Increased Identification of Extractable and Leachable Compounds by the Use of Chemical Ionization and Custom Databases

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Introduction

Extractables are chemical compounds that migrate from product contact material when exposed to an appropriate solvent under exaggerated conditions of time and temperature. Leachables are chemical compounds, typically a subset of extractables, that migrate into a drug formulation from any product contact material as a result of direct contact under normal process conditions or accelerated storage conditions[1]. Compound distribution among extractables, leachables and their controls helps to understand the origin of the compounds based on the overlap of their distributions. Although it is tedious to interpret the data in this manner manually, Mass Profiler Professional (MPP), a chemometrics software, facilitates differential analysis based on blank compound subtraction and aids in the visualization of significant differences between compounds and compound distribution across test samples.

Increased coverage by CI mode analysis:

In GC/MS analysis, electron ionization (EI) spectra may not provide a significant molecular ion, while chemical ionization (CI) spectra may include such a signal. This molecular ion signal combined with accurate mass information assists in obtaining the chemical formula, which aids in compound confirmation. With this approach, new compounds that are more amenable to soft ionization (such as CI) can also be detected. CI acquired data can be analyzed by the use of custom databases.

In this work, a high resolution accurate mass Gas Chromatograph Quadrupole Time-of-Flight (GC/Q-TOF) system was used to analyze semi-volatile extractables and leachables (E&L) from an Ophthalmic Drug Product (ODP). The workflow used in this study is shown in Figure 1.

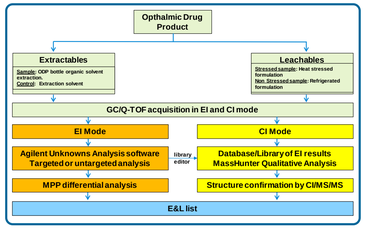

Figure 1. Extractable and leachables workflow for the analysis of semi-volatile compounds using a high resolution accurate mass GC/Q-TOF system.

Experimental

Leachables study: The leachable stressed ophthalmic drug product was prepared by heating 5 mL ODP formulation in its container closure system for 24 hours at 60°C. The leachable non-stressed samples were the drug formulation stored at manufacturer recommended conditions. Both stressed and non-stressed formulations were extracted in n-hexane using liquid-liquid extractions (LLE).

Sample preparation for extractables study: An empty ODP bottle was used in the extractable study. N-hexane was added and sonicated for 1.5 hrs. The extraction solvent, n-hexane, was used as the control/blank.

MPP analysis: The EI data were re-processed by Unknowns Analysis software and the results were exported into MPP software. Compound intensity within each sample was normalized to the intensity of a 1 µg/mL internal standard (triphenyl phosphate). The compounds found in the blank (n-hexane) were subtracted from all samples based on 2x intensity fold change.

Semi-quantitative estimation: Triphenyl phosphate relative response was used to estimate the amount of leachables using the procedure described by Jenke et al[2].

E&L Personal Compound Database (PCD): A custom database of literature reported extractables and leachables was created for reference search.

CI data analysis: The CI data were processed with possible adducts [M+H]+, [M+C2H5]+ and [M+C3H5]+. The EI .xml library was used as the formula database. The CI data were also searched for other extractables using the E&L PCD.

Structure elucidation using CI MS/MS: The CI MS/MS data files were processed using the "Find by Targeted MS/MS" feature within MassHunter Qualitative Analysis software. The fragment structures were drawn using ACD software (ACD Labs, Toronto).

GC	Agilent 7890A	MS	Agilent 7200
Injection port	Multimode Inlet (MMI)	Tune	Autotune
Mode	Splitless	Transfer line	280°C
Septum purge flow:	3 mL/min	MS Source (EI and CI)	300°C
Inlet Program	70°C (0.2 min) to 325°C (7 min) at 600°C/min.	MS Quad	175°C
Liner	Ultra Inert Splitless, single taper, glass wool (p/n 5190-3163)	Mass Range	55 to 700 amu
Carrier gas	Helium	Acquisition rate	5.00 spectra/sec
Flow	1.3 mL/min (constant)	Electon Ionization	
Purge flow to split vent	60 mL/min at 2.73 min	EI emission current	35 µA
Gas Saver	20 mL/min at 3 min	EI electron energy	70 eV
Oven Program	50°C (3 minutes) to 320°C (7 minutes) at 8°C/min. Equilibration time – 1 minute. Run time: 55 minutes.	Chemical Ionization	
Columns	Agilent DB-5ms, 30 m x 250 µm, 0.25 µm (p/n 122-5532)	CI emission current	240 µA
Injection volume	2 µL	CI gas flow	20% EPC
		CI electron energy	115 eV
		Mode	Positive
		CI Reagent gas	Methane
		Collision Cell EPC	Nitrogen, 1.5 mL/min

Table 1. Instrument parameters.

Results and Discussion

E&L analysis by EI mode GC/Q-TOF:

- Large numbers of compounds were identified in the extractable samples compared to the leachables study.
- Benzene 1,3-bis(1,1-dimethylethyl), an extractable compound used in polymer packaging, was identified at a retention time of 15.1 min (Figure 2).
- The Extracted Ions Chromatograms (EICs) of this deconvoluted component co-eluted and had the same peak shape, (Figure 2C) while its EI spectrum had a unit mass (NIST) library match with a score > 88 (Figure 2D).

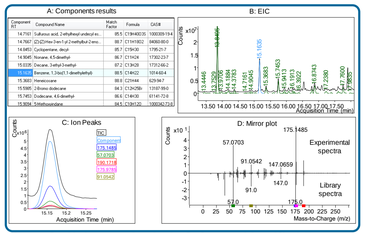

Figure 2. Unknown Analysis Software identified Benzene, 1,3-bis(1,1-dimethylethyl) by deconvolution and NIST library search. Components list (A), deconvoluted component chromatograms (B), overlay of EICs of individual component (C) and mirror plot of deconvoluted component spectrum and library hit D.

Sample analysis by MPP Software:

- Normalization: The normalization step normalizes the intensity of all compounds with respect to the intensity of the spiked internal standard within each sample.
- Fold change based blank subtraction: Fold change analysis as a subtraction technique, retains compounds which are found with greater than a 2 fold increase in intensity when compared to the blank.
- Visualization: Venn diagrams to visualize compound distribution across several samples.

Figure 3 shows the Venn diagram of the leachable stressed sample compared to the extractable (3A), and to the leachable non-stressed (3B). Analysis of the data reveals the leachable stressed sample and non-stressed sample (Figure 3B) contained 15 compounds in common.

To understand if any of those 15 common compounds could come from extractable conditions, an overlap display was produced (Figure 3C). The results show that 6 of the 15 compounds present in the non-stressed leachable sample originated from the container. One of these, benzene 1,3-bis(1,1-dimethylethyl), leached even under non-stressed conditions. (E)-3-Eicosene is a non-polar alkane found in the formulation and unaffected by heat stress, but did not originate as an extractable.

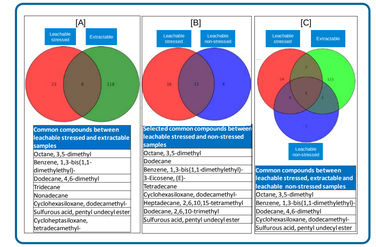

Figure 3. MPP Venn diagram showing the overlap of compounds found between leachable stressed and extractable (A), leachable stressed and non-stressed (B) and among all three – leachable stressed, extractable and non-stressed (C) samples. The table below each overlap results shows the selected list of compounds which were common among the samples compared.

Semi-quantification:

The semi-quantitative results of leachable stressed samples are shown in table 2. Four compounds are found to exceed 20 ppm mark of Analytical Evaluation Threshold (AET) and would require a safety assessment test.

Retention Time	Leachable stressed sample	Semi-quantitation estimation (ppm)*
8.75	Octane, 3,5-dimethyl	3
15.16	Benzene, 1,3-bis(1,1-dimethylethyl)-	132
15.75	Dodecane, 4,6-dimethyl	7
16.19	Tridecane	12
16.20	Nonadecane	8
16.87	Cyclohexasiloxane, dodecamethyl-	80
19.92	Sulfurous acid, pentyl undecyl ester	39
20.53	Cycloheptasiloxane, tetradecamethyl-	22

Table 2. The semi-quantitation estimation of compounds common between leachable stressed and extractable samples.*

***quantification values can vary up to 4 fold [2]**

Results and Discussion

Increased identification of E&L CI/MS and databases:

- EI derived database: The CI data were searched against a custom database created from the EI mode results. Benzene,1,3-bis(1,1-dimethylethyl) ($C_{14}H_{22}$) which was common between leachable stressed, non-stressed and extractable, had a library match score of 88. The CI results confirmed the presence of benzene,1,3-bis(1,1-dimethylethyl) with mass accuracy of 2 ppm.
- Custom databases: The CI extractable and leachable data were also processed using a custom PCD database containing literature reported extractable and leachable compounds. The results show that additional E&Ls were detected with an average mass accuracy of < 3.0 ppm (see Table 3).

Extractable	Leachables				
EI Database					
ID	Mass	Mass Error (ppm)	ID	Mass	Mass Error (ppm)
1-Octene	112.1252	5.0	3-Penten-2-one, 4-methyl-	98.0732	0.1
2,4-Diethyl-9H-thioxanthen-9-one	268.0922	2.9	2-Hexanone	100.0886	0.9
Acenaphthylene	152.0626	3.1	Propyl p-hydroxybenzoate; Propyl Paraben	180.0786	1.0
Benzo[def]phenanthrylene	252.0939	3.6	Benzoic acid, 4-ethoxy-, ethyl ester	194.0943	1.7
Benzaldehyde	106.0419	4.4	Phenol, isonyl- BHT	220.1827	2.1
Ethyl p-hydroxybenzoate	166.0630	2.5	3-Penten-2-one, 4-methyl-	98.0732	2.2
Ethylbenzoyl-formate	178.0630	2.0	2-Cyclopenten-1-one, 2-methyl-	96.0575	2.3
Squalene	410.3913	0.9	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	330.2770	2.4
Custom Databases					
Benzene, 1,3-dichloro-	145.9690	0.4	3-Carene	136.1250	4.2
Cyclopentane, decyl-	210.2350	1.1	o-methylbenzyl benzoate	240.0790	0.7
Stigmasta-3,5-diene	396.3760	0.1	9H-Thioxanthen-9-one, 2-(1-methylethyl)-	254.0770	0.8

Table 3. CI results showing a selected list of extractables and leachables confirming the EI mode compounds.

Structure confirmation by CI/MS/MS

- An eye irritant, aenzoic acid, 4-ethoxy-ethyl ester (ethyl 4-ethoxybenzoate, Figure 5) detected by the CI mode analysis was analyzed by CI MS/MS in order to confirm identification.
- The CI MS/MS analysis of ethyl 4-ethoxybenzoate, is shown in Figure 5, with assigned structures to the fragment ions at *m/z* 195.1016.

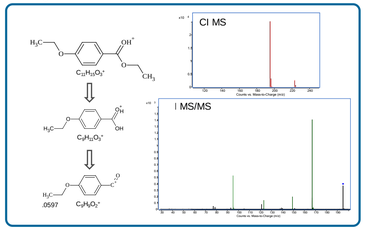

Figure 5. Structure confirmation of ethyl 4-ethoxybenzoate.

EI spectra of decylcyclopentane did not yield the molecular ion peak at 210 *m/z*, but CI and CI MS/MS spectra identified decylcyclopentane (Figure 6).

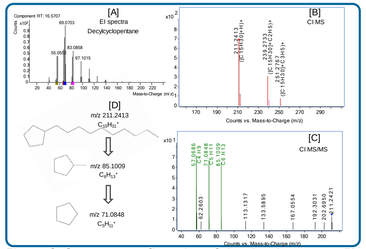

Figure 6. Structure confirmation of decylcyclopentane. EI spectra (A), CI MS spectra (B), CI MS/MS spectra (C), and proposed fragmentation pathway (D).

Conclusions

An Agilent 7200 GC/Q-TOF was used to perform high resolution accurate mass qualitative screening and identification of extractables and leachable compounds from ophthalmic drug products. The EI data from the analysis of E&L compounds were matched with the NIST 14.0 library to help compound identification. Data processing and interpretation was facilitated using Mass Profiler Professional software which enables differential analysis of sample sets. Venn Diagrams helped visualize unique and common compounds across sample groups. The custom databases that combine experimental as well as literature data were created and used to interrogate the CI data. The accurate mass CI data helped to confirm tentative hits and expand the list of identified compounds. The versatility of database and library creation and the use of CI GC/Q-TOF with accurate mass data increased the number of detected and identified compounds. Further information regarding this work can be obtained here[3].

References

1. "Recommendations for Extractables and Leachables Testing Part 1: Introduction, Regulatory Issues, and Risk Assessment" BioProcess International, Dec 2007
2. Jenke D, et.al., "Utilization of Internal Standard Response Factors to Estimate the Concentration of Organic Compounds Leached from Pharmaceutical Packaging Systems and Application of Such Estimated Concentrations to Safety Assessment," Journal of Chromatographic Science 50 (2012) 208-212
3. Lateef S S, et.al., "Differential Analysis in Screening Assays for an Extractables and Leachables study using an Agilent 7200 GC/Q-TOF System combined with Data Mining Software, Agilent Application Note, 5991-6688EN, March 2016