APPLICATION NOTE 000206

Non-targeted screening of extractables from vial septa using high-resolution Orbitrap GC-MS

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Goal

The aim of this work is to demonstrate the utility of the Thermo Scientific™ Orbitrap Exploris™ GC mass spectrometer for confident non-targeted screening of extractables from vial septa, with complementary detection using the Thermo Scientific™ TRACE™ 1310 gas chromatograph with flame ionization detection.

Introduction

The investigation of potentially toxic chemical impurities leaching from a wide variety of plastics, polymers, and packaging products destined for pharmaceutical products, food packaging, or personal care products is growing in importance due to increasing global regulations but remains a challenging analysis for chemists. Typical extractables and leachables (E/L) studies aim to identify, quantify, and ultimately minimize any impurities that can migrate from packaging into a final product or drug. The term “extractables” describes 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 exposure). The term “leachables” is used to define chemicals that can migrate from the packaging into a drug product over the course of its shelf life.

Extractable testing is primarily used to mitigate risk by identifying potentially toxic leachables very quickly and allowing the selection of the most appropriate packaging material. 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 product (e.g., drug) and packaging material and may continue to form during storage.

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. Packaging products may contain a large number of volatile and semi-volatile constituents, and therefore are well matched to GC-MS analysis.

There are several analytical challenges associated with the analysis of extractables and leachables using GC or GC-MS. To have a good coverage of the chemical content, a GC or GC-MS platform that can sensitively and selectively detect chemical constituents, taking into account the variety and complexity of possible matrices, should be used. GC coupled to high-resolution mass spectrometry is one of the most suitable approaches as it offers both sensitivity and selectivity. In particular, high-resolution accurate mass from GC-Orbitrap MS with sub-ppm mass accuracy, high resolving power, and versatility for sample introduction, combined with unique software algorithms for automated deconvolution and extensive spectral libraries, make it a suitable solution for both qualitative and quantitative assessments of extractables and leachable components.

This work aims to demonstrate the applicability of GC-FID in combination with GC-Orbitrap technology for the full-scan untargeted workflow, using high mass resolving power to obtain accurate mass measurements, thus enabling confident elemental composition proposals and structural elucidation to detect and identify chemical components of various vial septa. 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. For confident confirmation of compounds identified, softer ionization modes (chemical ionization, CI) were employed in addition to classical electron ionization (EI).

Experimental

Preparation of samples

Twenty-one septa were removed from caps, sourced from a range of suppliers, including septa material composed of PTFE/silicone and rubber.

Septa were removed from each cap and cut into four sections per septa (using clean, oil-free scissors and tweezers). Using tweezers, 0.3 g of the cut septa were placed into a screw top GC vial. 1 mL of the extraction solvent mixture (1:1:1 methanol/DCM/hexane) was added to each vial (using a glass syringe). The vials were sealed and left overnight (~18 hours). The extraction solvent from each sample was split and transferred to six vials (using GC vials with inserts, P/N 03-FIV(A); caps, P/N 11-AC-TST1)); procedural blanks were also prepared using the extraction solvent.

Instrument and method setup

An Orbitrap Exploris GC mass spectrometer, configured with a Thermo Scientific™ TriPlus™ RSH™ autosampler and a Thermo Scientific™ Instant Connect split/splitless (iC-SSL) injector, was used for all tests. A Thermo Scientific™ Instant Connect Flame Ionization Detector (iC-FID) was used GC-FID experiments.

The Orbitrap Exploris GC mass spectrometer was tuned, air leak checked, and calibrated in <1.5 min using FC43 (CAS 311-89-7) to achieve a mass accuracy of <2.0 ppm. The system was operated using electron ionization (EI), as well as positive chemical ionization (PCI) with methane as the reagent gas. Data were acquired in full-scan and 60,000 mass resolution (full width at half maxima FWHM, measured at *m/z* 200). Data acquired were lock-mass corrected using GC column bleed siloxane masses.

Compound separation was achieved for both GC-FID and GC-Orbitrap experiments on a Thermo Scientific™ TraceGOLD™ TG-5SilMS, 30 m x 0.25 mm × 0.25 µm column. Additional details of the instrument parameters are shown in Tables 1 and 2.

Table 1. GC and injector conditions

TRACE 1310 GC system parameters				
Injection volume (µL)	1			
Liner	Thermo Scientific™ LinerGOLD™ single taper with quartz wool, P/N 453A1925-UI			
Inlet temperature (˚C)	280			
Carrier gas, (mL/min)	He, 1.2			
Inlet module and mode	SSL, splitless			
Split ratio	87			
Splitless time	1.00 min			
Purge flow (mL/min)	5			
Column	TraceGOLD TG-5SilMS, 30 m × 0.25 mm × 0.25 µm P/N 26096-1420			
Oven temp. program	RT (min)	Rate (˚C/min)	Target temp. (˚C)	Hold time (min)
Initial	0	-	40	0.50
Final	0.5	15	320	5.00
Run time	25	-	-	-
FID parameters				
Detector temperature (˚C)	300			
FID air flow (mL/min)	350			
FID H_2 flow (mL/min)	40			
FID make up N_2 flow (mL/min)	20			
FID acquisition rate (Hz)	10			

Table 2. Mass spectrometer conditions

Ionization type	EI	PCI
Transfer line (˚C)	290	290
Ion source (˚C)	320	200
CI gas type	n/a	Methane
CI gas flow (mL/min)	n/a	1.0
Electron energy (eV)	70	
Acquisition mode	Full-scan	
Mass range (Da)	50–650	
Mass resolution	60,000 FWHM at *m/z* 200	
Data acquisition rate (Hz)	7	

Data processing

For GC-Orbitrap experiments, data were acquired using Thermo Scientific™ TraceFinder™ software. The TraceFinder single platform software integrates instrument control, method development functionality, and qualitative and quantitation workflows. TraceFinder software also contains accurate mass spectral deconvolution and spectral matching functionality. In addition, the acquired data were imported into Thermo Scientific™ Compound Discover™ version 3.2 software for spectral deconvolution, compound identification, and multivariate statistical analysis.

Thermo Scientific™ Mass Frontier™ Spectral Interpretation software, version 8.0 was used to elucidate the chemical structure of putatively identified compounds via NIST mass spectra library matching.

For the GC-FID experiments, data were acquired using Thermo Scientific™ Chromeleon™ 7.3 CDS software, which allows the analyst to set up acquisition, processing, and reporting methods with easy data reviewing and flexible data reporting.

Results and discussion

The object of this study was to analyze a range of different vial septa solvent leachates using the GC-Orbitrap system for the full-scan untargeted workflow and the high mass resolving power to obtain accurate mass measurements, thus enabling confident elemental composition proposals and structural elucidation to detect and identify chemical components of various vial septa. Fast acquisition speed, in combination with a high in-scan dynamic range and high sensitivity, facilitate the detection of both low- and high-intensity components. For confident confirmation of compounds identified, softer ionization modes (chemical ionization, CI) were employed, in addition to classical electron ionization (EI). GC-FID data was also acquired.

Non-targeted screening for unknown components in vial septa

For non-targeted qualitative screening of septa, full-scan data was first acquired using EI, followed by spectral deconvolution with library matching for putative compound identification. For additional confidence in the identification of unknowns, a confirmation step using positive chemical ionization (PCI) was employed. The workflow used for non-targeted screening is summarized in Figure 1.

Detect: Electron ionization, full scan

Full scan (FS) data (EI) was first acquired for all samples; example total ion chromatograms (TICs) are shown in Figure 2 for two of the analyzed septa (white silicon / blue PTFE, and natural rubber / TEF).

Isolate, search, and identify: Deconvolution

For the collected GC EI data, extraction, deconvolution, and identification of unknowns based on mass spectral library matching was performed using Compound Discover software. An example of the generated results is shown in Figure 3, with the overlaid extracted ion chromatograms of the peak eluting at 9.55 min, the results table, and the measured versus the NIST20 library EI spectrum. The component was putatively identified against the NIST20 library as tetradecamethyl-cycloheptasiloxane, with a total score of 92.1, library search index (SI) = 702, and high-resolution filter value (HRF) = 95.3.

Elemental composition

Mass Frontier structural elucidation software was used to aid the identification of the elemental composition for the ion *m/z* 340.2397 from the mass spectra for the peak at 19.63 min in the natural rubber / TEF septa sample (measured with 60,000 RP at FWHM 200 *m/z*), as illustrated in Figure 4.

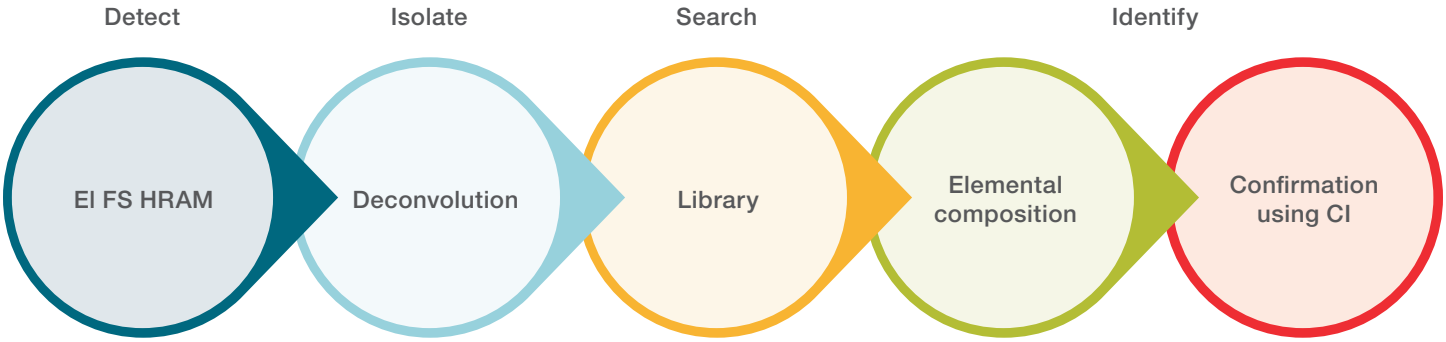


Figure 1. Workflow for non-targeted screening of vial septa. Full scan data were acquired using EI full scan HRAM; spectral deconvolution with library search for putative compound identification; and confirmation using chemical ionization (CI) data, for added specificity and selectivity.

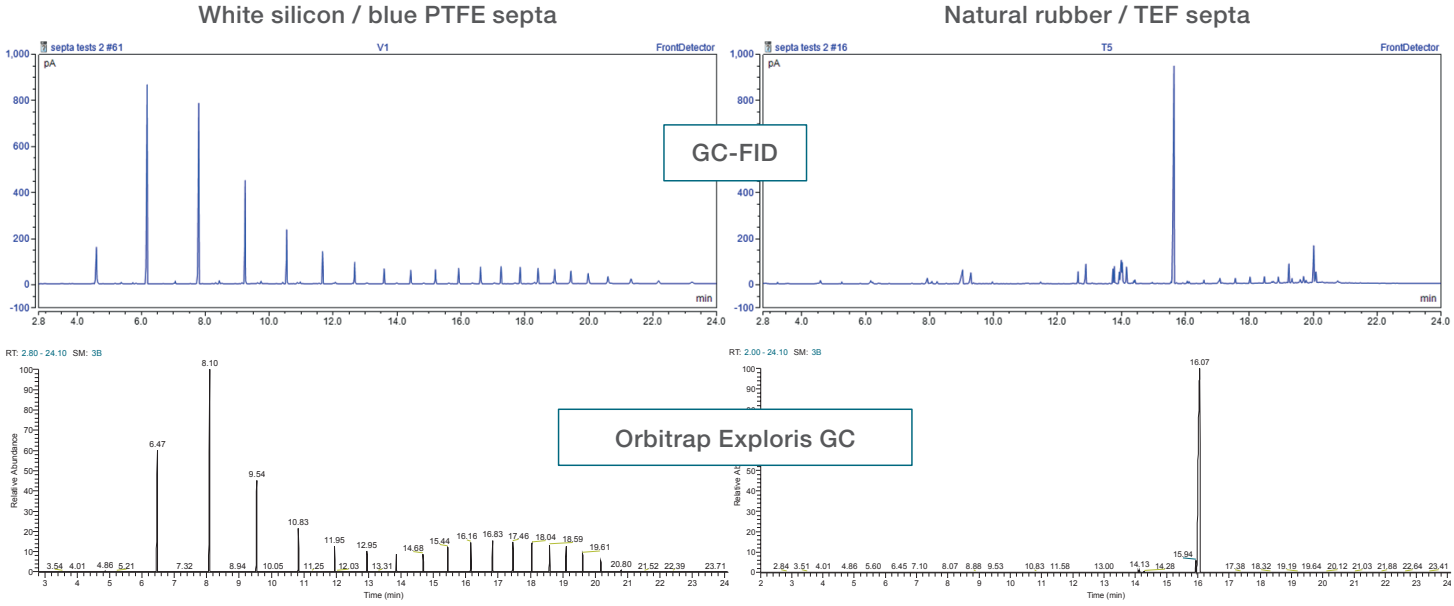


Figure 2. GC-MS TICs for EI full scan data (bottom) and GC-FID data (top), obtained for septa sample (white silicon / blue PTFE) and septa sample (natural rubber / TEF)

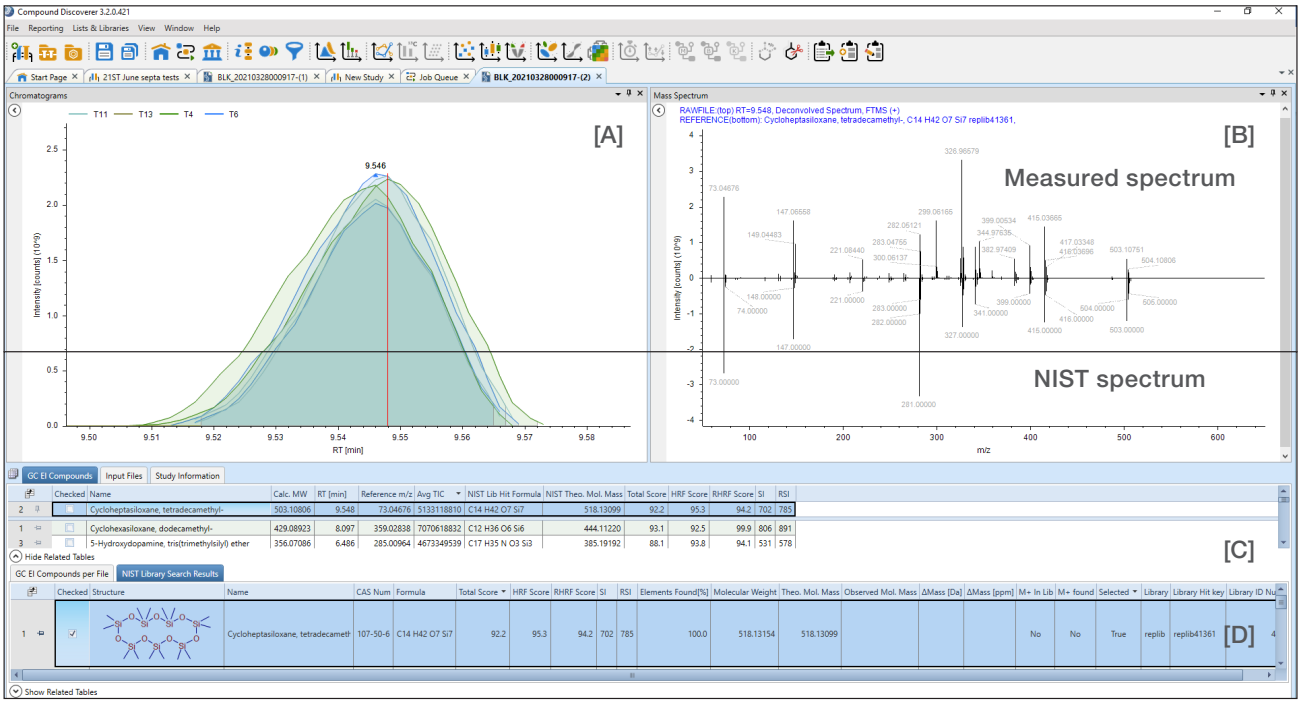


Figure 3. Compound Discoverer software deconvolution results for a range of the tested silicon / PTFE septa, for the compound eluting at RT= 9.55 min and putatively identified as tetradecamethyl-cycloheptasiloxane. [A] XIC for tetradecamethyl-cycloheptasiloxane; [B] Measured vs NIST20 library EI spectrum for tetradecamethyl-cycloheptasiloxane; [C] Result table with deconvoluted compound; [D] Library search results against NIST20.

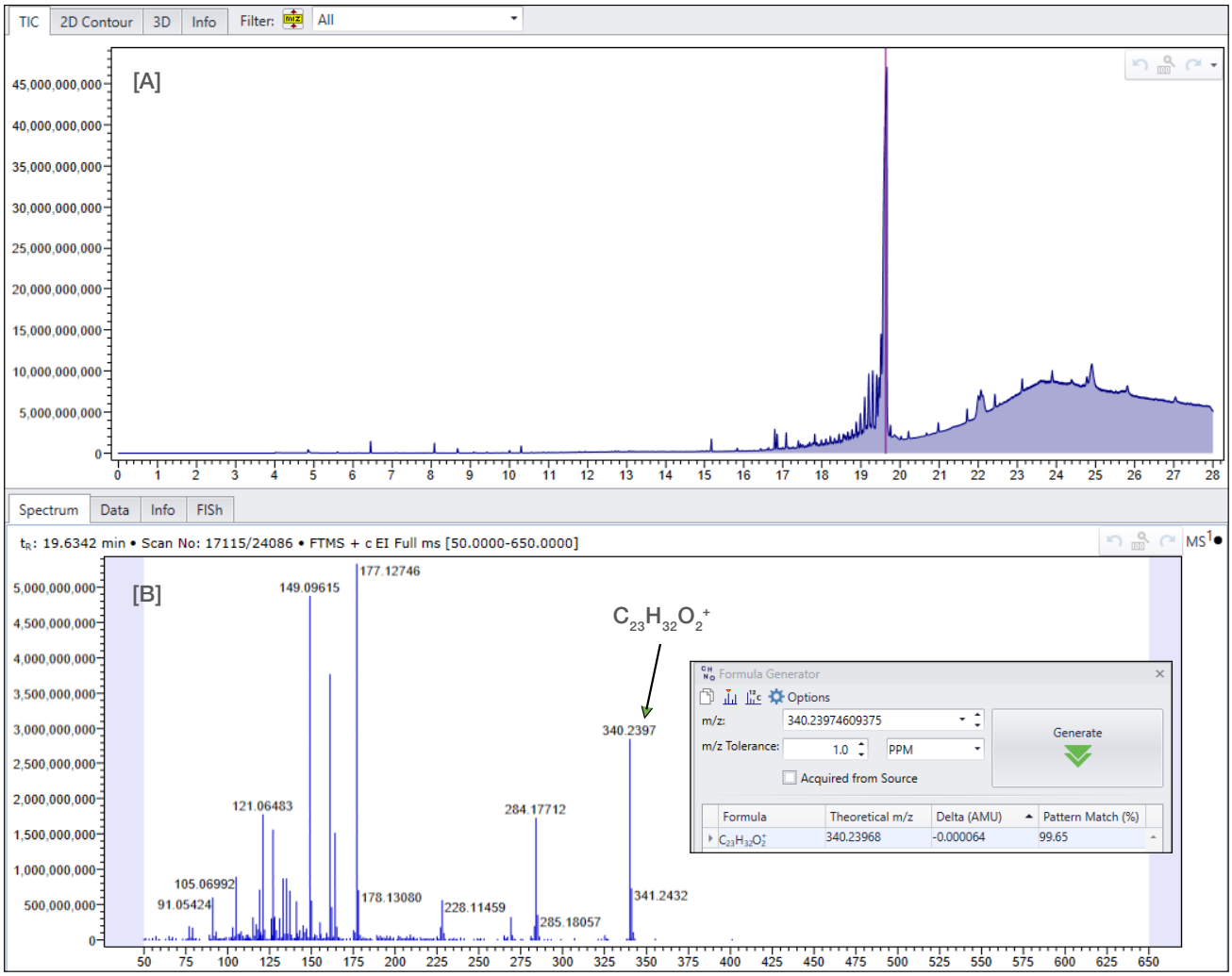


Figure 4. Mass Frontier structural elucidation software results used to aid identification of the elemental composition results for the peak at 19.63 min in the natural rubber / TEF septa sample. [A] TIC; [B] Mass spectra for the peak at 19.63 min, with Formula Generator results for the parent ion at *m/z* 340.2397.

Molecular ion and adduct confirmation

The NIST library results (Figure 5) generated for the mass spectra for the peak at 19.63 min in the natural rubber / TEF sample returned phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-, with a forward match of 882 and a reverse match of 887. Further confirmation in the identification of compounds was accomplished by assessing the spectra to identify the fragments achieved. Predicted fragments were generated using Mass Frontier software that match the fragment peaks present in the mass spectra for the peak at 19.63 min in the natural rubber / TEF sample, as shown in Figure 6 for the top 5 mass spectra peaks.

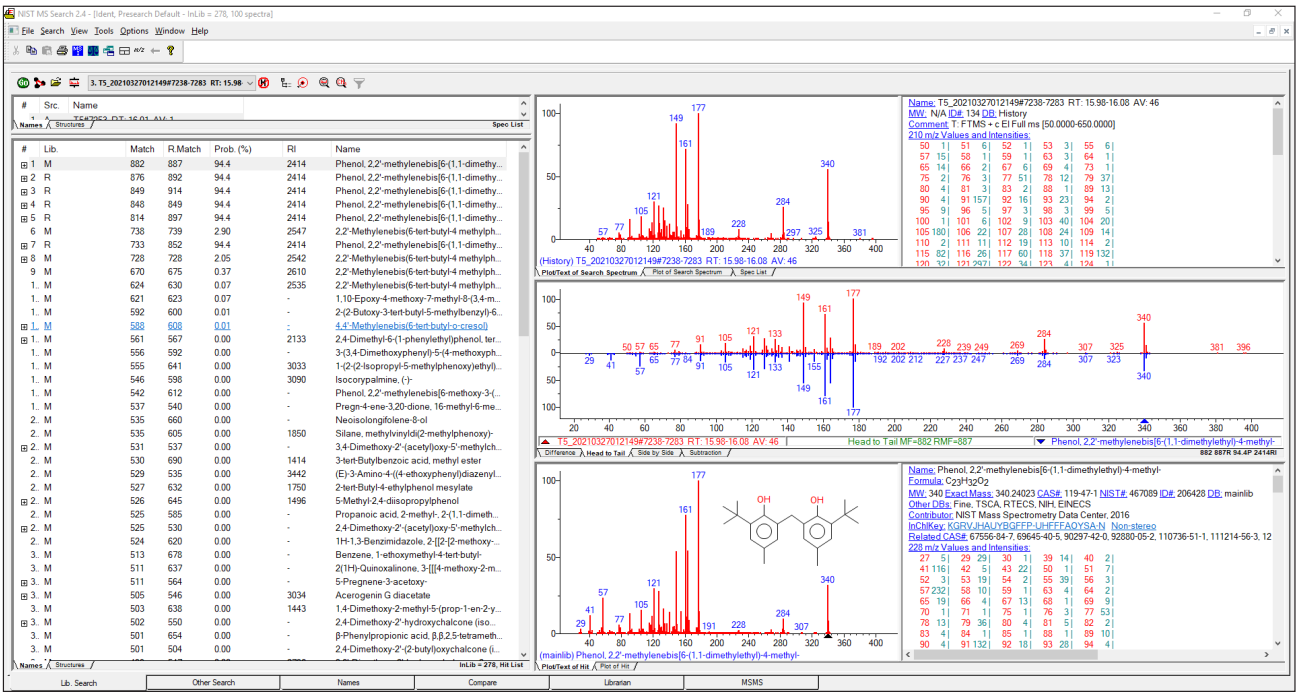
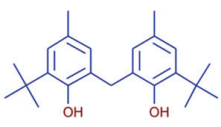
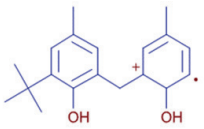
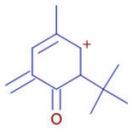
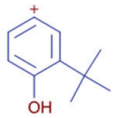
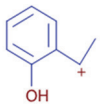


Figure 5. NIST library search results generated for the mass spectra for the peak at 19.63 min in the natural rubber / TEF sample with a top match of phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- returned

Peak number	1	2	3	4	5
m/z (theoretical)	340.2367	284.1771	177.1274	149.0961	121.0648
Proposed structure					

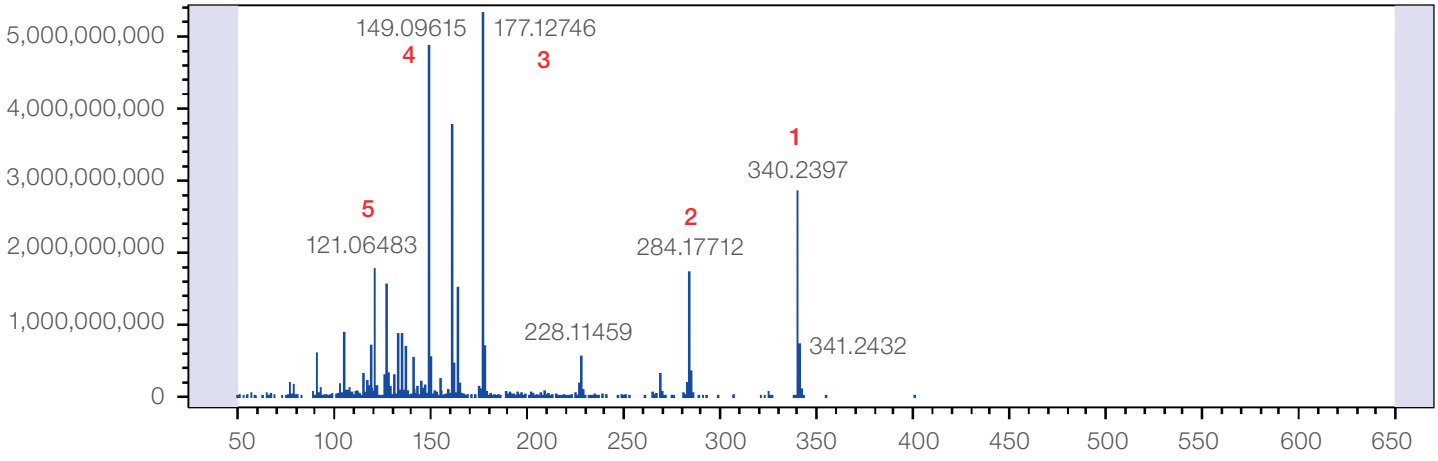


Figure 6. Mass Frontier software generated proposed structures for the ions generated in the mass spectra for the peak at 19.63 min in the natural rubber / TEF sample, against the top match of phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-

Multivariate statistical analysis: PCA and V-Plot

To quickly assess if significant differences between sample groups exist and what they are, full scan data were imported into Compound Discover software and a multivariate statistical analysis step was carried out on a range of septa. Principal component analysis (PCA) is a statistical approach that highlights variations between sample groups and allows visualization of strong patterns in complex datasets. The generated PCA plot is shown in Figure 7, highlighting the variation between different samples.

To isolate the chemical components responsible for these variations, differential analyses were carried out using volcano-plots (V-plots), which are useful to quickly identify changes in large data sets composed of replicate data. A V-plot comparing two of the analyzed samples is shown in Figure 8.

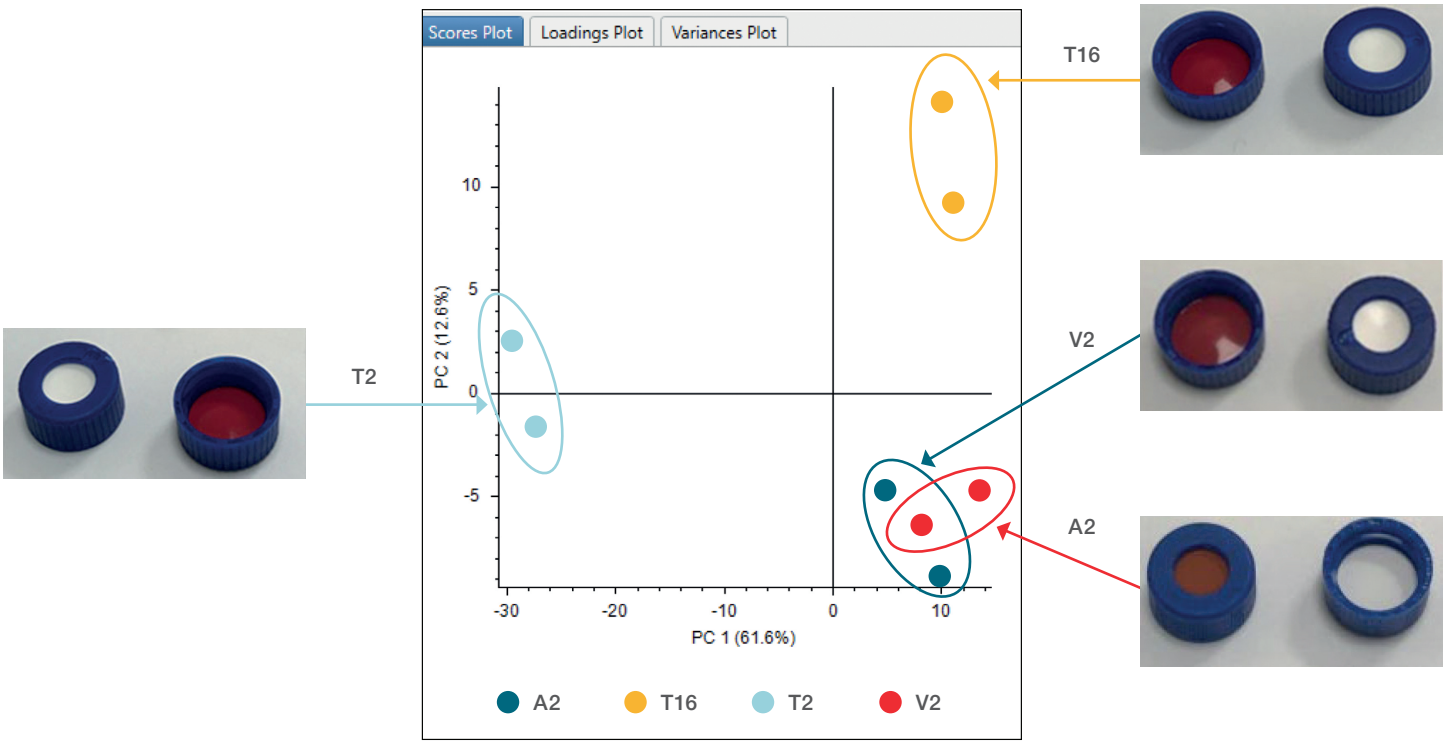


Figure 7. Centered PCA score plot obtained for a range of the tested vial septa. The PCA plot highlights the variations between different samples.

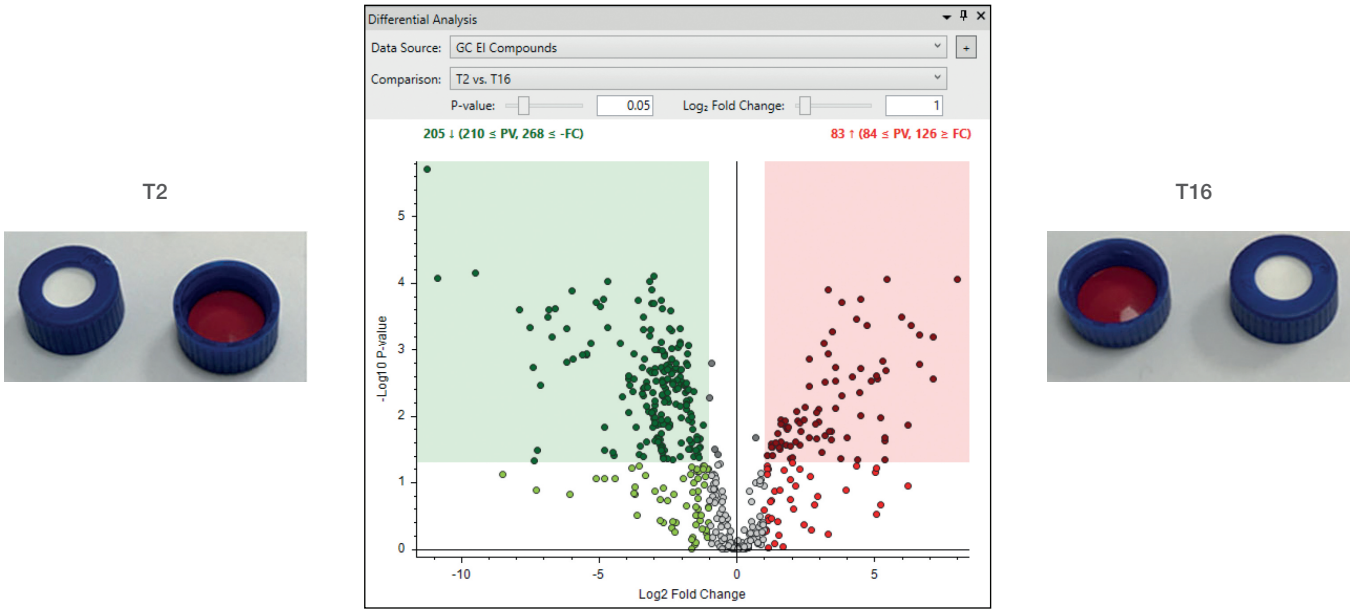


Figure 8. A volcano-plot scatterplot showing the statistical significance (p-value) versus the magnitude (fold change) when comparing two of the tested septa

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Conclusions

- The results of this study demonstrate that using the Orbitrap Exploris GC with unique intuitive software workflows for automated deconvolution and extensive spectral libraries provides excellent solutions for non-targeted screening, illustrated for the analysis of extractables from vial septa. The Orbitrap mass spectrometer delivers excellent mass accuracy for all components in a sample, leading to fast, confident characterization of samples.
- Reliable and robust chromatographic separation in combination with fast data acquisition speeds make the Orbitrap Exploris GC mass spectrometer an ideal platform for chemical profiling of complex samples.
- Compound Discoverer 3.2 software provides sophisticated algorithms for spectra deconvolution and library search combined with statistical analysis in one single platform for putative identification of compounds and streamlined data analysis. Moreover the predictive fragmentation and structural elucidation capabilities of Mass Frontier software allows for additional confidence in compound identification.
- The consistent sub 1-ppm mass accuracy, in combination with the excellent sensitivity, makes confident identification of all components in a sample possible. Routine resolving power of 60,000 FWHM and wide dynamic range eliminates isobaric interferences, which increases confidence in the results in complex matrix.
- Using statistical tools available within Compound Discover software, it is possible to distinguish the differences between different septa, highlighting for example the different proportion of high to low mass range siloxanes.

Find out more at **thermofisher.com/OrbitrapExplorisGC**

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