Identification and Comparison of Extractables in Drug Container Closure Systems

State-of-the-art Data Mining Process Taking Complete Advantage of Electron Ionization, Chemical Ionization, Collision-induced Dissociation, and Accurate Mass Information using an Agilent 7200 GC/Q-TOF System

Application Note

Pharmaceutical

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Abstract

Regulatory authorities and working groups provide guidelines on the evaluation of drug containers to ensure the safety and efficacy of drug products. The guidelines for container qualification suggest a risk-based approach when applying an extraction study. This approach identifies and classifies the toxicological relevance of nonvolatiles, semivolatiles, and elemental contaminants found in extracts from components or from the entire drug container closure system. In this work, four drug containers originating from different manufacturers have been investigated, generating profiles for semivolatile extractable compounds. Data were acquired on an Agilent 7200 GC/Q-TOF in both electron ionization (EI) and chemical ionization (CI) modes to ensure a comprehensive identification of the extracted compounds. EI data have been acquired to identify compounds using the NIST library. Compound distribution across the different samples was visualized based on a chemometric approach using Agilent MassHunter Mass Profiler Professional software. Compounds that could be only tentatively identified after EI were confirmed using parent ion mass data generated in CI mode. Approximately 170 compounds were identified in each container. The results indicate that the combination of EI and CI data increased the number of identified extractable compounds, enabling a thorough evaluation of container systems.

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Introduction

Extractable studies on drug containers provide valuable information that ensures the safety and efficacy of drugs. An extractable study of the drug container closure system during the early phase of the manufacturing process efficiently supports early material assessment and selection processes[1]. Changes in the manufacturing process when using containers from different vendors change the extractable profiles. Evaluation of the toxicity of extractable compounds from the drug container closure systems is performed using a risk-based approach outlined by the Food and Drug Administration (FDA)[2,3]. This approach allows for patient population, route of drug administration, and the potential for interaction between formulation and container systems. For ophthalmic drug products (ODP), the degree of concern associated with the route of administration, and the likelihood of interaction between the liquid formulation and the packaging material has been defined as high.

The approach to evaluate the toxicity is to rely on available literature on the absorption, distribution, metabolism, and elimination (ADME) of the compound. If such information is not available, the compounds must be classified based on their structure using the Cramer classification approach. The Cramer classification classifies compounds into different classes:

- **Class I** – low toxicity
- **Class II** – intermediate toxicity
- **Class III** – significant toxicity

Known reactive functional groups that fall under Class III are aliphatic secondary amino-, cyano-, N-nitroso-, diazo-, triazeno-, quarternary N, strain-ringed lactones, epoxides, quinones, and α,β-unsaturated ketones[4]. Recently, Jenke[3] developed and justified a semiquantitative risk evaluation matrix used to determine the amount and testing necessary to establish whether the container is suitable for its intended use.

An extractable study, like any untargeted study, attempts to identify compounds without prior knowledge of the sample contents. Compounds showing strong fragmentation in electron ionization (EI) mode will increase the number of unknowns detected. However, by applying an orthogonal soft ionization technique, such as chemical ionization (CI), highly labile compounds amiable to softer ionization can be more easily identified. Experiments performed on accurate mass instrumentation while using custom-made accurate mass databases would invariably increase the number of compounds identified. Accurate mass instrumentation would also facilitate the identification of unknowns by formula generation of molecular peaks and their fragments.

This Application Note shows that a high resolution Agilent 7200 GC/Q-TOF system was used for extractable studies on four different drug container systems. The sample preparation aimed to analyze only the semivolatile compounds. The chromatograms were acquired in both EI and CI modes. Using the Agilent MassHunter Unknown Analysis software, the EI spectra were automatically deconvoluted and matched to NIST 14 library. The data acquired from the extracts of four different containers were visualized using a Venn diagram, which is a feature of Agilent Mass Profiler Professional (MPP) software. Compounds that were tentatively identified due to low EI library matching scores were converted to a custom database. These low custom databases were used to mine CI accurate mass data to confirm some of the compounds. Figure 1 shows the workflow used for this study.

Workflow

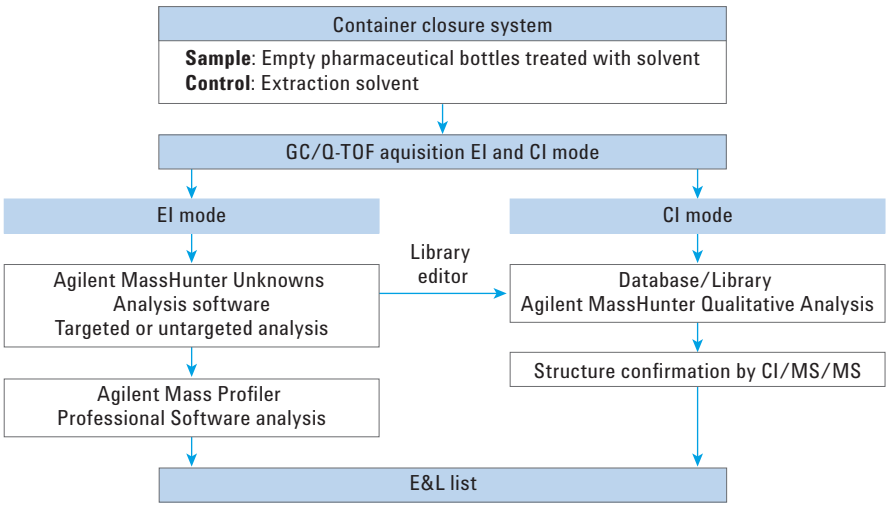

Figure 1. Extractable and leachables workflow for analysis of semivolatiles using accurate mass GC/Q-TOF system.

Experimental

HPLC grade *n*-hexane, 99%, was purchased from RCI Labscan (Thailand).

Sample preparation

Four empty formulation bottles were purchased locally. Two were made of low-density polyethylene (containers 1 and 2), and two were made of polyethylene (containers 3 and 4). Five milliliters of *n*-hexane was added to each, and sonicated for 1.5 hours. After sonication, the solvent was analyzed. *n*-hexane extraction solvent was used as a solvent blank.

Data acquisition and processing

The following Agilent software was used for data acquisition and processing:

- Agilent MassHunter Acquisition Software (B.07.02)
- Agilent MassHunter Qualitative Analysis Software including PCDL Manager Standalone tool (B.07.00).
- Agilent MassHunter Quantitative Analysis Software including Library Editor and Unknown Analysis standalone tools (B.07.01).
- Agilent Mass Profiler Professional Software (Ver. 13.1)

Instrument parameters

Table 1 shows the instrument parameters used in this analysis.

Data analysis

EI source data analysis

The data files were processed using MassHunter Unknowns Analysis software for deconvolution, and matched against the NIST 14 library match. A match score of >80 was used to select identified compounds.

Table 1. GC/Q-TOF instrument parameters used in this experiment.

GC conditions	
GC	Agilent 7890A
Injection port	Multimode inlet (MMI)
Mode	Splitless
Septum purge flow	3 mL/min
Inlet program	70 °C (0.2 minutes) to 325 °C (7 minutes) at 600 °C/min
Liner	Ultra Inert Splitless, single taper, glass wool (p/n 5192-3163)
Carrier gas	Helium
Flow	1.3 mL/min (constant)
Purge flow to split vent	60 mL/min at 2.73 minutes
Gas saver	20 mL/min at 3 minutes
Oven program	50 °C (3 minutes) to 320 °C (7 minutes) at 6 °C/min
Equilibration time	1 minute
Run time	55 minutes
Columns	Agilent DB-5ms, 30 m × 250 µm, 0.25 µm (p/n 122-5532)
Injection volume	2 µL
MS conditions	
MS	Agilent 7200
Tune	Autotune
Transfer line	280 °C
MS source (EI and CI)	300 °C
MS quad	175 °C
Mass range	55 to 700 amu
Acquisition rate	5.00 spectra/sec
Election ionization	
EI emission current	35 µA
EI electron energy	70 eV
Chemical ionization	
CI emission current	240 µA
CI gas flow	20 % EPC
CI electron energy	115 eV
Mode	Positive
CI reagent gas	Methane
Collision cell EPC	Nitrogen, 1.5 mL/min

Creating an accurate mass EI library

The EI results with scores from 50 to 79 were exported to Library Editor Software to form a low score EI library. Scores ≥ 80 were exported to form a high score EI library. The library (in .xml format) contained compound information such as name, formula, retention time (RT), and spectra.

MPP analysis

The EI data were reprocessed using the Unknowns Analysis tool to deconvolute and match spectra and RT using the accurate mass, high score EI library. This step helped to filter the results to be exported into MPP software.

Agilent Personal Compound Database (PCD)

A custom database of literature-reported extractables and leachables was created. The database entries consisted of chemical formula, accurate mass, and CAS ID.

CI data analysis

The CI data were processed in MassHunter Qualitative Analysis software using the Find by Formula algorithm with possible adducts $[M+H]^+$, $[M+C_2H_5]^+$, and $[M+C_3H_5]^+$. The low score EI library was used as a formula database. The CI data were also searched for other extractables using the custom user-created extractable library.

Structure elucidation using CI/MS/MS

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

Toxicological evaluation

In silico prediction for Cramer classification was performed using Toxtree v 2.6.13[5]

Results and Discussion

EI mode analysis

The data acquired in EI mode were processed using the Unknowns Analysis tool for chromatographic deconvolution and library matching. Although height-based filtering of compounds can be performed by Unknowns Analysis Software, no filtering was applied. Benzene, (1-ethylundecyl)-, also called 2-phenyl tridecane, is an extractable compound identified at 27.3 minutes (Figure 2) in container 2. The Extracted Ions Chromatograms (EIC) of this deconvoluted component coeluted, and had the same peak shape (Figure 2C) while its EI spectrum had a unit mass (NIST) library match with a score 85.3.

A Components results

Component RT	Compound Name	Match Factor	Formula	CAS#
27.0796	Heptadecane, 3-methyl-	81.6	C18H38	6418-44-6
27.1722	Heptadecane, 3-methyl-	86.7	C18H38	6418-44-6
27.3067	Phthalic acid, hept-4-yl isobutyl ester	84.2	C19H28O4	1000356-78-3
27.3317	Benzene, (1-ethylundecyl)-	85.3	C19H32	4534-52-5
27.6710	Heneicosane	83.9	C21H44	629-94-7
27.7912	Nonadecane	88.2	C19H40	629-92-5

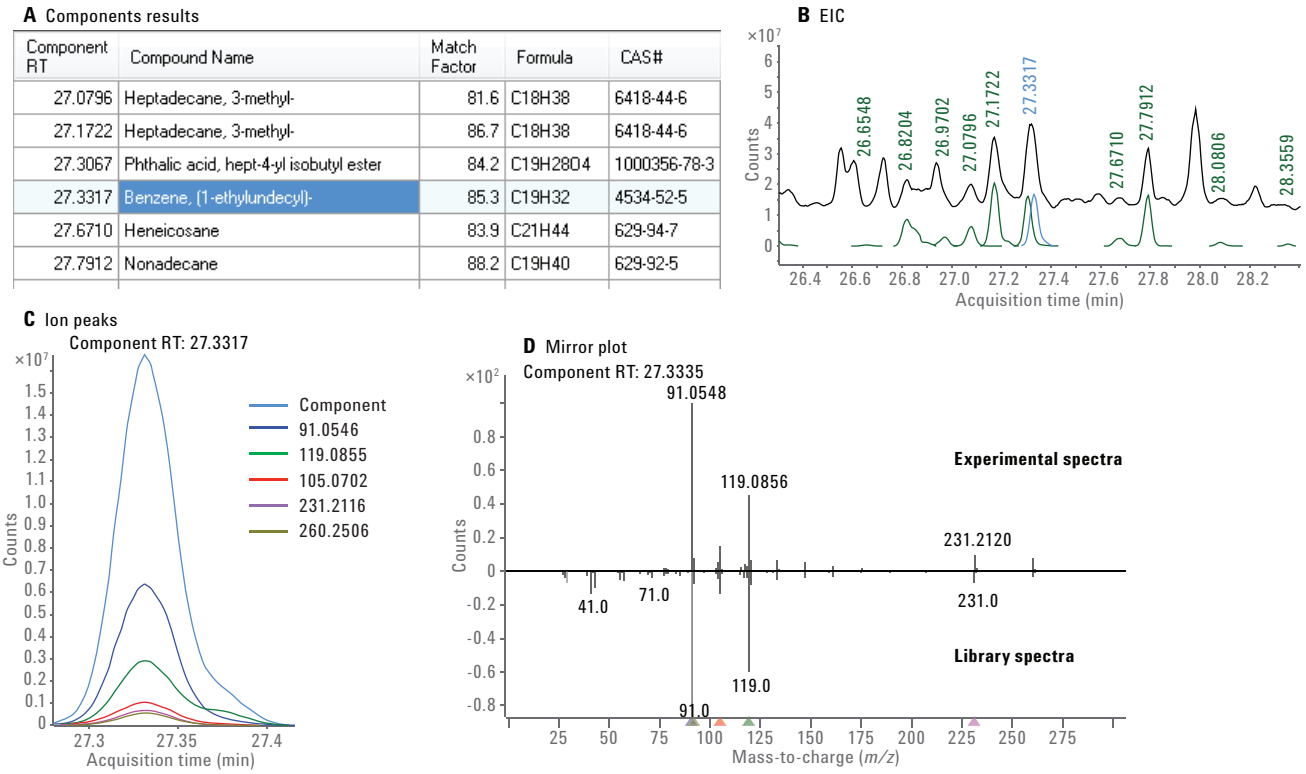

Figure 2. Agilent MassHunter Unknown Analysis Software identified Benzene (1-ethylundecyl), by deconvolution and NIST library search. Component results (A), deconvoluted component chromatograms (B), overlay of EICs of individual component (C), and mirror plot of deconvoluted component spectrum and library hit (D).

Compounds with acceptable spectral matches with a score >80, were exported to MPP software for data interpretation. The compound distribution across samples was visualized using a Venn diagram in MPP software. Figure 3 shows the Venn diagram of the distribution of identified extractables from the four different container systems. The Venn diagram shows 22 compounds (identified) commonly found in all containers independent of the manufacturer. Comparison of extractables distribution shows more than 200 compounds present in containers 3 and 4. Approximately 150 compounds have been extracted from containers 1 and 2, wherein container 2 shows the lowest number of compounds. The study shows that containers 3 and 4 are less suitable as a container system, compared to containers 1 and 2. Table 2 shows a selected list of extractables identified in container 2, and the compounds found common to all containers.

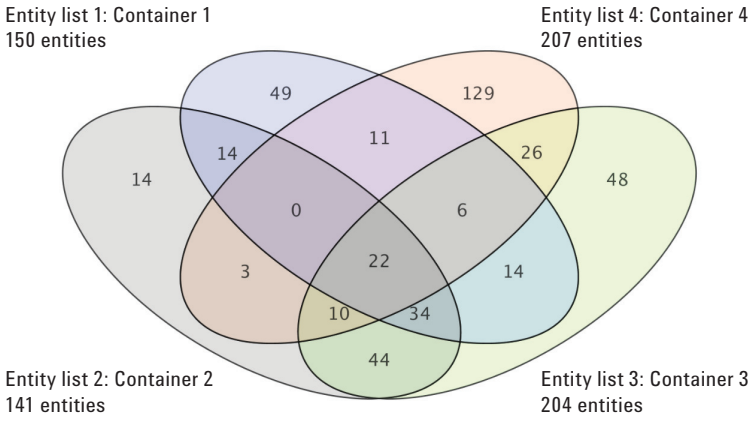

Figure 3. An Agilent Mass Profiler Professional Venn diagram showing the distribution of EI identified compounds among the four containers.

Table 2. Selected list of compounds identified in extract of container 2 (14 compounds from the Venn diagram in Figure 3) and compounds common between all samples.

Extractable compound list found only in container 2
Octadecane
Benzene, (1-methylundecyl)-
Pentadecane, 2,6,10,14-tetramethyl-
Heptadecane, 3-methyl-
Benzene, (1-ethylundecyl)-
Eicosane, 2-methyl-
1,2-Benzenedicarboxylic acid, *bis*(2-methylpropyl) ester
Heneicosane
Nonadecane
Tetradecane
Pentacosane
Commonly found extractables in all containers
Pentadecane
trisiloxane, 1,1,1,5,5,5-hexamethyl-3-[(trimethylsilyl)oxy]-
Heptadecane, 3-methyl
Pentadecane, 2,6,10-trimethyl-
Nonane, 4,5-dimethyl
Tetracosane, 11-decyl
Pentadecane, 8-hexyl
(E)-Hex-3-enyl (E)-2-methylbut-2-enoate
Dodecane
3-Ethyl-3-methylnonadecane
Cyclohexasiloxane, dodecamethyl-
Dodecane, 2,6,10-trimethyl
Octane, 3,5-dimethyl-
Tridecane
Nonane, 5-(1-methylpropyl)-
Tridecane, 6-methyl-
Sulfurous acid, 2-ethylhexyl isohexyl ester
Cycloheptasiloxane, tetradecamethyl-
6,6-Diethylhoctadecane

CI mode analysis

The CI data acquisition mode increased the number of identified compounds that were not considered as identified after EI data acquisition due to low library matching scores. To confirm the presence of low score EI results, a library of low score hits from the EI data analysis were made using the Library Editor Software. For example, benzoic acid, 4-ethoxy-, ethyl ester had an EI matching score of 67 of 100 points because it was buried in the chemical noise. The compound was confirmed using the CI mode with a 0.58 ppm mass error for the most abundant molelcular ion (Table 3A). Additionally, a database of EI high score results could be made to mine CI data to confirm EI results, as previously shown[6]. To identify more compounds, an in-house built database was compiled containing extractables reported in the literature. Accurate mass information helps to identify compounds by formula. Table 3B shows the list of contaminants identified by data mining the CI files, using the literature-derived custom database. Accurate mass CI data can be used to distinguish between compounds that cannot be determined using CI mode unit resolution single quad data. For example, *m/z* 194.094 and 194.0577 have been identified as two different compounds: benzoic acid, 4-ethoxy-ethyl ester and 1,3-benzenedicarboxylic acid, 1,3,-dimethylester, respectively. This identification of compounds having the same nominal *m/z* requires high resolution accurate mass data.

Table 3. CI-MS data were searched against low score EI results (A) and extra compounds detected using in-house databases (B) from container 2. The mass error refers to the most abundant molecular ion.

A) Extractable by low score EI databases			B) Extractable by literature-derived custom databases		
ID	Mass	Mass error (ppm)	ID	Mass	Mass error (ppm)
(-)-Aristolene	204.1880	1.4	1,2-(1,8-napthalenediyl)benzene	202.0784	-0.6
(2S,6R,7S,8E)-(+)-2,7-Epoxy-4,8-megastigmadiene	192.1510	-3.3	1,3-Benzenedicarboxylic acid	166.0266	0.1
(3S,4aR,8aR)-1,1,3,6-Tetramethyl-3-vinyl-3,4,4a,7,8, 8a-hexahydro-1H-isochromene	220.1830	3.6	1,3-Benzenedicarboxylic acid, 1,3-dimethyl ester	194.0577	0.9
(4aS,8aS)-8-Isopentyl-4,4,7,8a-tetramethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	262.2660	4.9	1-Heptadecanol, 1-acetate	298.2865	2.4
1,1′-Bicyclooctyl	222.2350	1.6	1-Heptene	98.1094	1.1
1,2-Dimethoxy-4-(adamantyl-1)benzene	272.1780	-2.5	1-Octene	112.1252	-0.1
1,3-di-iso-propylnaphthalene	212.1570	3.8	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethyl)-, methy	314.2233	4.1
1,3-Dimethyl-5-*n*-decylcyclohexane	252.2820	2.6	2,5-Cyclohexadiene-1,4-dione	108.0207	3.7
1,4,5,8-Tetramethylnaphthalene	184.1250	0.5	2-Cyclohexen-1-one, 3,5,5-trimethyl-	138.1043	1.1
10,18-Bisnorabieta-5,7,9(10),11,13-pentaene	238.1720	1.9	2-Cyclopenten-1-one, 2-methyl-	96.0572	3.1
13,15-Octacosadiyne	386.3910	3.2	2-Hexanone	100.0884	4.6
1-Naphthalenol, 1,2,3,4,4a,7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-	222.1980	1.5	2-Naphthol	144.0569	4.4
1-Nonadecene	266.2970	1.6	2-Nonenal, 2-pentyl-	210.1978	2.6
1R,2c,3t,4t-Tetramethyl-cyclohexane	140.1570	3.4	2-Propanol, 1-ethoxy-	104.0840	-2.6
1-Undecene, 9-methyl-	168.1880	0.8	3(2H)-Furanone, 5-(1,2-dihydroxyethyl)-	144.0424	-0.9
2,2,3-Triethyloxirane	128.1200	1.6	3-Octanone	128.1198	2.5
2,2′-Dimethylbiphenyl	182.1100	3.9	4,7-Methano-1H-indene, 3a,4,7,7a-tetrahydro-	132.0939	-0.2
2H-1-Benzopyran-5-carboxylic acid, 3,4-dihydro-2-methyl-4-oxo-	206.0580	0.3	4-Methylbenzophenone	196.0884	1.9
2-Methyl-6-methyleneoct-7-en-4-one	152.1200	0.7	4-Octylphenol; *p*-Octylphenol	206.1675	-1.9
Benzoic acid, 4-ethoxy-, ethyl ester	194.0940	-0.6	Benzoic acid, 4-ethoxy-, ethyl ester	194.0941	0.9

Confirmation by CI/MS/MS

The accurate mass CI/MS/MS was used to confirm the structures for diethyl phthalate detected in EI. Phthalates, in general, have a missing molecular peak and a dominant fragment peak at *m/z* 149. Therefore, it is a challenge to assign correct identification to phthalates. The GC/Q-TOF provides not only accurate mass for the molecular and fragment ions, but also for MS/MS fragments, which enables formula generation for product ions. Figures 4A and 4B show the CI and CI/MS/MS analyses of diethyl phthalate. The CI/MS/MS spectra were interpreted (Figure 4C) using ACD software (ACD Labs, Toronto), and the product ions of precursor at *m/z* 223.0937 were identified. The characteristic fragment peak of *m/z* 149.0239 for phthalates was also observed (Figure 4B). The fragments and their accurate mass helped to confirm the compound identity.

Table 4. Cramer classification of compounds from four different containers exceeding 20 µg/mL concentration.

Compound	Container	Cramer classification
5,5-Diethylpentadecane	4	Class I (Low)
Hexadecane	1,2,3,4	Class I (Low)
3-Methyloctacosane	4	Class I (Low)
1-Hexadecanol	4	Class I (Low)
Eicosane	4	Class I (Low)
cis-11-Eicosenamide	4	Class III (High)
1-Decanol, 2-hexyl-	4	Class I (Low)
3,3,13,13-Tetraethylpentadecane	4	Class I (Low)
1-Hexacosanol	4	Class I (Low)
2-Methylpentacosane	4	Class I (Low)
Cyclotetradecane	4	Class I (Low)
3,3-Diethylpentadecane	4	Class I (Low)
2-Methylheptacosane	4	Class I (Low)
Cyclopentane, undecyl-	4	Class I (Low)
1-Octadecanol	4	Class I (Low)
3-Methyltriacontane	4	Class I (Low)
5,5-Diethylheptadecane	4	Class I (Low)
Octacosanol	4	Class I (Low)
3-Methylhexacosane	4	Class I (Low)
3,3-Diethylheptadecane	4	Class I (Low)
Heptadecane	1,2,3	Class I (Low)
Dodecane	1	Class I (Low)
Tetradecane	1	Class I (Low)
2,2,4,4, tetramethyloctane	1	Class I (Low)
Octadecane	3	Class I (Low)

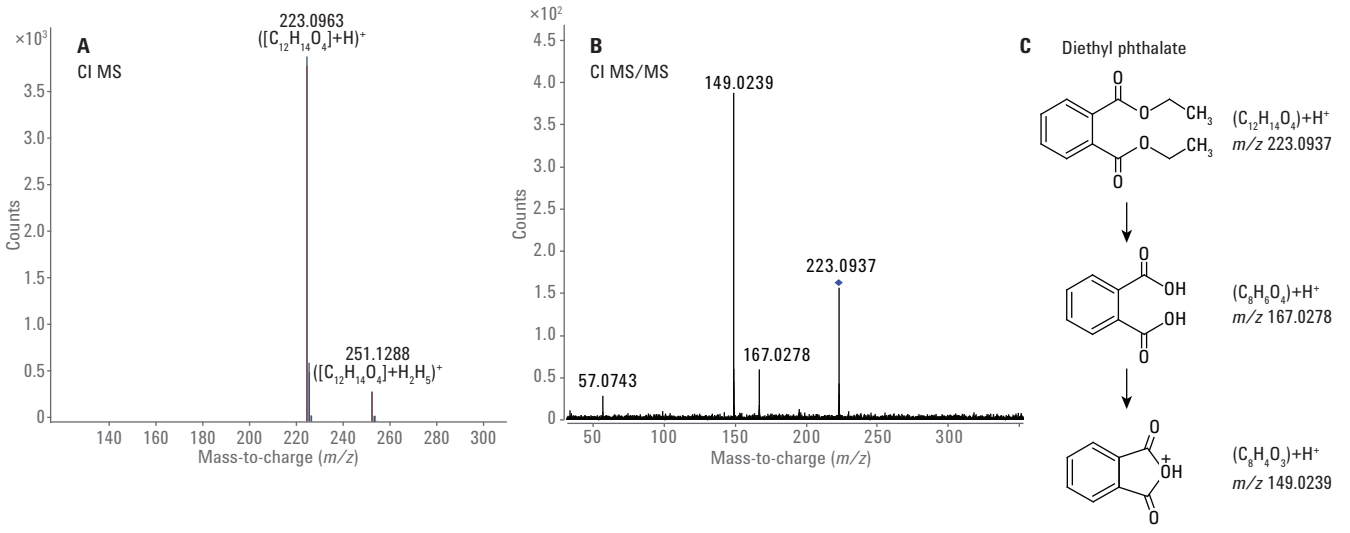

Figure 4. Structure elucidation of diethyl phthalate.

Toxicity evaluation

The concentration of compounds from all containers detected by both EI and CI mode were determined semiquantitatively using triphenyl phosphate as an internal standard[7]. In the evaluation of ophthalmic drug containers, extractables present at >20 µg/mL would be considered for risk evaluation. In this study, compounds exceeding 20 µg/mL were categorized based on the Cramer classification to determine their potential toxicity, assuming that the containers are being used for ophthalmic drug products. Table 4 shows the results. One high risk compound was found in container 4, which also included many more extractables than the other containers. Therefore, container 4 is a less suitable choice for formulation.

Conclusion

An Agilent 7200 GC/Q-TOF system was used to perform qualitative screening and identify extractables from four different container closure systems. An extended number of identified compounds was obtained by acquiring data in both EI and CI mode. Agilent MassHunter Mass Profiler Professional software provided a versatile tool in automated data mining workflows. Unique and common compounds within the different group of samples were determined. EI spectral data were identified using the NIST 14.0 library. Compounds with low library match scores were stored in a custom library to mine CI data, providing an increase of 14 % in compounds identified in one of the samples. The CI data files were also used to search against an in-house database by formula containing common extractables known from literature. Additionally, CI/MS/MS was performed to confirm the identity of these compounds. The Cramer classification and MPP distribution plot of the detected extractables show that container 2 had the lowest number of extractables and no significant amounts of Cramer Class III compounds.

References

1. Mire-Sluis, A.; *et al.* Extractables and Leachables. Challenges and Strategies in Biopharmaceutical Development. *BioProcess International* Feb **2011**.

2. Guidance for Industry, Container Closure Systems for Packaging Human Drugs and Biologics. U.S. Department of Health and Human Services, Food and Drug Administration: Rockville, MD, May **1999**.

3. Jenke, D. Development and Justification of a Risk Evaluation Matrix to Guide Chemical Testing Necessary To Select and Qualify Plastic Components Used in Production Systems for Pharmaceutical Products. *PDA Journal of Pharmaceutical Science and Technology* **2015**, *69*, 677–712.

4. Li, K.; *et al.* Creating a Holistic Extractables and Leachables (E&L) Program for Biotechnology Products. *PDA Journal of Pharmaceutical Science and Technology* **2015**, 69590–619.

5. IdeaConsult Ltd. Toxtree (Estimation of Toxic Hazard—A Decision Tree Approach) v.2.6.13. http://toxtree.sourceforge.net/.

6. 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 Technologies Application Note*, publication number 5991-6688EN, **2016**.

7. 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* **2012**, 50206–212.

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