Detection and Identification of Extractable and Leachable Compounds from Ophthalmic Drug Product using High Resolution LC/MS

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Introduction

Drug substances and products may become contaminated by chemical compounds from primary and secondary packaging materials. Compounds which can be extracted directly from the container closure systems are called "extractables" while compounds, which are found within the formulation are called "leachables" which are often a subset of extractables. The US FDA has issued guidance on container closure systems for packaging human drugs and biologics[1], due to the potential risk impurities pose to consumer health. The guidance document includes protection, safety and compatibility guidelines.

In general, profiling extractables and leachables (E&L) is a complex analytical challenge due to the following factors:

- The wide range of materials used in making of primary and secondary containers.
- The diversity of physicochemical properties of the extracted and leached impurities.
- Varying concentration levels in samples (ranging from pg/mL to µg/mL).
- Detection of these compounds in a wide range of different matrices.

To overcome these challenges, multiple and often complementary analytical techniques such as LC/MS, GC/MS, and ICP/MS are required. Here we present LC/MS technique.

During screening for impurities, it is likely that E&Ls are present in the blank solvent originating from its container. Typically, a simple background subtraction during the data process will remove potential E&Ls also from the samples. Therefore, it is important to perform sample-to-sample comparison to retain compounds based on their intensity differences. This is performed by Mass Profiler (MP) software. MP is a statistical program that helps to compare two individual samples, replicates of the single sample, or replicates of two samples groups.

The methodology shown in Figure 1 was used for analysis of E&Ls in an ophthalmic drug product (ODP). This methodology enables the rapid and accurate identification of extractables and leachables.

Sample Preparation	Extraction conditions
Acquisition	• MS for statistical comparison and identification • Auto MS/MS for structure confirmation or elucidation
Statistical comparison and identification	• Mass Profiler Software. • Untargeted and targeted analysis. • Formula generation and database search on significant compounds.
Structure confirmation and elucidation	Molecular Structure Correlator software for "known" structure confirmation or "unknown" structure elucidation

Figure 1. Data analysis workflow using Agilent MassHunter Acquisition, Mass Profiler and Molecular Structure Correlator Software used in this study.

Experimental

Sample Preparation

Extractable sample
An ophthalmic medicine bottle was purchased from a local stores. It was washed with water and filled with extraction solvent (1:1 methanol: water) and incubated in an oven at 55 °C for 72 hours. The extract was used for a direct injection into the LC-MS/MS system. A second sample was analyzed as a blank, which contained the pure extraction solvent.

Leachable samples
A first leachable samples, designated as the "stressed sample" was obtained by heating the ophthalmic drug formulation and its container to 60 °C for 24 hours. The heated formulation was injected directly into the LC-MS/MS system. A second leachable sample designated as the" non-stressed sample" was the ophthalmic drug formulation stored at recommended conditions and was also directly injected into the system.

Instrument
Agilent 1290 Infinity Binary LC System and an Agilent Q-TOF G6540A System with a Dual Agilent Jet Stream source were used for LC/MS/MS analysis. The Agilent 1290 Infinity Binary UHPLC System comprised of a binary pump (p/n G4220A), autosampler (p/n G4226A), ALS Thermostat (p/n G1330B), and TCC (p/n G1316A).

Table 1: LC and MS method parameters

LC conditions			MS conditions	
Column	Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 X 100 mm, 1.8 µm (p/n 959758-306)		Ionization mode	Dual Spray AJS-ESI operated in positive and negative mode
Column temperature	50 °C		Drying Gas	10 L/min @ 150 °C
Mobile phase A	100 mg/L Ammonium acetate in water		Nebulizer pressure	30 Psi
Mobile phase B	Methanol		Sheath gas	11 L/min @ 200 °C
Flow rate	0.5 mL/min		Capillary voltage	3500 V
Gradient	Time (min)	% Methanol	Nozzle voltage	300 V
	0	40	Fragmentor	145 V
	8	100	Acquisition mode	MS and Auto MS/MS
	11	100	Scan Segment #	CE (V)
	Stop	11 min	1	5
	Post	1.5 min	2	15
Injection volume	5 µL		3	30
Needle wash	1:1 Methanol:Water for 10s		Mass range	50-1300 m/z
Autosampler temperature	6 °C		Reference ions	Positive: 121.0507 and 922.0098 Negative: 112.9856 and 1033.9881

Experimental

Data Analysis:

PCDL (Personal Compound Data Library) Manager Software: An user-generated, custom database, containing E&Ls reported in the literature, was created in this software using molecular formulae and structure.

Mass Profiler Software: The experimental group was either the extractable or leachable stressed sample while the control group was either solvent blank or leachable non-stressed sample. A statistical analysis and fold change was performed on the replicate groups. Compound occurrence frequency with > 50% in at least one group and 2.0 fold change, was considered The differential features obtained from the fold change analysis were matched against the custom database with a mass accuracy criteria < 5 ppm.

Targeted Leachable Analysis: In addition to untargeted analysis, targeted analysis was performed on the leachable samples to identify "known" leachable impurities. The formulae in E&L custom database were used for "Batch Target Feature Extraction" analysis by Mass Profinder Software and exported to MP software.

Molecular Structure Correlator (MSC) analysis: MSC analysis has been performed to identify unknown compounds which were not included in the PCDL. The auto MS/MS results were exported to the MSC software. Here, "ChemSpider" was selected as the structure source for unknown analysis.

Results and Discussion

Data comparison and identification using MassHunter Mass Profiler Software:

Extracts from the empty ophthalmic bottle analyzed in positive ion mode revealed 200 compounds of which the abundance of 45 compounds were significantly higher compared to the solvent blank. Due to the criteria of a fold change of greater than 2.0, the presence of dinonyl phthalate has been confirmed and not eliminated from the sample. Figure 2 shows a logarithmic abundance plot of compounds found in the extractables study with one, two, and four fold change intensity cut-offs indicated.
By combining the positive and negative ionization analysis results 54 compounds have been detected in the leachable study. The negative ionization mode contributed with 30% to this number of detected compounds.

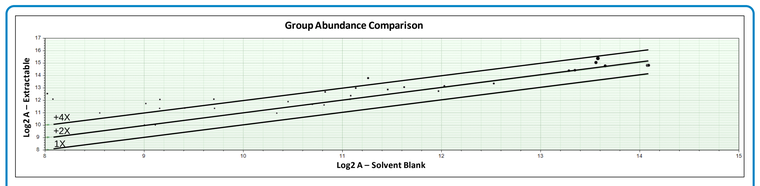

Figure 2: Mass Profiler plot of logarithmic abundance of extractable compounds versus solvent blank. A one, two, and four fold abundance line marks the abundance threshold for experimental compounds above the solvent blank control.

Identification

The identification functionality within MP software was used to identify the compounds based on their accurate mass. Figure 3 shows the identity, isotopic distribution and structure of dinonyl phthalate, which was identified in the empty ophthalmic bottle extract. 11 extractable compounds were identified with the use of the user generated database. Table 2 shows the list of identified extractable and leachable compounds from positive and negative ionization modes

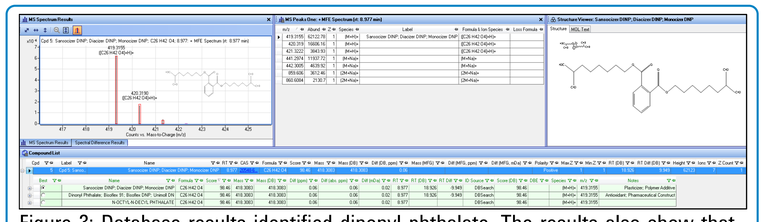

Figure 3: Database results identified dinonyl phthalate. The results also show that isomers, diisononyl phthalate and n-octyl-n-decyl phthalate are also possible targets.

Table 2. Accurate mass (user created) custom database assisted identification of E&Ls

Compound name	Mass Error (ppm)	CAS Number	Sample
Diethylene Glycol	1.42	111-46-6	Extractable
Sodium Ricinoleate	4.89	5323-95-5	Extractable
1,3-propanedione, 1,3-diphenyl (Rhodiastab 83)	0.39	120-46-7	Extractable
Isopropyl-9H-thioxanthen-9-one	0.89	75081-21-9	Extractable
Irgacure 651	1.17	24650-42-8	Extractable
Iso-Octyl methacrylate (A58)	0.37	28675-80-1	Extractable
1-docosene	1.37	1599-67-3	Extractable
2 Ethyl hexyl 4-(dimethylamino)-benzoate	1.03	21245-02-03	Extractable
Irgacure 907	0.74	71868-10-5	Extractable and Leachable
13-Docosenamide, (13Z) (Erucamide)	0.11	112-84-5	Extractable and Leachable
Dinonyl phthalate	0.06	84-76-4	Extractable and Leachable
Myristyl dimethylamine oxide	0.33	3332-27-2	Leachable
Acetic acid, propyl ester	1.92	109-60-4	Leachable

Results and Discussion

Targeted Leachable Analysis

leachable samples were analyzed in a targeted way by applying the extractable information stored in the database. Figure 4 shows the logarithmic abundance plot of significant extractables detected in the leachables samples. The data show that, from 45 significant extractables, 16 were found in the leachables sample. Within stressed and non-stressed samples, the concentration of the found compounds do not change (lie on 1X abundance line). The abundance plot reveals significant change in concentration of only three compounds due to heat stress.

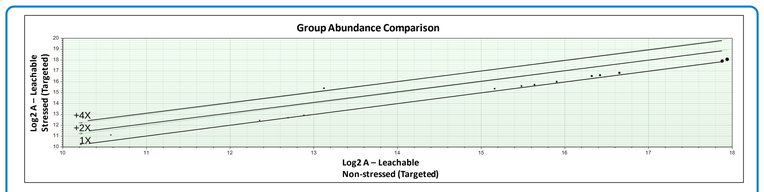

Figure 4. Mass Profiler results showing plot of logarithmic abundance of leachable compounds stressed versus leachable non-stressed, targeted compounds. Several compounds lay on the one fold abundance line marks.

Untargeted compound identification, compound confirmation and structural elucidation

In E&L studies, reference standards MS/MS spectra may not be readily available. Therefore, software assisted structure elucidation offers a viable means of determining the chemical structures of unknown compounds. Agilent Molecular Structure Correlator (MSC) software facilitates the identification of compounds tentatively for which reference standards are not available. Here, accurate mass/formula of experimental MS/MS fragments is correlated with in silico fragment ions from a structure database. Custom PCDL (user generated) , "ChemSpider" or "PubChem" databases were used in this study.

The PCDL database structures were used for compound confirmation while web-based databases were used to determine possible unknown compounds. Figure 5 shows confirmation of the compound di-isononyl phthalate (DINP, a potential endocrine disruptor) and associated isomers. Other compounds confirmed are: erucamide, isopropyl-9H-thioxanthene-9-one, and irgacure 907 (a photo-initiator used as photo-polymerization). Some of the unknown compounds identified as E&L included: (9E)-N-butyl–9–octadecenamide (0.1 ppm, MSC score: 85), 3-oxo-2,3-diphenylpropanal (0.4 ppm, MSC score: 75), derivate of pyrrolidine carboxylate (0.2 ppm, MSC score: 81), and the derivative of 3,4-O-isopropylidene-D-ribitol (0.3 ppm, MSC score: 84).

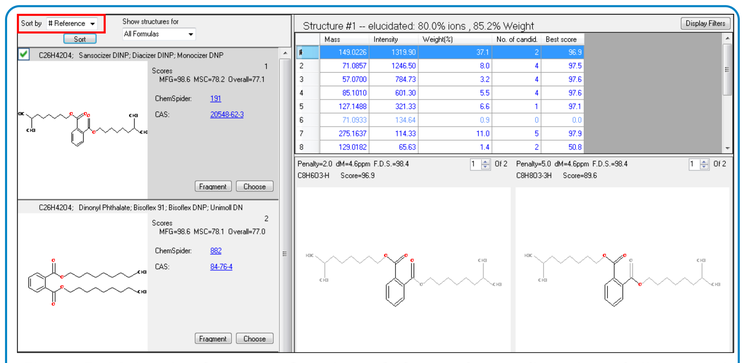

Figure 5: Molecular Structure Correlator Software for confirmation of di-isononyl phthalate (DINP). Experimentally observed MS/MS fragments matched to theoretical structure fragments and results sorted based on maximum number of reference citations.

Conclusions

- In this work, an Agilent 1290 Infinity UHPLC System coupled to an Agilent 6540 Q-TOF was used to analyze E&L compounds from ophthalmic drug products and their container.
- Statistical comparison to differentiate E&L compounds from control samples was performed using Agilent Mass Profiler Software.
- The compounds that differed significantly between samples were identified using an accurate mass PCDL database.
- The analysis of MS/MS data using MSC software facilitated the structural elucidation of unknown compounds.
- Approximately 50 compounds were found to be present in each E&L sample.
- The results of this study identified several compounds that could pose a potential health risk. Semi-quantification results (data not shown) of leachables study show that 3 compounds – diisononyl phthalate, n-dioctyl phthalate, and erucamide, could leach in stressed drug product and were found in excess of 1 µg/mL concentration and therefore are to be reported to regulatory authorities.
- For more information please see the app note[2].

References

1. 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). Container Closure Systems for Packaging Human Drugs and Biologics; Guidance for Industry; May (1999).
2. Lateef, S S., "Extractables and Leachables Detected in Ophthalmic Drug Products," Agilent Application Note, 5991-6828EN (2016).