Identification of Leachable Impurities from Pharmaceutical Container Closure Materials Using Orbitrap based GC-MS

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ABSTRACT

The results of this study show that the Q Exactive GC system is an ideal analytical tool for extractables and leachables studies where both known and unknown compounds are encountered. Software tools enable fast and accurate differential analysis to be performed to isolate unique features in extracts. Routine mass resolution of 60,000 FWHM and consistent sub-ppm mass accuracy ensures selective and confident compound detection and identification.

INTRODUCTION

The investigation of potentially toxic chemical impurities leaching from a wide variety of plastics, polymers and packaging products destined for pharmaceutical products has received a great deal of attention and remains a challenging analysis for chemists. Often termed extractables and leachables (E/L) studies, their aim is to identify, quantify and ultimately minimize any impurities that can migrate from packaging into a final product or drug. "Extractables" are those chemicals that can extract from components of a container closure system into solvents under accelerated laboratory conditions, such as elevated temperature and aggressive solvent, with the aim to extract the maximum compounds without deforming or degrading the material. "Leachables" are defined as chemicals that can migrate from the packaging into a drug product over the course of its shelf life.

Gas Chromatography-Mass Spectrometry (GC-MS) has been widely used in extractables studies as it provides analytical advantages of chromatographic resolution, reproducibility, peak capacity and importantly extensive spectral libraries to aid in identification. As packaging products can contain a large number of volatile and semi-volatile constituents it is well suited to analysis by GC-MS. In this study we seek to take advantage of a new class of GC-MS system with high mass resolution performance and exceptional mass accuracy for the detection and identification of compounds in polymer gaskets (O-rings) used in container closure systems and production seals. This work aims to demonstrate the application of a complete untargeted workflow to detect and identify chemical components in the O-rings. This work focuses on analyzing the samples using a full scan non targeted acquisition and using high mass resolving power to obtain accurate mass measurements.

MATERIALS AND METHODS

Samples

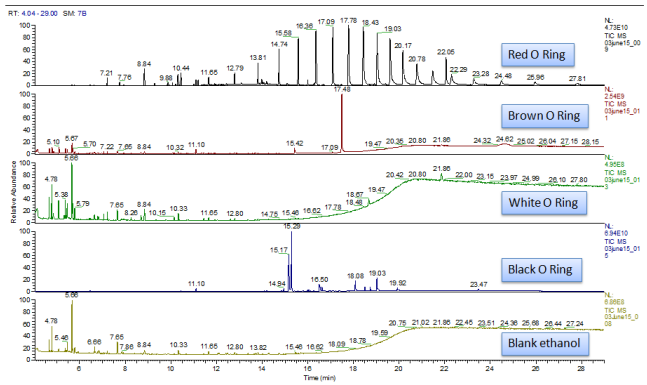
A total of four O-ring samples were included in the leachable study; A - Red Ring, B - Brown Ring, C - White Ring and D - Black Ring.
An accelerated leachable study was performed, following BPOG guidelines. Samples were cut into 20 mm sections and submerged in 10 mL 100% ethanol, 50% ethanol, WFI and 5M NaCl for 30 days at 40°C in a sealed crimped cap vial. A solvent blank was used, for chromatographic comparison, was also treated following the same protocol. An aliquot of each sample extract was transferred to GC vial for analysis.

Gas Chromatography-Mass Spectrometry

1 µL of sample was injected into a splitless injector and compound separation was achieved using a Thermo Scientific™ TRACE™ 1310 gas chromatograph and a Thermo Scientific™ TraceGOLD™ TG-5SILMS
30 m length × 0.25 mm inner diameter × 0.25 µm film thickness column. A Thermo Scientific™ TriPlus™ RSH autosampler was used for sample introduction (Table 1). High resolution EI spectra were acquired using 60,000 FWHM resolution (measured at *m/z* 200) with a mass range of 50–600 *m/z*. An internal lock mass was used throughout the acquisition.

RESULTS

Figure 1. GC-MS total ion chromatograms of the 100% ethanol leachate from four O-rings and blank (control).



Isolating Unique Components

Full scan chromatograms were obtained for each sample and the total ion chromatograms (TICs) are shown in figure 1. The first step in this analysis was to quickly identify the peaks in each of the samples that were unique or significantly elevated when compared to the blank.

This was achieved by binary comparison between the test sample and the blank in TraceFinder. The software first performs an accurate mass deconvolution of the data. An example deconvoluted peak cluster for ethyl octanoate is shown in figure 2. A heat map is shown in TraceFinder to quickly identify the peaks that are elevated in the test sample (figure 3). For example, the peak at 17.49 minutes with a base peak of *m/z* 277.07800 is a peak elevated in the brown O-ring.

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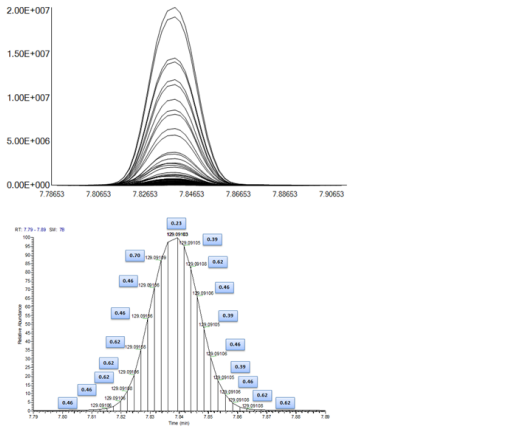

Figure 2. Deconvoluted peak cluster (upper) identified as ethyl octanoate from the black O-ring (D). Extracted ion chromatogram (lower) for ethyl octanoate ion *m/z* 129.0910 (±5 ppm mass window) in black O-ring showing 18 scans/ peak. Excellent accurate mass stability is shown for each individual scan (ppm mass error).

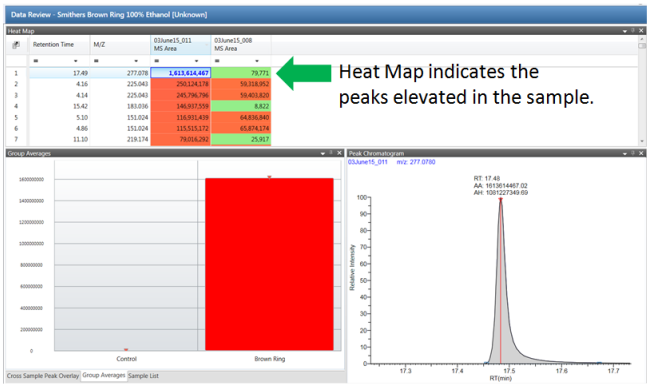

Figure 3. TraceFinder unknown screening window showing a section of the peak list for the Brown O-ring (B) and blank (control). The heat map (upper window) is used to isolate the peaks that are elevated in the sample. The group averages window (bottom left) shows the intensity of the peak at 17.49 minutes with base peak of m/z 277.078 in the two samples.

Identifying compounds with confidence

The deconvoluted spectrum is first searched against commercial nominal mass spectral libraries (e.g. NIST 2014) and the list of hits are scored based on a combination of the search index (SI) score and high resolution filtering (HRF) value (figure 4). The HRF value is the percentage of the spectrum that can be explained by the chemical formula in the library search. The top hit for the peak at 17.49 minutes was for the compound triphenylphosphine oxide, where 98.8% of the spectrum can be explained based on accurate mass. The fragments observed are matched to the elements in the proposed compound with sub 1ppm mass accuracy which adds confidence in the identification.

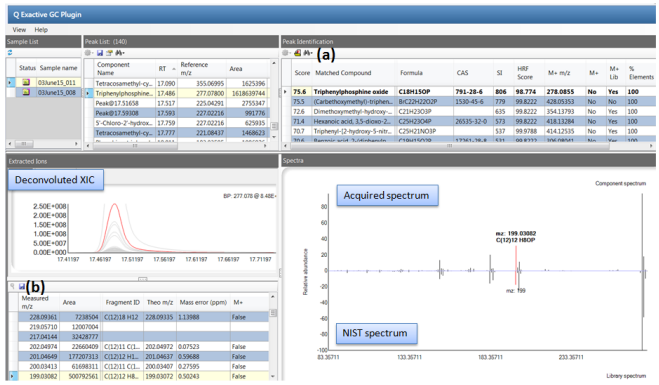

Figure 4. Identification of peak at 17.49 minutes as triphenylphosphine oxide. Screenshot of the deconvoluted data and library match in TraceFInder. (a) List of library hits sorted by score (combination of SI and HRF). (b) List of fragment ions from EI spectrum and elemental composition based on elements in top hit.

Identifying compounds without a spectral library match

However, when the spectral library match from the EI spectrum is inconclusive, then the PCI data can be used to confirm the elemental composition of the parent molecule using accurate mass information. This is where excellent mass accuracy becomes essential to limit the list of possible chemical formulae and to provide confidence when a proposed identification is made. In the black O-ring sample a peak was observed at 15.17 minutes that did not have a satisfactory match to any compound in the NIST library. Assessment of the possible library hits that were suggested could be eliminated as the accurate mass data did not support them. The PCI data was then used to establish the molecular ion by assessment of the adducts (figure 5). This spectrum showed the adducts $[M+H]^+$ (0 ppm) for ion *m/z* 325.14344, $[M+C_2H_5]^+$ (0.3 ppm) for ion *m/z* 353.17483. The presence of these adducts indicated that the *m/z* 324.13541 was the molecular ion in the EI spectrum. From this ion an elemental composition of the parent molecule could be proposed.

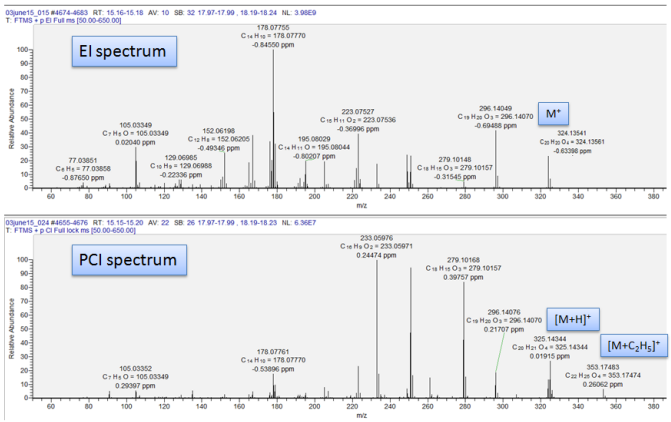

Figure 5. Unknown EI and PCI spectra at 15.17 minutes in black O-ring (D). Identification of the molecular ion and adducts in the PCI spectrum suggest a formula $C_{20}H_{20}O_4$.

This is a critical stage in the process and it is where excellent mass accuracy can be used to limit the number of possible chemical formulae. For example, when a 10 ppm mass accuracy window is used 16 possible formulae are proposed for the M^+ ion using the elements Carbon (1-30), Hydrogen (1-60), Nitrogen (1-5), Oxygen (1-5), Phosphorus (1-2) and Sulphur (1-2). This is compared to a 1 ppm mass accuracy window that suggests only one possible formula, $C_{20}H_{20}O_4$. The identification is further supported by the mass accuracy and elemental formula for the $[M+H]^+$ and $[M+C_2H_5]^+$ adducts in the PCI spectrum.

All of the four O-ring samples were evaluated using the same workflow and the results are summarized in table 1. The most intense unique peaks for each sample were assessed and tentative identifications made based on library matching and the accurate mass of the molecular ion or adducts in the EI and CI spectra. The black and brown O-rings showed the greatest number of components that were extracted into the ethanol and the white O-ring showed the least. The red O-ring sample showed contamination with cyclic siloxanes, as clearly seen in the TIC and these were not included in the results table.

Sample	RT (min)	Base Peak (m/z)	Compound Name	Formula	Base Peak Mass Accuracy (ppm)	Molecular ion Mass Accuracy (ppm)
Black O Ring	15.17	178.07754	$C_{20}H_{20}O_4$	$C_{20}H_{20}O_4$	0.9	0.7
	15.29	178.07754	$C_{20}H_{20}O_4$	$C_{20}H_{20}O_4$	0.1	0.2
	18.08	171.13806	Tetraethylene glycol bis (2-ethylhexanoate)	$C_{24}H_{46}O_7$	0.6	-
	23.47	219.17435	Irganox 1076	$C_{35}H_{62}O_3$	0.0	1.0
	14.94	280.10939	ethyl1-hydroxy-2,3-diphenylcycloprop-2-ene-1-carboxylate	$C_{18}H_{16}O_3$	0.4	0.4
	16.50	126.09145	9-Octadecenamide	$C_{18}H_{35}NO$	0.9	0.6
Brown O Ring	17.48	277.07790	Triphenylphosphine oxide	$C_{18}H_{15}OP$	0.9	0.1
	15.42	183.03595	Triphenylphosphine	$C_{18}H_{15}P$	0.7	0.9
	11.10	219.1743	4-tert-butyl-2,6-diisopropylphenol	$C_{16}H_{26}O$	0.2	0.2
	11.35	149.02341	Diethyl phthalate	$C_{12}H_{14}O_4$	0.6	0.9
	13.57	185.04198	Diphenyl sulfide	$C_{12}H_{10}S$	0.2	0.1
White O Ring	11.93	263.20074	1,4 Dihydrophenacetic acid, 3,5-di-t-butyl, ethyl ester	$C_{18}H_{30}O_2$	0.7	0.4
	7.65	101.02344	Butanedioic acid, diethyl ester	$C_8H_{14}O_4$	0.5	-
Red O Ring	10.44	163.07549	Ethanone, 1-[4-(1-hydroxy-1-methylethyl)phenyl	$C_{11}H_{14}O_2$	0.9	0.4
	15.09	87.044	Methyl stearate	$C_{19}H_{38}O_2$	1.3	0.1
	16.00	155.07025	di(butoxyethyl)adipate	$C_{18}H_{34}O_6$	0.1	1.0

Table 1 Summary of the peaks elevated in the four O ring samples and the tentative identification of the compounds

CONCLUSIONS

GC Orbitrap mass spectrometer in combination with TraceFinder is an extremely effective tool for the profiling of extractables samples and in the identification of unknown peaks.

• Reliable and robust chromatographic separation in combination with fast data acquisition speeds make the Q Exactive GC an ideal platform for chemical profiling of complex samples.

• The consistent sub 1 ppm mass accuracy in combination with excellent sensitivity makes confident identification of all components in a sample possible. Routine resolving power of 60,000 FWHM and a wide dynamic range eliminates isobaric interferences, increasing confidence in results when compounds are identified in complex matrices.

• The EI and PCI data obtained was used for tentative compound identification against commercial libraries. Where no library match was made the mass accuracy allowed for elemental compositions to be proposed with a high degree of confidence. Proposed identifications can be quickly confirmed or eliminated based on accurate mass.

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