Application of an Accurate Mass Database and Library for the Screening and Verification of Extractables and Leachables in Pharmaceutical Drug Products

Application Note

Pharmaceuticals

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Abstract

This Application Note describes the creation of an accurate mass library for extractables and leachables (E&L), and its application in the E&L studies on ophthalmic drug products. Accurate mass spectra were acquired for a large collection of E&L compounds in either one or both ionization modes and for all relevant ion species.

Ophthalmic drug product (ODP) containers were extracted to study extractables while ODP formulation samples were treated under suitable conditions to study leachables. The samples were analyzed using an Agilent 1290 Infinity LC coupled to an Agilent 6540 Q-TOF LC/MS system for MS and untargeted (Data-Dependent MS/MS) acquisition. The LC/MS system was operated in positive and negative electrospray ionization mode using dual spray Agilent Jet Stream technology. This work demonstrates the data analysis workflow, and use of the Extractables and Leachables LC/MS Accurate Mass Personal Compound Database and Library (PCDL) for the screening and verification of E&L in drug products.

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Introduction

Extractables are chemical compounds that migrate from container closure systems 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 container closure system as a result of direct contact under normal process conditions or accelerated storage conditions[1].

Drug substances and products can be contaminated by these leachables migrating from primary and secondary packaging materials. Due to the potential impact of these impurities on patient health, the US FDA has issued guidance on container closure systems for packaging human drugs and biologics[2]. As part of a risk evaluation, it is necessary to identify these compounds and ensure that the drugs are suitable for their intended use[3]. Identifying extractables and leachables (E&L) is a complex task due to diversity in their physico-chemical properties and the availability of searchable database and libraries.

In this study, an Agilent 1290 Infinity LC and an Agilent 6540 Q-TOF LC/MS operated with an Agilent Jet Stream (electrospray ionization) source in both positive and negative ionization modes were used. Quadrupole time-of-flight (Q-TOF) mass spectrometers are suitable for this study due to their high resolving power and accurate mass measurement capabilities. The data were acquired using full scan MS-only followed by data-dependent MS/MS acquisition. Agilent Mass Profiler software was used to statistically compare the MS data of the extract and control samples. The compounds that differed significantly between samples and controls were identified using the Agilent Extractables and Leachables LC/MS Accurate Mass Personal Compound Database and Library (PCDL). The MS/MS spectra from the data-dependent acquisition were then matched against the curated MS/MS spectra in the PCDL. The Agilent LC/MS E&L PCDL contains a relevant list of more than 1,000 extractables and leachables

for database searching with more than 350 compounds with accurate mass LC/MS/MS spectra for compound confirmation. Compounds whose spectra were not matched were analyzed further using the Agilent Molecular Structural Correlator (MSC) software. This workflow enabled identification by accurate mass followed by confirmation by accurate mass MS/MS spectra.

Experimental

Reagents and standards

All reagents and solvents were LC/MS grade. Ammonium acetate and methanol were purchased from Sigma-Aldrich (Bangalore, India). Ultrapure water was produced using a Milli-Q system (Millipore, India). E&L analytical standards were either purchased from Sigma-Aldrich (St. Louis, MO, USA), or AccuStandards (New Haven, CT, USA).

Sample preparation

Extractable samples

Ophthalmic medicines formulated in plastic bottles were purchased from a local store in India. The contents were discarded, and the bottles were washed with water, then filled with extraction solvent (1:1 methanol: water), and incubated in an oven at 55 °C for 72 hours. The extracts were transferred to an HPLC vial 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 leachable samples, designated as the stressed samples, were obtained by heating the ophthalmic drug formulation in its container at 60 °C for 24 hours. The heated formulation after incubation, was sonicated for 10 seconds, and transferred to an HPLC vial and injected directly into the LC/MS/MS system. Other leachable samples, designated as the nonstressed samples, were created from the ophthalmic drug formulation stored at recommended conditions, then transferred to an HPLC vial and injected directly into the LC/MS/MS system. All samples were analyzed in triplicate.

LC/MS/MS Analysis of ophthalmic drug product (ODP) E&L analysis

A lack of standardization for E&L analysis has led to groups developing many different LC and MS conditions. Since there are many ways to run the instrument, the conditions used in this study should not be considered as being validated for optimal performances for all types of samples. LC separation was carried out using an Agilent 1290 Infinity LC, consisting of an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity High-Performance Autosampler (G4226A), an Agilent 1290 Infinity Thermostat (G1330B), and an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C). The UHPLC system was coupled to an Agilent G6540A Q-TOF LC/MS equipped with a dual-spray Agilent Jet Stream electrospray ionization source for ODP E&L analysis. Reference mass ions were delivered using an Agilent 1260 Infinity Isocratic Pump (G1310B) for long worklist. Spectra were acquired using Agilent MassHunter acquisition Software revision B.06.01. The Q-TOF LC/MS instrument was operated in 2 GHz extended dynamic range and in positive or negative ionization mode with two consecutive analytical runs: (i) MS acquisition with a data rate of 7 scans/second in MS and (ii) Auto MS/MS acquisition with a data rate of 7 scans/second in MS and 6 scans/second in MS/MS. Table 1 shows the chromatographic conditions and major MS conditions. Note, in this study, to prove the robustness of the PCDL, we used data from our published work where the collision energies used were operated at 5, 15, and 30 V[4]. The ideal collision energies to use are 10, 20, and 40 V, to give better scores in database searching.

Creation of the Agilent Extractable and Leachables LC/MS PCDL

Accurate mass spectra of single analyte solutions were acquired using flow injection or a short column in target MS/MS mode at collision energies of 10, 20, and 40 V, using an Agilent 6545 Q-TOF LC/MS. If precursor ion stability required either lower or higher collision energies, additional spectra were acquired in a second run. Typically, MS/MS spectra were acquired for the $[M+H]^+$ and $[M-H]^-$ ion species for each analyte. When highly abundant additional adduct ion species were observed, accurate mass MS/MS spectra were also acquired for the $[M+NH_4]^+$, $[M+Na]^+$, or $[M+Cl]^-$ species. In addition, fragment loss spectra were acquired for some compounds. In either positive or negative ionization mode, MS/MS spectra were acquired for more than 350 relevant E&L compounds. For many compounds, MS/MS library spectra were captured in both ionization modes, and for more than one precursor ion species. To eliminate mass assignment errors, fragment masses in the acquired spectra were compared to the theoretical fragment formulae, and all ion peaks were corrected to their theoretical masses. All MS/MS spectra were curated for spectral noise, and a minimum base peak threshold was applied to ensure good ion statistics for all fragment ions. The corrected spectra were included in the Agilent PCDL for E&L LC/MS Analysis (p/n G6890 CA). The Agilent E&L PCDL was then used for the screening and identification of E&L compounds in the extracts of three ophthalmic drug products.

Table 1. LC/MS experimental conditions.

Parameter	Value	
LC Conditions		
UHPLC 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 B) Methanol	
Gradient program	Min %B 0.0 40 8.0 100 11.0 100 Stop time: 11.0 minutes Post time: 1.5 minutes	
Flow rate	0.50 mL/min	
Injection volume	5 µL	
MS Conditions		
Gas temperature	150 °C	
Gas flow	10 L/min	
Nebulizer	30 psig	
Sheath gas temperature	200 °C	
Sheath gas flow	11 L/min	
Polarity	Positive	Negative
Capillary voltage	3,500 V	3,500 V
Nozzle voltage	300 V	300 V
Reference mass correction	121.05087 922.00980	112.9856 1,033.9881
MS: Mass range	50 to 1300 amu	
MS: Scan rate	7 spectra/s	
Auto MS/MS MS mass range	50 to 1300 amu	
Auto MS/MS MS/MS mass range	50 to 1300 amu	
Scan rate	7 spectra/s (MS) 6 spectra/s (MS/MS)	

Figure 1 shows a screen capture of the Agilent MassHunter PCDL Manager software along with the accurate mass MS/MS spectrum of the standard Irganox 1425 acquired in positive ionization at 10 V collision energy with ammonia adduct during PCDL creation.

Data Analysis

Agilent Mass Profiler software

The experimental group consisted of extractable samples, while the control group consisted of solvent blank. A statistical analysis and fold change was performed on the replicate groups using Agilent Mass Profiler software (rev. B.07.01) on MS scan data. Compound occurrence frequency with > 50 % in at least one group was considered, Q score > 80, differential score > 85. The two-way batch comparison was performed with the criteria of a fold change of > 2.0. This was a two-times higher abundance in the experimental group compared to control group. In leachables analysis, the experimental groups were the leachable stressed sample, while the control group were the nonstressed samples. Since potential extractable compounds could also be found in the control group, the fold change setting, in leachable samples, was kept at both 1.0 and 2.0. The differential features obtained from the fold change analysis were matched against the E&L accurate mass PCDL with a mass accuracy criteria < 5 ppm. The differential list from MS data, was exported as a generic CSV file, to be used as the formula and RT source for processing MS/MS data. This information was not brought in for acquisition but used in data analysis. Alternatively, to guarantee generating MS/MS at the correct RT, this CSV list can also be used as a preferred ion on an inclusion list for acquisition in either auto MS/MS or targeted MS/MS.
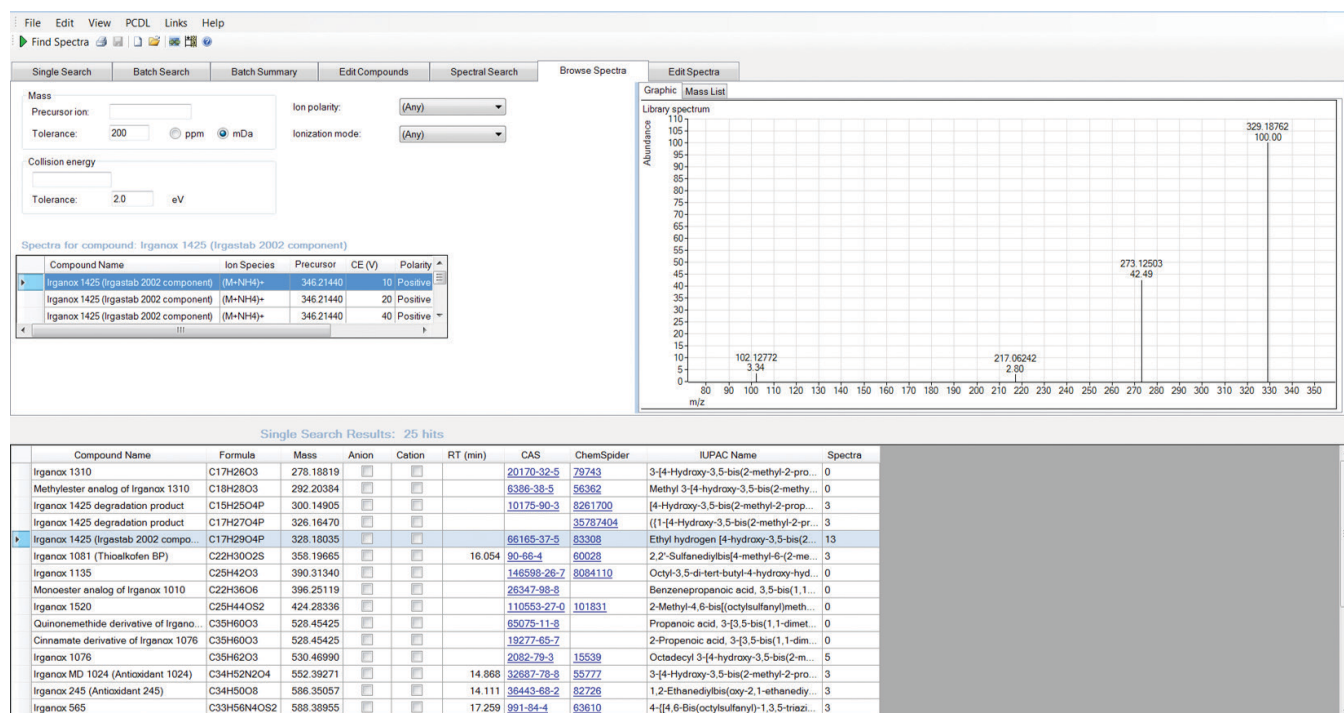

Figure 1. Agilent MassHunter PCDL Manager software showing the Agilent E&L LC/MS PCDL and the accurate mass MS/MS spectrum of Irganox 1425.

MS/MS Data analysis

To confirm the compounds detected with a database search against the accurate mass E&L PCDL, the MS/MS data were evaluated using Agilent MassHunter Qualitative Analysis software revision B.07.00. Positive identifications of E&Ls were reported if the compound was detected by the find-by-formula data mining algorithm, using the exported CSV file as input file, with a mass error <5 ppm and with a sufficient score (including isotope abundance and isotope spacing). A retention time window of ±1 minute was specified for peak detection to compensate for retention time shifts due to system to system variability but with RT required to be matched, since this would choose only those precursors from MS/MS spectra that were found in differential analysis.

Library search

The MS/MS extracted spectra were matched against the E&L PCDL using MassHunter Qualitative Analysis software. A library match score of >75 was considered as compound confirmation, while compounds not identified by PCDL were exported for analysis using MSC.

MSC analysis

MSC analysis was performed to identify unknown compounds that were not included in the PCDL or showed poor library scores. PCDL as well as ChemSpider were chosen as the structure source in MSC, to determine possible identification.

Results and Discussion

Screening of E&L in drug products by MS acquisition

A UHPLC/Q-TOF/MS method has been developed to perform E&L analysis for ODP samples. Mass Profiler software was used to determine a differential list of E&L compounds found in samples compared to controls[4], and the Agilent E&L LC/MS PCDL was used to identify the E&L compounds. The results were scored based on the agreement of the accurate monoisotopic mass, the isotope ratio, and the isotope spacing of all the adducts detected.

Figure 2 shows the peak spectrum for Erucamide, a common slip agent, found in the ophthalmic drug container extractable sample. The measured m/z 338.3417 signal was in good agreement with the expected isotope ratio (red boxes). The software could assign five ions to the $[M+H]^+$, $[M+Na]^+$, $[M+NH_4]^+$, $[2M+H]^+$, and $[2M+Na]^+$ species of Erucamide including their isotope signals. The good mass accuracy and isotope pattern matching was reflected in a good target score of 97.85 (out of 100).
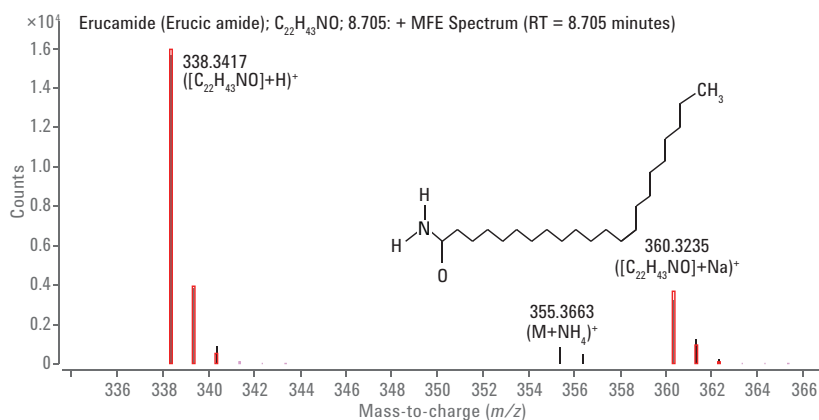

Figure 2. Peak spectrum obtained by the ID Browser feature of Agilent Mass Profiler software for Erucamide found in an ODP container extractable sample.

Confirmation of E&L in drug products by MS/MS acquisition

The differential list was exported as a .txt file and converted to a generic CSV file containing information on compound name, formula, retention time, and accurate mass. This generic CSV file was then used as the formula source to extract MS/MS spectra from auto MS/MS data in a find-by-formula algorithm. After MS/MS extraction, the spectra were matched against the library spectra contained in the Agilent E&L LC/MS PCDL. Figure 3 shows the MS/MS spectrum for Irgacure 907 in the extractable sample in comparison to the library spectrum from the PCDL (3C). Figure 3B shows a mirror plot of the difference spectrum. All major fragment ions listed in the library spectrum of Irgacure 907 were found in the measured spectrum within a narrow mass extraction window and in a similar ratio as in the reference spectrum. Thus, the reverse search against the exact mass library resulted in a score of 86.63 out of 100, and verified the presence of Irgacure 907 in the sample. The detailed compound identification results are shown in the compound table in Figure 4.
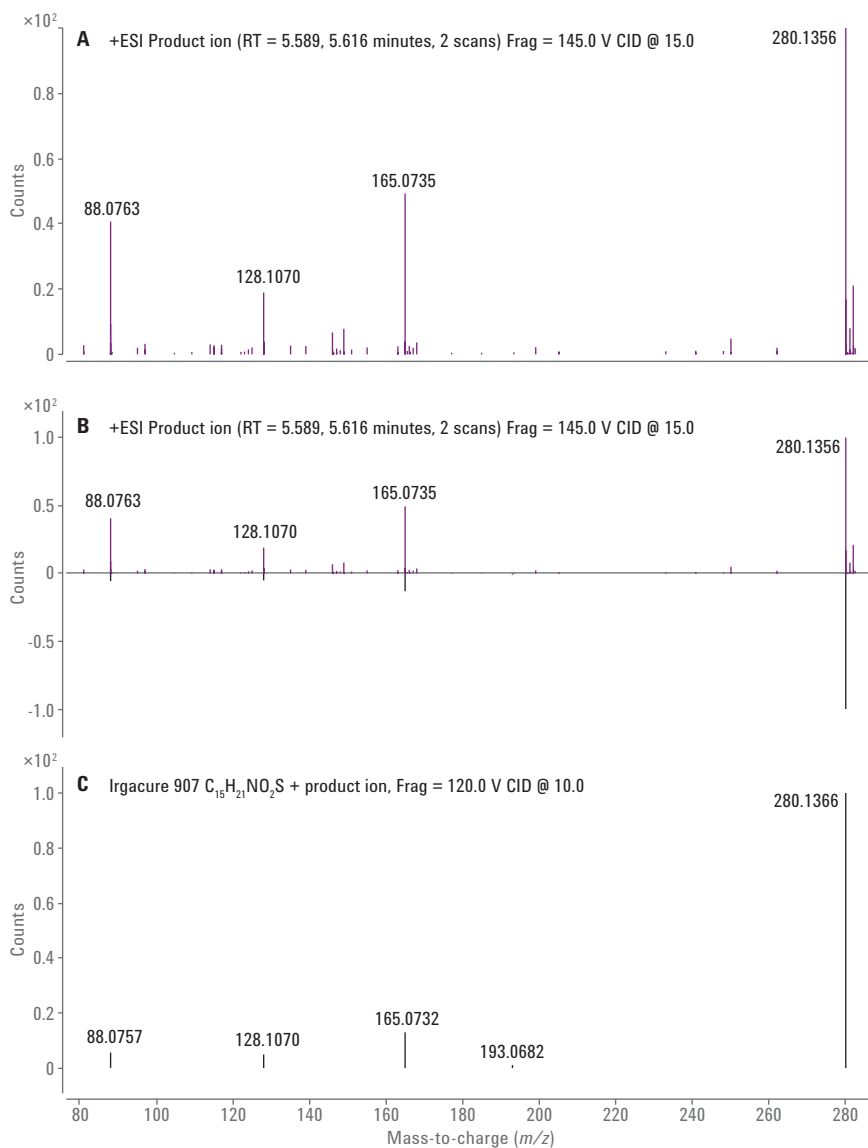

Figure 3. Comparison of the measured spectrum of Irgacure 907 at 15 V collision energy in the extractable samples (A), with the reference spectrum at 10 V collision energy (C) from the Agilent E&L Analysis PCDL and the difference plot (B).

Figure 4. Compound identification results for Irgacure 907 in the extractable sample including mass accuracy and isotope information from MS and MS/MS spectra.

PCDL MS/MS spectra acquired from standards were used for spectral matching and confirmation. However, when standard spectra are not available, compound confirmation can be performed using the Agilent MSC software[4]. MSC software correlates accurate mass/formula of experimental MS/MS fragments with *in-silico* fragment ions from a structure database and proposes possible matches. The sources of possible structures were >1,000 compounds present in E&L LC/MS PCDL, PubChem, or ChemSpider databases. Table 2 shows the overall results of accurate mass screening for E&Ls combined with confirmation of the identified compounds by MS/MS library searching for ODP. Tetraethyleneglycol had a low library score since it was acquired at 5 V, but it was also identified by MSC software (Table 2A, 2B), thus confirming the compound. As many as three extractables were also found in leachable (nonstressed) control samples. Approximately 80 % of the compounds identified by accurate mass were confirmed by library matching.

Table 2A. Selected compound table for extractables and leachables confirmed with positive or negative auto MS/MS acquisition.

Compound	Type	Formula	Ion species	Mass	Mass deviation (ppm)	Score (Lib)	Source
Irgacure 907	Extractable and leachable (control)	$C_{15}H_{21}NO_2S$	$[M+H]^+$	279.1293	0.9	86.6	PCDL
Tetraethyleneglycol	Extractable	$C_8H_8O_5$	$[M+H]^+$	194.1154	1.43	54.0	PCDL
Octyldimethyl PABA	Extractable	$C_{17}H_{27}NO_2$	$[M+H]^+$	277.2042	1.34	96.3	PCDL
Erucamide	Extractable and leachable (control)	$C_{22}H_{43}NO$	$[M+H]^+$	337.3342	0.76	87.0	PCDL
4-Isopropylthioxanthone	Extractable	$C_{16}H_{14}OS$	$[M+H]^+$	254.0763	1.12	99.3	PCDL
Irgacure 651 (degradant, -CH$_3$O)	Extractable	$C_{16}H_{16}O_3$	$[M+H]^+$	256.1098	0.57	90.0	PCDL
OBM/Methyl-2-benzoylbenzoate	Extractable and leachable (control)	$C_{15}H_{12}O_3$	$[M+H]^+$	240.0786	2.33	96.0	PCDL
Stearamide	Leachable (control)	$C_{18}H_{37}NO$	$[M+H]^+$	283.2875	1.47	100	PCDL
Lignoceric acid	Leachable (control)	$C_{24}H_{48}O_2$	$[M+H]^-$	368.3654	0.19	100	PCDL

Table 2B. Selected compound table for extractables and leachables confirmed with MSC.

Compound	Type	Formula	Ion species	Mass	Mass deviation (ppm)	Score (MSC)	Source
Tetraethyleneglycol	Extractable	$C_8H_8O_5$	$[M+H]^+$	194.1154	1.43	64.0	MSC
Polypropyleneglycol glycerol ether triacrylate	Leachable	$C_{21}H_{32}O_9$	$[M+H]^+$	428.205	0.41	65.8	MSC
Acetic acid propyl ester	Leachable	$C_5H_{10}O_2$	$[M+H]^+$	102.068	0.48	62.5	MSC

Conclusions

An accurate mass MS/MS PCDL for extractables and leachables was created and applied for the detection and confirmation of E&L in ODPs. MS mode acquisition was used to create a differential compound list of compounds, while MS/MS acquisition was used for compound confirmation of those differential compounds. The Agilent E&L LC/MS PCDL was used for spectral matching for compound confirmation. For efficient data review, Agilent Qualitative Analysis software was used to visualize the results including mass accuracy, library match scores, and retention time matching. Agilent Molecular Structural Correlator software was used to confirm additional compounds not found in the PCDL or had low library matching scores. The combination of statistical analysis software and compound confirmation tools provides an effective tool for E&L analysis of drug products.

References

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