Detection and Identification of Extractable Compounds from a Drug Container Closure System Using High Resolution LC/MS and Mass Profiler Software

Application Note

Authors

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Abstract

An Agilent 1290 Infinity LC system coupled to an Agilent 6530 Q-TOF system, in combination with data mining software was used to identify extractable compounds from an ophthalmic drug container closure system. Extractables from pharmaceutical packaging materials are mostly harmful compounds with a large diversity of physico-chemical properties. Orthogonal analytical technologies are often needed to ensure wide detection coverage of all analytes. Some application notes have demonstrated the effectiveness of GC/MS methodologies[1,2,3]. This Application Note illustrates the ability to perform this analysis by LC/MS. The Agilent 6530 Q-TOF was operated in both positive and negative ionization modes using the data independent All Ions MS/MS acquisition mode. An Agilent MassHunter Profinder combined with Agilent Mass Profiler Software were used to distinguish significant extractables from the solvent control. The database search feature within Mass Profiler Software identified extractable compounds using a customized personal compound accurate mass database specific for extractable/leachable compounds.

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Introduction

Drug substances and products can get contaminated by chemicals migrating from primary and secondary packaging materials. Due to the potential impact of these impurities on patient health, the US FDA has issued guidance to the industry on container closure systems for packaging human drugs and biologics[4]. Profiling compounds that can be extracted from the packaging materials, or that have leached into a drug substance or product is a complex task due to:

- Wide range of materials used for the construction of primary and secondary containers
- Diversity of the physico-chemical properties of the extracted and leached impurities
- Varying detection levels in samples ranging from ng to µg
- Challenges in the detection of these compounds in a wide range of different matrices

To overcome these challenges, multiple and often orthogonal analytical techniques are required. For example, Norwood *et al.*, have reviewed numerous HPLC and LC/MS methods developed for the analysis of extractable and leachable compounds[5].

In this study, a generic method was developed to detect a set of analytes belonging to different classes of compounds such as plasticizers, photo-initiators, stabilizers, antioxidants, and so forth. An Agilent 1290 Infinity LC system in combination with an Agilent 6530 Q-TOF Mass Spectrometer was used to separate, detect, and identify these compounds. Agilent MassHunter Profinder and Agilent Mass Profiler Software programs have been used in conjunction with a customized Extractable and Leachable database to help rapidly analyze data and identify detected compounds with a high degree of confidence.

Agilent MassHunter Profinder Software can rapidly mine multiple high resolution MS data files. For untargeted data analysis, the recursive batch molecular feature extraction data mining capability of this software minimizes false positive and negative identifications. Extracted ion chromatograms from the processed data can be seen as color-coded groups. Data can be easily exported to the Mass Profiler program as compound exchange format (.cef) files for subsequent differential analysis. This readily allows different samples to be compared, and differences determined. In the case of targeted data analysis, where the targeted extractable compounds are monitored, a Find by Formula algorithm is chosen within the Profinder software. The data can then be exported as an identified list to the Profiler software for sample-to-sample comparison.

Mass Profiler software is a statistical program that helps compare feature similarities and differences between data sets, which may be:

- Two individual samples,
- Replicates of the sample, or
- Replicates of two samples groups.

Furthermore, the software allows principal component analysis (PCA) of samples with student t-test and sample correlation. Identification of features for untargeted analysis uses a combination of database search and molecular formula generation in the built-in ID Browser program.

In this Application Note, the untargeted workflow shown in Figure 1 was followed, enabling the rapid and accurate identification of extractable compounds.

Experimental

Table 1 lists the chemicals used to create a personnel compound database. They were purchased from Sigma-Aldrich. MS grade methanol, isopropyl alcohol (Fluke), DI water (Milli-Q, Millipore).

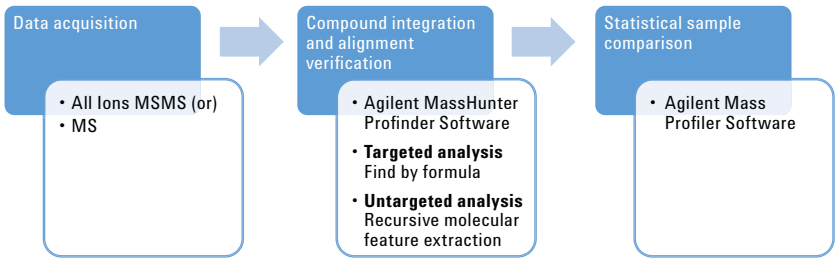

Figure 1. Data processing workflow using Agilent MassHunter Acquisition, Agilent Profinder, and Agilent Mass Profiler Software.

Table 1. Target analytes used in spike samples.

No.	Analyte	CAS	Empirical formula	Monoisotopic mass
1	Ethyl paraben	120-47-8	$C_9H_{10}O_3$	166.063
2	Irgacure 184	947-19-3	$C_{13}H_{16}O_2$	204.115
3	Irgacure 651	24650-2-8	$C_{16}H_{16}O_3$	256.1099
4	Dipropyl phthalate	131-16-8	$C_{14}H_{18}O_4$	250.1205
5	4-*n*-Octyl phenol	1806-26-4	$C_{14}H_{22}O$	206.1671
6	Diethyl hexyl phthalate	117-81-7	$C_{24}H_{38}O_4$	390.277
7	Irganox 1010	6683-19-8	$C_{73}H_{108}O_{12}$	1176.7841
8	Irganox 1076	2028-79-3	$C_{35}H_{62}O_3$	530.4699
9	Iragafos 168	31570-04-4	$C_{42}H_{63}O_3P$	646.4515

Sample preparation

Stock solution

A 1,000 ppm stock solution of each analyte (Table 1) was prepared by dissolving the standards in IPA. Stock solutions were further diluted in water to prepare a mix containing all nine analytes.

Standard mix solution

Stock solutions were mixed and diluted to obtain ~1 ppm of each target analyte.

Extracted sample

An empty ophthalmic medicine bottle, purchase locally, was washed with water and filled to the brim with 1:1 methanol:water. This was incubated in an oven at 55 °C for 72 hours. An aliquot (200 µL) was taken for analysis.

Spiked sample

To obtain a target concentration of ~0.05 ppm, 10 µL of the standard spike solution was added to 190 µL of the extracted sample.

Instrumentation

Table 2 shows the details of LC and MS conditions used in this experiment.

- Agilent 1290 Infinity Binary LC System comprising:
    - Agilent 1290 Infinity Binary Pump (G4220A)
    - Agilent 1290 Infinity Autosampler (G4226A)
    - Agilent 1200 Series ALS Thermostat (G1330B)
    - Agilent 1200 Series Thermostatted Column Compartment (G1316A)
    - Agilent 1290 Infinity Diode-Array Detector (G4212A)
    - Agilent Max Light Cartridge with 60-mm path length flow cell (G4212-60007)
- Agilent 1260 Binary Pump VL G1312C for introducing reference mass using a 1:100 splitter (G1607-60000)
- Agilent Q-TOF G6530A and Agilent Dual Spray Jet Stream Source (G1959A)

Table 2. LC and MS method parameters.

LC conditions	
Column	Agilent ZORBAX RRHD Eclipse Plus C8, 3.0 × 100 mm, 1.8 µm (p/n 959758-306)
Column temperature	50 °C
Mobile phase A	100 mg/L ammonium acetate in water
Mobile phase B	Methanol
Flow rate	0.5 mL/min
Gradient	Time (min) % Methanol 0 40 8 100 11 100
Stop time	11 minutes
Post time	1.5 minutes
Injection volume	5 µL
Needle wash	1:1 methanol:water for 10 seconds
Autosampler temperature	6 °C
MS conditions	
Ionization mode	Dual Spray AJS-ESI
Drying Gas	10 L/min at 150 °C
Nebulizer pressure	30 psi
Sheath gas	11 L/min at 200 °C
Capillary voltage	3,500 V
Nozzle voltage	300 V
Fragmentor	145 V
Acquisition parameters	
Acquisition mode	All Ions MS Scan
Segments and CE (V)	Experiment segment no. CE (V) 1 0 2 15 3 40
Polarity	Positive and Negative
Mass range	50–1,300 *m/z*
MS Scan rate	7 spectra/s
Reference ions	Positive: 121.0507 and 922.0098 Negative: 112.9856 and 1033.9881

Software

- Agilent MassHunter Data Acquisition B.05.01
- Agilent MassHunter Qualitative Analysis B.07.00
- Agilent MassHunter Profinder B.06.00
- Agilent MassHunter Mass Profiler B.07.00

Results and Discussion

A new high resolution LC/MS method has been developed for the detection of extractable compounds from drug containers using a spike mix of compounds. The spiked sample was also used as a system performance test. The test mix data analysis ensures system sensitivity and separation. The compounds spiked in the extracted solvent are listed in Table 1, and cover a broad polarity range. After optimizing the method parameters as shown in Table 2, three replicates each of blanks, spiked samples, and extracted sample were injected, and data was collected in both positive and negative ionization modes. Figure 2 shows the extracted compound chromatogram obtained in the positive Figure 2A and negative Figure 2B ionization mode. Many analytes were found in positive mode, although a few showed better response in negative mode as shown in Figures 2A and 2B.

Data analysis of extracted sample

The LC/MS method was used to analyze an extract obtained from an empty ophthalmic medicine bottle. Figure 3 shows the total compound chromatogram (TCC) of the sample for both positive and negative modes.

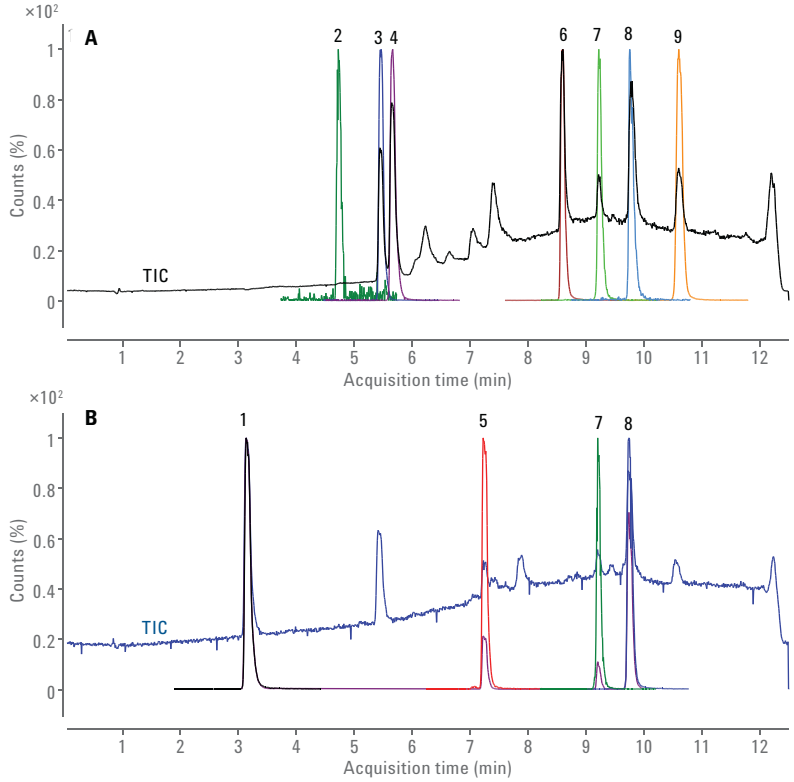

Figure 2. Total ion chromatograms of extracted sample spiked with known standards extracted compound chromatogram of target analytes in positive mode (A) and negative mode (B).

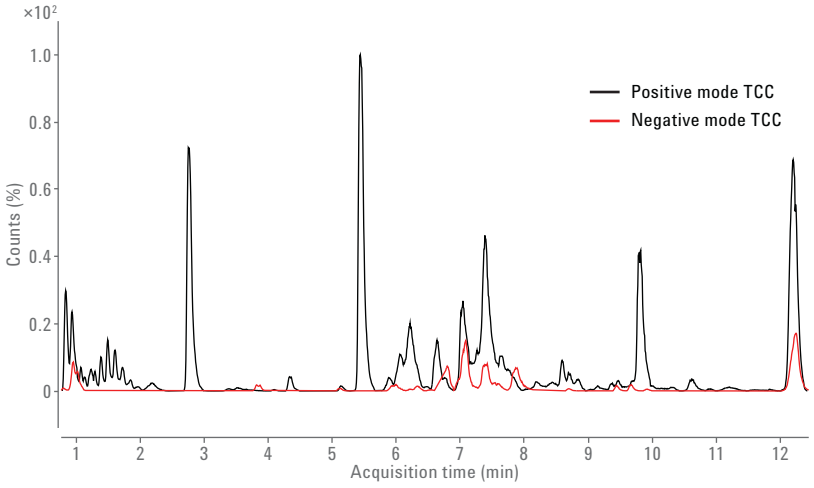

Figure 3. The positive and negative TCC from an empty ophthalmic bottle extract.

Data alignment using Agilent MassHunter Profinder Software

The chemical features in the acquired data was extracted, aligned, and integrated using MassHunter Profinder Software. Any features that failed to integrate can be detected visually and integrated. A total of 175 features from both positive and negative acquisition modes were extracted and integrated from the samples.

Data comparison and identification using Agilent MassHunter Mass Profiler Software

The extracted features were imported into Mass Profiler Software. Mass Profiler Software helps to statistically compare data and run database searches for compound identification. Figure 4 shows the composite features list which tentatively identifies the differential compounds and a plot of logarithmic abundances of features in experimental versus control replicates as analyzed by Mass Profiler. A 4-fold change (abundance of height) and abundance cut off filter of > 3,000 was applied. The analysis revealed 66 features in both positive and negative ionization modes present in extracted samples at significant levels.

The ID Browser functionality with Mass Profiler helped confirm the identity of compounds by database searching and formula generation. Figure 5 shows the identity, isotopic distribution, and structure of a plasticizer, diethylene glycol dibenzoate, found in the empty ophthalmic bottle extract.

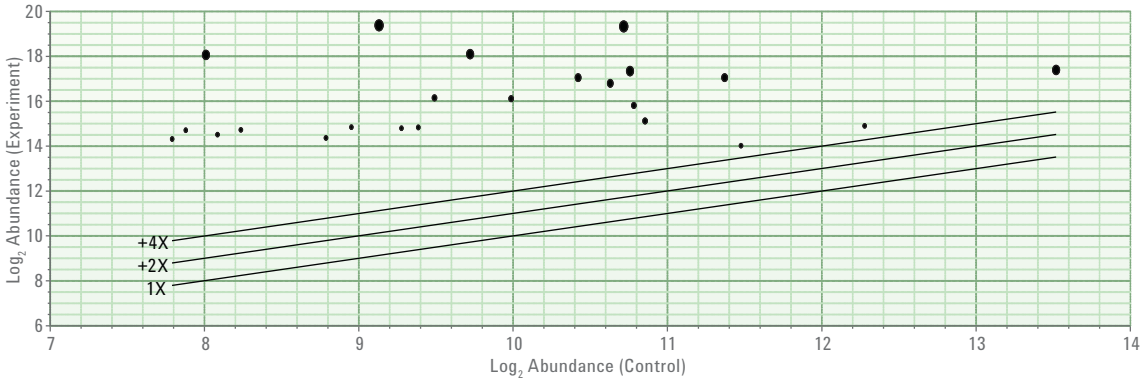

Figure 4. Agilent Mass Profiler results showing database identified compounds (top) and plot of logarithmic abundance of compounds in sample versus blank (bottom). A 4-fold abundance line marks the abundance threshold for experimental compounds above the solvent blank control.

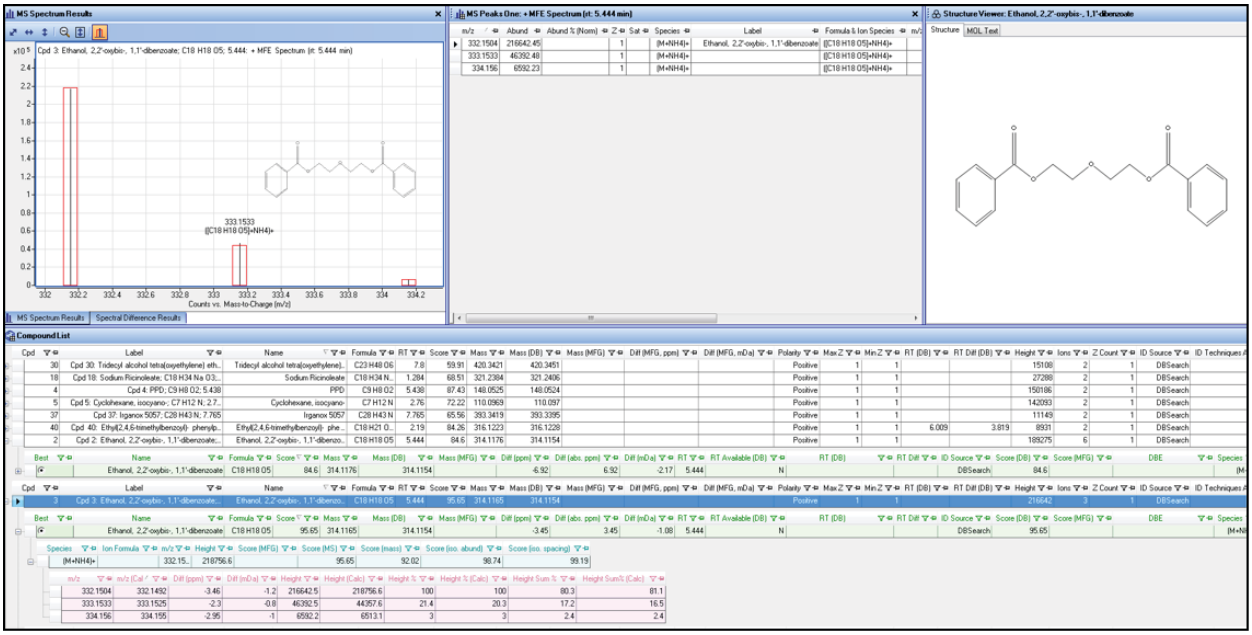

Figure 5. Compound identification by database search and formula generation, highlighted compound shows diethylene glycol dibenzoate.

A customized database of 1,560 compounds was used for the database search. From 66 differentially compounds, some of the compounds identified through accurate mass database were, tridecyl alcohol (oxyethylene) ethanol, sodium ricinoleate, irganox 5057, ethyl(2,4,6-trimethylbenzoyl)-phenylphosphinate, isocyano cyclohexane, degradant of irganox, hexadecanoic palmitic acid, and many others were detected.

Conclusion

In this Application Note, an Agilent 1290 Infinity LC system coupled to an Agilent 6530 Q-TOF was used to analyze extractables from a drug container closure system. The analysis of a standard mix of nine plasticizers showed that both positive and negative electrospray ionization are required to detect many extractable compounds. The data was efficiently analyzed by a two-step workflow. The first step involved integration and alignment verification between replicates of the extractable and its blank solvent using Agilent MassHunter Profinder Software, and the second step involved statistical comparison of the two groups using Agilent MassHunter Mass Profiler Software. Further database search and molecular formula generation feature of Mass Profiler was used to putatively identify and determine the formulae of compounds. The results of this study show that 66 compounds, including an antioxidant additive such as irganox and skin irritants such as ricinoleate were significantly present in the empty bottle extract. This study can be applied to a vendor qualification for a drug container closure system or an extractable and leachable study of drug formulations.

References

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