

Confident Identification of Leachable Impurities from Pharmaceutical Container Closure Materials using Orbitrap-Mass-Spectrometer-Based GC-MS

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Key Words

Extractables and Leachables, Q Exactive GC, Orbitrap mass spectrometry, differential analysis, unknown identification, container closure system, pharmaceutical.



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 amount 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.

The potential, versus the actual, impact of the product on its user:

- Extractable = possible impact.
- Leachable = actual impact

The object on which the testing is performed:

- Extractable = the container material
- Leachable = the final product

Extractable testing is primarily used to mitigate risk by identifying potentially toxic leachables very quickly and allowing the selection of a different packaging material. In general, for most dosage forms, any material that is in direct contact with an API (Active Pharmaceutical ingredient) should be considered for extractable and leachable analysis and in some cases, secondary or tertiary packaging, e.g., labels should also be considered. Leachables can come from the container closure system and any components used in the manufacturing process. They may also be the product of reactions between the drug and packaging material and may continue to form during storage.¹ A controlled extractables experiment is accomplished by exposing the material to extremes of solvents, pH, and temperatures to test the product under worst case scenarios. The confident detection and identification of compounds present is a very demanding task and it is essential that analysts use the available technology to accurately and comprehensively characterize products.

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 may contain a large number of volatile and semi-volatile constituents, they are well suited to GC-MS analysis. 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. It focuses on analyzing the samples using full-scan non-targeted acquisition and using high mass resolving power to obtain accurate mass measurements. This resolving power is important to enable confident elemental composition proposals, structural elucidation, and discrimination of co-eluting and isobaric compounds. Fast acquisition speeds, in combination with a high in-scan dynamic range and high sensitivity, facilitate the detection of both low- and high-intensity components. These features, in combination with unique software algorithms for automated deconvolution and sample comparison, create a powerful solution for comprehensive product characterization.

Experimental Conditions

Samples and Preparation

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 BioPhorum Operations Group (BPOG) guidelines. Samples were cut into 20 mm sections and submerged in 10 mL 100% ethanol, 50% ethanol, water for injection (WFI), and 5M sodium chloride (NaCl) for 30 days at 40 °C in a sealed, crimped cap vial. A solvent blank for chromatographic comparison, was treated following the same protocol. An aliquot of each sample extract was transferred to a GC vial for analysis. For the aqueous samples, a liquid:liquid into dichloromethane extraction was performed prior to the GC analysis.

Instrument and Method Setup

A Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system was used in all experiments. Sample introduction was performed using a Thermo Scientific™ TriPlus™ RSH autosampler, and chromatographic separation was obtained using a Thermo Scientific™ TRACE™ 1310 GC and a Thermo Scientific™ TraceGOLD™ TG-5SiIMS 30 m × 0.25 mm I.D. × 0.25 μm film capillary column with a 10 m guard. (P/N 26096-1425). Additional details of instrument parameters are displayed below.

CONDITIONS

TRACE 1310 GC Parameters

Injection Volume (μL):	1
Liner:	Single gooseneck
Inlet (°C):	280
Carrier Gas, (mL/min):	He, 1.2

Oven Temperature Program

Temperature 1 (°C):	40
Hold Time (min):	1
Temperature 2 (°C):	320
Rate (°C/min):	15
Hold Time (min):	10

Q Exactive GC Mass Spectrometer Parameters

Transfer line (°C):	280
Ionization type:	EI/PCI
Ion source (°C):	230
Electron energy (eV):	70
Acquisition Mode:	Full scan
Mass range (Da):	50–650
Resolving power (FWHM):	60,000 @ m/z 200
Lockmass, column bleed (m/z):	207.03235

The Q Exactive GC system was operated in EI full scan mode using 60,000 (FWHM at m/z 200) resolving power. Additional experiments were run using positive chemical ionization (PCI) with methane as reagent gas (1.5 mL/min) to obtain information on the molecular ions and to support the identification of unknown component peaks.

Data Processing

Data were acquired using the Thermo Scientific™ TraceFinder™ software. This single platform software package integrates instrument control, method development functionality, and qualitative and quantitation-focused workflows. The TraceFinder software includes accurate mass spectral deconvolution and spectral matching functionality.

Results and Discussion

The objective of this study was to analyze O-ring solvent leachates using non-targeted, full-scan data acquisition and to identify, by binary comparison with the control (blank ethanol), the significant peaks that are unique in the O-ring extract in order to propose an identity to any differences observed. In addition, the study aimed to quickly identify the compounds present in a sample using accurate mass deconvolution and spectral matching. In order to achieve these objectives, a complete workflow was used. Details of this workflow are summarized in Figure 1.

Isolating Unique Components

Full-scan chromatograms were obtained for each sample and the total ion chromatograms (TICs) are shown in Figure 2. The Q Exactive GC system acquires accurate mass data with a wide dynamic range, so compounds can be detected at both very high and very low concentrations without the loss of accurate mass information. This ability is very powerful for identifying unknown peaks in a complex sample with a high degree of confidence. The first step in this analysis was to quickly identify the unique or significantly elevated peaks in each sample when compared to the blank. Although peaks can be seen in the TICs, it is essential that all peaks are extracted from the data to ensure that a full characterization of a sample is performed and that no potentially toxic compound is missed.

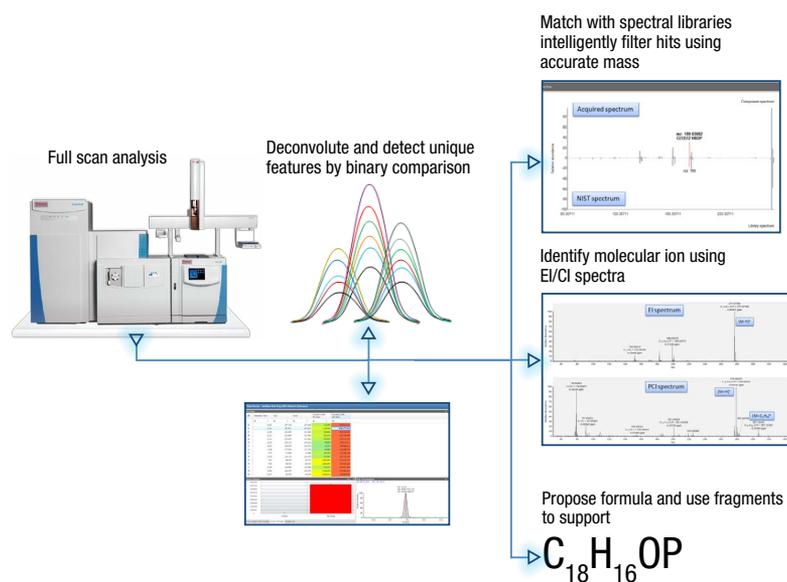


Figure 1. Workflow for the Q Exactive GC system for chemical profiling and identification of unknown peaks.

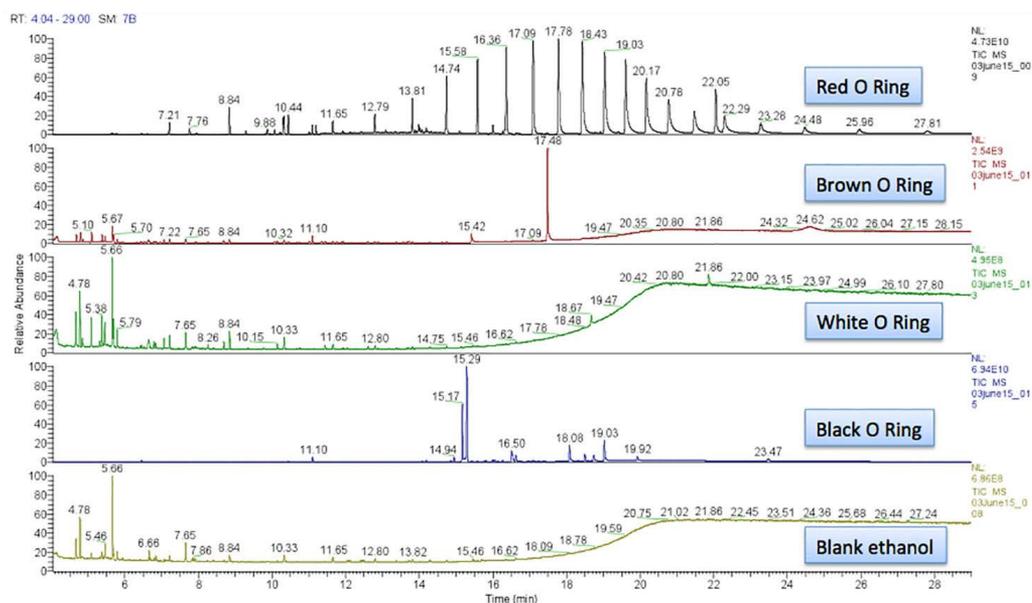


Figure 2. GC-MS total ion chromatograms or the 100% ethanol leachate from four O-rings and blank (control).

This characterization was achieved using TraceFinder software to conduct a binary comparison between the test sample and the blank. The software first performs an accurate mass deconvolution of the data with the aim of detecting all of the peaks above a signal to noise threshold of 30:1, and to clean up the spectra so that only ions that maximize at the same retention time remain for library matching. An example deconvoluted peak cluster for ethyl octanoate is shown in Figure 3, along with the number of scans across the peak and their accurate mass error for the base peak (m/z 129.0910). The peak list is then compared to the blank and sorted to show the peaks that are unique to the sample. The TraceFinder software creates a heat map to quickly identify elevated peaks in the test sample (Figure 4). For example, the peak at 17.49 minutes with a base peak of m/z 277.0780 is elevated in the brown O-ring.

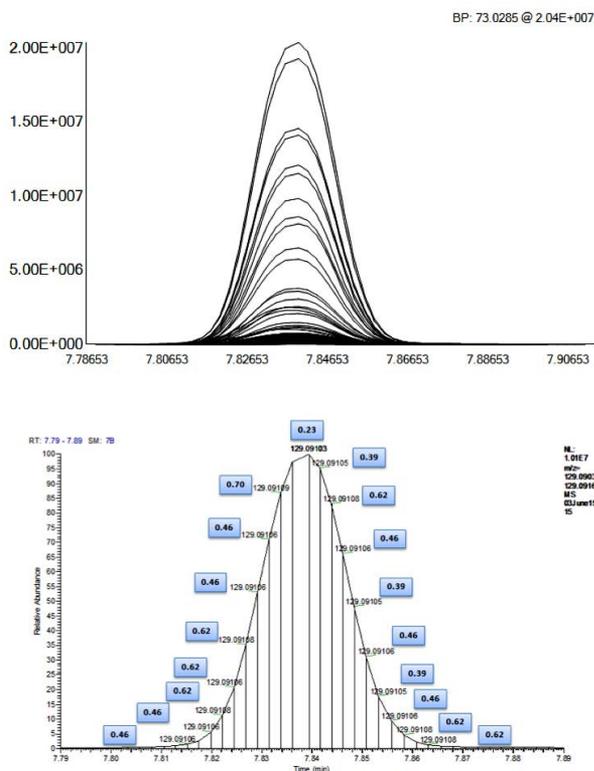


Figure 3. Deconvoluted peak cluster (upper) identified as ethyl octanoate from the black O-ring (D). Extracted ion chromatogram (lower) for ethyl octanoate ion m/z 129.0910 (± 5 ppm mass window) in black O-ring showing 18 scans/peak. Excellent accurate mass stability is shown for each individual scan (ppm mass error).

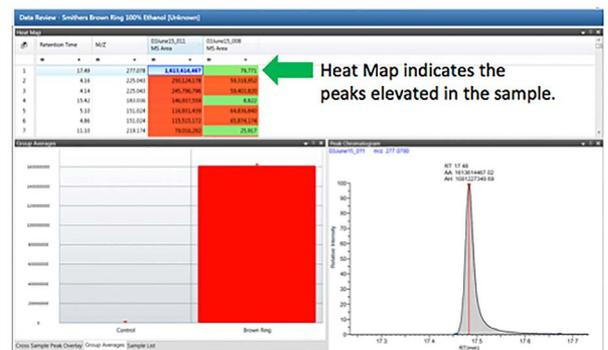


Figure 4. TraceFinder unknown screening window showing a section of the peak list for the Brown O-ring (B) and blank (control). The heat map (upper window) is used to isolate the peaks that are elevated in the sample. The group averages window (bottom right) shows the intensity of the peak at 17.49 minutes with base peak of m/z 277.078 in the two samples.

Identifying Compounds with Confidence

Having isolated a peak of interest, the final phase is to identify the compound. This identification is performed automatically in the TraceFinder software (Figure 5). The deconvoluted spectrum is first searched against commercial nominal mass spectral libraries (e.g., NIST 2014) and hits are scored based on a combination of the search index (SI) score and high resolution filtering (HRF) values. The HRF value is the percentage of the spectrum that can be explained by the chemical formula in the library search.



Figure 5. 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.

The combination of accurate mass matching and explaining the ions observed in the spectrum provides a fast and confident route to the identification of unknown compounds. 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-1-ppm mass accuracy, which adds confidence in the identification. The base peak m/z 277.07790 corresponds to the $[M-H]^+$ ion with a mass difference of 0.8 ppm from the theoretical m/z 277.07768 for the formula $C_{18}H_{15}OP$. If only a traditional search index was used to sort the list of hits, several of the other suggested compounds would also provide a good spectral match (>700). However, these suggestions can be automatically eliminated as the elements they contain do not match with those observed from the accurate mass data.

Further confirmation in the identification of triphenylphosphine oxide can be obtained by assessing the PCI spectrum (Figure 6). The PCI data can be used to identify the elemental composition of the parent molecule by looking for common adducts such as $[M+H]^+$ or $[M+C_2H_5]^+$ and assessing whether the proposed elemental formula is within the expected mass accuracy (<1 ppm). In the PCI spectrum for triphenylphosphine oxide, the adducts $[M+H]^+$ (0 ppm, mass error), $[M+C_2H_5]^+$ (0.2 ppm) and $[M+C_3H_5]^+$ (0.4 ppm) were observed with outstanding mass accuracy.

Identifying Compounds Without a Spectral Library Match

When the spectral library match from the EI spectrum is inconclusive, 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 an identification is made. In the black O-ring sample, a peak, which did not have a satisfactory match to any compound in the NIST library, was observed at 15.17 minutes. The library hits suggested could be eliminated as the accurate mass data did not support those hits. The PCI data was then used to establish the molecular ion by assessing the adducts (Figure 7). 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. This proposal represents a critical stage in the identification process. It is where excellent mass accuracy

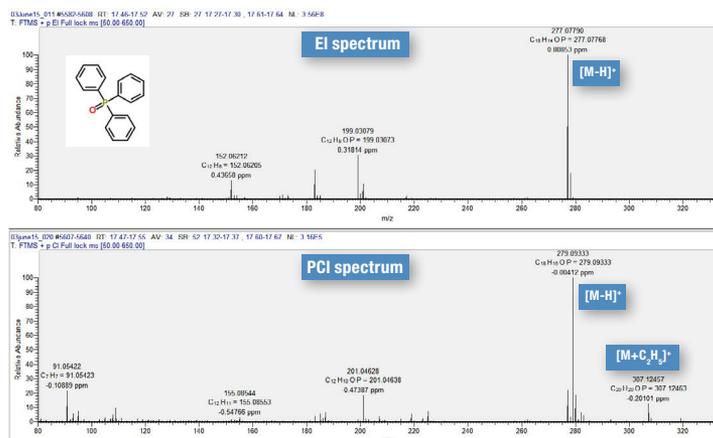


Figure 6. Triphenylphosphine oxide ($C_{18}H_{15}OP$) EI and PCI spectra at 17.86 minutes in brown O-ring. PCI data supports identification of parent with formula above with sub 1 ppm mass accuracy.

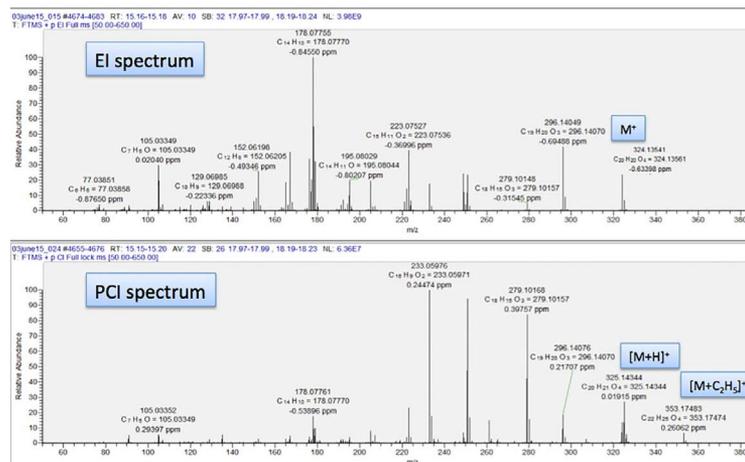


Figure 7. Unknown EI and PCI spectra at 15.17 minutes in black O-ring (D). Identification of the molecular ion and adducts in the PCI spectrum suggest a formula $C_{20}H_{20}O_4$.

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, $C_{20}H_{20}O_4$. This level of mass accuracy significantly reduces the number of formulae that need to be investigated and also increases the confidence in any proposed assignment. 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. In addition to the peak at 15.17 minutes, there was a peak at 15.29 minutes with identical EI and PCI spectra. This peak is likely to be an isomer of the same compound.

One final stage to support the proposed formula and to derive structural information is to use the accurate mass fragments. To achieve this, we can use either the fragments in the EI spectrum or an additional MS/MS experiment can be performed to confirm that the fragments are from the molecular ion. The ion m/z 325.14 was isolated in the quadrupole and fragmentation induced in the HCD cell to generate fragments from this molecular ion. Figure 8 shows the resulting MS/MS spectrum for m/z 325.14. The fragments observed contain the elements in the proposed parent with sub-1-ppm mass accuracy. Therefore, even when we cannot give a chemical name to a peak, we can extract detailed information of a component with a high degree of confidence.

All of the four O-ring samples were evaluated using the same workflow. The results are summarized in Table 1. The most intense unique peaks for each sample were assessed. Putative identifications were 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 extracted into the ethanol and the white O-ring showed the fewest. The red O-ring sample showed contamination with cyclic siloxanes, as clearly seen in the TIC. These contaminants were not included in the results table.

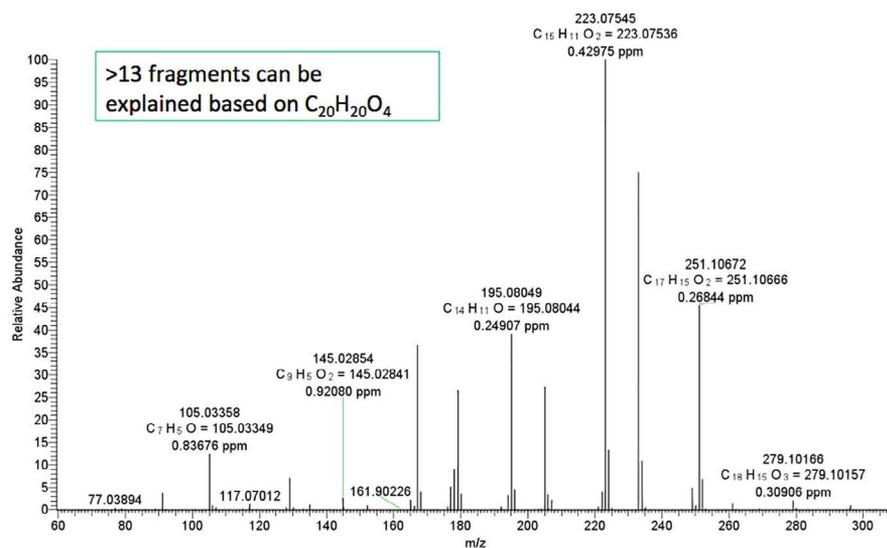


Figure 8. MS/MS spectrum of m/z 325.14 showing all fragments and elemental compositions that support proposed compound formula.

Table 1. Summary of the peaks elevated in the four O-ring samples and the putative identification of the compounds. Low mass error for both base peaks and molecular ion adds confidence to proposed identities.

Sample	RT (min)	Base Peak (m/z)	Search Index	Compound Name	Formula	Base Peak Mass Accuracy (ppm)	Molecular ion Mass Accuracy (ppm)
Black O-Ring	15.17	178.07754	No match	$C_{20}H_{20}O_4$	$C_{20}H_{20}O_4$	0.88	0.66
	15.29	178.07754	No match	$C_{20}H_{20}O_4$	$C_{20}H_{20}O_4$	0.11	0.22
	18.08	171.13806	673	Tetraethylene glycol bis (2-ethylhexanoate)	$C_{24}H_{46}O_7$	0.64	-
	23.47	219.17435	777	Irganox 1076	$C_{35}H_{62}O_3$	0.03	1.02
	14.94	280.10939	536	ethyl 1-hydroxy-2,3-diphenylcycloprop-2-ene-1-carboxylate	$C_{18}H_{16}O_3$	0.39	0.39
Brown O-Ring	16.50	126.09145	652	9-Octadecenamide	$C_{18}H_{35}NO$	0.87	0.63
	17.48	277.07790	806	Triphenylphosphine oxide	$C_{18}H_{15}OP$	0.85	0.06
	15.42	183.03595	831	Triphenylphosphine	$C_{18}H_{15}P$	0.68	0.91
	11.10	219.1743	796	4-tert-butyl-2,6-diisopropylphenol	$C_{16}H_{26}O$	0.18	0.21
	11.35	149.02341	831	Diethyl phthalate	$C_{12}H_{14}O_4$	0.60	0.9
White O-Ring	13.57	185.04198	813	Diphenyl sulfide	$C_{12}H_{10}S$	0.21	0.05
	11.93	263.20074	711	1,4 Dihydrophenacetic acid, 3,5-di- <i>t</i> -butyl, ethyl ester	$C_{18}H_{30}O_2$	0.72	0.43
	7.65	101.02344	781	Butanedioic acid, diethyl ester	$C_8H_{14}O_4$	0.54	-
Red O-Ring	10.44	163.07549	775	Ethanone, 1-[4-(1-hydroxy-1-methylethyl)phenyl]	$C_{11}H_{14}O_2$	0.85	0.38
	15.09	87.044	740	Methyl stearate	$C_{19}H_{38}O_2$	1.26	0.06
	16.00	155.07025	690	di(butoxyethyl)adipate	$C_{18}H_{34}O_6$	0.13	1.02

Conclusions

The results of this study demonstrate that the Q Exactive GC hybrid quadrupole-Orbitrap mass spectrometer, in combination with TraceFinder software, is an extremely effective tool for profiling complex samples and identifying unknown peaks. The Orbitrap mass spectrometer delivers excellent mass accuracy for all components in a sample, leading to fast, confident characterization of samples, regardless of the concentration.

- Reliable and robust chromatographic separation in combination with fast data acquisition speeds make the Q Exactive GC system 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 eliminate isobaric interferences, increasing confidence in results when compounds are identified in complex matrices.
- TraceFinder software allowed for a fast and comprehensive characterization of the O-ring samples, isolating and identifying compounds with confidence.
- The EI and PCI data obtained were 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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