thermo scientific

APPLICATION NOTE 10704

Analytical solutions for challenges in headspace GC-MS analysis of volatile extractable and leachable compounds

Authors

Xiaoteng Gong[1], Dujuan Lu[1], Danny Hower[1], Julian Gulbinski[1], Daniela Cavagnino[2], and Cristian Cojocariu[3]

[1]SGS, Fairfield, NJ, USA
[2]Thermo Fisher Scientific, Milan, IT
[3]Thermo Fisher Scientific, Runcorn, UK

Keywords

Extractables and leachables, E&L, Orbitrap GC-MS, HRAM, valve and loop, static headspace, gas chromatography, GC, gas chromatography-mass spectrometry, GC-MS, flame ionization detector, FID

Goal

This work illustrates the headspace GC-MS method development and optimization for volatile extractable and leachable (E&L) compounds. In particular, the focus has been on the partition coefficient of common organic volatile impurities (OVIs) in a variety of matrices, phase ratio, and equilibrium time, which provide theoretical foundations for incubation temperature, salt addition, and sample/vial volume ratio. In addition, different headspace (HS) sampling strategies for GC-MS analysis of OVIs in packaging components were compared and optimized.

This work highlights the simplicity of the static headspace method set up and optimization, making this sampling technique very suitable and convenient for the study of volatiles extractables in polymeric materials. Additionally, the coupling of the headspace sampling technique with a high performing HRAM GC-MS system is expected to open advanced detection and identification capability for better understanding and characterization of volatiles impurities profiling in polymeric materials.

ThermoFisher SCIENTIFIC

Introduction

Pharmaceutical products come into contact with a wide range of polymeric materials on their journey from the production line to patients. Plastic and rubber contact surfaces are present at almost every stage of a product's lifecycle: in the single-use systems such as filters and tubing employed in manufacturing processes, in the packaging components that protect medicines during transport, and in the delivery devices such as syringes, pens, and inhalers used to administer treatments to patients. While these materials are essential to ensure the sterility and quality of medicines during their manufacture and storage, they can also pose a serious risk to human health, through the leaching of potentially dangerous substances into products, with the risk to compromise the stability or even worse the pharmacological activity of the product. These substances, known as extractables and leachables, are the focus of rigorous testing workflows to ensure therapeutics are safe for use and meet regulatory requirements.

Given the broad range of materials that may be present in a single device, packaging unit, or storage container, identification of the contact component from which they originate is essential. Plastics can contain a wide range of extractables and leachables derived from additives and storage aids such as antioxidants, plasticizers, emulsifiers, and colorants. Due to the wide range of volatility and polarity of such chemicals, different chromatographic techniques are used for a comprehensive study. Commonly, gas chromatography equipped with a headspace sampler and coupled to mass spectrometry (headspace GC-MS) is used to analyze the volatile fraction of extractable and leachable compounds. In extractable and leachable studies, it can be challenging to achieve sufficient sensitivity and selectivity because of the diversity of unknown analytes potentially present and the complex matrix of the drug products. To address the challenges in the headspace GC-MS analysis of OVIs, a method development study based on the theory of gas phase chemistry was performed to optimize the headspace sampling parameters. The new Thermo Scientific™ TriPlus™ 500 Gas Chromatography Headspace Autosampler has been used in conjunction with a high-resolution, accurate mass (HRAM) GC-MS/FID, based on the Thermo Scientific™ Orbitrap™ technology. This results in a powerful technique to assess and quantify potentially dangerous untargeted volatile extractables at trace level.

Experimental

For the experiments, a TriPlus 500 Headspace autosampler was coupled to a Thermo Scientific TRACE™ 1310 GC-FID and Thermo Scientific™ Q Exactive™ GC Orbitrap™ GC-MS/MS system (Figure 1). The headspace method optimization has been performed with the GC-FID configuration, as the first step of the project, before data acquisition with the HRAM GC-MS system. The experimental conditions for the headspace autosampler and the GC-FID system are reported in Table 1.

Figure 1. TriPlus 500 Headspace Autosampler coupled to the TRACE 1310 GC and Q Exactive GC Orbitrap GC-MS/MS system

Table 1A. TriPlus 500 Headspace Autosampler operating conditions

TriPlus 500 Headspace Autosampler Operating Parameters	
Incubation Temperature (°C):	80
Incubation Time (min):	30
Shaking:	Fast
Pressurization Mode:	Pressure
Vial Pressure (kPa):	100
Pressure Equilibration Time (min):	0.2
Loop Volume (mL):	1
Loop/Sample Path Temp. (°C):	150
Loop Pressure (kPa):	40
Loop Equilibrium Time (min):	1
Needle Purge Flow:	2
Injection Time (min):	1.5
Injection Mode:	Standard
Vial Volume (mL):	20

Table 1B. Trace 1310 GC-FID operating conditions

TRACE 1310 GC Operating Parameters		
Carrier Gas:	Helium	
Carrier Gas Flow (mL/min):	1	
Oven Temperature Program:		
Rate (°C/min)	*Temperature (°C)*	*Hold Time (min)*
–	40.0	1.0
7.0	200.0	7.0
FID		
Air Flow (mL/min):	350	
Hydrogen (mL/min):	35	
Nitrogen (mL/min):	40	
Column:	Thermo Scientific™ TraceGOLD™ TG-624 30 m × 0.32 mm ID 1.8 µm P/N 26085-3390	

Table 2. Q Exactive GC Orbitrap mass spectrometer parameters

Q Exactive GC Orbitrap GC-MS/MS System Parameters	
IMS Acquisition Mode:	Full MS or SIM
Ionization Mode:	EI
Polarity:	Positive
Resolution:	60,000 (FWHM at *m/z* 200)
AGC Target:	10E6
Scan Range:	35 to 500 *m/z*
MS Transfer Line Temp.(°C):	250
Ion Source Temp. (°C):	200
Lock Mass Ions (*m/z*):	73.04680, 133.01356, 207.03235, 281.05114, 355.06990

Samples considered for this project are pre-filled syringe plunger stoppers for the determination of possible extractable compounds. The plunger stoppers are rubber made components coming into contact with liquid medication for injection when loaded into a disposable syringe, and therefore represent a possible source of contamination.

After the HS method optimization, identification of unknowns in the test samples was possible due to the full scan HRAM data acquired with the Orbitrap-based GC-MS system. The system parameters used for the Q Exactive GC Orbitrap Mass Spectrometer are reported in Table 2.

Thermo Scientific™ TraceFinder™ software was used for both FID and HRMS data acquisition and to control the entire HS-GC-MS system.

Sample preparation

Different approaches for sample preparation were evaluated for the extractable study of rubber plunger stoppers, with the purpose to optimize the extraction efficiency (Figure 2).

Figure 2. Samples prepared for extractable study from rubber plunger stoppers

Procedure A

Plunger stoppers (2 stoppers/vial) were put into 20 mL HS vials. The instrument method was the same as reported in Table 1, except for the HS oven incubation temperature, which was set at 160 °C.

Procedure B

Thirty plunger stoppers were immersed in 30 mL of LC-MS grade water (extraction solvent) in a solvent bottle. Incubation was performed at 50 °C for 24 hours. Then, 2 mL of extract were transferred into a 20 mL HS vial. This extract amount corresponds to two stoppers/vial. The instrument method was the same as reported in Table 1.

Procedure C

Plunger stoppers (two stoppers/vial) were put into a 20 mL HS vial with 2 mL of LC-MS grade water (extraction solvent). The instrument method was the same as reported in Table 1.

Procedure D

Thirty plunger stoppers were immersed in 30 mL of LC-MS grade water (extraction solvent) in a solvent bottle. Incubation was performed at 50 °C for 24 hours. Five hundred milligrams of sodium chloride (NaCl) were put into a 10 mL HS vial. Two milliliters of extract were transferred into the 10 mL HS vial with salt. The extract amount corresponds to two stoppers/vial. The instrument method was the same as reported in Table 1, except for the HS vial volume. A 10 mL HS vial was used in this case.

Extraction blank

Two milliliters of LC-MS grade water (extraction solvent) were transferred into a 20 mL HS vial. A blank solution including 500 mg of NaCl was also prepared by adding 2 mL of LC-MS grade water in a 10 mL HS vial, to compare with procedure D. The instrument method was the same as reported in Table 1.

Results and discussion

The risk of polymer-derived extractables entering pharmaceutical products has increased in recent years due to the growing adoption of single-use technologies and novel packaging solutions with unknown formulations. The volatile fraction of E&L compounds can be conveniently analyzed with a simple sample preparation using the headspace sampling technique, which can extract the volatile fraction and leave behind heavier non-volatile matrix compounds, thus preventing possible contamination of the analytical system.

The main challenges of this type of analysis are related to the complexity of the matrices, the diversity of extractable and leachable compounds, and the required sensitivity of the analytical method. The headspace extraction technique allows the injection of only the volatile fraction, leaving behind the non-volatile matrix and helping to reduce the complexity of the analysis. By optimizing the extraction procedure and increasing the selectivity of the analytical system through high-resolution, accurate mass acquisition, it was possible to develop an efficient method for the determination of known and unknown volatile extractables in rubber material such as the pre-filled syringe plunger stoppers.

Sample extraction optimization

The headspace sampling technique is based on the equilibrium of volatile compounds' concentration established in a closed vial between a condensed phase (the sample) and the gas phase above it (the headspace) (Figure 3). At equilibrium, the volatile compounds are characterized by a partition coefficient (K) representing the ratio between the concentration of the analyte in the sample and the concentration of the analyte in the gas phase. Some key factors that influence the K value in favor of the gas phase, such as the temperature of the vial and the matrix composition,[1] allow for the maximization of the concentration of the target analytes in the headspace, increasing the efficiency of the extraction and the sensitivity of the method.

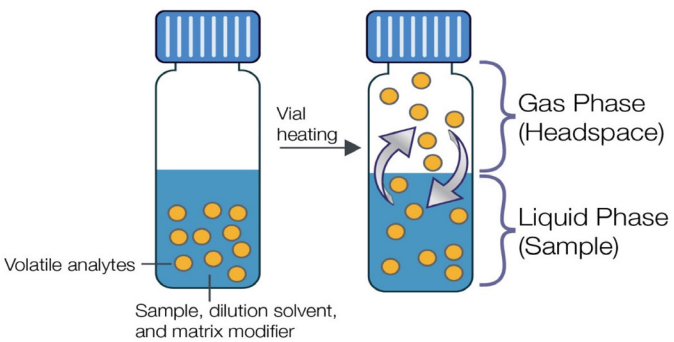


Figure 3. Principle of the static headspace sampling technique

In this study, the most significant gain in sensitivity was achieved by acting on the matrix modification and reducing the phase ratio. A common way to modify an aqueous sample matrix to favor volatile organic compounds into the gas phase is the addition of inorganic salt. In this case, the addition of NaCl showed an evident improvement in terms of sensitivity. With the same purpose, the phase ratio was reduced by using a 10 mL vial in place of a 20 mL vial. In Figure 4, the FID chromatograms obtained with the sample preparation procedures B (standard method) and D (optimized method) are compared.

Comparing the sample chromatogram obtained with procedure D with the blank solution, some peaks could be attributed to impurities in salt or in water and therefore were not considered as extractables (Figure 5).

Procedure C was found to be the least efficient procedure for water extraction of the sample during this study.

Unknowns identification

Determining the identity of compounds not present in commercial libraries was once a complex challenge, requiring a significant amount of time and a good deal of analytical detective work. Thanks to highly accurate mass measurements delivered by the Orbitrap GC-MS, it is possible to accurately determine the exact mass of the analytes and their MS fragments, confirm their chemical composition, and elucidate unknown compound chemical structures with confidence. Assessment of measured vs. theoretical isotopic pattern is also critical for unambiguous confirmation of elemental composition of unknowns.

The optimization of the headspace method allowed for the identification of several extracted compounds, as shown in Figure 6, through improvement of sensitivity on MS.

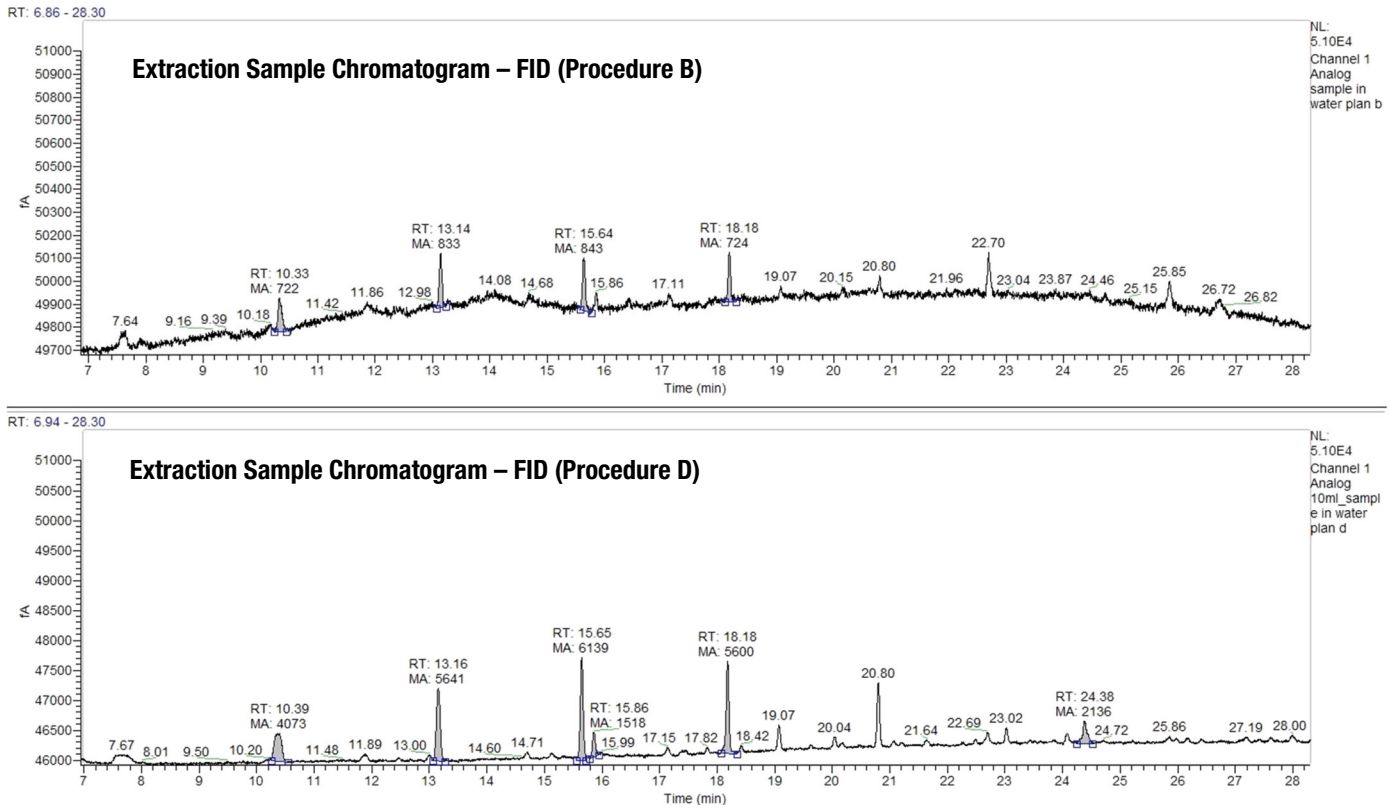


Figure 4. HS-GC-FID chromatograms of sample prepared with procedure B (top) and sample prepared with procedure D (bottom)

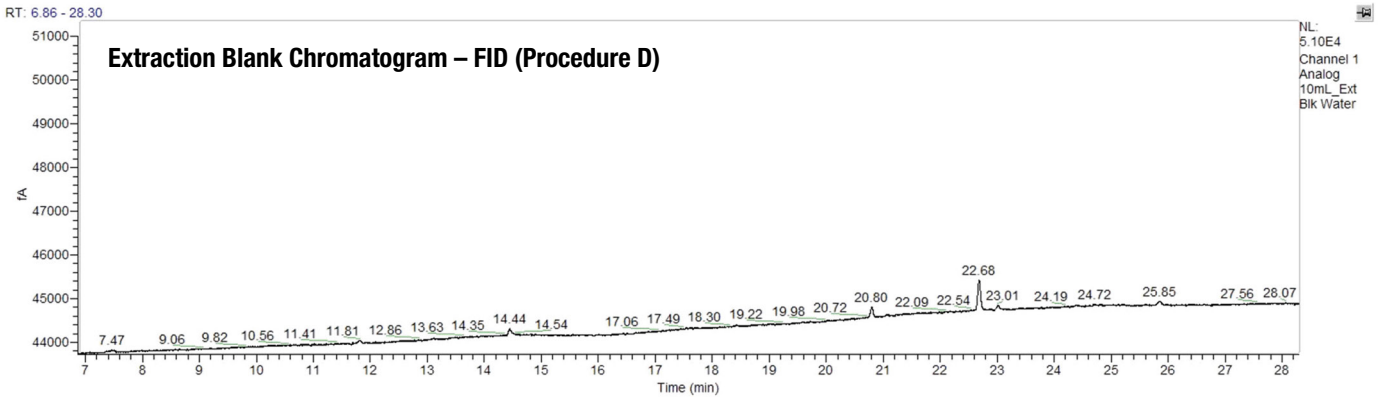


Figure 5. Peaks with RT 20.8, 22.68, 23.02, and 25.86 are not considered as extractables as found in the blank solution.

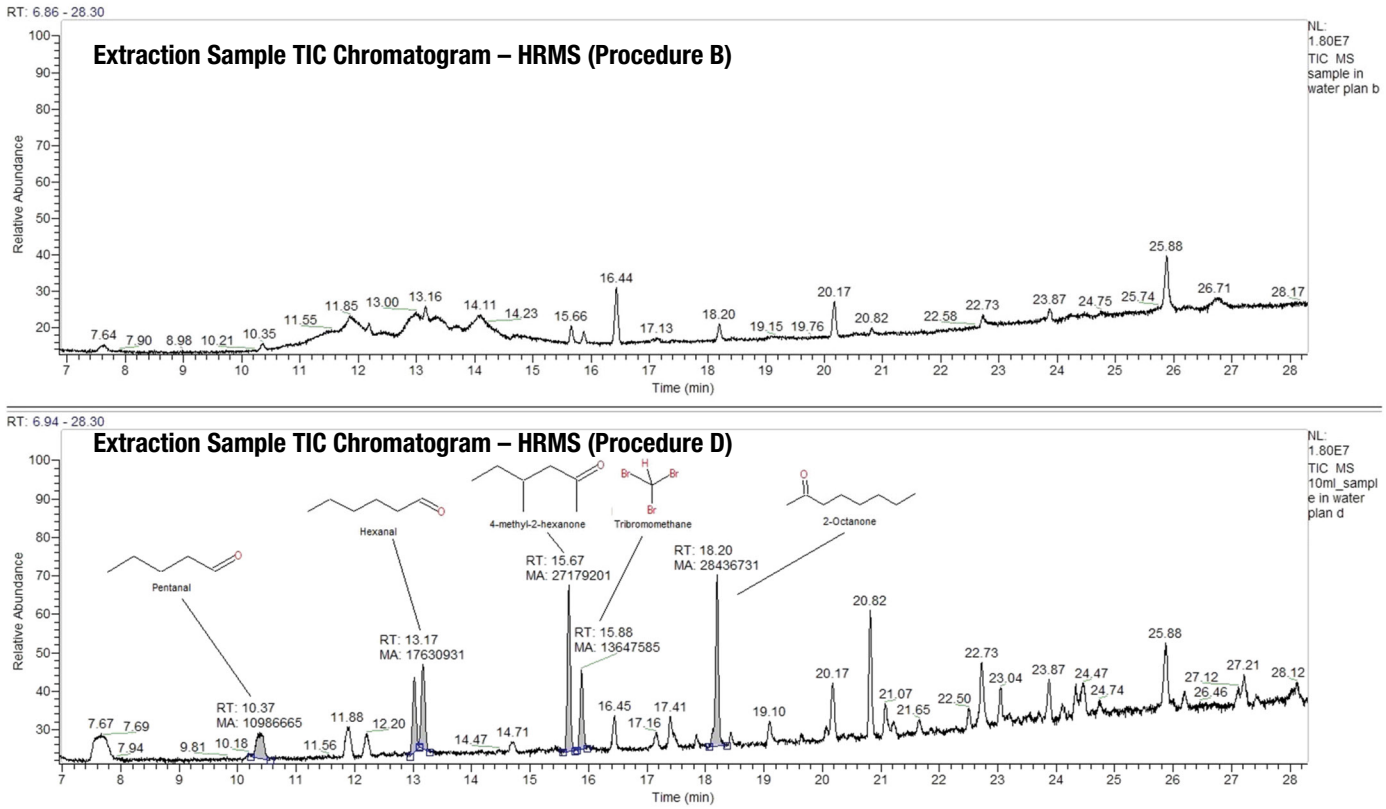


Figure 6. HS-HRAM GC-MS chromatograms (TIC, EI full-scan) of sample prepared with procedure B (top) and sample prepared with procedure D (bottom)

Additional unknown compounds could be extracted by using stronger extraction conditions. A different procedure involving dry heating of the plunger stoppers without water extraction permitted an increase of the incubation temperature up to 160 °C. Under those conditions, additional unknown compounds could be identified, providing a deeper understanding of the extractable profile of the sample. A comparison of the MS chromatograms of the water and dry extracted headspace can be seen in Figure 7. In particular, a typical oligomer formed in the copolymerization of isoprene and isobutylene,[2] 1-isopropenyl-2,2,4,4-tetramethylcyclohexane ($C_{13}H_{24}$), was clearly visible in the chromatogram of the dry solid sample headspace. Due to hydrophobic structure, it was not detected in the water extract of the plunger stoppers. With the accurate mass information of the molecular ion and all the fragments in the mass spectrum, it was possible to acquire a confident elucidation of the structure (Figure 8).

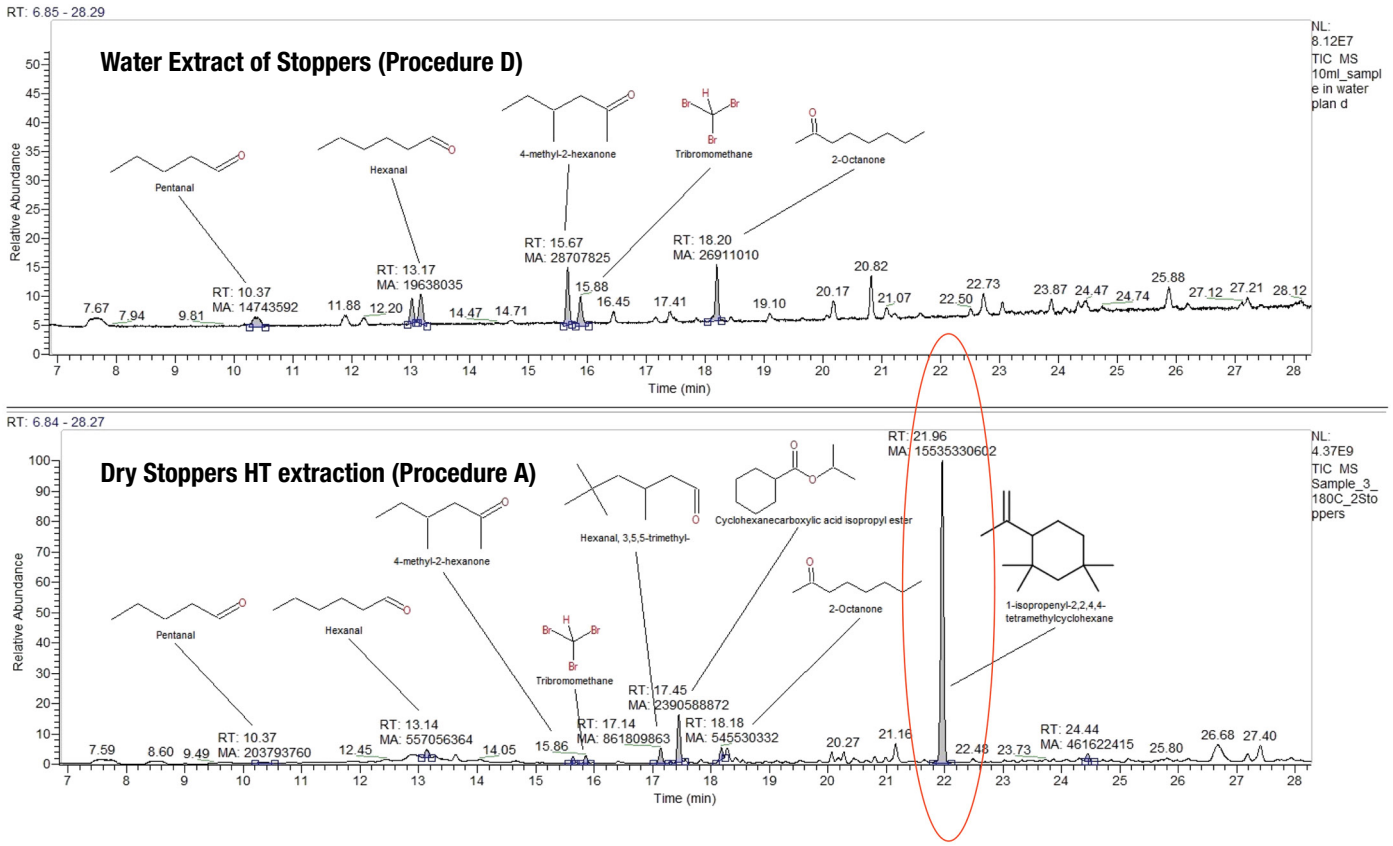
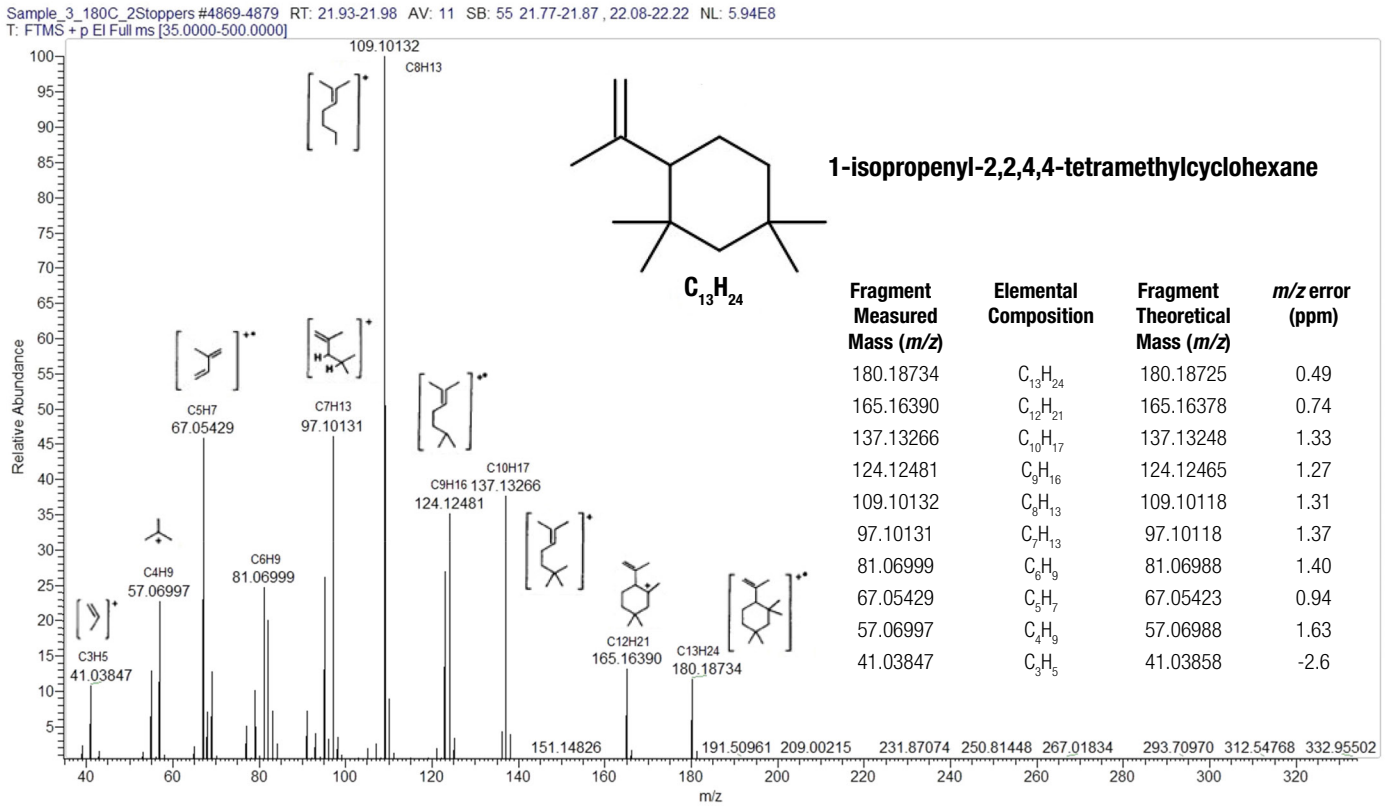


Figure 7. HS-HRAM GC-MS chromatograms of sample prepared with procedure D (top) and sample prepared with procedure A (bottom). Highlighted peak indicates an oligomer that was visible in the dry solid sample headspace.



Fragment Measured Mass (*m/z*)	Elemental Composition	Fragment Theoretical Mass (*m/z*)	*m/z* error (ppm)
180.18734	$C_{13}H_{24}$	180.18725	0.49
165.16390	$C_{12}H_{21}$	165.16378	0.74
137.13266	$C_{10}H_{17}$	137.13248	1.33
124.12481	$C_{9}H_{16}$	124.12465	1.27
109.10132	$C_{8}H_{13}$	109.10118	1.31
97.10131	$C_{7}H_{13}$	97.10118	1.37
81.06999	$C_{6}H_{9}$	81.06988	1.40
67.05429	$C_{5}H_{7}$	67.05423	0.94
57.06997	$C_{4}H_{9}$	57.06988	1.63
41.03847	$C_{3}H_{5}$	41.03858	-2.6

Figure 8. HRAM spectrum of the oligomer 1-isopropenyl 2,2,4,4-tetramethylcyclohexane ($C_{13}H_{24}$). Accurate mass for all the fragments is reported in the table with elemental composition and the mass accuracy (ppm).

Unknown compounds are identified based on library search, ion formulation with high resolution masses, and retention index. Additional work is on-going to get further ID confirmation with CI experiments.

The accurate mass was around 1 ppm for almost all the identified compounds, as reported in Table 3.

Conclusions

The results of this study demonstrate that the integration of the TriPlus 500 Headspace Autosampler with the Q Exactive GC Orbitrap mass spectrometer and TraceFinder software is a powerful solution for extracting, profiling, and identifying unknown peaks in complex samples.

- An efficient and robust static headspace sampling method was optimized to generate a high number of candidate peaks for compound identification. The key parameters of incubation temperature, matrix composition, and phase ratio were evaluated.
- During method optimization with integrated FID, significant sensitivity gains were observed by the addition of NaCl, combined with the use of smaller sample vial volume. While water extraction was found to be the least efficient extraction protocol, the dry heating of the plunger stopper samples to 160 °C increased the detection of unknown compound peaks.
- Robust chromatographic separation in combination with high mass resolution of 60,000 FWHM make the Q Exactive GC system an ideal platform for chemical profiling of complex samples. The low ppm mass accuracy, in combination with excellent sensitivity, makes confident identification of all components in a sample possible. NIST-searchable EI spectra and retention index scoring enabled several compounds to be identified with speed and confidence in the syringe plunger samples analyzed.

References

1. Kolb, B.; Ettre, L.S. Static Headspace-Gas Chromatography: Theory and Practice. Wiley-VCH, Hoboken, NY, 1997.
2. Kuntz, I.; Powers, K. W.; Hsu, C. S.; Rose, K. D. *Cyclic oligomer formation in the copolymerization of isoprene with isobutylene.* Makromol. Chem., Macromol. Symp. 13/14, 337-362 (1988).

Table 3. HRAM MS compound identification in dry headspace extract of rubber plunger stoppers

Identified Compound	Formula	RT (min)	Observed Base Peak or Molecular Ion	Mass Accuracy Δm (mDa)	Mass Accuracy (ppm)
Pentanal	$C_5H_{10}O$	10.37	58.04135	-0.03	0.58
Hexanal	$C_6H_{12}O$	13.14	82.07763	0.07	-0.88
4-methyl-2-hexanone	$C_7H_{14}O$	15.64	114.10399	-0.07	0.64
Tribromomethane	$CHBr_3$	15.86	172.84187	0.04	-0.20
3,5,5-trimethylhexanal	$C_9H_{18}O$	17.14	142.13538	-0.16	1.15
Cyclohexanecarboxylic acid isopropyl ester	$C_{10}H_{18}O_2$	17.45	129.09096	0.05	-0.36
2-Octanone	$C_8H_{16}O$	18.18	128.11970	-0.13	1.04
1-isopropenyl 2,2,4,4-tetramethylcyclohexane	$C_{13}H_{24}$	21.96	180.18732	-0.07	0.38

Find out more at **thermofisher.com/TriPlus500**

ThermoFisher SCIENTIFIC