[APPLICATION NOTE]

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

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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 C_{18} Column, 130 Å, 3.5 µm, 4.6 mm × 150 mm

X-Bridge BEH C_{18} 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: X-Bridge 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

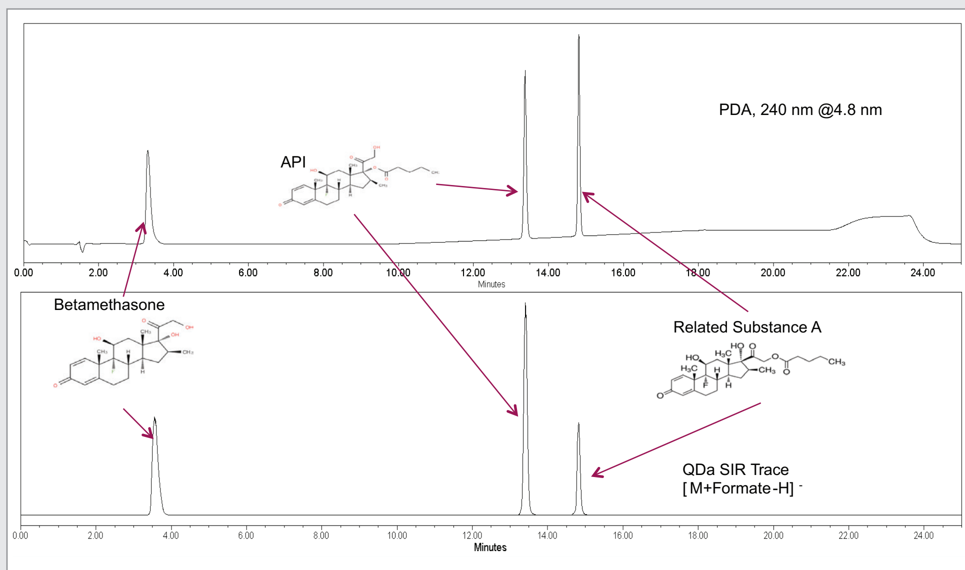

Figure 1. PDA chromatogram (top) and ACQUITY QDa SIR chromatogram (bottom) of solvent standards for betamethasone, betamethasone valerate, and Related Substance A.

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.

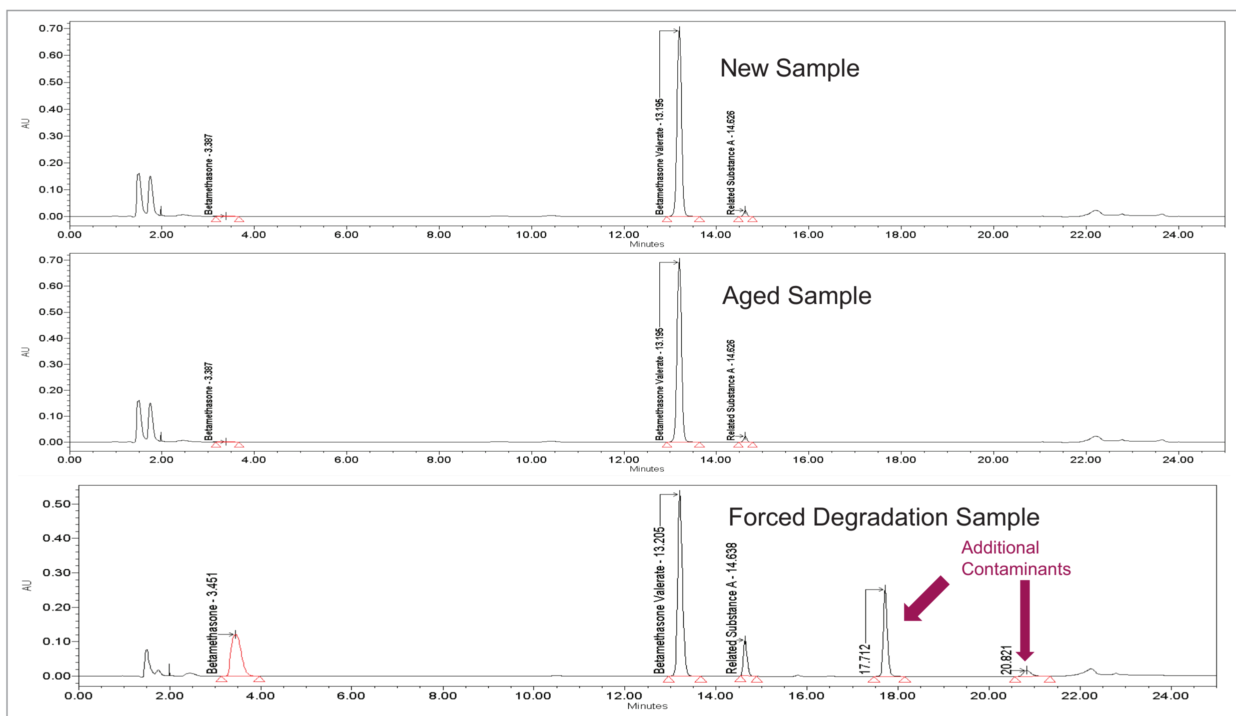

Figure 2. PDA chromatograms of the New Sample BMV Scalp application (top), Aged Sample (middle), and Forced Degradation Sample (bottom).

New sample

Peak Results

	Name	RT	Area	Height	Amount	Units	% Impurity
1	Betamethasone	3.440					
2	Betamethasone Valerate	13.291	4569675	255824	0.02920	ug/mL>	
3	Related Substance A	14.692	63544	6200	0.00030	ug /mL>	1.0
4		21.166	81859	7629			

Aged Sample

Peak Results

	Name	RT	Area	Height	Amount	Units	% Impurity
1	Betamethasone	3.440					
2	Betamethasone Valerate	13.269	4971263	283740	0.03177	ug/mL>	
3	Related Substance A	14.674	131583	13230	0.00062	ug /mL>	1.9
4		21.147	80501	7229			

Forced Degradation Sample

Peak Results

	Name	RT	Area	Height	Amount	Units	% Impurity
1	Betamethasone	3.479	1814046	102618	0.01053	ug/mL>	43.7
2	Betamethasone Valerate	13.246	3766222	472458	0.02407	ug/mL>	
3	Related Substance A	14.667	557653	100910	0.00262	ug /mL>	10.9
4		17.250	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.

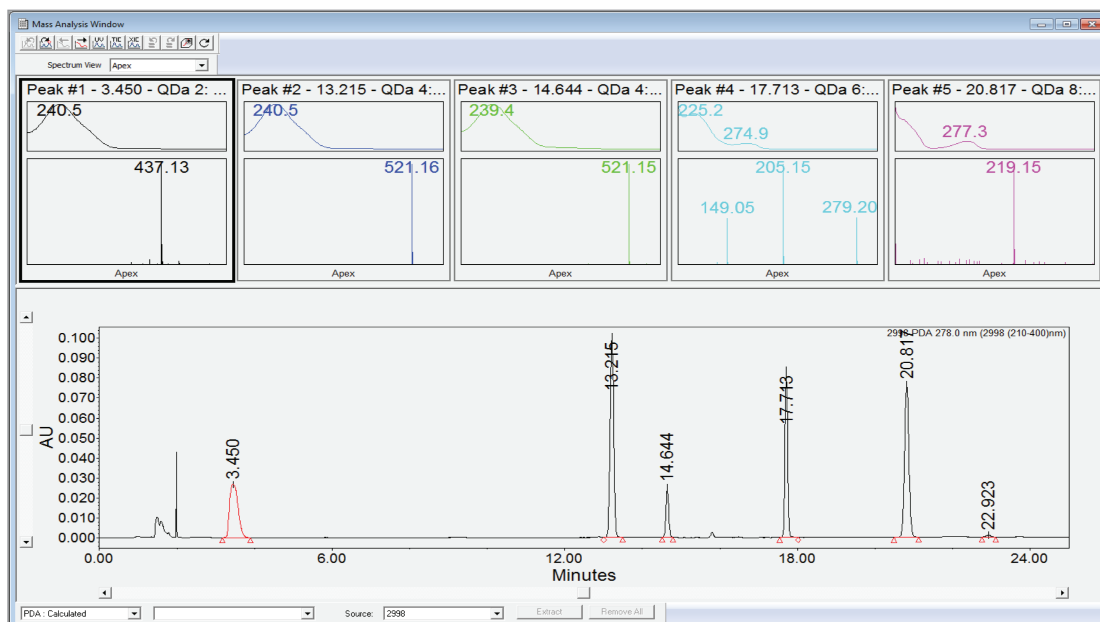

Figure 4. Empower 3 Software Mass Analysis window for the Forced Degradation sample showing UV spectra and MS scan data for peaks 1–5 in the chromatogram.

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.

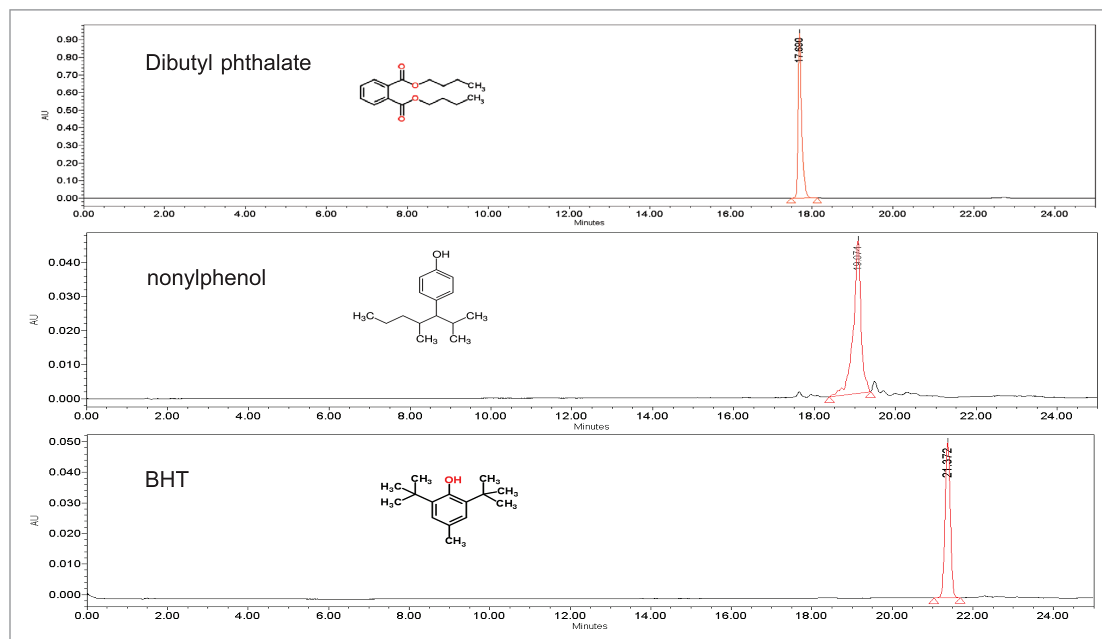

Figure 5. UV Chromatograms of solvent standards of dibutyl phthalate (top), nonylphenol (middle), and butylated hydroxyl toluene (BHT) (bottom).

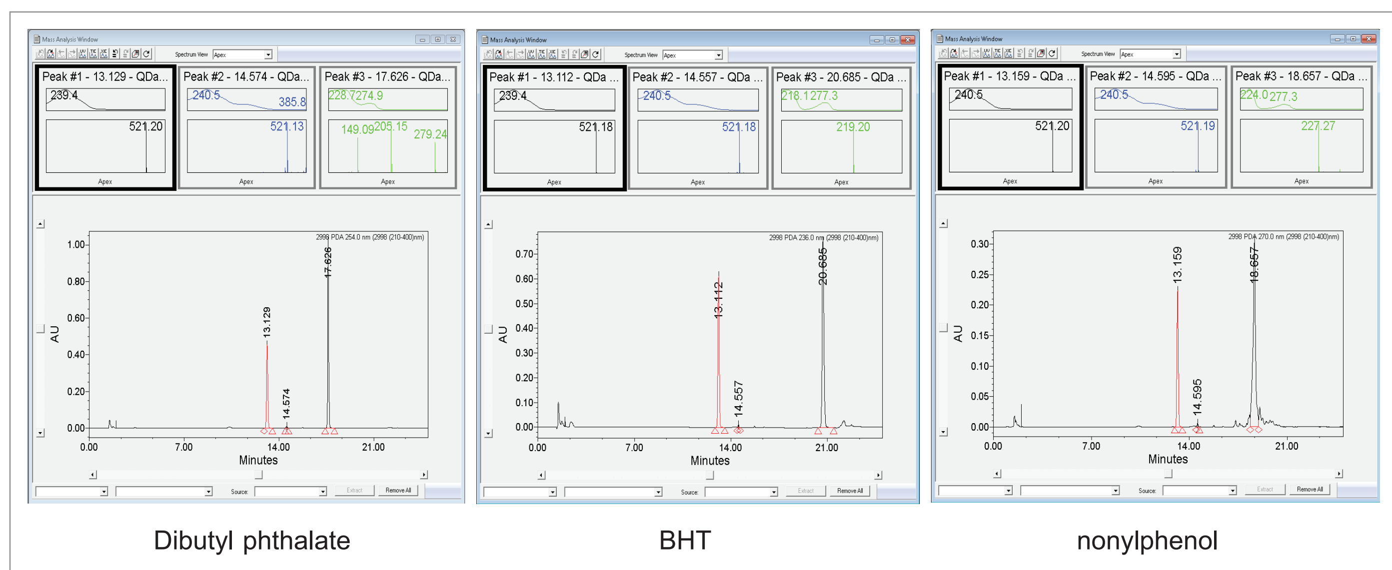

Figure 6. Empower Mass Analysis window summaries of matrix samples spiked with dibutyl phthalate (left), BHT (middle), and nonylphenol (right).

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).

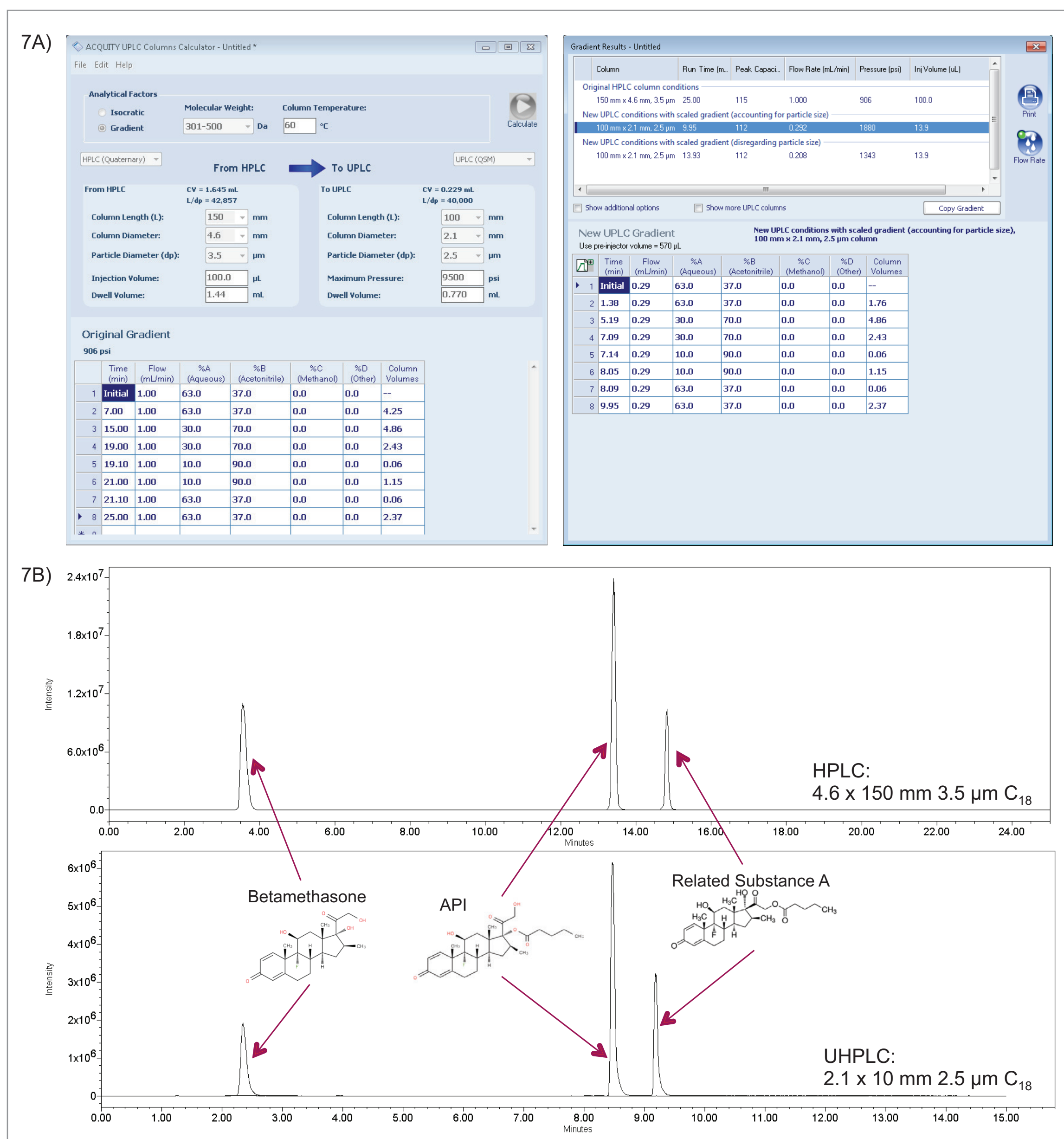

Figure 7A. Automated gradient scaling with the ACQUITY Column Calculator; 7B. Comparison of HPLC and UHPLC chromatograms.

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.

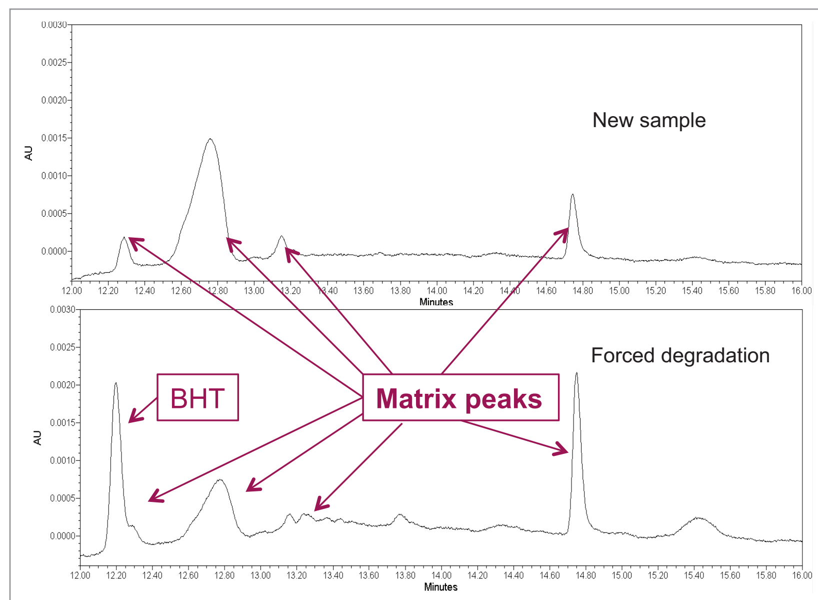

Figure 8. UV Chromatograms of the New Formulation sample (top) and Forced Degradation sample (bottom) showing matrix features common to both samples.

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.

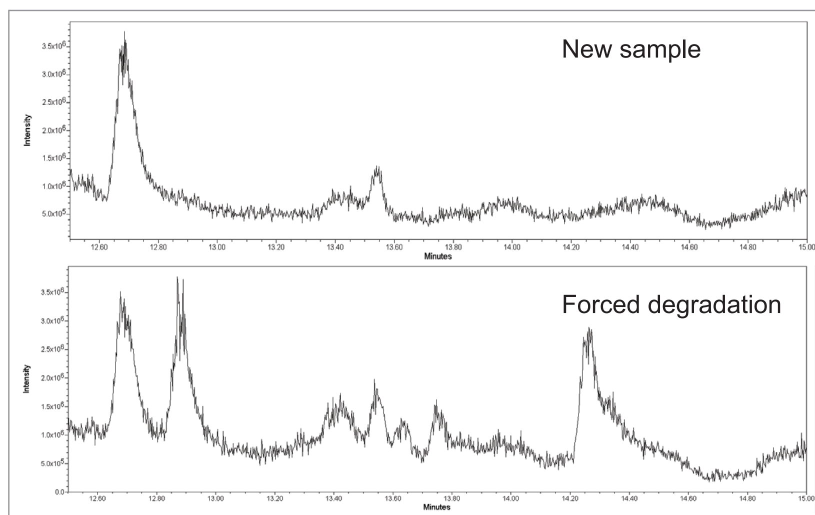

Figure 9. Comparison of the TIC (positive scan mode) for the New Formulation and Forced Degradation samples.

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.

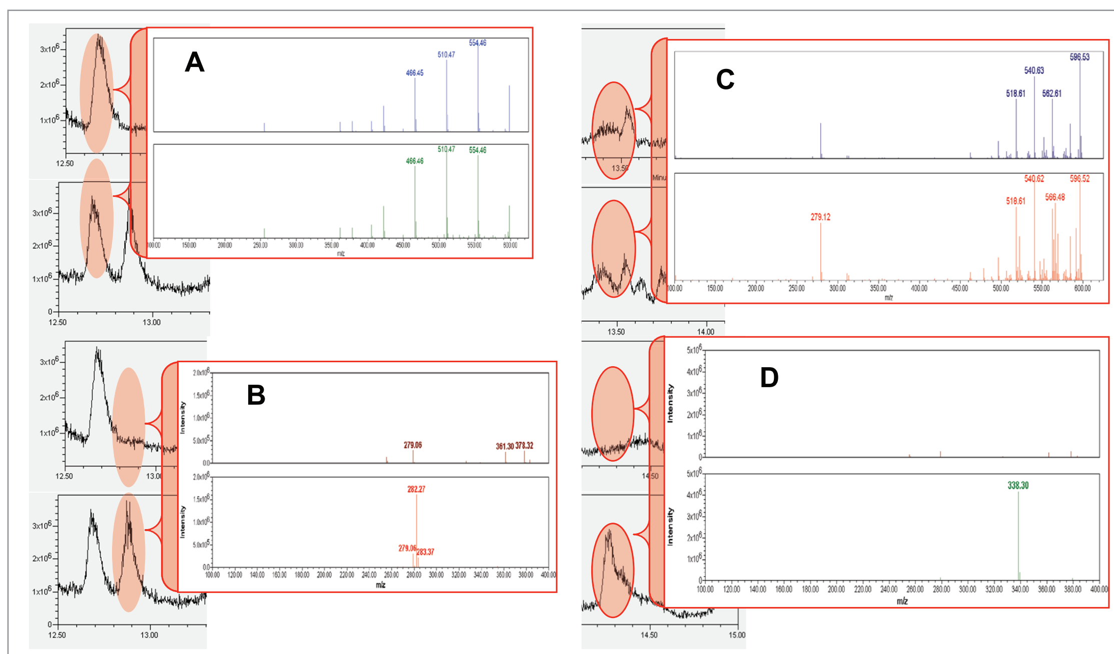

Figure 10. Probing the mass spectral data for differences between the New Formulation and Forced Degradation samples.

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.

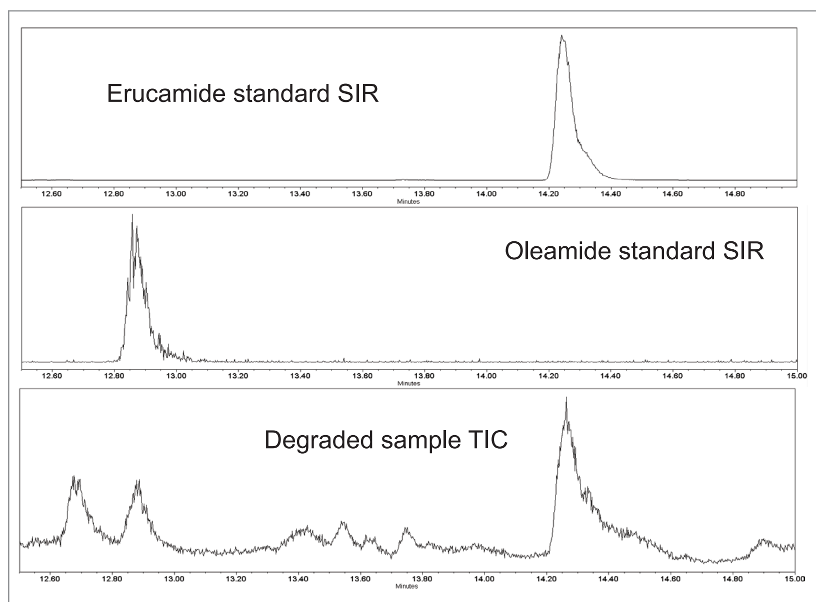

Figure 11. Comparison of erucamide and oleamide standards with degraded sample TIC.

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.

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