Extractables and Leachables Detected in Ophthalmic Drug Products

Detection and Identification Using High-Resolution LC/MS/MS

Application Note

Pharmaceuticals

Author

Syed Salman Lateef

Agilent Technologies, Ltd.

India

Abstract

Compounds leaching from container closure systems can cause contamination to drug substances or products. As part of a risk evaluation, it is necessary to identify these compounds and ensure that the drugs are suitable for their intended use. Typically, such compounds are present at low concentrations and are masked by the drug matrix. Therefore, highly sensitive and selective methods are required to detect and identify these compounds. Quadrupole time-of-flight (Q-TOF) mass spectrometers are suitable for this purpose due to their high resolution and accurate mass measurement capabilities. An Agilent 1290 Infinity LC System and an Agilent 6540 Q-TOF system combined with statistical analysis software were used to detect and identify extractable and leachable (E&L) impurities from ophthalmic drug products. Statistical data analysis was performed using Agilent Mass Profiler Software (MP) to determine the compounds present in the samples compared to controls. The database search tool within MP software helped to identify E&Ls using a customized accurate mass database. For the identification of unknown E&Ls, MS/MS data together with the structure prediction software, Molecular Structural Correlator, was used. In this study, 50 compounds were detected in each of the E&L samples.

Agilent Technologies

Introduction

Drug substances and products may become contaminated by chemical compounds from primary and secondary packaging materials. Compounds that can be extracted directly from container closure systems are called extractables, while compounds found within the formulation are called leachables, and are often a subset of extractables. The US Food and Drug Administration (FDA) has issued guidance on container closure systems for packaging human drugs and biologics[1], due to the potential risk that impurities pose to consumer health. The guidance document includes protection, safety, and compatibility guidelines. In general, profiling extractables and leachables (E&Ls) is a complex analytical challenge due to the following factors:

- The wide range of materials used for the construction 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. Recent Application Notes have demonstrated the effectiveness of GC/MS[2,3,4] methodologies. Also, Norwood *et al.*, have reviewed numerous LC and LC/MS methods for the analysis of E&Ls[5].

During screening for impurities, it is likely that E&Ls are present in the blank solvent originating from its container. Typically, eliminating the compounds detected in the blank solvent with a simple background subtracted during the data process will also remove potential E&Ls from the samples. Therefore, it is important to perform sample-to-sample comparison to retain compounds based on their intensity differences. Agilent Mass Profiler (MP) is a statistical program that helps to compare similarities and differences between data sets. These data sets may be two individual samples, replicates of a single sample, or replicates of two sample groups. The identification of compounds as part of untargeted analysis uses a combination of database searching and molecular formula generation based on high resolution mass spectrometry.

In this study, an Agilent 1290 Infinity UHPLC system with an Agilent 6540 Q-TOF mass spectrometer was used to separate, detect, and identify E&Ls. Agilent MP was used together with a (user-generated) customized E&L database. For both known and unknown compounds, MS/MS spectra were matched with theoretical best structures using Agilent Molecular Structure Correlator (MSC) Software.

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

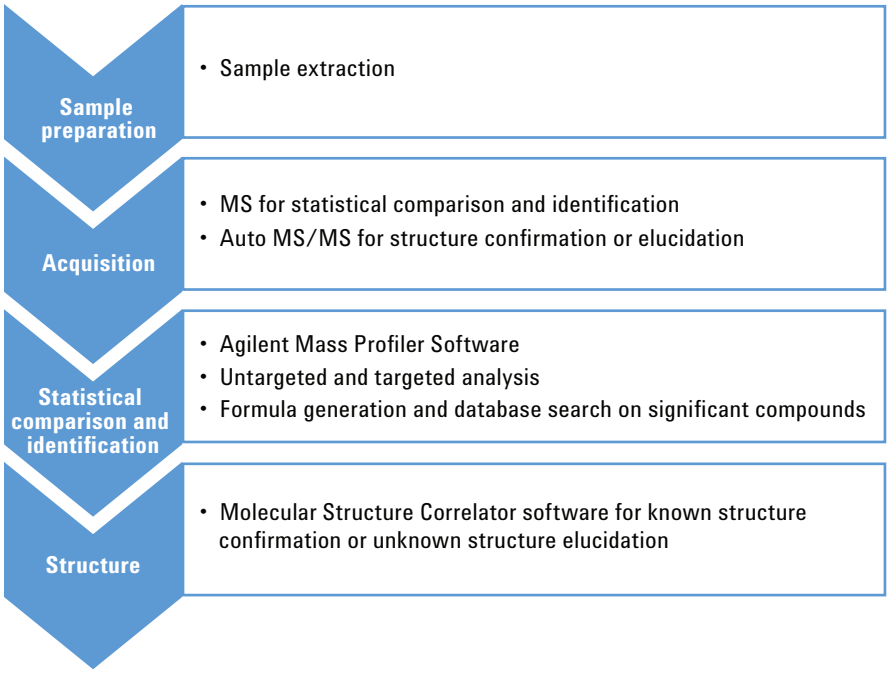

Figure 1. Data analysis workflow using Agilent MassHunter Acquisition, Agilent Mass Profiler, and Agilent MassHunter Molecular Structure Correlator Software used in this study.

Experimental

Table 1 lists the chemicals used to create a personnel compound database. They were purchased from Sigma-Aldrich. MS-grade methanol and deionized water (Milli-Q, Millipore) were used in the study.

Sample preparation

Extractable sample

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

Leachable samples

The first leachable sample, 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 nonstressed sample, was the ophthalmic drug formulation stored at recommended conditions, and also injected directly into the system.

All samples were analyzed in triplicate.

Instrument setup

An 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:

- Agilent 1290 Infinity Binary Pump (p/n G4220A)
- Agilent 1290 Infinity ALS Thermostat (p/n G4226A)
- Agilent 1290 Infinity ALS Thermostat (p/n G1330B)
- Agilent 1260 Infinity thermostatted column compartment (p/n G1316A)

Table 1. 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 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	MS and Auto MS/MS
Segments and CE (V)	Scan segment no. CE (V) 1 5 2 15 3 30
Polarity	Positive and Negative
Mass range	50–1,300 *m/z*
Reference ions	Positive: 121.0507 and 922.0098 Negative: 112.9856 and 1033.9881

Table 1 shows the LC and MS conditions used in these analyses. The ion source conditions were optimized to enable the sensitive detection of E&Ls. LC/MS analysis was performed in both positive and negative ionization modes.

Data analysis

The following software was used for data analysis:

- Agilent MassHunter Data Acquisition B.06.01
- Agilent MassHunter Qualitative Analysis B.07.00
- Agilent MassHunter PCDL Manager B.07.00
- Agilent MassHunter Mass Profiler B.07.01
- Agilent MassHunter Profinder B.06.00
- Agilent MassHunter Molecular Structure Correlator Software B.07.00

Personal Compound Database and Library (PCDL)

An user-generated, custom database, containing E&Ls reported in the literature, was created using both molecular formulae and structure. This database contained 1,840 compounds.

Agilent MassHunter Mass Profiler Software

The experimental group was either the extractable or leachable stressed sample, while the control group was either solvent blank or leachable nonstressed sample. A statistical analysis and fold change was performed on the replicate groups. Compound occurrence frequency with >50 % in at least one group was considered for fold change analysis. The two-way batch comparison was performed with the criteria of a fold change of greater than 2.0, which is a 2x higher abundance in the experimental group compared to the control group. 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 the custom E&L database were used for Batch Target Feature Extraction analysis by MassHunter Profinder Software. Profinder software pictures the extracted ion chromatograms from the processed data as color-coded groups, and displays peak integration. The integration of the peaks was verified, and the results exported to MP for statistical analysis (as described above).

MSC analysis

MSC analysis was performed to identify unknown compounds that were not included in the PCDL. The auto MS/MS data were processed in Agilent MassHunter Qualitative Analysis software using Find by Formula. The differential list of compounds generated by Mass Profiler was used as the formula source within the Find by Formula algorithm to extract MS/MS fragments from selected precursors of the compounds in the differential list. The results of auto MS/MS fragments were exported to the MSC software. Here, the PCDL database was chosen as the structure source for known structure confirmation, and the online database ChemSpider was selected as the structure source for unknown analysis.

Semiquantitation

A calibration curve was prepared using dioctyl phthalate standard in extraction solvent. The concentration range was from 1 pg/µL to 50 ng/µL. The calibration curve was used to estimate an approximate concentration of leachables containing aromatic rings. Since no true standards were used for each compound, an error of 30 % was assumed for the concentration values.

Results and Discussion

Data comparison and identification using Agilent 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. Dioctyl phthalate and dinonyl phthalate were found in the extractable sample and the solvent blank control, but exhibited 0.05 and 2 fold change in intensity difference, respectively. A simple blank subtraction would eliminate both of these compounds from the sample. Due to the criteria of a fold change of >2.0 used by the Mass Profiler software, the presence of dinonyl phthalate was confirmed in 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 were detected in the leachable study. The negative ionization mode made a 30 % contribution to this number of detected compounds.

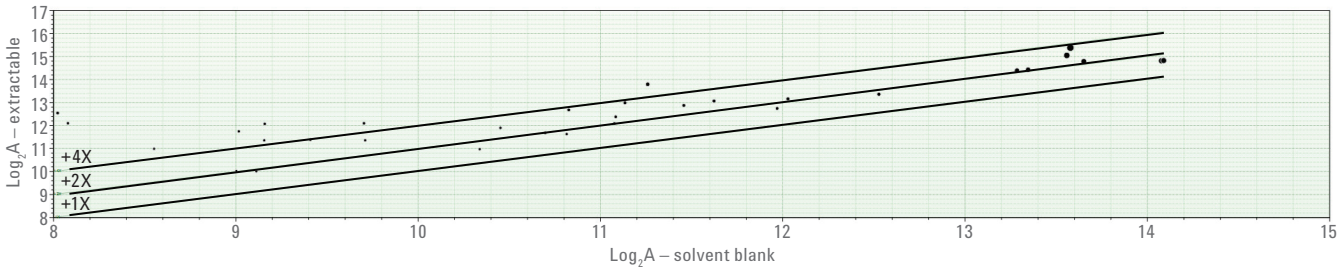

Figure 2. An Agilent Mass Profiler plot of logarithmic abundance of extractable compounds versus solvent blank. A one, two, and four-fold abundance line marks the abundance threshold for experimental compounds above the solvent blank control.

The identification functionality within MP software was used to identify the compounds based on their accurate mass. Using MassHunter, it is easy to create your own PCDLs with existing (literature derived) data. Figure 3 is an example of a user-generated database, showing the identity, isotopic distribution, and structure of dinonyl phthalate identified in the empty ophthalmic bottle extract. Eleven extractable compounds were identified with the user-generated database. Table 2 shows the list of identified extractable and leachable compounds from positive and negative ionization modes.

Targeted leachable analysis

Untargeted analysis helps to identify unknown newly generated compounds (degradants or reactants) that may be formed due to stress conditions. Such compounds would also be potential leachable compounds (Table 2). However, the leachable samples can also be 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 nonstressed samples, the concentration of the found compounds do not change (lie on 1x abundance line). The abundance plot reveals significant change in concentration, due to heat stress, of only three compounds.

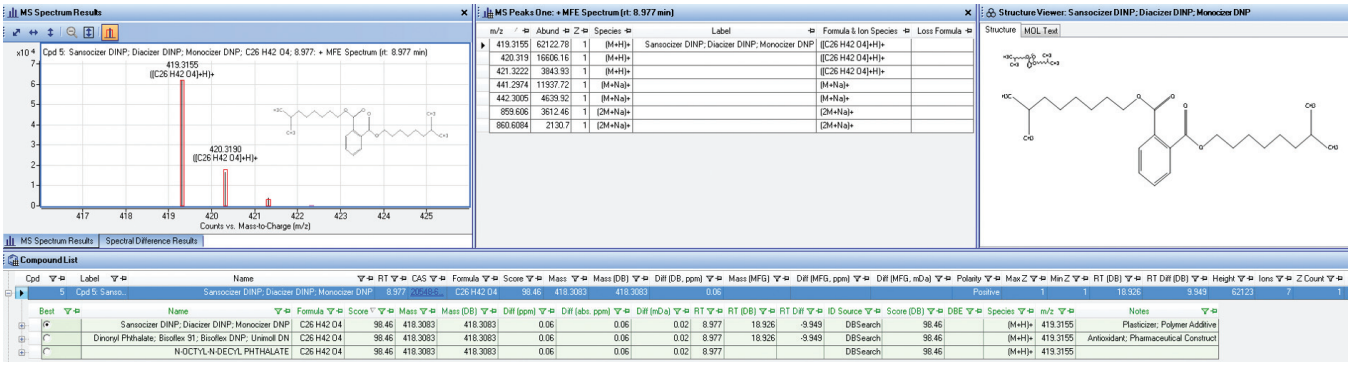

Figure 3. Database results identified dinonyl phthalate. The results also show that isomers diisononyl phthalate and *n*-octyl-*n*-decyl phthalate are also possible targets.

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

Compound	Mass error (ppm)	CAS number	Sample type
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

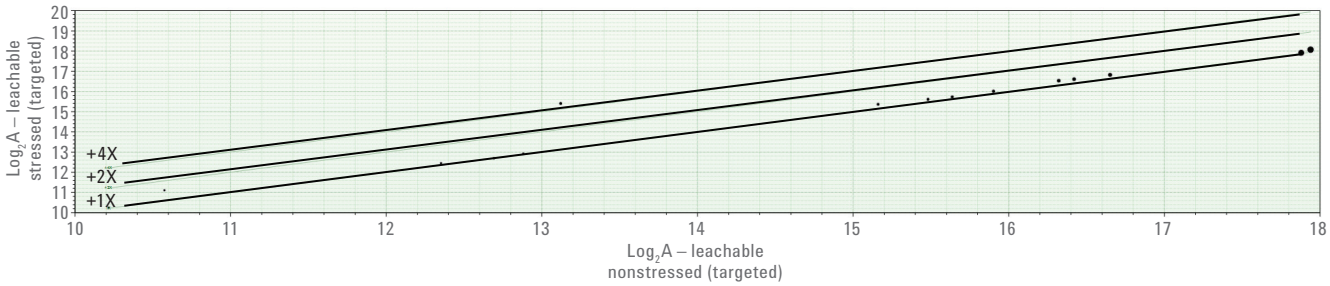

Figure 4. Agilent Mass Profiler results showing a plot of logarithmic abundance of leachable compounds stressed versus leachable nonstressed, targeted compounds. Several compounds lay on the one fold abundance line marks.

Untargeted compound identification, compound confirmation, and structural elucidation

MS/MS spectra acquired from standards were used for both structure confirmation and unknown structure elucidation. However, in E&L studies, reference standards may not be readily available. Therefore, software assisted structure elucidation offers a viable means of determining the chemical structures of unknown compounds. Agilent MSC software facilitates the tentative identification of compounds for which reference standards are not available. MSC software correlating accurate mass/formula of experimental MS/MS fragments with *in silico* fragment ions forms a structure database, and proposes possible matches. The sources of possible structures are the user-generated custom PCDL, ChemSpider, or PubChem databases.

The PCDL database structures were used for compound confirmation, while web-based databases were used to determine unknown compounds. Figure 5 shows confirmation of the compound di-isononyl phthalate (DINP, a potential endocrine disruptor), and associated isomers.

Other compounds confirmed were erucamide, isopropyl-9H-thioxanthene-9-one, and irgacure 907 (a photo-initiator used as photo-polymerization). Some of the unknown compounds identified as E&Ls 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)
- Derivative of 3,4-O-isopropylidene-D-ribitol (0.3 ppm, MSC score: 84)

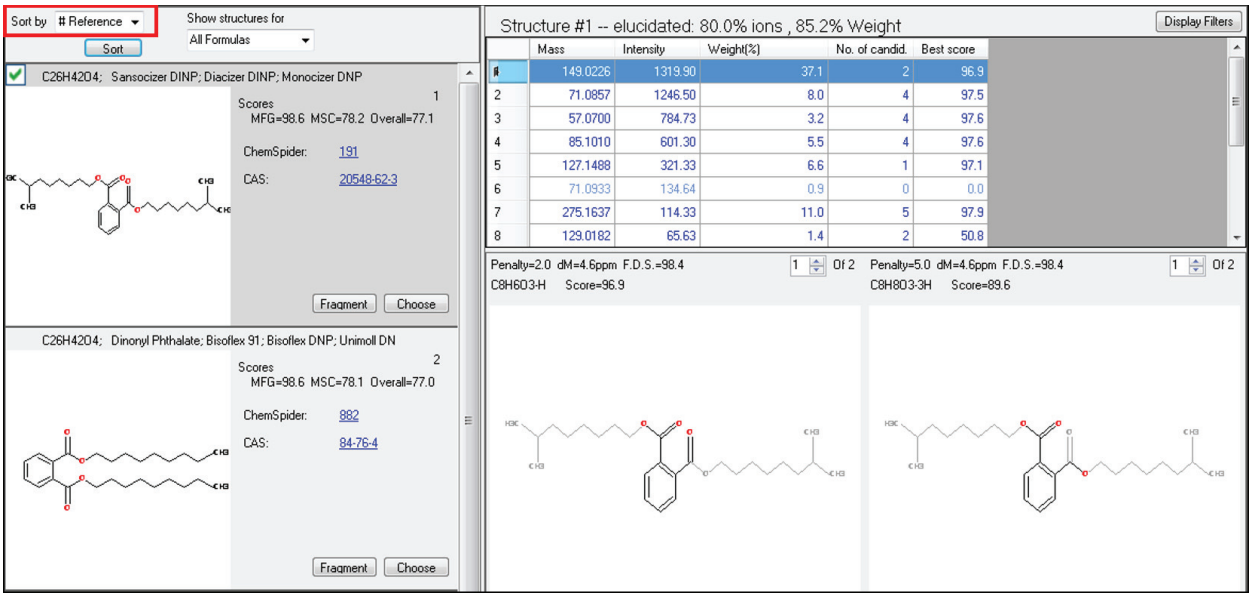

Figure 5. Agilent Molecular Structure Correlator Software for confirmation of di-isononyl phthalate (DINP). Experimentally observed MS/MS fragments match theoretical structure fragments, and results are sorted based on maximum number of reference citations.

Semiquantitation

Semiquantitation was performed using an external phthalate standard to obtain a rough estimate of the concentration of leachables in the leachable stressed sample. The Analytical Evaluation Threshold (AET) is the threshold above which the analyst would report the need for a toxicological assessment. According to the latest PQRI working group report[6], AETs for ODPs are reported based on concentrations (ppm). Leachables found in excess of 1 ppm are to be reported to regulatory authorities, above 10 ppm are identified, while above 20 ppm are used for risk assessment. The semiquantitative results of leachable stressed samples are shown in Table 3. The results show that three leachables: diisononyl phthalate, *n*-dioctyl phthalate, and erucamide, exceed the 1 ppm level, and are required to be reported as part of the extractable and leachable study.

Table 3. Semiquantitative estimation of leachables in stressed ophthalmic solution.

Leachables	ppm ± 30 % (µg/mL)
Diisononyl phthalate (DINP)	1.4 ±0.4
n-Dioctyl phthalate (DOP, DEHP)	2.5 ± 0.7
Phthalic anhydride	0.14 ± 0.04
Methyl-2-benzoylbenzoate	0.11 ± 0.03
Irgacure 907	0.02 ± 0.005
Hexyl amine	0.04 ± 0.01
Ionox 100	0.03 ± 0.01
Erucamide	1.68 ± 0.50
Glycerol dilaurate	0.08 ± 0.02
1,2-Benzenedicarboxylic acid, 1,2-*bis*(8-methylnonyl)ester	0.16 ± 0.05
Myristyl dimethylamine oxide	0.0009 ± 0.0003
Acetic acid, propyl ester	0.10 ± 0.03

Conclusion

This Application Notes shows that 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. The data were rigorously analyzed in a two-step workflow. The first step was a statistical comparison to differentiate E&L compounds from control samples using Agilent Mass Profiler Software. The compounds that differed significantly between samples were identified using an accurate mass PCDL database. In the second step, the analysis of both known and unknown compounds was performed using MSC software. This enabled the confirmation of known compounds, and 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. The semiquantitation results of the leachables study show that three 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.

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. Wong, D. M.; Firor, R. L. Analysis of Extractable/Leachable Compounds from Transdermal Patches Using GC/MSD Systems, *Agilent Technologies Application Note*, publication number 5991-5605EN, **2015**.

3. Wong, D. M.; Firor, R. L. Analysis of Extractable/Leachable Compounds From Plastic Intravenous Bag Sets Using GC/MSD Systems, *Agilent Technologies Application Note*, publication number 5991-5616EN, **2015**.

4. Wong, D. M.; Firor, R. L. Analysis of Extractable/Leachable Compounds from Generic Liquid Drug Formulations Using GC/MSD Systems, *Agilent Technologies Application Note*, publication number 5991-5632EN, **2015**.

5. Norwood, D. L.; *et al.* HPLC and LC/MS Analysis of Pharmaceutical Container Closure System Leachables and Extractables. *Journal of Liquid Chromatography & Related Technologies* **2009**, 32, 1768–1827.

6. Houston, C. T. The PQRI PODP Approach: Understanding Threshold Concepts and Their Application To Different Routes of Administration. *Talk present at E&L Europe 2015*, November **2015**.

www.agilent.com/chem

Published in the USA, May 1, 2016
5991-6828EN

Agilent Technologies