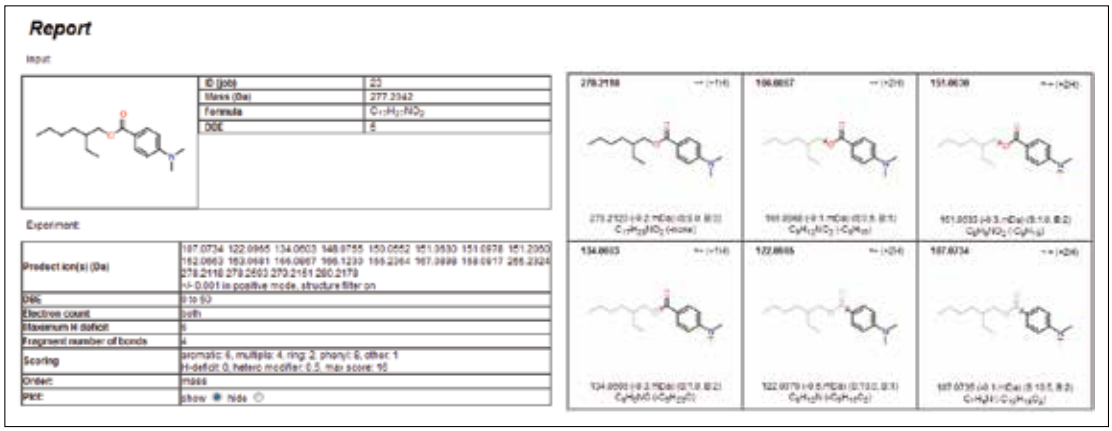


Figure 4. MassFragment output report showing five fragment ions have been assigned to the proposed structure, adding confidence to the identification.

Each of the five fragment ions measured demonstrates that plausible structures have been suggested based on breaking various bonds in the proposed precursor 2-ethylhexyl-4-(dimethylamino)benzoate, increasing confidence to the assignment of this identity to the peak at 13.6 minutes. Figure 5 shows the MS^E spectra with annotated MassFragment structures. This compound is most likely derived from the ink applied to the paper and board,[7] but compounds of a similar chemical type have been shown to be persistent after the recycling process. Now that the fragments and retention time have been assigned to this compound, they can be fed back into the database for greater confidence in future identifications.

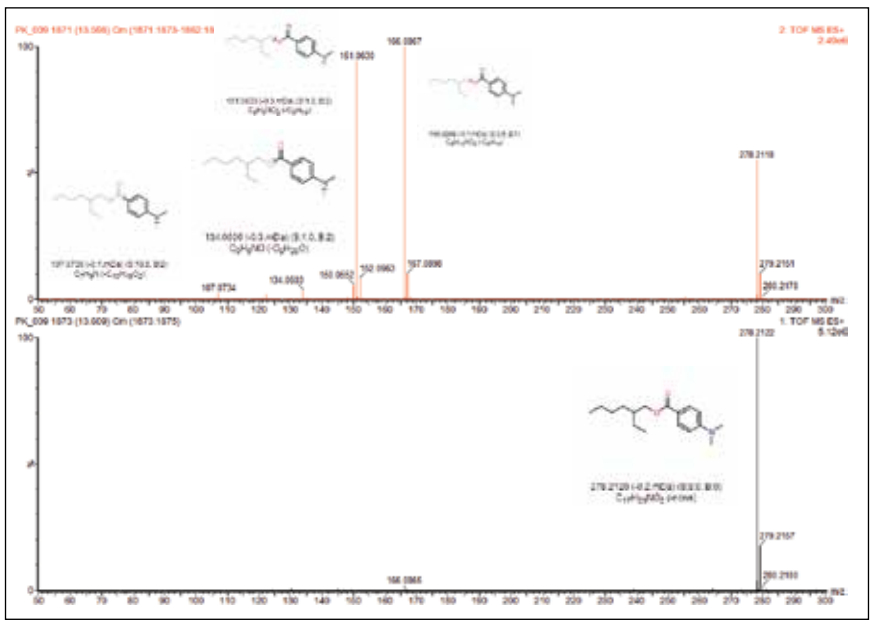


Figure 5. MS^E mass spectra for the peak at 13.6 minutes with MassFragment identifications annotated.

CONCLUSIONS

The chromatographic separation, high resolution, and accurate mass capabilities of the ACQUITY UPLC/SYNAPT G2 HDMS System have been used to perform analysis of paper and board food packaging extracts. This enabled confident identification of previously unknown compounds with the potential to migrate into foodstuffs. Both molecular species and fragment ion information collected using MS^E acquisition were processed with ChromaLynx XS and MassFragment software, resulting in high levels of confidence in the resulting identifications.

References

1. Dima G, Verzera A , Grob K. Migration of mineral oil from party plates of recycled paperboard into foods: 1. Is recycled paperboard fit for the purpose? 2. Adequate testing procedure. *Food Additives and Contaminants Part A.* 2011; 28(11): 1619-1628.
2. Vollmer A, Biedermann M, Grundbock F, Ingenhoff JE, Biedermann-Brem S, Altkofer W, Grob K. *European Food Research and Technology.* 2011; 232:175-182.
3. Gartner S, Balski M, Koch M, Nehls I. Analysis and migration of phthalates in infant food packed in recycled paperboard. *Journal of Agricultural and Food Chemistry.* 2009; 57(22): 10675-10681.
4. Koivikko R, Pastorelli S, deQuiros ARB, Paseiro-Cerrato R, Paseiro-Losada P, Simoneau C. *Food Additives and Contaminants Part A.* 2010; 27(10): 1478-1486.
5. Driffield M, Lloyd AS, Lister L, Leak J, Speck D, Bradley EL. Manuscript in preparation. 2013.
6. Driffield M, Bradley EL, Castle L, Coulier L. Identification of unknown migrants from food contact materials. *Mass Spectrometry in Food Safety, Methods and Protocols.* 2011; 357-372.
7. Food Standards Agency (2011) Food Survey Information Sheet 03/11. Migration of selected ink components from printed packaging materials into foodstuffs and screening of printed packaging for the presence of mineral oils.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com

[NOTES]

Sales Offices:

Australia 61 2 9933 1777

Austria 43 1 877 18 07

Belgium and Luxembourg 32 2 726 1000

Brazil 55 11 4134 3788

Canada 1 800 252 4752

China 86 21 6156 2666

Czech Republic 420 2 617 11384

Denmark 45 46 59 8000

Finland 358 9 5659 6288

France 33 1 30 48 72 00

Germany 49 6196 400 600

Hong Kong 852 2964 1800

Hungary 36 1 350 5086

India 91 080 49292200 03

Ireland 353 1 448 1500

Israel 9723 3731391

Italy 39 02 265 0983

Japan 81 3 3471 7191

Korea 82 2 6300 9200

Malaysia 603 7841 1688

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

Singapore 65 6593 7100

Spain 34 93 600 9300

Sweden 46 8 555 115 00

Switzerland 41 56 676 7000

Taiwan 886 2 2508 5500

UAE 971 4 214 62 38

UK 44 208 238 6100

US 1 800 252 4752

For your local sales office, please visit www.waters.com/contact

www.waters.com/extractables

Waters

THE SCIENCE OF WHAT'S POSSIBLE.™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com