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A UHPLC-DAD-Q-TOF MS Method for Detection and Accurate Identification of Extractable and Leachable Compounds

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Introduction

Drug substances and products can get contaminated by chemicals migrating from container closure systems. Contamination can occur during the entire life cycle of the pharmaceutical compound. To assess the presence of such impurities, especially for certain drug classes such as OINDP or to evaluate the materials used for the construction of container closure systems several different analytical techniques are used.

LC with UV an/or MS is effective for the analysis of non-volatile impurities. In the present study, an Agilent 1290 UHPLC with a 6530 QTOF in series with a DAD was used. The data analysis process included library searching and formula generation.

	Analyte	CAS	Empirical Formula	Monoisotopic Mass
1.	4-Hydroxy benzoic acid ethyl ester	120-47-8	C9H10O3	166.062988
2.	Irgacure 184	947-19-3	C13H16O2	204.115036
3.	Benzophenone	119-61-9	C13H10O	182.073166
4.	Irgacure 651	24650-42-8	C16H16O3	256.109955
5.	Dipropyl phthalate	131-16-8	C14H18O4	250.120514
6.	Diethyl hexyl phthalate	117-81-7	C24H38O4	390.277008
7.	Irganox 1010	6683-19-8	C73H108O12	1176.784058
8.	Irganox 1076	2028-79-3	C35H62O3	530.469910
9.	Iragafos 168	31570-04-4	C42H63O3P	646.451477
10.	4-n-Octyl phenol	1806-26-4	C14H22O	206.167068
11.	Diphenyl butadiene	538-81-8	C16H14	206.109543

Preparation of Stock solution and Empty Ophthalmic bottle extraction

A 1000 ppm stock solution of each analyte was prepared by dissolving the standards in IPA. Stock solutions were further diluted in water to prepare a mix containing all the 11 analytes. The mix was further diluted to get a solution of working standards containing 1 ppm each of dipropyl phthalate, diethyl hexyl phthalate and Irgacure 651, 2 ppm of Irganox 1010, Irganox 1076, Irgafos 168, 4-Ethyl benzoate, 4-n-octyl phenol, and DPBD and 4 ppm each of Benzophenone, Irgacure 184. An empty ophthalmic medicine bottle was washed with water and filled with 1:1 Methanol:Water. It was oven incubated at 55 °C for 3 days. An aliquot (200 µL) was taken for analysis. 10 µL of the stock solution of E and L mix was added to another 190 µL aliquot to get a spiked sample.

Experimental

LC conditions

Mobile phase A: 0.01% Ammonium acetate
Mobile phase B: 100% Methanol

Flow rate 0.5 mL/min

Gradient: 40 % B initially; ramp up to 100%B over 8 minutes; hold at 100% B for 3 minutes; bring to 40%B in 0.1 min; stop data collection at 11.0 min and hold for a post time of 1.5 min

Column: Agilent ZORBAX RRHD Eclipse Plus – C8 3.0 X 100 mm, 1.8 µm (P.N. 959758-306); Column temperature: 50 °C

Injection volume: 5 µL; Autosampler temperature: 6 °C;

MS conditions

Ionization mode: Dual Spray AJS-ESI

Drying Gas: 10 L/min @ 150 °C

Nebulizer pressure: 30 Psi

Sheath gas: 11 L/min @ 200 °C,

Capillary voltage: 3500 V

Nozzle voltage: 300 V

Fragmentor: 145 V

Acquisition parameters

All Ions approach

Acquisition mode: MS Scan

Polarity: Positive and Negative

Mass range: 50-1300 m/z

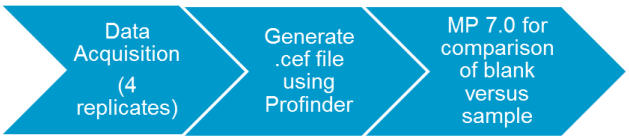
MS Scan rate: 7 spectra/s

Scan Segment #	CE (V)
1	0
2	15
3	40

ES-TOF reference solution introduced using 1:100 splitter (G1607-60000)

Reference mass stream nebulized with gas inlet through port 'H' on the splitter, Positive polarity: 121.0507 and 922.0098; Negative polarity: 112.9856 and 1033.988109

Workflow



'Extractable and Leachable' compounds database used to identify compounds

Results and Discussion

An Extracted Compound Chromatogram (ECC) of an aqueous mix of standards is shown in Figure 1.

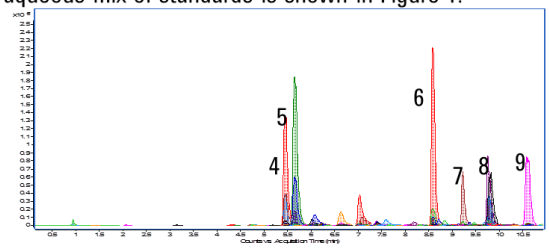


Figure 1: ECC of a mix of standards in positive polarity

With the All-Ions approach data is collected at different collisional energies. Typically MS spectra is obtained at the lower or zero collisional energy and MS/MS spectra are obtained at two higher collisional energies.

The extracted MS spectra at CE = 0 V and MS/MS spectra at CE = 15 and 40 V of diethyl hexyl phthalate are shown in the Figure 2.

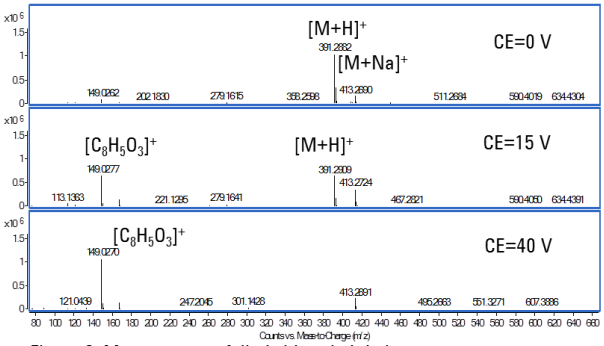


Figure 2: Mass spectra of diethyl hexyl phthalate

Diphenyl butadiene which does not ionize in the electrospray MS source and hence cannot be detected by QTOF can be detected by its characteristic UV absorbance at 330 nm. Simultaneous acquisition of diode array (DAD) data makes the method amenable for routine analysis in a QA/QC environment. The figure 3 shows the UV absorbance of the standards at 220 nm, 240 nm and 330 nm respectively.

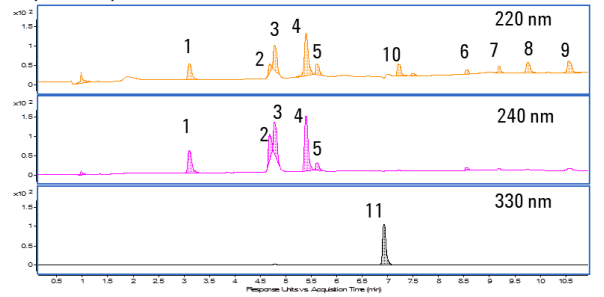


Figure 3: UV absorbance of standards

Analysis of empty ophthalmic bottle extract

Polymer additives leaching from primary and/or secondary container closure systems into drug substances or products can impact their safety. Of particularly high concern is the contamination of ophthalmic solutions and suspensions from impurities migrating from the LDPE packaging materials that the typically used. Figure 4 shows the ECC of an empty ophthalmic bottle extract.

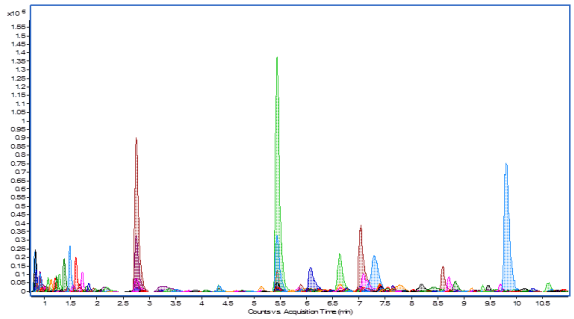


Figure 4: ECC of a empty ophthalmic bottle extract

A wide variety of materials are used in the packaging products. Different classes of chemicals such as plasticizers, anti-oxidants, photo-initiators, dyes etc., can leach from the primary and secondary containers. Besides generic methods and sensitive techniques, sophisticated software tools are required for identification and characterization of these contaminants.

In the present study, Profinder software was used for batch feature extraction to capture specific and important variables from the replicate data. The generated feature files were converted to .cef (Compound Exchange Format) files for exporting to Mass Profiler for further statistical analysis, database search and formula generation. The custom 'Extractables and Leachables' database was used in this study to identify compounds with a high degree of confidence.

Figure 5 shows the Extracted Ion Chromatogram (EIC) and mass spectra of a compound found in the empty ophthalmic bottle extract (bottom red trace) but not in the blank (upper black trace).

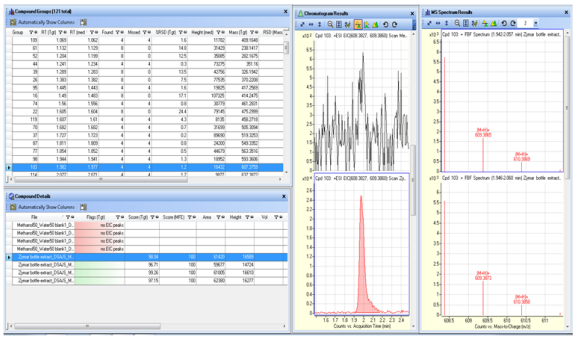


Figure 5: Profinder analysis of sample and solvent blank

Results and Discussion

Database search and formula generation

The .cef files from the blanks and samples were compared in order to identify the relative abundance of features. The following Figure 6 shows the identified compounds and the plot of logarithmic abundances of compounds in the sample versus the blank.

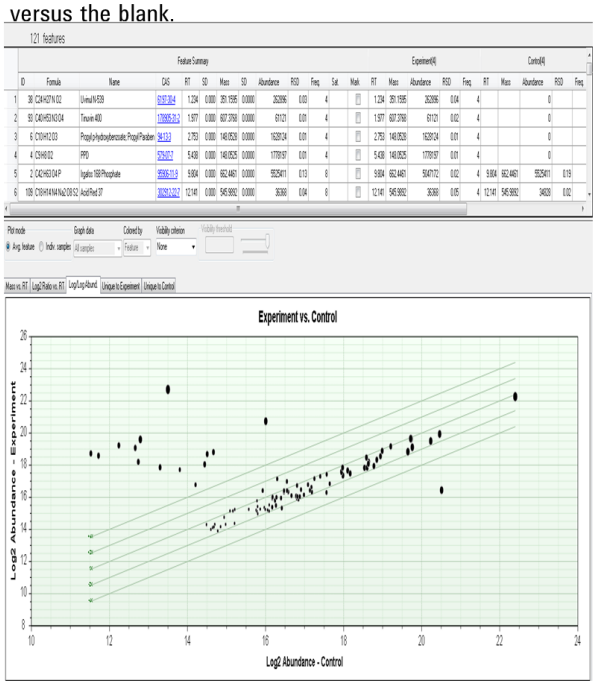


Figure 6: Mass Profiler analysis of .cef files of sample and solvent blank imported from Profinder

Mass Profiler also helps to identity compounds by searching for features in the database and provides structures of identified compounds as shown in Figure 7.

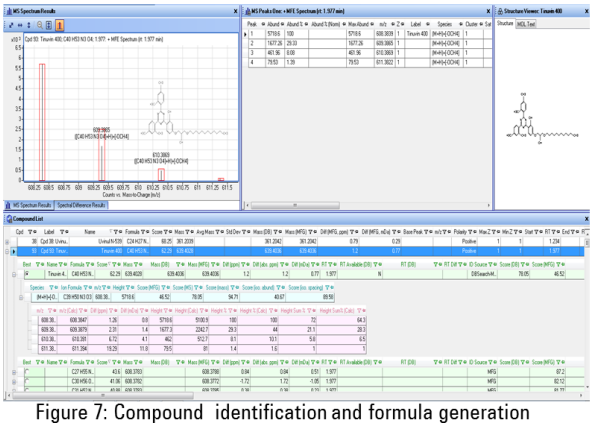


Figure 7: Compound identification and formula generation

PCA and CC Plots

Figure 8 shows the PCA analysis of the data was able to clearly separate samples (red dots) from blanks (blue dots) with PC1 capturing 93.3% of the total variance.

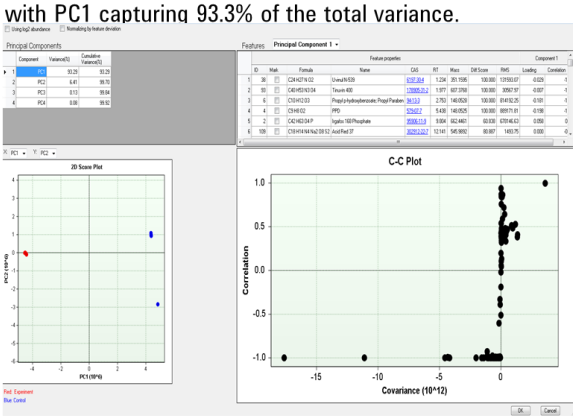


Figure 8: PCA showing separation of samples and solvent blanks.

Some compounds such as 4-hydroxy ethyl benzoate and 4-n-octyl phenol were detected in the negative ionization mode as shown in Figure 9. Profinder and Mass Profiler were also successful in identifying compounds with a high degree of accuracy in the negative ionization mode.

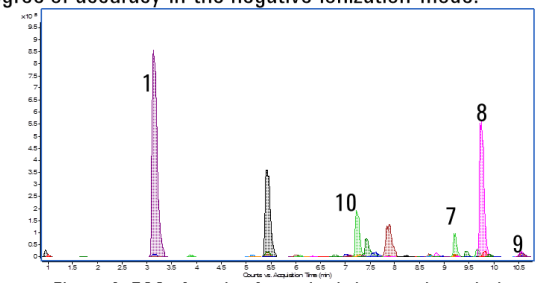


Figure 9: ECC of a mix of standards in negative polarity

Conclusions

- A UHPLC-DAD-QTOF method for the analysis of extractables and leachables was developed.
- The workflow for data analysis includes generation of .cef files using MassHunter Profinder and Mass Profiler for comparison of experimental and control samples
- '*Extractables and Leachables*' database was used for compound identification.
- The 'Molecular Formula Generator' feature of Mass Profiler was used to determine the formulae of compounds.