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Application Note

Food Testing and Agriculture

Determination of 2-MCPD and 3-MCPD Fatty Acid Esters in Infant Formula Using an Agilent 8890 GC System with an Agilent 5977B GC/MSD

Authors

Youjuan Zhang, Xia Yang, and Shuai Wang
Agilent Technologies (Shanghai) Co. Ltd. Shanghai 200131 P. R. China

Abstract

This application note describes a reliable analytical method for determining the fatty acid esters of 3-monochloropropane-1,2-diol (3-MCPD) and 2-monochloropropane-1,3-diol (2-MCPD) in infant formula. Two different derivatization reagents, heptafluorobutyrylimidazole (HFBI) and phenylboronic acid (PBA), were evaluated for sample preparation. An Agilent 8890 GC system coupled with an Agilent 5977B GC/MSD was used for qualitative and quantitative analyses. Results demonstrated the benefits of the workflow solution for the analysis of monochloropropanediols in infant formula. Great peak shape and resolution were obtained. Satisfactory recoveries were achieved, ranging from 86.9 to 106.7%. Precision was also good, with the relative standard deviations less than 15%.

Introduction

Monochloropropanediols (MCPDs) are commonly monitored in edible fats and oils. MCPDs are food contaminants that are generated during food processing. Studies have found that many of the MCPDs found in food exist in the form of chloropropanediol fatty acid esters (MCPDEs). Free forms of MCPDs are released from their esterified forms during digestion. The chemical structures of 2- and 3-MCPD and their esters are shown in Figure 1.

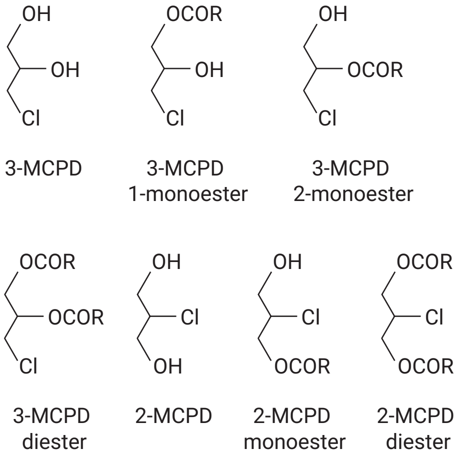


Figure 1. Chemical structures of 2- and 3-MCPD and their esters.

In general, analytical methods for the determination of MCPDEs follow two distinct routes: direct and indirect determination. The direct analysis of MCPDEs by LC/MS does not destroy the molecular structure. Due to the variety of MCPDEs and the difference of MCPDEs in different matrices, the corresponding standards and internal standards are needed for quantitative analysis using the direct LC/MS approach. The indirect determination of MCPDEs entails the cleavage of the MCPD from its esterified form, and therefore fewer standards are required. Several analytical methods have been standardized for the indirect analysis of MCPDEs in foods, such as: AOCS 2013a[1], AOCS 2013b[2], AOCS 2013c[3], ISO 18363-1[4], ISO 18363-2[5], ISO 18363-3[6], GB 5009.191-2016[7], and SN/T 5220-2019[8]. Those indirect methods all follow a similar protocol: cleavage of MCPD, sample cleanup, derivatization, and GC/MS analysis. The challenging steps are the cleavage of the MCPD from its esterified form (transesterification) and the derivatization reaction. The cleavage of MCPDs is carried out under acidic or alkaline conditions to form fatty acid methyl esters and MCPDs. Samples must be derivatized before GC/MS analysis due to the low volatility and high polarity of MCPDs. Despite the challenges and complex sample preparation, the indirect method is more popular than the direct method. The indirect determination is more desirable because of the low cost for standards and method versatility.

MCPDEs can potentially be found in various types of processed food. Many investigations have been reported about MCPDs analysis in oils.[9] However, analysis in milk matrices has been seldom reported. This application note specifies a procedure for the simultaneous determination of 2-MCPD and 3-MCPD fatty acid esters in infant formula in a single assay. The method is based on acid catalyzed ester cleavage and derivatization of the cleaved (free) analytes with HFBI or PBA prior to GC/MS analysis. Both the sample preparation and GC/MS conditions were optimized, and the performance of the analytical method was evaluated.

Experimental

Chemicals and reagents

All reagents and solvents were HPLC or analytical grade. Water was ultrapure, which was obtained using a purification system. *n*-Hexane, tetrahydrofuran (THF), heptane, sulfuric acid (purity ≥95%), methanol, acetone, heptafluorobutyrylimidazole (HFBI, purity ≥99%), rac 1,2-*bis*-palmitoyl-3-chloropropanediol (purity ≥98%), rac 1,2-*bis*-palmitoyl-3-chloropropanediol-d_5 (purity ≥99%), 1,3-distearoyl-2-chloropropanediol (purity ≥98%), 1,3-distearoyl-2-chloropropanediol-d_5 (purity ≥98%), and phenylboronic acid (PBA, purity ≥98%) were purchased from ANPEL Laboratory Technologies (Shanghai) Inc. Sodium hydrogen carbonate (purity ≥99.5%) was purchased from J&K Scientific Ltd. Sodium sulfate (purity ≥99%) was purchased from Sinopharm Chemical Reagent Co. Ltd.

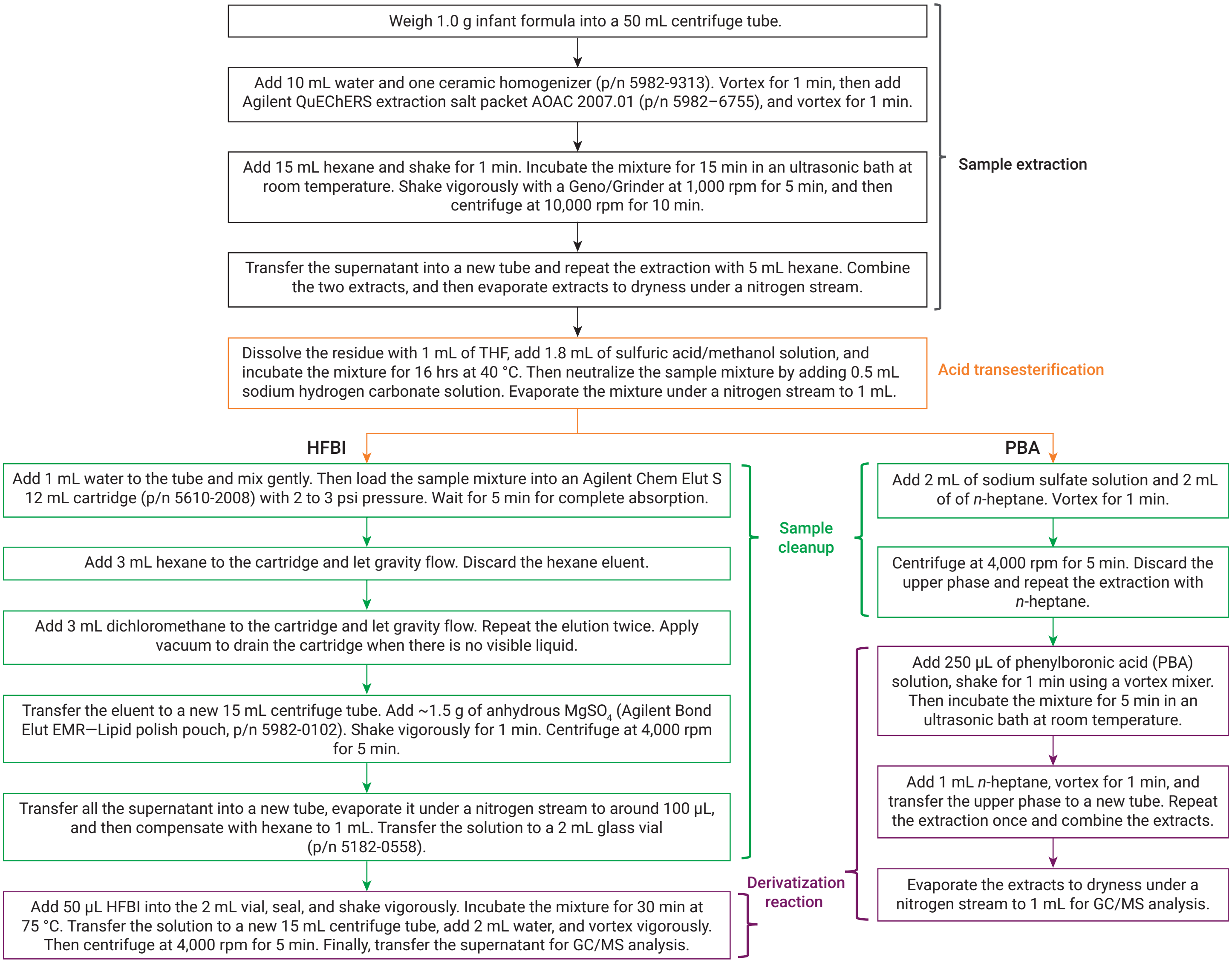
Solutions and standards

- The stock solution of MCPD esters was prepared using hexane as the solvent.
- The sulfuric acid/methanol solution (1.8%, volume fraction) was prepared by pipetting 1.8 mL of sulfuric acid into a 100 mL volumetric flask and filling it up to the mark with methanol.
- The sodium hydrogen carbonate solution (9.6%, mass concentration) was prepared by weighing 9.6 g of sodium hydrogen carbonate into a 100 mL volumetric flask and filling it up to the mark with ultrapure water. An ultrasonic bath was used to ensure the complete dissolution of the reagent.
- The phenylboronic acid solution (PBA solution) was prepared by weighing 2.5 g of phenylboronic acid and adding 20 mL of an acetone/ultrapure water mixture (19/1, volume fraction). The mixture was then shaken vigorously.
- The sodium sulfate solution (20%, mass concentration) was prepared by weighing 20 g of sodium sulfate into a 100 mL volumetric flask and filling it up to the mark with ultrapure water. An ultrasonic bath was used to ensure the complete dissolution of the reagent.

Samples and calibration standard preparation

The infant formula samples were purchased from a local grocery store. The entire sample preparation workflow is shown in Figure 2. Four major parts are included in the sample preparation process: sample extraction, acid transesterification, sample cleanup, and derivatization of cleaved (free) analytes with HFBI or PBA. The first two steps, sample extraction and acid transesterification, are the same for all samples. The final two steps, cleanup and derivatization, are different based on which derivatization reagent is used. Users can choose one of the two processes for sample preparation according to their preference and laboratory situation.

Matrix blanks were created by taking clean infant formula samples through the entire sample preparation procedure. Matrix matched calibration standards were prepared by spiking a standard solution into the matrix after sample extraction and before acid transesterification. The calibration standards correspond to 10, 20, 50, 100, 200, 500, and 1,000 µg/kg (equivalent to free form). The concentration levels mentioned in this study are all equivalent to MCPD free form. Rac 1,2-*bis*-palmitoyl-3-chloropropanediol-d_5 and 1,3- distearoyl-2-chloropropanediol-d_5 were used as the internal standards for 3-MCPD and 2-MCPD esters respectively. Internal standards were prepared with a concentration of 100 µg/kg. Prespiked quality control (QC) samples were made by spiking the appropriate standard working solution into infant formula,



Weigh 1.0 g infant formula into a 50 mL centrifuge tube.

Add 10 mL water and one ceramic homogenizer (p/n 5982-9313). Vortex for 1 min, then add Agilent QuEChERS extraction salt packet AOAC 2007.01 (p/n 5982–6755), and vortex for 1 min.

Add 15 mL hexane and shake for 1 min. Incubate the mixture for 15 min in an ultrasonic bath at room temperature. Shake vigorously with a Geno/Grinder at 1,000 rpm for 5 min, and then centrifuge at 10,000 rpm for 10 min.

Transfer the supernatant into a new tube and repeat the extraction with 5 mL hexane. Combine the two extracts, and then evaporate extracts to dryness under a nitrogen stream.

Dissolve the residue with 1 mL of THF, add 1.8 mL of sulfuric acid/methanol solution, and incubate the mixture for 16 hrs at 40 °C. Then neutralize the sample mixture by adding 0.5 mL sodium hydrogen carbonate solution. Evaporate the mixture under a nitrogen stream to 1 mL.

HFBI

Add 1 mL water to the tube and mix gently. Then load the sample mixture into an Agilent Chem Elut S 12 mL cartridge (p/n 5610-2008) with 2 to 3 psi pressure. Wait for 5 min for complete absorption.

Add 3 mL hexane to the cartridge and let gravity flow. Discard the hexane eluent.

Add 3 mL dichloromethane to the cartridge and let gravity flow. Repeat the elution twice. Apply vacuum to drain the cartridge when there is no visible liquid.

Transfer the eluent to a new 15 mL centrifuge tube. Add ~1.5 g of anhydrous $MgSO_4$ (Agilent Bond Elut EMR—Lipid polish pouch, p/n 5982-0102). Shake vigorously for 1 min. Centrifuge at 4,000 rpm for 5 min.

Transfer all the supernatant into a new tube, evaporate it under a nitrogen stream to around 100 µL, and then compensate with hexane to 1 mL. Transfer the solution to a 2 mL glass vial (p/n 5182-0558).

Add 50 µL HFBI into the 2 mL vial, seal, and shake vigorously. Incubate the mixture for 30 min at 75 °C. Transfer the solution to a new 15 mL centrifuge tube, add 2 mL water, and vortex vigorously. Then centrifuge at 4,000 rpm for 5 min. Finally, transfer the supernatant for GC/MS analysis.

PBA

Add 2 mL of sodium sulfate solution and 2 mL of of *n*-heptane. Vortex for 1 min.

Centrifuge at 4,000 rpm for 5 min. Discard the upper phase and repeat the extraction with *n*-heptane.

Add 250 µL of phenylboronic acid (PBA) solution, shake for 1 min using a vortex mixer. Then incubate the mixture for 5 min in an ultrasonic bath at room temperature.

Add 1 mL *n*-heptane, vortex for 1 min, and transfer the upper phase to a new tube. Repeat the extraction once and combine the extracts.

Evaporate the extracts to dryness under a nitrogen stream to 1 mL for GC/MS analysis.

Figure 2. The step-by-step procedure for sample preparation using HFBI or PBA as the derivatization reagent.

vortexing for 1 minute, then allowing them to settle for 5 minutes to achieve equilibrium for the sample extraction process. QC samples were quantified against calibration curves at the level of 200 µg/kg with five replicates.

Equipment and material

Equipment and material used for sample preparation include:

- SPEX SamplePrep 2010 Geno/Grinder (Metuchen, NJ, USA)
- Eppendorf Centrifuge (Hamburg, Germany)
- Agilent Vac Elut 20 manifold (part number 12234101)
- Agilent QuEChERS extraction salt packets, AOAC 2007.01 method (part number 5982-6755)
- Agilent Bond Elut EMR—Lipid polish pouch, 3.5 g anhydrous $MgSO_4$ (part number 5982-0102)
- Agilent ceramic homogenizer for 50 mL (part number 5982-9313)
- Agilent Chem Elut S, 3 mL sample, 12 mL tube (part number 5610-2008)

Instrument conditions

Analyses were performed on an Agilent 8890 GC system with an Agilent 5977B GC/MSD. The instrument conditions are listed in Table 1.

Table 1. Conditions for MCPD analysis.

Parameter	Value
Injection Volume	1 µL
Inlet	Split/splitless; temperature: 280 °C; splitless mode, purge flow 60 mL/min at 0.75 min
Inlet Liner	Agilent Ultra Inert, splitless, single taper, glass wool (p/n 5190-2293)
Column	Agilent J&W DB-5ms Ultra Inert GC column, 30 m × 0.25 mm, 1 µm (p/n 122-5533UI)
Carrier Gas	Helium, 1 mL/min, constant flow
Oven Program	**HFBI derivatives:** 50 °C (1 min), 20 °C/min to 90 °C, 2 °C/min to 100 °C (4 min), then 30 °C/min to 300 °C; post run temperature: 310 °C, post run flow: 3 mL/min, post run time: 5 min **PBA derivatives:** 50 °C (1 min), 25 °C/min to 180 °C (2 min), 2 °C/min to 190 °C (2 min), then 30 °C/min to 230 °C (10 min) post run temperature: 310 °C, post run flow: 3 mL/min, post run time: 5 min
Transfer Line Temperature	280 °C
Source Temperature	230 °C
Quadrupole Temperature	150 °C
Acquisition Mode	SIM
EM Voltage Gain Mode	2
Solvent Delay	5 min
Tune File	Etune.u

Results and discussion

Chromatogram

This study was performed on an 8890 GC system equipped with a split/splitless inlet and a 5977B GC/MSD with an electron ionization (EI) source. The MSD data were collected in selected-ion monitoring (SIM) mode and analyzed by Agilent MassHunter software. The quantification of 2- and 3-MCPD is based on the 2- and 3-MCPD-d5 signal ratios, respectively. Figures 3 and 4 show the extracted-ion chromatogram (EIC) traces for target compounds in infant formula at the spiking level of 200 µg/kg with HFBI derivatization. The system shows great resolution. The target compounds, including the internal standards, are baseline separated. Figure 5 shows the EIC traces at the spiking level of 200 µg/kg with PBA derivatization.

Compared to HFBI, the internal standards and target compounds are not well separated when using PBA. The coeluting compounds do not share common MSD fragments, so the ions that are unique to each compound can be extracted and processed separately. Many nontarget peaks can be seen eluting before and after the target compounds in the chromatograms, which are caused by the multistep reaction in the sample preparation. The postrun function of MassHunter software was used with higher oven temperature and column flow. This postrun function can reduce the analysis time, increase the sample throughput, and also reduce the contamination for the MSD. Although the post column backflush device was not used in this study, it is still recommended because it effectively protects and reduces the contamination for the column and MSD.

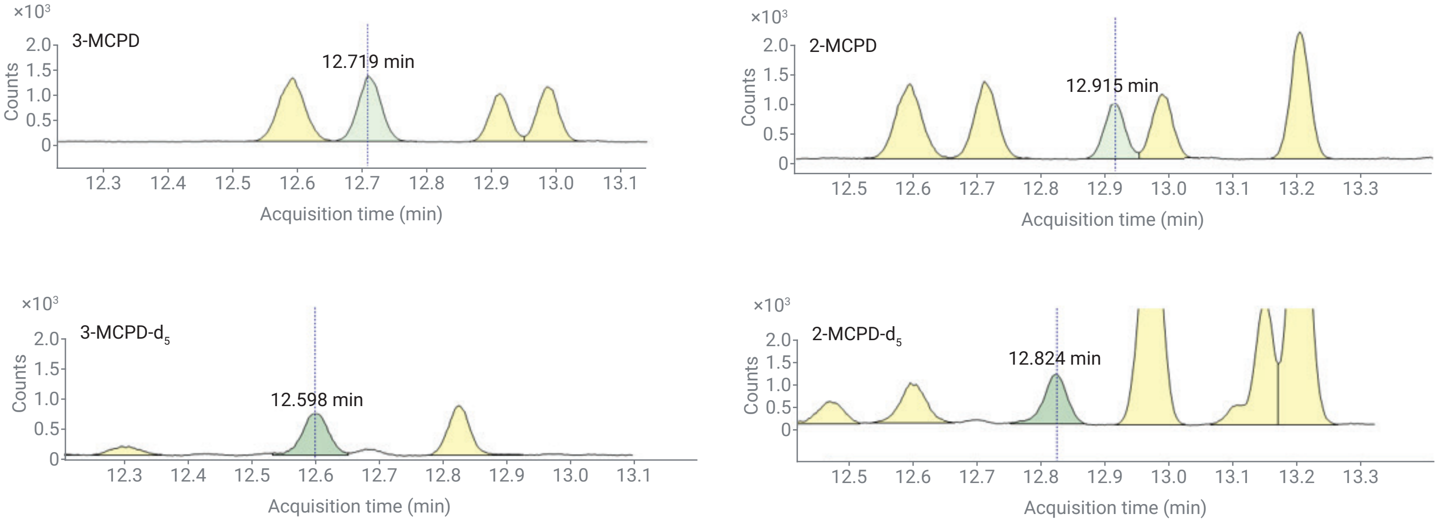


Figure 3. GC/MSD EIC trace of target compounds with HFBI derivatization at the spiking level of 200 µg/kg in infant formula.

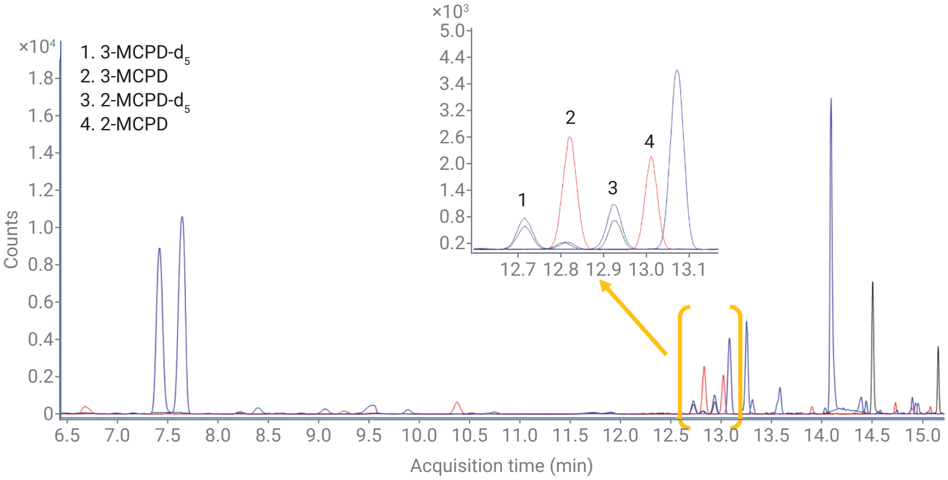


Figure 4. GC/MSD EIC trace of HFBI derivatives at the spiking level of 200 µg/kg in infant formula.

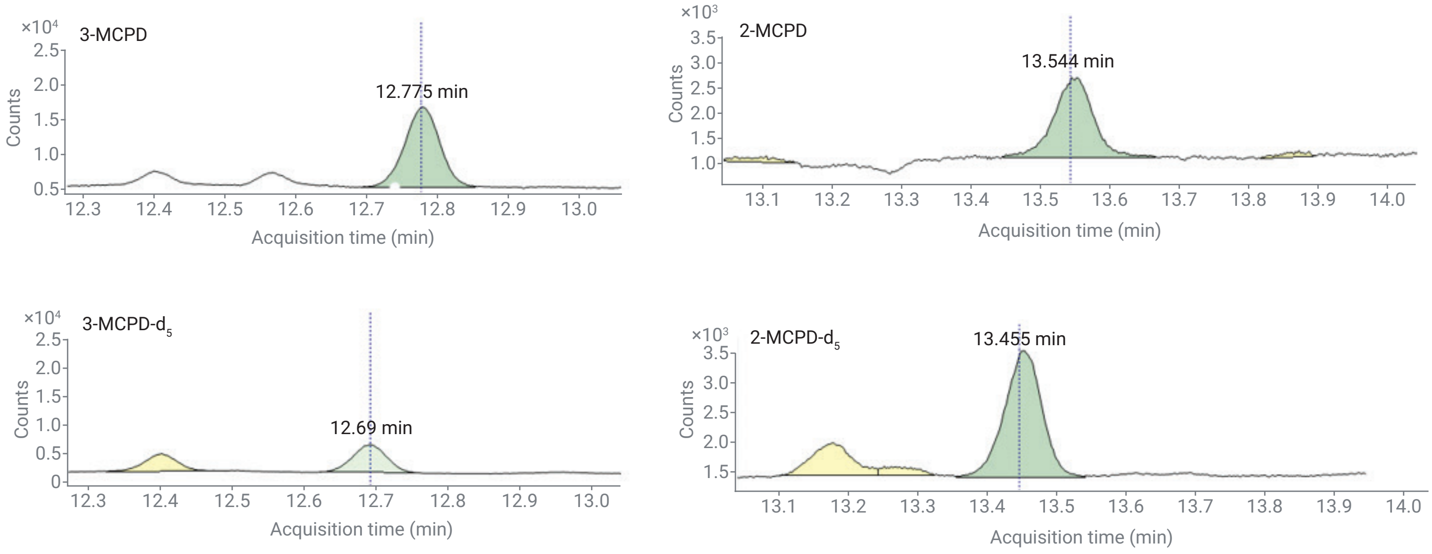


Figure 5. GC/MSD EIC trace of target compounds with PBA derivatization at the spiking level of 200 µg/kg.

Method optimization

Column evaluation: Stationary phase and dimensions are the most important factors in choosing the best capillary GC column. A low polarity column with dimensions of 30 m × 0.25 mm, 0.25 µm was recommended in methods GB5009.191-2016 and SN/T5220-2019. However, when using HFBI as the derivatization reagent, 3-MCPD and 2-MCPD could not be baseline separated on an Agilent J&W HP-5ms Ultra Inert, 30 m × 0.25 mm, 0.25 µm column, and 3-MCPD and 2-MCPD shared a quantitative ion, *m/z* 289 for high sensitivity. In this study, a column with thicker film, the Agilent J&W DB-5ms Ultra Inert, 30 m × 0.25 mm, 1 µm column, was used to improve resolution.

Salt packet evaluation: In the sample extraction process, 10 mL water and one ceramic homogenizer were added to 1 g of infant formula, and then 15 mL hexane was added for extraction. Emulsification appeared during the hexane extraction. However, a clear separation was observed between the aqueous and organic phases when salt was added to the sample. The addition of salt increases the ionic strength of the aqueous phase, which helps the lipids distribute into the organic phase, effectively reducing emulsification. In this study, the QuEChERS extraction salt packet (AOAC 2007.01 method) was used, which showed clear layers of separation, consistent extraction efficiency, and good recoveries.

Cleanup evaluation with HFBI derivatization: There are different processes available for sample cleanup. The Agilent Chem Elut S 12 mL supported liquid extraction (SLE) cartridge and a traditional liquid/liquid extraction (LLE) were compared. The step-by-step operation using the Chem Elut S is detailed in Figure 2. LLE was conducted in a centrifuge tube, with dichloromethane as the elution solvent. To improve extraction efficiency, a vortex mixer was used to mix the samples thoroughly. After centrifugation, the dichloromethane stayed at the lower layer for collection. The upper water layer was transferred to a new tube to repeat the extraction, which needs extra caution. This process was conducted three times to extract the target analytes efficiently, and the dichloromethane layers were combined. The response results from the two cleanup processes were similar, but LLE was more time-consuming and labor-intensive. Compared to LLE, SLE showed a walk-away extraction workflow without the need for special precautions. The less labor-intensive SLE method made it easier to extract the samples, and it also allowed for significant improvements to productivity and throughput. Thus, SLE was used in this study to provide a simplified workflow.

Solvent evaluation with HFBI derivatization: Dichloromethane was used as the elution solvent during the cleanup process, prior to derivatization. Two different solvents, hexane and dichloromethane, were investigated for the derivatization reaction and for use as the final sample solvent for the MCPD derivatives. As demonstrated in Figure 6, a higher response was obtained with hexane as the sample solvent for both 3-MCPD and 2-MCPD, which means improved sensitivity. Therefore, after SLE sample cleanup and water removal, the collected eluent (dichloromethane) was evaporated under a nitrogen stream to around 100 µL. The sample was then reconstituted with hexane to 1 mL before the derivatization reaction.

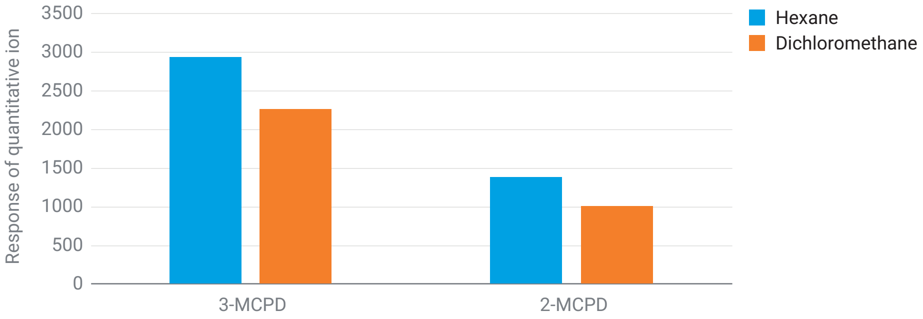


Figure 6. Comparison of different sample solvents with HFBI derivatization.

Method validation

MCPD compounds are highly polar and have high boiling points, which can cause peak shape issues. To improve the peak shape and sensitivity, MCPD compounds are usually derivatized before GC analysis. The selection of the best derivatization reagent is critical to the method success. The reaction speed, ease of operation, production of potentially interfering by-products, and selectivity are all factors that need to be considered. HFBI is recommended as the derivatization reagent in GB5009.191-2016 method, while PBA is used in ISO 18363-3, AOCS Cd 29a and SN/T5220-2019 methods. For HFBI methods, the derivatization reagent is sensitive to water, and the derivatization reaction will fail in the presence of water. Therefore, it is a critical step to remove water completely before HFBI derivatization. Also, attention should be paid to the storage of HFBI to prevent failure due to moisture absorption. The advantage of the HFBI method over the PBA method is that PBA only reacts with diols to form nonpolar cyclic products, while HFBI can react with all nucleophilic molecules. So, PBA is often selected as the derivatization reagent in the determination of 2-MCPD and 3-MCPD. However, HFBI is more suitable for the simultaneous determination of MCPD and dichloropropanol. When mass spectrometry is used for detection, high fragment ions could be chosen for HFBI derivatives to avoid interference with low molecular weight compounds. The advantage of the PBA method is that it is simpler and less time consuming than the HFBI method. For PBA, the derivatization reaction can occur in the presence of water and needs less reaction time. Both HFBI and PBA are evaluated in this study, and satisfactory performance is obtained with both.

HFBI method: Linearity, repeatability, detection limit, and recovery were tested in this study to evaluate the quantitative method with HFBI derivatization. Matrix matched calibration standards prepared at concentration levels from 10 to 1,000 µg/kg yielded correlation coefficient values (R^2) ≥ 0.997 for the two analytes. Calibration curves are shown in Figure 7. The repeatability measurements (concentration % RSD) were evaluated using eight injections of spiked samples at 10, 100, and 1,000 µg/kg. Table 2 lists the concentration RSDs, which were in the range of 0.5 to 3.1%, showing excellent performance. Eight repeat injections of matrix matched calibration standard with a low concentration level of 10 µg/kg were analyzed to calculate the detection limit. The detection limit for 3-MCPD and 2-MCPD were 1.35 and 1.30 µg/kg, respectively. Recoveries and precisions were determined at the spiking level of 200 µg/kg with five replicates. Mean recoveries were 86.9% for 3-MCPD and 106.7% for 2-MCPD with RSD <15%, as shown in Table 3.

PBA method: For the PBA method, the same spiking level of 200 µg/kg was analyzed as a QC sample for method validation. The detailed quantitation results are shown in Table 3. Acceptable recoveries of 80 to 120% were achieved for the two analytes with RSD <10%.

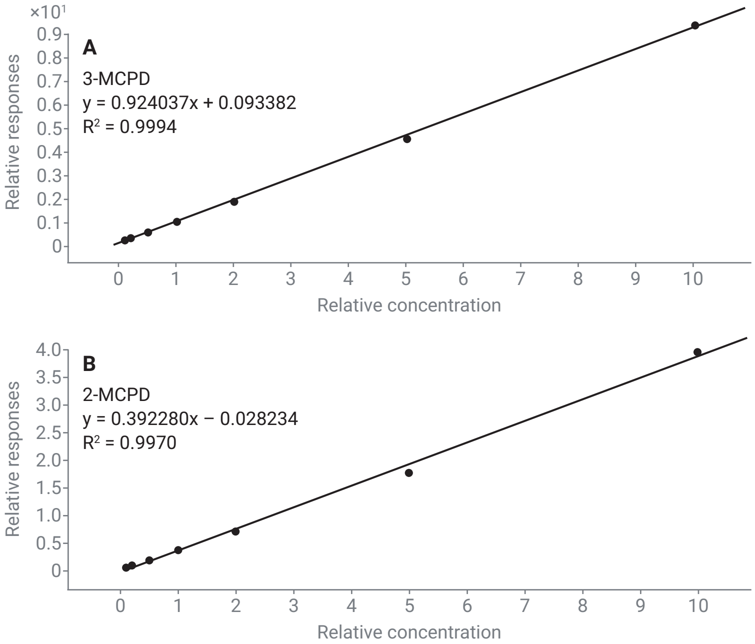


Figure 7. Calibration curve from 10 to 1,000 µg/kg for A) 3-MCPD HFBI derivative, and B) 2-MCPD HFBI derivative.

Table 2. Method quantitation results for HFBI derivatives.

Compound Name	Retention Time (min)	Concentration % RSD (n = 8)			Detection Limit (µg/kg)
		10 µg/kg	100 µg/kg	1,000 µg/kg	
3-MCPD	12.820	3.1	1.3	0.5	1.35
2-MCPD	13.012	2.1	1.6	1.3	1.30

Table 3. Recovery percentages and precision results for QC samples at 200 µg/kg (n = 5).

	HFBI			PBA		
Compound Name	Quant Ion	Mean Recovery %	% RSD	Quant Ion	Mean Recovery %	% RSD
3-MCPD	289	86.9	14.3	147	103.4	9.2
2-MCPD	289	106.7	5.8	198	100.9	4.2

Conclusion

This application note describes a solution for the simultaneous determination of 2-MCPD and 3-MCPD fatty acid esters in infant formula. Two reliable and robust workflows using different derivatization reagents for sample preparation were developed. The Agilent 8890 GC system with the Agilent 5977B GC/MSD was used for data acquisition and analysis. The method was validated by linearity, detection limit, recovery, and precision for HFBI derivatives. Recovery and precision results were also evaluated for PBA derivatives. The satisfactory results obtained from two sample preparation processes serve as a useful reference for MCPD esters analysis in infant formula.

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Analysis of 1,4-Dioxane in Consumer Products by Headspace-Gas Chromatography/Mass Spectrometry

Authors

Sujan Fernando and
Alesia Haddad-Carroll
Clarkson University

Ron Honnold, Simon Jones,
Shannon Coleman,
Joe Hendrick, and
Tim Conjelko
Agilent Technologies, Inc.

Abstract

1,4-Dioxane is an industrial chemical contaminate that may be found at trace levels in consumer products.[1–6] Government jurisdictions are beginning to regulate the amount of 1,4-dioxane allowed in consumer products. The allowable concentrations are expected to vary from state to state and country to country. Meanwhile, it has already been banned and deemed unsafe in cosmetics in Canada.[1–6] There have been several methods developed to test for 1,4-dioxane, but none of these methods are adequate to detect 1,4-dioxane in consumer products with complex mixtures and solutions.[7,8]

The current study shows methodology for low level detection of 1,4-dioxane in consumer products including cosmetics, liquid soaps, shampoos, and cleaning products. The extraction and analysis were initially performed using an Agilent 7694E headspace sampler attached to an Agilent 7890 GC with an Agilent 5977A MSD using Agilent MassHunter software. Then, the analysis was reproduced (with minor method modifications) on a newer system consisting of an Agilent 7697A headspace sampler attached to an Agilent Intuvo 9000 GC with a 5977B MSD using MassHunter software.

The quantitation of the target analyte 1,4-dioxane was performed using isotope dilution by adding deuterated 1,4-dioxane-d_8 as an internal standard to all samples, controls, and calibrators. This method has a linear quantitation range from 10 ng/g to 20,000 ng/g (ppb). Sample size for liquid detergents was typically 2 mL and for solids was in the range of 0.1 to 2.0 g, depending on the matrix. The method detection limit was determined to be 7.1 ppb on the older system and 2.3 ppb on the newer system. Alternate qualifiers were needed for confirmation in matrix due to interferences at low levels.

Introduction

1,4-Dioxane is a heterocyclic organic compound, classified as an ether. 1,4-Dioxane ($C_4H_8O_2$) is a colorless liquid or solid at cool temperatures (below 53 °F) with a sweet odor like that of diethyl ether. 1,4-Dioxane is highly water-soluble, making it mobile in the environment, and it does not readily biodegrade.

Due to the myriad of problems attributed to 1,4-dioxane in the environment, it has been identified as a chemical warranting further research toward its removal from consumer products (cosmetic, personal care, and cleaning products) by California DTSC (Department of Toxic Substances Control).[9] In New York state, there is a law in place that restricts the amount of 1,4-dioxane allowed in personal care, cleaning, and cosmetic products. The state of New York has also adopted a first-in-the-nation drinking water standard for 1,4-dioxane and set the maximum contaminant level of 1 part per billion (ppb).[11]

1,4-Dioxane is a trace by-product formed during the synthesis of ethoxylated ingredients used in finished consumer products (cosmetic, personal care, and cleaning products). 1,4-Dioxane can form inadvertently by undesirable side reactions during ethoxylation. It can form when two consecutive ethylene oxide units are cleaved from a chain of ethylene oxides and form a ring of 1,4-dioxane. Or, it can form when the ethylene oxide ring opens to form ethylene glycol, and two ethylene glycols dimerize to form 1,4-dioxane. Both reaction pathways are shown in Figure 1. These ingredients include certain detergents, foaming agents, emulsifiers, and solvents identifiable by the prefix, suffix, word, or syllables: PEG, polyethylene, polyethylene glycol, polyoxyethylene, -eth- (e.g. laureth sulfate), or -oxynol-. The compound 1,4-dioxane is not used as an ingredient in consumer products but may be present as a trace contaminant in some.

The New York State Environmental Conservation Law has been amended to include limits on the maximum allowable concentration of 1,4-dioxane in consumer products. The limit for household cleaning and personal care products is set to 2 ppm effective December 31, 2022 and 1 ppm effective December 31, 2023. The maximum allowable concentration of 1,4-dioxane in cosmetic products will be 10 ppm effective December 31, 2022.[10]

There have been several methods developed to test for 1,4-dioxane, primarily for soil, water, air, cleaning products, and cosmetics. Without modification, none of the standard methods can quantify 1,4-dioxane at the single-digit parts per million (ppm) level. Consumer products are challenging to analyze due to the complex matrices associated with viscous and foaming products like gels and detergents. Due to these method demands, there is a need for developing a testing method using headspace GC and single ion monitoring (SIM) technology.[7–8,12,13]

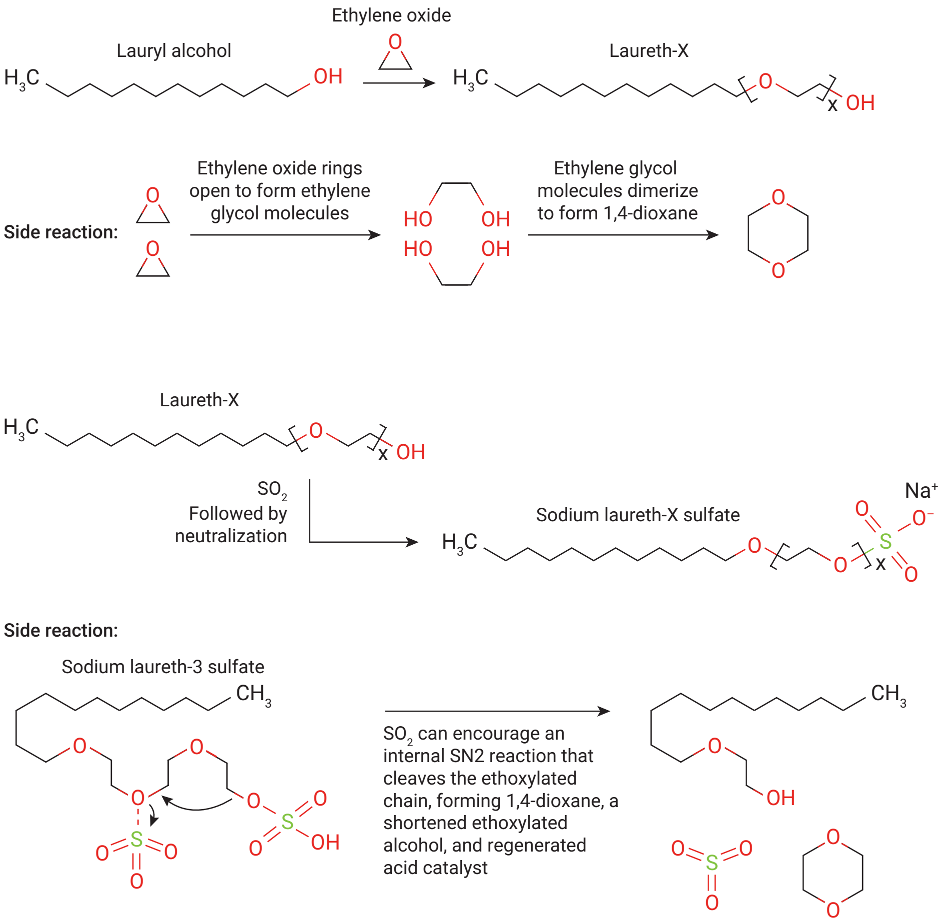


Figure 1. Two reaction pathways for synthesizing 1,4-dioxane (Environment Canada and Health Canada 2010).

Headspace has been used in several applications for analyzing volatile organic compounds with the aid of gas chromatography. In headspace analysis, the ambient volume above a sample matrix is sampled where the volatile compounds exist in gaseous form at predictable levels. In this case, the sample is typically heated to volatilize the compounds of interest into the headspace and allowed to reach equlibrium, which leads to accurate and reproducible analytical results when sampled. A major advantage of this approach is that it enables the analysis of only the volatile subset of compounds present in a sample. The complex matrix that may otherwise interfere in the case of direct injection of the sample is avoided.

Extraction techniques such as liquid-liquid extraction or solid-phase extraction may yield cleaner extracts for direct injection. However, these techniques are time and solvent-consuming and thus are unattractive compared to headspace.

The consumer products analyzed using the headspace method are highly complex and contain a myriad of volatile and semivolatile compounds that are co-extracted with the 1,4-dioxane. To compensate for these matrix effects, the isotope dilution quantitation technique is used.[12] Isotope dilution involves the addition of a known and constant amount of deuterated 1,4-dioxane (1,4-dioxane-d_8) as an internal standard to each sample, quality control, calibrator, and calibration verification. The deuterated analog of 1,4-dioxane behaves the same as 1,4-dioxane, both physically and chemically, allowing for the reproducible and accurate quantitation of 1,4-dioxane in complex matrices.

Experimental

Acquisition method

All analyses were performed on two systems. The first system was an Agilent 7890 GC equipped with an Agilent 7694E headspace sampler attached to an Agilent 5977A MSD. The second system was an Agilent Intuvo 9000 GC system equipped with an Agilent 7697A headspace sampler attached to an Agilent 5977B MSD. SIM was used to enhance sensitivity and selectivity.

After initial full autotune, a passing check tune must be performed before the start of a batch or every 24 hours. If the check tune does not pass, corrective action must be performed, and then a full autotune must be run.

GC method parameters are shown in Table 1. Headspace method details are in Table 2. MSD parameters are listed in Tables 3 and 4.

Supplies

- Volumetric flasks, Class A, 1 mL and 10 mL with ground glass stoppers
- Glass Pasteur pipets
- Volumetric air-displacement pipettes 50 to 1,000 µL with disposable tips
- 20 mL glass headspace vials with magnetic caps/PTFE-lined septa
- 12 mL screw cap glass vials
- Column dimensions: 30 m × 0.25 mm, 0.25 µm
- Ultrahigh purity helium
- Ultrahigh purity nitrogen
- Methanol: pesticide residue grade, purge and trap grade

Standard preparation

Make sure that clean glassware is used in the preparation of standards and samples. Rinse all glassware with Milli-Q water prior to use. Use certified vials to transfer neat standards if available.

Table 1. GC method parameters used for the analysis of 1,4-dioxane.

	Agilent 7890 GC System	Agilent Intuvo 9000 GC System
Inlet	60 °C	160 °C
Hold	0.1 min	
Ramp 1	500 °C/min	
Final Temperature	250 °C	
Septa Purge	3 mL/min	3 mL/min
Mode	Split 1:1	Split 10:1
Liner	Agilent inlet liner, Ultra Inert, splitless, single taper, glass wool (p/n 5190-3171)	Agilent inlet liner, Ultra Inert, splitless, straight 2 mm (p/n 5190-6068)
Oven	40 °C	40 °C
Hold	0 min	0.5 min
Ramp 1	10 °C /min	10 °C/min
Final Temperature	140 °C	140 °C
Hold	0 min	0 min
Column Flow	2 mL/min	2 mL/min
Column	GC column, like the DB-5, 30 m × 0.25 mm, 0.25 µm	Agilent J&W DB-5ms Ultra Inert Intuvo GC column module, 30 m × 0.25 mm, 0.25 µm (p/n 122-5532UI-INT)

Internal standard (1,4-dioxane-d_8)

1. Transfer an aliquot (~1 mL) of the dioxane-d_8 neat standard into a clean vial. Add 25 µL of this solution to a 25 mL volumetric flask, fill to mark with Milli-Q water, and shake well. Label this solution as 1,000 ppm dioxane-d_8 internal standard.
2. Next, transfer 25 µL of the 1,000 ppm dioxane-d_8 standard to a 25 mL volumetric flask, fill to mark with Milli-Q water, and shake well. Label this solution as 1 ppm dioxane-d_8 internal standard.

Native standard (1,4-dioxane)

1. Transfer an aliquot (~1 mL) of 1,4-dioxane neat standard into a clean vial. Add 25 µL of this solution to a 25 mL volumetric flask, fill to mark with Milli-Q water, and shake well. Label this solution as 1,000 ppm dioxane standard.
2. If needed, transfer 1 mL of the 1,000 ppm dioxane standard to a 50 mL volumetric flask, fill to mark with Milli-Q water, and shake well. Label this solution as 20 ppm dioxane standard (calibration standard 0).
3. Next, transfer 250 µL of the 1,000 ppm dioxane standard to a 25 mL volumetric flask, fill to mark with Milli-Q water, and shake well. Label this solution as 10 ppm dioxane standard (calibration standard 1).

Perform a serial dilution as described in the following protocol to make the remaining calibration standards for a seven-point calibration curve:

Table 2. Headspace method parameters used for the analysis of 1,4-dioxane.

	Agilent 7694E Headspace Sampler	Agilent 7697A Headspace Sampler
Incubation Temperature	100 °C	80 °C
Incubation Time	10 min	10 min
Loop Temperature	120 °C	80 °C
Transfer Line	120 °C	140 °C
Loop Fill and Pressure	0.2 min	
Fill Pressure		15
Loop Ramp		20 psi/min
Loop Final Pressure		10
Loop Equilibrium		0.05
Vial Size	20	20
Vial Shake		5

Table 3. MSD method parameters used for the analysis of 1,4-dioxane.

	Agilent 5977A MSD	Agilent 5977B MSD
Transfer Line Temperature	280 °C	280 °C
Source Temperature	230 °C	300 °C
Tune File	Atune.u	Etune.u
Gain Factor		10

Table 4. Compound-specific parameters for the analysis of 1,4-dioxane.

Compound Parameters	SIM Ions	Retention Times
Target 1,4-Dioxane	58,87,88	2.2 & 2.27 min
IS 1,4-Dioxane-d8	96	2.22 & 2.25 min

1. Transfer 5 mL of calibration standard 1 to a vial, add 10 mL of Milli-Q water, and mix well. Label this solution as 3.3 ppm dioxane standard (calibration standard 2).
2. Transfer 5 mL of calibration standard 2 to a vial, add 10 mL of Milli-Q water, and mix well. Label this solution as 1.1 ppm dioxane standard (calibration standard 3).
3. Transfer 5 mL of calibration standard 3 to a vial, add 10 mL of Milli-Q water, and mix well. Label this solution as 0.37 ppm dioxane standard (calibration standard 4).
4. Transfer 5 mL of calibration standard 4 to a vial, add 10 mL of Milli-Q water, and mix well. Label this solution as 0.12 ppm dioxane standard (calibration standard 5).
5. Transfer 5 mL of calibration standard 5 to a vial, add 10 mL of Milli-Q water, and mix well. Label this solution as 0.04 ppm dioxane standard (calibration standard 6).
6. Transfer 5 mL of calibration standard 6 to a vial, add 10 mL of Milli-Q water, and mix well. Label this solution as 0.013 ppm dioxane standard (calibration standard 7).
7. If desired, transfer 5 mL of calibration standard 6 to a vial, add 5 mL of Milli-Q water, and mix well. Label this solution as 0.0065 ppm dioxane standard (calibration standard 8).

Transfer 2 mL of each calibration standard into a headspace vial. To each vial, add 2 mL of 1 ppm dioxane-d_8 internal standard and mix well. The standards are ready for analysis.

Initial calibration verification (ICV)

A second source standard should be analyzed to verify the accuracy of the calibration. A certified reference material (CRM) containing 2,000 µg/mL of 1,4-dioxane (Sigma-Aldrich part number CRM48367) is used. The analyst may prepare any one (or all) of the following ICV standards for analysis.

- **ICV at 10 ppm:** Transfer 10 µL of CRM stock to a headspace vial containing 1990 µL of Milli-Q water. Add 2 mL of 1 ppm dioxane-d_8 internal standard and mix well.
- **ICV at 1 ppm:** Dilute the CRM stock to 20 µg/mL by combining 10 µL of stock with 990 µL of Milli-Q water in a 2 mL autosampler vial Transfer 100 µL of CRM at 20 µg/mL to a headspace vial containing 1,900 µL of Milli-Q water. Add 2 mL of 1 ppm dioxane-d_8 internal standard and mix well.
- **ICV at 0.1 ppm:** Dilute the CRM stock to 20 µg/mL by combining 10 µL of stock with 990 µL of Milli-Q water in a 2 mL ASV. Transfer 10 µL of CRM at 20 µg/mL to a headspace vial containing 1,990 µL of Milli-Q water. Add 2 mL of 1 ppm dioxane-d_8 internal standard and mix well.

Continuing calibration verification

A midlevel calibration standard (standard 4) should be injected after every 10 samples and at the end of the sequence.

Method blank

A method blank must be analyzed with each preparatory batch of samples. In this case, 2 mL of Milli-Q water is mixed with 2 mL of 1 ppm dioxane-d_8 internal standard in a headspace vial and analyzed.

Laboratory control spike (LCS)

An LCS should be analyzed per preparatory batch. The concentration of the LCS should be greater than LOQ and less than the midlevel calibration. A spiked water sample at 0.5 ppm concentration is prepared and analyzed as follows:

Transfer 100 µL of 1,4-dioxane standard at 10 ppm to 1,900 µL of Milli-Q water. Add 2 mL of 1 ppm dioxane-d_8 internal standard and mix well.

Matrix spike and matrix spike duplicate

A matrix spike should be performed one per preparatory batch. The following procedure describes the spiking of a sample with 1 ppm of 1,4-dioxane.

Combine 100 µL of the 1,000 ppm 1,4-dioxane standard with 900 µL of Milli-Q water in a 2 mL ASV to prepare a 100 ppm 1,4-dioxane standard. Next, spike 2 mL of a sample with 20 µL of the 100 ppm standard in a headspace vial. Add 2 mL of 1 ppm dioxane-d_8 internal standard and mix well.

Prepare a duplicate spiked sample following the same procedure for analysis.

Sample preparation and analysis

For liquid samples, measure 2 mL into a headspace vial along with 2 mL of 1 ppm dioxane-d_8 internal standard and mix well.

For semisolid or solid samples, measure between 0.1 to 2 g depending on the matrix into a headspace vial. It may be necessary to add water to the sample to form a slurry if the sample is thick. Add 2 mL of 1 ppm dioxane-d_8 internal standard to the sample and mix well prior to analysis.

Results and discussion

7890 GC system with 7694E headspace sampler

A seven-point calibration curve was used for quantification in the range of 13 ppb to 10 ppm. Based on five calibration curves (spanning three years), the R^2 value was greater than 0.997 in each calibration. The average response factor of the curves was 1.13 with a relative standard deviation (RSD) of 11.6%. Calibration data are shown in Figure 2.

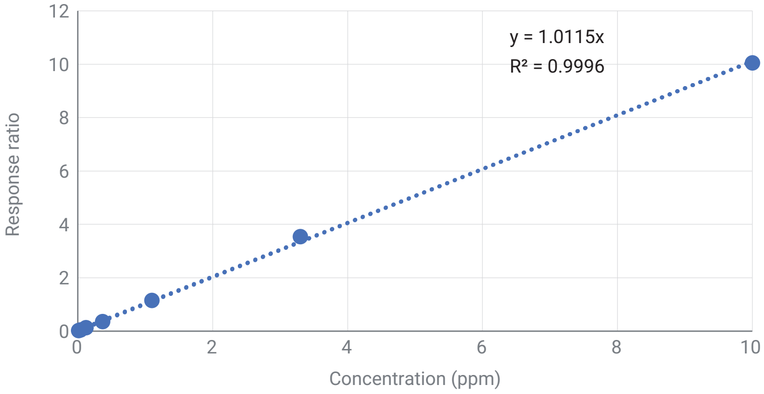


Figure 2. Calibration curve for 1,4-dioxane analysis on the Agilent 7890 GC system with Agilent 7694E headspace sampler.

The initial calibration was verified with the use of a certified reference material that was diluted and analyzed at 10, 1, and 0.1 ppm. In each case, the percent difference was less than 10%. For continuing calibration verification, a midlevel calibration standard (standard 4) was repeatedly injected over a period of five days from the initial calibration. The percent difference was less than 13.5%.

Quality control for this method was monitored throughout data collection. Method blanks yielded nondetectable levels to ensure that there was no carry over. Three laboratory control spikes were analyzed, and the accuracy ranged from 90 to 110%. Finally, a matrix spike and matrix spike duplicate were analyzed. The matrix spike samples were analyzed after spiking with a known amount (1 ppb) of 1,4-dioxane. The results are shown in Table 5.

The method detection limit (MDL) for 1,4-dioxane was calculated based on EPA methodology (EPA 821-R-16-006). The MDL was determined by spiking a detergent (predetermined to contain nondetectable levels of 1,4-dioxane) at a concentration of 50 ppb 1,4-dioxane. Ten replicates of the spiked sample were injected over three days, an example chromatogram is shown in Figure 3. The MDL was determined to be 7.1 ppb, as detailed in Table 6.

Table 5. Matrix spike and matrix spike duplicate results for 1,4-dioxane analysis on the Agilent 7890 GC system with Agilent 7694E headspace sampler.

Type	Concentration (ppm)	Percent Recovery	Percent Deviation
Sample	1.69		
Matrix Spike	2.63	94	
Matrix Spike Duplicate	2.58	90	1.6%

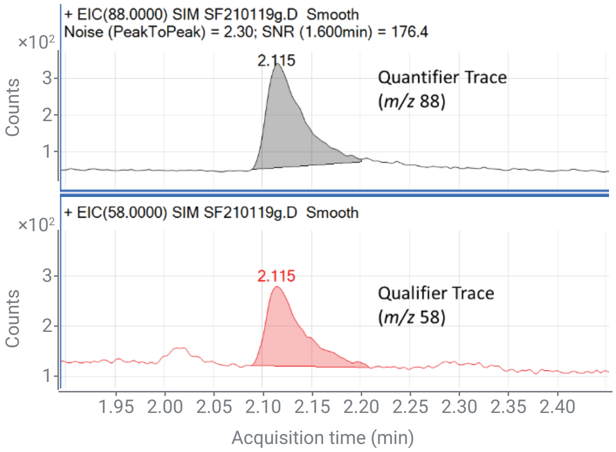


Figure 3. Example chromatography from one of the ten spiked sample injections used for method detection limit (MDL) calculation of 1,4-dioxane on the Agilent 7890 GC system with Agilent 7694E headspace sampler.

Table 6. Method detection limit (MDL) for the analysis of 1,4-dioxane on the Agilent 7890 GC system with Agilent 7694E headspace sampler.

Name	Retention Time (min)	SIM Ions	Concentration Injected	Average Measured Concentration	Standard Deviation	Method Detection Limit	Limit of Quantitation
1,4-Dioxane	2.115	88, 58	50 ppb	49 ppb	0.0025	7.1 ppb	13 ppb

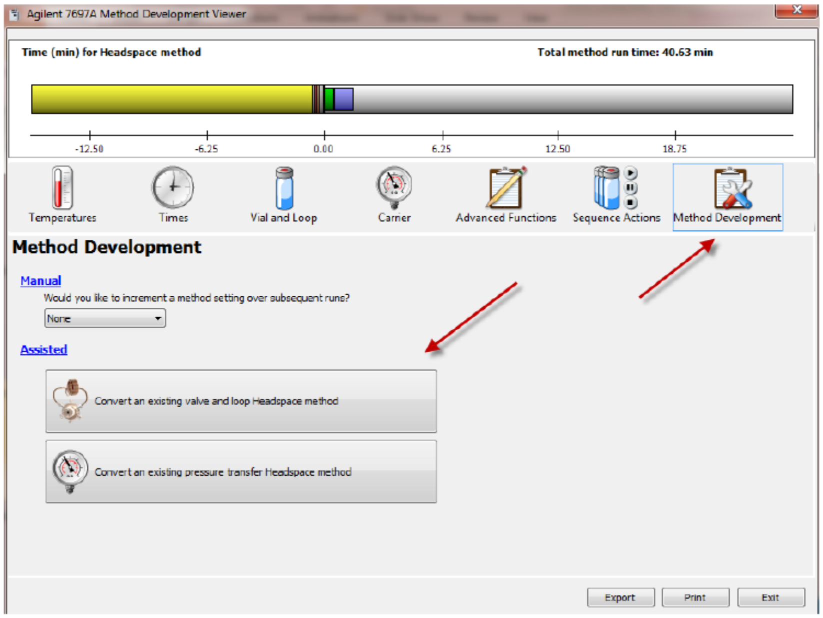
Intuvo 9000 GC system with 7697A headspace sampler

Method parameters from the 7890 GC system with 7694E headspace sampler can be transferred to the Intuvo 9000 GC system with 7697A headspace sampler. The conditions for the 7697A headspace sampler need minimal optimization. The 7697A has onboard pneumatics control to achieve active backpressure control. This difference changes the loop fill behavior and requires new method parameters. The use of the method conversion wizard (Figure 4) will give recommended starting conditions, which then can be optimized.[14]

A nine-point calibration curve was used for quantification in the range of 6.5 ppb to 20 ppm. Based on five calibration curves (spanning nine days), the R^2 value was greater than 0.9979, as shown in Figure 5. Average response factor of the curves was 1.1 with an RSD of 5.44%.

The initial calibration was verified with the use of a certified reference material that was diluted and analyzed at 10, 1, and 0.1 ppm. In each case, the percent difference was less than 10%. For continuing calibration verification, a midlevel calibration standard (standard 4) was repeatedly injected over a period of nine days from the initial calibration, and the percent difference was less than 10%.

Quality control for this method was also monitored throughout data collection. Method blanks yielded nondetectable levels, proving there was no system carry over. Three laboratory control spikes were analyzed, and the accuracy ranged from 90 to 110%. A matrix spike and matrix spike duplicate were also analyzed. The matrix spike samples were analyzed after spiking with a known amount (1 ppm) of 1,4-dioxane. The results are shown in Table 7.


Agilent 7697A Method Development Viewer

Time (min) for Headspace method

Total method run time: 40.63 min

Method Development

Manual

Would you like to increment a method setting over subsequent runs?

None

Assisted

Convert an existing valve and loop Headspace method

Convert an existing pressure transfer Headspace method

Standalone or as part of headspace driver

Figure 4. Screen capture from the method conversion wizard for the Agilent 7697A headspace sampler.

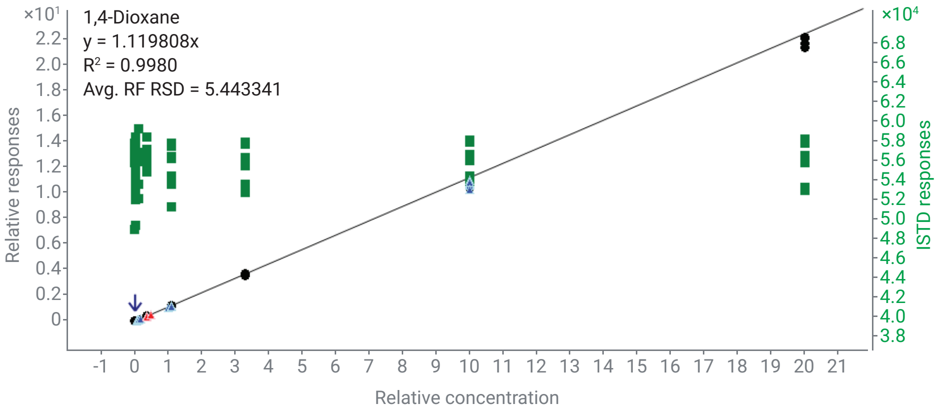


Figure 5. Calibration curve for 1,4-dioxane analysis on the Agilent Intuvo 9000 GC system with an Agilent 7697A headspace sampler.

Table 7. Matrix spike and matrix spike duplicate results for 1,4-dioxane analysis on the Agilent Intuvo 9000 GC system with Agilent 7697A headspace sampler.

Type	Concentration (ppm)	Percent Recovery
Sample	0.0	
Matrix Spike	0.994	94.2
Matrix Spike Duplicate	0.995	95.4

The MDL for 1,4-dioxane was calculated based on EPA methodology (EPA 821-R-16-006). The MDL was determined by spiking a detergent (predetermined to contain nondetectable levels of 1,4-dioxane) at a concentration of 50 ppb 1,4-dioxane. Thirty replicates of the spiked sample were injected over nine days, representative data are shown in Figure 6. The MDL was determined to be 2.3 ppb (Table 8).

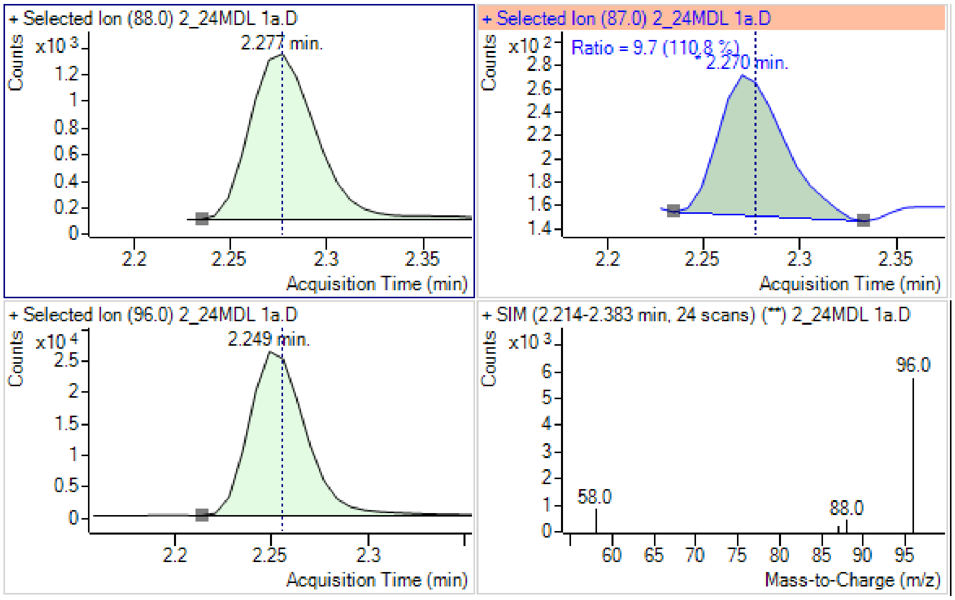


Figure 6. Example chromatography from one of the 30 spiked sample injections used for method detection limit (MDL) calculation of 1,4-dioxane on the Agilent Intuvo 9000 GC system with an Agilent 7697A headspace sampler.

Table 8. Method detection limit (MDL) for the analysis of 1,4-dioxane on the Agilent Intuvo 9000 GC system with an Agilent 7697A headspace sampler.

Name	Retention Time (min)	SIM Ions	Concentration Injected	Average Measured Concentration	Standard Deviation	Method Detection Limit	Limit of Quantitation
1,4-Dioxane	2.277	88, 87, 58	50 ppb	52.55 ppb	0.9	2.3 ppb	9.3 ppb

Best practices

Table 9. Best practices for the analysis of 1,4-dioxane.

Instrument Measure	Frequency	Requirement	Correction
Initial Calibration Verification (ICV)	Immediately after calibration	ICV ±30% true value	Reanalyze ICV, rerun calibration, corrective action
Continuing Calibration Verification (CCV)	Before batch and after every 10 analytical runs excluding blanks	CCV ±30% true value	Reanalyze CCV, rerun calibration, corrective action
Internal Standard (ISTD)	Added to every sample, standard, blank, and QC sample		
Retention Time (RT)	Evaluate in every sample	ISTD RT ±0.33 min Analyte RT <10 s To midpoint ICAL or first CCV	Inspect and perform instrument maintenance
Matrix Blank (MB)	With every batch of 10 or fewer samples	Analyte <LOQ	Reanalyze, corrective action
Laboratory Control Spikes (LCS)	With every batch of 10 or fewer samples	Spike recovery ±30%	Reanalyze, corrective action
Matrix Spike and Matrix Spike Duplicate (MS/MSD)	With every batch of 10 or fewer samples	Spike recovery ±30% RDP of MS/MSD <20%	Reanalyze, corrective action

Replace reference materials when responses do not pass criteria, are low compared to past calibrations, or reach their expiration date.

Recalibrate when the continuing calibration verification no longer passes within 20% of true value, or when maintenance has been performed.

Conclusion

These methods present a sensitive, robust, and selective method to determine 1,4-dioxane in consumer products including: cosmetic, personal care, and cleaning products. The benefits of using the Agilent 5977 MSD SIM capabilities with the headspace sampler cannot be underestimated in reducing sample matrix interference and improving signal-to-noise. This instrument configuration provides high selectivity and sensitivity with a more confidence-driven solution for the analysis of 1,4-dioxane. Lower detection limits cannot be achieved due to the myriad of interferences in the gel, viscous, and foaming mixtures. One approach to reduce matrix interferences and achieve lower detection limits would be the use of a triple quadrupole GC/MS system.

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Analysis of Free Volatile Phenols in Smoke-Impacted Wines by SPME

Authors

Jessica Westland and
Vanessa Abercrombie
Agilent Technologies, Inc.

Abstract

Ever since the 2003 wildfires in Australia and British Columbia, smoke impact has been a global concern for wine production.[1] With the increase in wildfires over various regions around the globe, many growers and wineries continue to worry about smoke impact in grapes and their wine. Agilent has developed a solid phase microextraction (SPME) gas chromatography/mass spectrometry (GC/MS) method to analyze the free-form volatile phenols associated with smoke impact. The Agilent SPME-GC/MS/MS method for the analysis of free-form volatile phenols associated with smoke impact allows for confident identification and reliable quantitation.

Introduction

Research has shown that smoke compounds can be absorbed by vines and grapes causing off-flavors in wines. While there is strong evidence that these compounds are mostly present in grapes and juice as nonvolatile forms, analysis of their free fraction has been used as a tool for screening grapes and assessing impacts in wines.[2] In the wine making process, the growth and maturation of the grape is arguably the most important step. During the period of veraison, acid concentration decreases, and sugar concentration increases while aromatic and flavor compounds start to develop. There are many external factors, weather conditions being the most influential, that determine when grapes have matured and are ready for harvest. Other environmental conditions, unrelated to temperature, such as smoke from nearby fires, can have a large and negative impact on the sensory quality of the wine.[3]

Guaiacol and 4-methylguaiacol have been identified as the primary volatile aromatics that contribute to the undesirable smoke impact characteristic. While aging wine in oak barrels can also contribute to the concentration of guaiacol and 4-methylguaiacol, the ratio of these two compounds will differ. Smoke-impacted berries contain almost four times as much guaiacol as 4-methylguaiacol.[2] The aroma contributed by oak barrels will be perceived as smoke and char. In contrast, when the two compounds are present due to smoke impact, it will be more reminiscent of campfires and ashtrays, which is not desirable in wine.

Detection limits for the analysis of smoke impact compounds must be sensitive enough to detect below 1 ppb, which is why selected ion monitoring (SIM) or multiple reaction monitoring (MRM) are commonly used in GC/MS analyses. Direct analysis of wine can be challenging because of the sugars, organic acids, and other aromatic compounds with higher retentions. To simplify the extraction and analysis of these volatiles, SPME has become the extraction method of choice. Its popularity for use stems from its operational simplicity, suitability for automation, reduced use of organic solvents, and direct thermal desorption into a gas chromatograph.

Experimental

Target volatiles

The main volatile phenols in smoke, guaiacol and 4-methylguaiacol, are useful markers of smoke impact in wines. Their respective concentrations correlate with the degree of perceived smoke impact, particularly in wines not exposed to toasted oak. However, they are not the only two compounds that are found and analyzed in smoke-affected wines, Table 1 lists the target free form volatile phenols that were analyzed in this experiment.

Table 1. Target free form volatile phenols.

CAS Number	Compound
74495-69-5	Guaiacol-d3
90-05-1	Guaiacol
93-51-6	4-Methylguaiacol
95-48-7	o-Cresol
13127-88-3	Phenol-d6
108-95-2	Phenol
95-87-4	2,5-Xylenol
2785-89-9	4-Ethylguaiacol
90-00-6	2-Ethylphenol
108-68-9	3,5-Xylenol
106-44-5	p-Cresol
108-39-4	m-Cresol
123-07-9	4-Ethylphenol
91-10-1	2,6-Dimethoxyphenol

Method

Sample preparation:

- 20 mL headspace vial and cap (part numbers 5188-6537 and 5188-2759)
- 10 mL sample with 4 g NaCl (Figure 1)
  - Addition of NaCl to saturation increases response for target compounds in smoke-affected grapes and wine by an average of 95%[4]

Figure 1. 20 mL amber headspace vials with water and wine samples.

- Samples spiked with calibrators and/or internal standards (ISTDs)
  - ISTDs spiked in at 10 ppb
- Agilent SPME Arrow DVB/carbon WR/PDMS, 1.10 mm, 120 µm (part number 5191-5861)
  - DVB/carbon WR/PDMS SPME phase was chosen for its selective extraction of odor and flavor compounds
  - SPME Arrow was used because of its significant benefit in extraction efficiency due to its larger sorption phase volume, compared to a traditional SPME fiber[5]

An Agilent PAL3 autosampler with robotic tool change (RTC) was installed on an Agilent 8890 GC system with an Agilent 7000D triple quadrupole GC/MS. The SPME headspace parameters, GC method settings, and MS conditions are listed in Tables 2, 3, and 4, respectively. Table 5 provides the MRM transitions used for GC/MS/MS analysis.

Table 2. SPME headspace parameters.

Parameter	Setting
Predesorption Time	3 min
Predesorption Temperature	250 °C
Incubation Time	5 min
Heatex Stirrer Speed	1,000 rpm
Heatex Stirrer Temperature	40 °C
Sample Extract Time	10 min
Sample Desorption Time	3 min

Table 3. Agilent 8890 GC settings.

Parameter	Setting
Inlet Liner	Agilent Ultra Inert inlet liner, splitless, straight, 0.75 mm id, recommended for SPME injections (p/n 5190-4048)
Injection Mode, Temperature	Splitless, 250 °C
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W DB-HeavyWAX GC column, 30 m × 0.25 mm, 0.25 µm (p/n 122-7132)
Oven Program	120 °C (hold 1 min); 10 °C/min to 250 °C (hold 0 min); 60 °C/min to 280 °C (hold 0 min)

Table 4. Agilent 7000D triple quadrupole GC/MS conditions.

Parameter	Setting
Transfer Line	280 °C
Acquisition Mode	dMRM
Solvent Delay	3.0 min
Tune File	Atune.eiex
Gain	10
MS Source Temperature	280 °C
MS Quadrupole Temperature	150 °C

Table 5. MRM transitions for free form volatile phenols.

CAS Number	Compound	Precursor Ion (*m/z*)	Product Ion (*m/z*)	CE (V)
74495-69-5	Guaiacol-d3	124.1	109	15
		124.1	81	15
90-05-1	Guaiacol	127	109	15
		126.9	109	15
93-51-6	4-Methylguaiacol	138.1	95	15
		138	123	15
95-48-7	*o*-Cresol	108.1	107.1	15
		107.1	77	15
13127-88-3	Phenol-d6	99.1	71	10
		71	69	10
108-95-2	Phenol	94	66	10
		66	65	10
95-87-4	2,5-Xylenol	122	107	15
		122	94	15
2785-89-9	4-Ethylguaiacol	152	137	15
		137.1	122	15
90-00-6	2-Ethylphenol	122.1	107.1	15
		107.1	77	15
108-68-9	3,5-Xylenol	121.1	107.1	15
		121.1	77	15
106-44-5	*p*-Cresol	108.1	107.1	15
		107.1	77	15
108-39-4	*m*-Cresol	108.1	107.1	15
		107.1	77	15
123-07-9	4-Ethylphenol	122.1	107	15
		108.1	78	15
91-10-1	2,6-Dimethoxyphenol	154	139	15
		139.1	83	15

Results and discussion

Calibration

Blanks are important for quality control and robust quantitative analytical methods. In this experiment, Milli-Q (18.2 Ω) water was used as a blank to simulate a clean matrix without any interferences. However, since wine includes many components that can affect the measurement of the target analytes, white wine was used as a matrix blank.

Table 6 provides the calibration ranges and linearity values for the target free form volatiles when calibrated in Milli-Q water. Figure 2 shows guaiacol and 4-methylguaiacol Milli-Q water calibration curves together.

To account for matrix effects in quantitating guaiacol and 4-methylguaiacol, a bag-in-a-box white wine was chosen. The reasons this matrix was chosen were:

- The skins, where smoke impact compounds reside, are separated from the juice before the fermentation process.
- It is an unspecified blend, which represents a broader matrix.
- The packaging removes the exposure of oak and cork from the wine.

Table 6. Agilent 7000D triple quadrupole GC/MS calibration range and R^2 in Milli-Q water.

Compound	Calibration Range (ppb)	R^2
Guaiacol	0.2 to 50.3	0.999
4-Methylguaiacol	0.1 to 25	0.999
o-Cresol	0.2 to 50	0.996
Phenol	0.5 to 125.5	0.997
2,5-Xylenol	0.1 to 25	0.998
4-Ethylguaiacol	0.1 to 25	0.998
2-Ethylphenol	0.03 to 7.5	0.995
3,5-Xylenol	0.1 to 5	0.998
p-Cresol	0.1 to 25	0.997
m-Cresol	0.1 to 25	0.998
4-Ethylphenol	0.1 to 25	0.998
2,6-Dimethoxyphenol	0.1 to 25	0.998*

* Type = quadratic, origin = force; weight = 1/x.

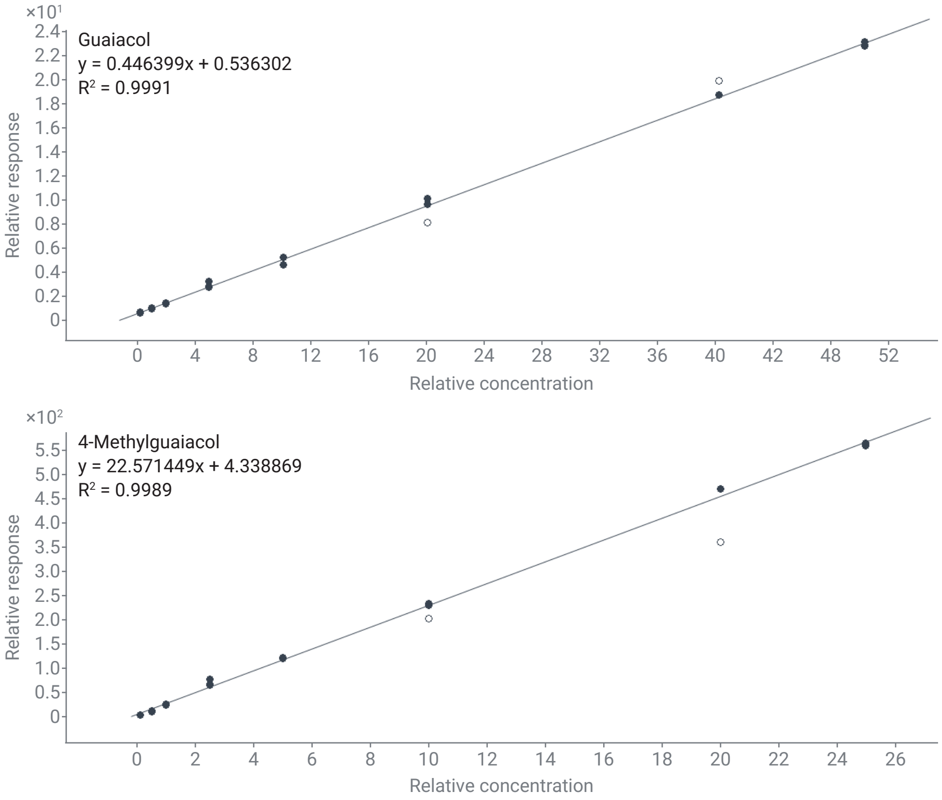

Figure 2. Calibration curves for guaiacol and 4-methylguaiacol in Milli-Q water.

Table 7 provides the calibration ranges and linearity values for the target free form volatiles when calibrated in the bag-in-a-box white wine. Figure 3 shows guaiacol and 4-methylguaiacol white wine calibration curves together.

Table 7. Agilent 7000D triple quadrupole GC/MS calibration range and R^2 in white wine.

Compound	Calibration Range (ppb)	R^2
Guaiacol	0.2 to 50.3	0.993
4-Methylguaiacol	0.1 to 25	0.996
o-Cresol	0.2 to 50	0.996
Phenol	0.5 to 125.5	0.997
2,5-Xylenol	0.1 to 25	0.996
4-Ethylguaiacol	0.1 to 25	0.996
2-Ethylphenol	0.03 to 7.5	0.995
3,5-Xylenol	0.1 to 5	0.998
p-Cresol	0.1 to 25	0.995
m-Cresol	0.1 to 25	0.995
4-Ethylphenol	0.1 to 25	0.996
2,6-Dimethoxyphenol	0.1 to 25	0.995

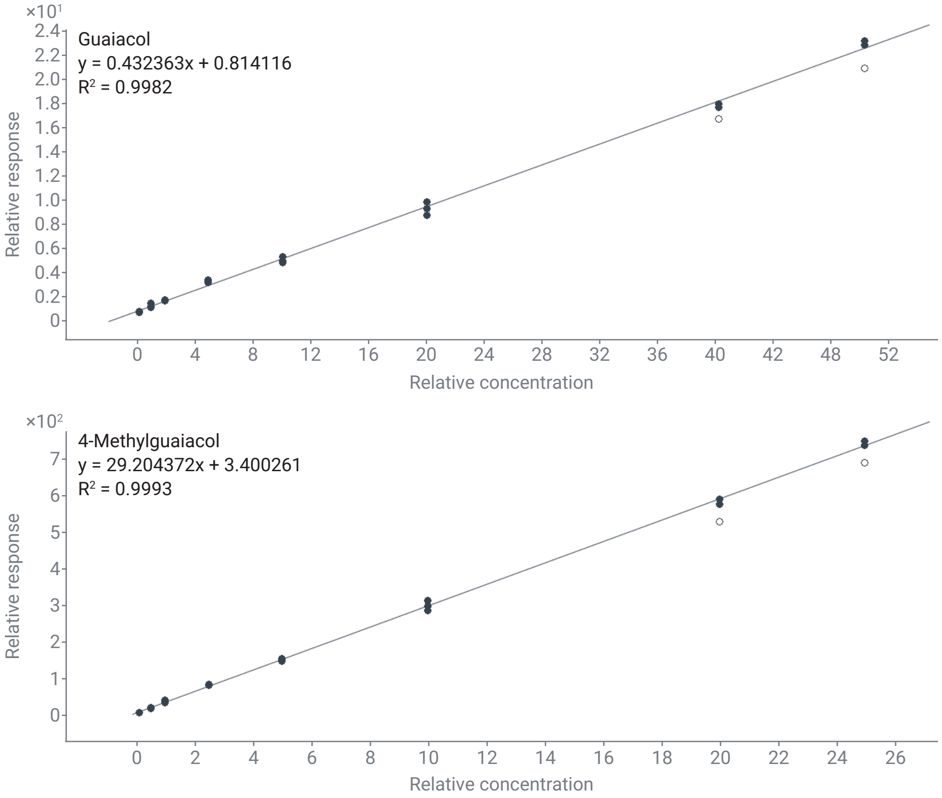

Figure 3. Calibration curves for guaiacol and 4-methylguaiacol in white wine.

Quantitation of smoke impact markers

In grapes not exposed to smoke, levels of 0.1 to 0.3 ppb for both guaiacol and 4-methylguaiacol can be observed. Guaiacol levels above 1 ppb could suggest exposure to smoke, and levels of guaiacol in smoke-exposed grapes have been as high as 55 ppb. On average, a ratio of 3.7/1 guaiacol/4-methylguaiacol is observed in undesirable smoke-impacted grapes and wine.[2]

Guaiacol and 4-methylguaiacol levels in all wine samples and the white wine blank, signals were quantitated based on the Milli-Q water calibration curve (Table 8). No sample had a quantitative level of 4-methylguaiacol.

Target free form volatile phenols were quantitated by white wine calibration from three replicates of each red wine sample (Table 9). Note that 4-methylguaiacol and 3,5-xylenol were below limit of quantitation (LOQ) for all samples, and therefore are not included in the table. The slight decrease in concentration of guaiacol from the Milli-Q water calibration to the white wine calibrations (standard deviation = 0.82 and RSD = 9.35%) indicates the matrix effects that wine has on the quantitation.

Table 8. Guaiacol levels identified in wine matrices.

Guaiacol	Franzia White Wine	Franzia Red Wine	CA Pinot Noir	OR Pinot Noir	Red Wine Sample
Average Concentration, n = 3 (ppb)	0.64	6.74	10.27	5.16	9.15
Standard Deviation	0.33	0.65	1.17	0.42	0.80
% RSD	51.80	9.57	11.40	8.13	8.72

Table 9. Average concentration (ppb) of targets identified in red wine samples.

Sample	Guaiacol	*o*-Cresol	Phenol	2,5-Xylenol	4-Ethylguaiacol	2-Ethylphenol	*p*-Cresol	*m*-Cresol	4-Ethylphenol	2,6-Dimethoxyphenol
Franzia Red Wine	6.32	0.41	2.73	<LOQ	0.09	<LOQ	1.61	0.38	< LOQ	0.77
CA Pinot Noir	9.97	1.90	5.58	0.23	0.22	0.01	0.75	0.68	0.08	1.05
OR Pinot Noir	4.68	2.05	6.20	16.23	10.81	<LOQ	1.73	1.44	24.81	0.60
Red Wine Sample	8.81	5.70	16.35	<LOQ	<LOQ	0.03	4.61	2.30	0.16	0.57

Conclusion

Consumers tend to respond negatively to smoke-affected wines. Since there are no effective ways to remove smoke compounds from grapes or wines, smoke impact can be a major problem for a vineyard. This contamination can be a significant financial impact for the grape-grower, as no harvest would mean no income. There is also a reputational risk, not only for the grape-grower but for the region.[6] The Agilent SPME-GC/MS/MS method for the analysis of free-form volatile phenols associated with smoke impact allows for confident identification and reliable quantitation.

References

1. Smoke Impact in Grapes and Wine. ETS Laboratories, **2020**.
2. Herve, E.; Price, S.; Burns, G. Free Guaiacol and 4-Methylguaiacol as Markers of Smoke Taint in Grapes and Wines: Observations from the 2008 Vintage in California. *ETS Laboratories*, **2011**.
3. Abercrombie, V. Fast Analysis of Smoke Taint Compounds in Wine with an Agilent J&W DB-HeavyWax GC Column. *Agilent Technologies application note*, publication 5994-0081EN, **2018**.
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Application Brief

Food Testing and Agriculture

Use of Salt to Increase Analyte Concentration in SPME Headspace Applications

Author

Jessica Westland
Agilent Technologies, Inc.

Abstract

Static headspace gas chromatography is one of the most frequently used techniques for the analysis of flavor components in foods and beverages. Samples must be prepared to maximize the concentration of the volatile components in the headspace and minimize unwanted contamination from other compounds in the sample matrix. The use of solid phase microextraction (SPME) allows for a fast, solvent-less, selective analysis of the headspace compounds. The addition of salt to the sample matrix will often lower the partitioning coefficient (K) for some target analytes, thus increasing the concentration of analytes in the headspace, which is the key advantage of this methodology.

Experimental

Amount of salt

The magnitude of the salting-out effect on K is not the same for all compounds. Compounds with K values that are already relatively low will experience little change in partition coefficient after adding a salt to an aqueous sample matrix. The addition of salt, however, will assist by lowering the compounds with higher K values and increase their concentration in the headspace. Each application is different. As a rule, the amount of salt added should be enough to saturate the sample (20 to 40% wt/wt salt/sample ratio). Saturation will maintain the same ionic strength from sample-to-sample and ensure reproducibility.

For example, water salinity is 35 g/L, which equates to 3.5 g in 10 mL of sample. In this case, 4 g (±0.5 g) of salt to a 10 mL water-based sample will ensure that enough salt has been added to saturate the sample.

Type of salt

Sodium chloride (NaCl) is the most used salt to adjust ionic strength. However, other salts such as ammonium chloride (NH_4Cl), sodium sulfate (Na_2SO_4), or sodium hydroxide (NaOH) may have different salting out capabilities, particularly when dealing with complex matrices such as food. It is important to note that while salt may improve the SPME extraction of the desired analytes, it could also cause co-extraction of more matrix interferences or undesired compounds.

Method

Guaiacol and 4-methylguaiacol are main target compounds implicated in smoke-affected grapes and wines. The use of the DVB/carbon WR/PDMS SPME phase was chosen due to its selective extraction of odor and flavor compounds.

Sample preparation

- 20 mL headspace vial and cap (part numbers 5188-6537 and 5188-2759)
- 10 mL sample with 4 g of NaCl
- Samples (n = 5) spiked at 50 ppb
- Agilent SPME Arrow DVB/carbon WR/PDMS, 1.10 mm, 120 µm (part number 5191-5861)

Table 1. SPME headspace parameters.

Parameter	Setting
Predesorption Time	3 min
Predesorption Temperature	250 °C
Incubation Time	5 min
Heatex Stirrer Speed	1,000 rpm
Heatex Stirrer Temperature	40 °C
Sample Extract Time	10 min
Sample Desorption Time	3 min

Table 2. Agilent 8890 GC settings.

Parameter	Setting
Inlet Liner	Agilent Ultra Inert inlet liner, splitless, straight, 0.75 mm id, recommended for SPME injections (p/n 5190-4048)
Injection Mode, Temperature	Splitless, 250 °C
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W DB-HeavyWAX GC column, 30 m × 0.25 mm, 0.25 µm (p/n 122-7132)
Oven Program	120 °C (hold 1 min); 10 °C/min to 250 °C (hold 0 min); 60 °C/min to 280 °C (hold 0 min)

Table 3. Agilent 7000D triple quadrupole GC/MS conditions.

Parameter	Setting
Transfer Line	280 °C
Acquisition Mode	dMRM
Solvent Delay	3.0 min
Tune File	Atune.eiex
Gain	10
MS Source Temperature	280 °C
MS Quadrupole Temperature	150 °C

An Agilent PAL3 autosampler with robotic tool change (RTC) was installed on an Agilent 8890 GC system with an Agilent 7000D triple quadrupole GC/MS. The SPME headspace parameters, GC method settings, and MS conditions are listed in Tables 1, 2, and 3, respectively.

Results and discussion

The increase of response of smoke impact volatiles is seen with the addition of 4 g of NaCl. Figure 1 shows the TIC scan of multiple smoke impact compounds when analyzed with and without the addition of NaCl. Figures 2 and 3 show the area differences of guaiacol and 4-methylguaiacol by analyzing their MRM transitions. Table 4 provides the area counts for both guaiacol and 4-methylguaiacol with and without the addition of NaCl.

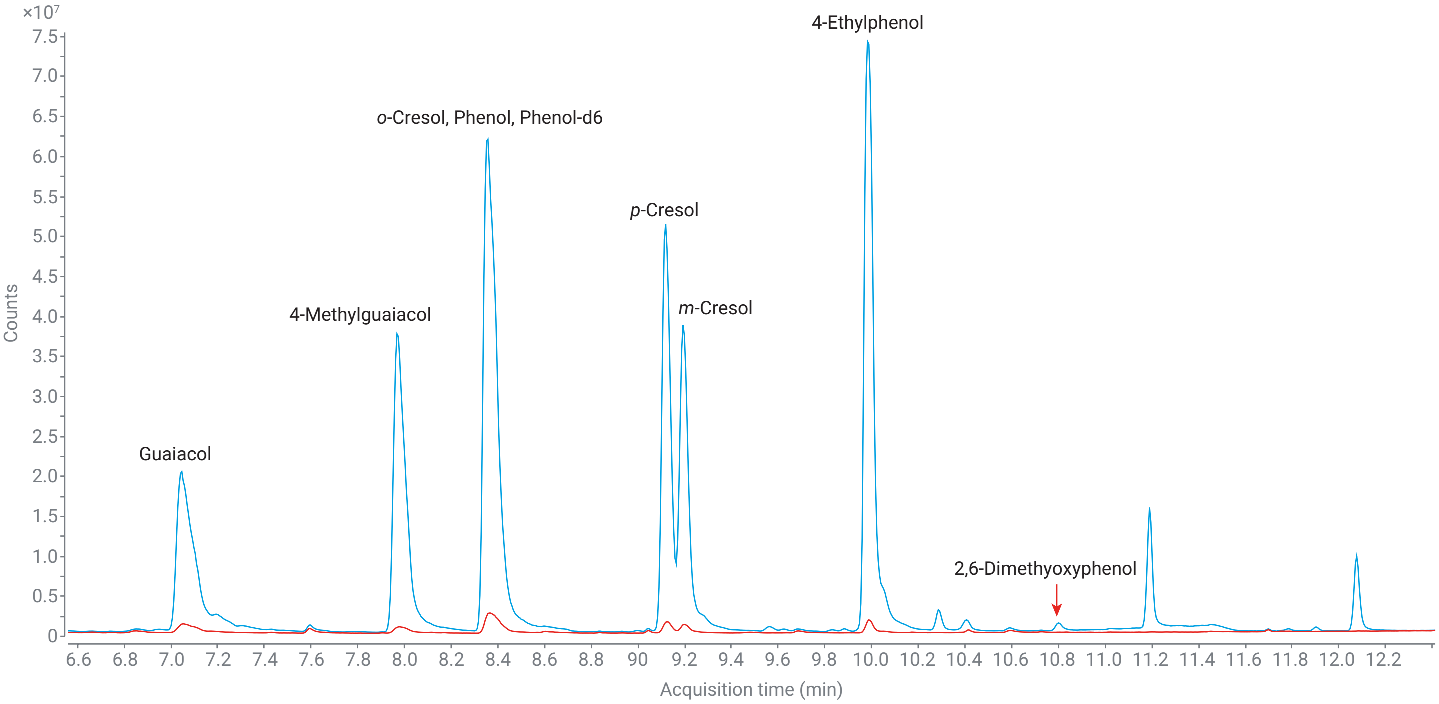
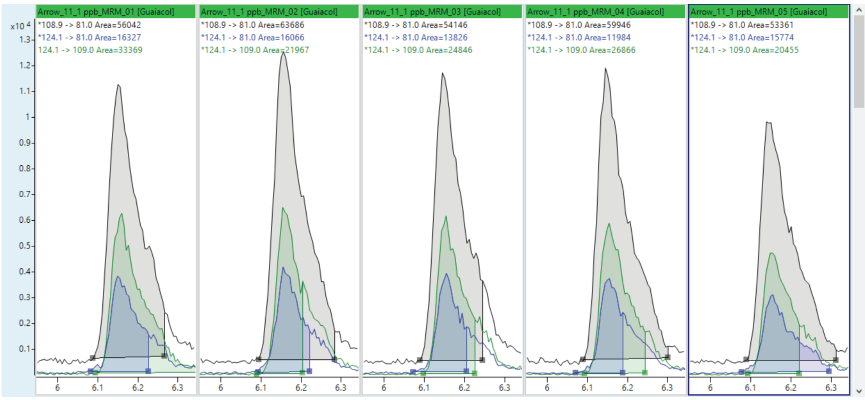


Figure 1. TIC scan of smoke impact compounds at 50 ppb extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861). The red trace indicates standards that were run without salt, and the blue trace indicates standards that were run with 4 g NaCl.

A) 1 ppb guaiacol with no NaCl



```
Arrow_11_1 ppb_MRM_01 [Guaiacol]
*108.9 -> 81.0 Area=56042
*124.1 -> 81.0 Area=16327
124.1 -> 109.0 Area=33369

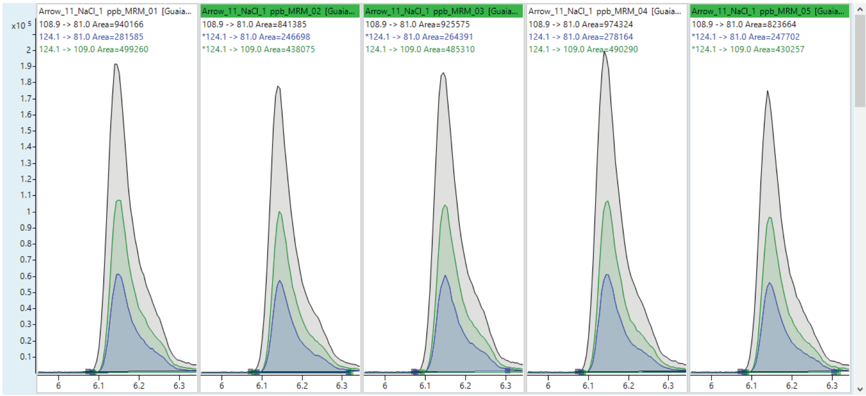
Arrow_11_1 ppb_MRM_02 [Guaiacol]
*108.9 -> 81.0 Area=63686
*124.1 -> 81.0 Area=16066
*124.1 -> 109.0 Area=21967

Arrow_11_1 ppb_MRM_03 [Guaiacol]
*108.9 -> 81.0 Area=54146
*124.1 -> 81.0 Area=13826
*124.1 -> 109.0 Area=24846

Arrow_11_1 ppb_MRM_04 [Guaiacol]
*108.9 -> 81.0 Area=59946
*124.1 -> 81.0 Area=11984
*124.1 -> 109.0 Area=26866

Arrow_11_1 ppb_MRM_05 [Guaiacol]
*108.9 -> 81.0 Area=53361
*124.1 -> 81.0 Area=15774
*124.1 -> 109.0 Area=20455
```

B) 1 ppb guaiacol with 4 g NaCl



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Arrow_11_NaCl_1 ppb_MRM_01 [Guaia...
108.9 -> 81.0 Area=940166
124.1 -> 81.0 Area=281585
124.1 -> 109.0 Area=499260

Arrow_11_NaCl_1 ppb_MRM_02 [Guaia...
108.9 -> 81.0 Area=841385
*124.1 -> 81.0 Area=246698
*124.1 -> 109.0 Area=438075

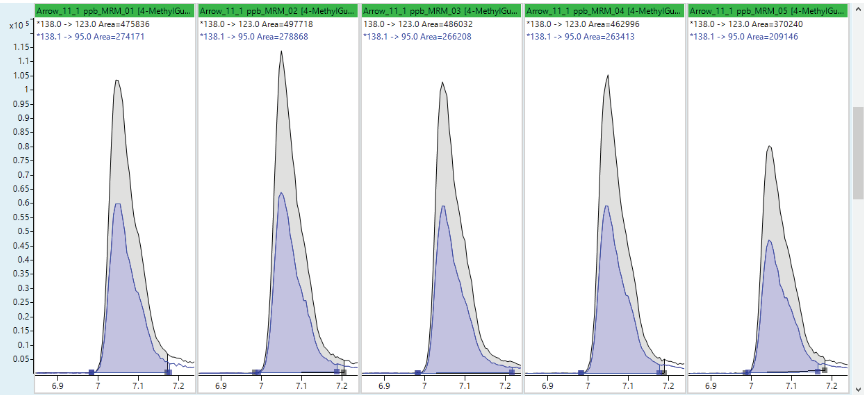
Arrow_11_NaCl_1 ppb_MRM_03 [Guaia...
108.9 -> 81.0 Area=925575
*124.1 -> 81.0 Area=264391
124.1 -> 109.0 Area=485310

Arrow_11_NaCl_1 ppb_MRM_04 [Guaia...
108.9 -> 81.0 Area=974324
124.1 -> 81.0 Area=278164
124.1 -> 109.0 Area=490290

Arrow_11_NaCl_1 ppb_MRM_05 [Guaia...
108.9 -> 81.0 Area=823664
*124.1 -> 81.0 Area=247702
*124.1 -> 109.0 Area=430257
```

Figure 2. MRM comparison with area counts for 1 ppb guaiacol replicates with A) no addition of salt and B) 4 g NaCl. Extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

A) 1 ppb 4-methylguaiacol with no NaCl



B) 1 ppb 4-methylguaiacol with 4 g NaCl

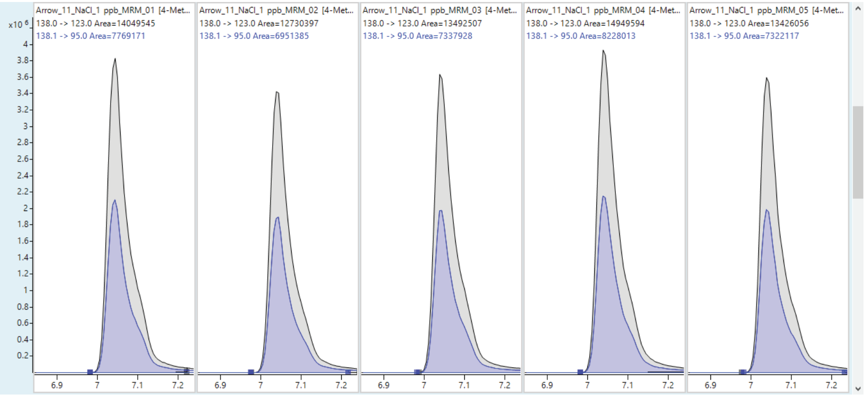
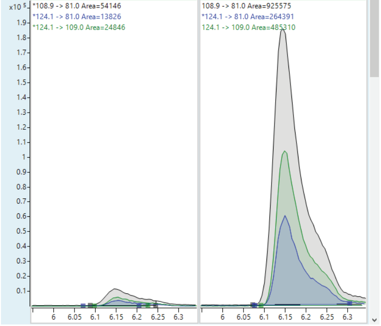


Figure 3. MRM comparison with area counts for 1 ppb 4-methylguaiacol replicates with A) no addition of salt and B) 4 g NaCl. Extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

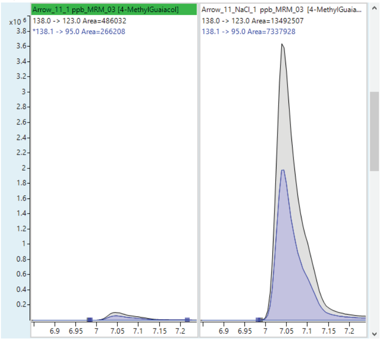
A) Guaiacol



0 g NaCl 4 g NaCl

94% increase in response with NaCl

B) 4-Methylguaiacol



0 g NaCl 4 g NaCl

96% increase in response with NaCl

Figure 4. SPME comparison of wine impact compounds with and without NaCl for A) guaiacol and B) 4-methylguaiacol.

Table 4. Area counts of 1 ppb guaiacol and 4-methylguaiacol extracted with the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

Compound	Amount of NaCl	Replicate 01	Replicate 02	Replicate 03	Replicate 04	Replicate 05	% RSD
Guaiacol	0 g	56,042	63,686	54,146	59,946	53,361	7.04
	4 g	940,166	841,385	925,575	974,324	823,664	6.50
4-Methylguaiacol	0 g	475,836	497,718	486,032	462,996	370,240	10.67
	4 g	14,049,545	12,730,397	13,492,507	14,949,594	13,426,056	5.40

Conclusion

With the addition of NaCl to saturation, there is an average of 95% increase in response for the target compounds implicated in smoke-affected grapes and wines.

References

1. Westland, J.; Abercrombie, V. Analysis of Free Volatile Phenols in Smoke-Impacted Wines by SPME. *Agilent Technologies application note*, publication number 5994-3161EN, **2021**.

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Application Brief

Food Testing and Agriculture

Response Comparison of Agilent SPME Arrows and Agilent SPME Fibers with DVB/Carbon WR/PDMS Phase for Free Volatile Phenols

Author

Jessica Westland
Agilent Technologies, Inc.

Abstract

Solid phase microextraction (SPME) has become one of the most widely used extraction technologies for volatile aromatics. Its popularity for use stems from its operational simplicity, suitability for automation, reduced use of organic solvents, and direct thermal desorption into a gas chromatograph. Additionally, SPME combines the matrix separation of analytes with a concentrating step. With the introduction of the Agilent SPME Arrows, the choice between an Agilent SPME fiber or SPME Arrow has become a common application question. This application demonstrates the significant benefit in extraction efficiency of the Agilent DVB/carbon WR/PDMS SPME Arrow (1.1 mm) larger sorption phase volume.

Experimental

For both SPME fibers and SPME Arrows, there are similar parts that are worth noting (Figure 1). The hub is the colored top of the SPME fiber or SPME Arrow, which signifies the phase type. The adjustable needle guide controls the septum piercing needle. The septum piercing needle has two purposes. The first purpose is to pierce the septa for extraction and desorption. The second purpose is to house and protect the phase. For the SPME Arrow, the septum piercing needle is only used for housing and protecting the phase. The arrow-shaped septum-piercing tip pierces the septa when using an SPME Arrow.

SPME fiber properties

The SPME fiber has a 10 mm phase length, a 9.4 mm^2 sorption phase surface area, and a 0.6 µL sorption phase volume.

SPME Arrow properties

SPME Arrows combine trace level sensitivity with high mechanical robustness. The SPME Arrow has an outside diameter of 1.10 or 1.50 mm, resulting in large sorption phase surfaces and volumes (Figure 2). The arrow-shaped tip allows smooth penetration of the vial and injector septa. In contrast to traditional SPME fibers, the SPME Arrow design fully protects the sorptive material, minimizing adverse influences and loss of analytes during the transfer processes.[1]

Method

Headspace solid phase microextraction has been shown to be a fast and effective sampling method for gas chromatography/mass spectrometry (GC/MS) analysis. The technique is used extensively for the determination of volatile compounds in wine. Guaiacol and 4-methylguaiacol are main target compounds implicated in smoke-affected grapes and wines.

The use of the DVB/carbon WR/PDMS SPME phase was chosen due to its selective extraction of odor and flavor compounds. An Agilent PAL3 autosampler with robotic tool change (RTC) was installed on an Agilent 8890 GC system with an Agilent 5977B GC/MSD. The SPME headspace parameters, GC method settings, and MS conditions are listed in Tables 1, 2, and 3, respectively.

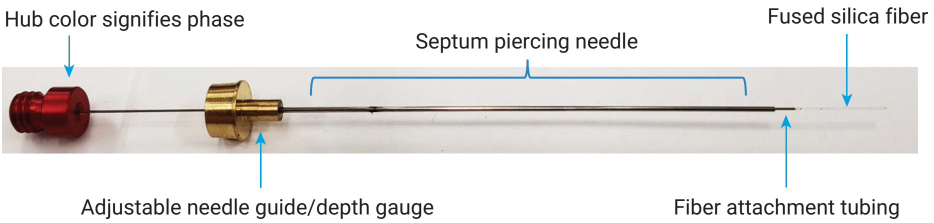

Figure 1. Characteristics of a classical 100 µm PDMS SPME fiber.

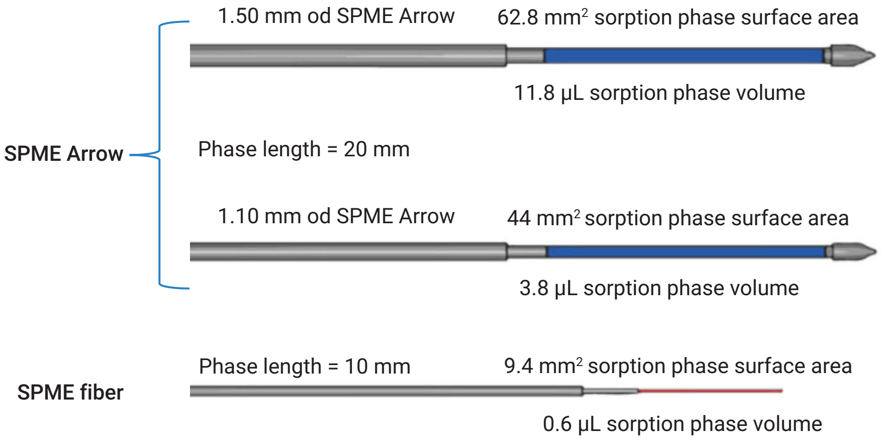

Figure 2. Sorption phase surface area and sorption phase volume comparisons for SPME Arrows and SPME fibers.

Table 1. SPME headspace parameters.

Parameter	Setting
Predesorption Time	3 min
Predesorption Temperature	250 °C
Incubation Time	5 min
Heatex Stirrer Speed	1,000 rpm
Heatex Stirrer Temperature	40 °C
Sample Extract Time	10 min
Sample Desorption Time	3 min

Table 2. Agilent 8890 GC settings.

Parameter	Setting
Inlet Liner	Agilent Ultra Inert inlet liner, splitless, straight, 0.75 mm id, recommended for SPME injections (p/n 5190-4048)
Injection Mode, Temperature	Splitless, 250 °C
Control Mode	Constant flow (1.2 mL/min)
Column	Agilent J&W DB-HeavyWAX GC column, 30 m × 0.25 mm, 0.25 µm (p/n 122-7132)
Oven Program	120 °C (hold 1 min); 10 °C/min to 250 °C (hold 0 min); 60 °C/min to 280 °C (hold 0 min)

Table 3. Agilent 5977B GC/MSD conditions.

Parameter	Setting
Transfer Line	280 °C
Acquisition Mode	SIM
Solvent Delay	3.0 min
Tune File	HES_Atune.u
Gain	1
MS Source Temperature	280 °C
MS Quadrupole Temperature	150 °C

Sample preparation

- 20 mL headspace vial and cap (part numbers 5188-6537 and 5188-2759)
- 10 mL sample with 4 g of NaCl
- Samples spiked at 50 ppb
- Agilent SPME fiber, DVB/C-WR/PDMS/10 (part number 5191-5874)
- Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (part number 5191-5861)

Results and discussion

In addition to guaiacol and 4-methylguaiacol, there are other target analytes that are often included the analysis of smoke affected wines (Figure 3). The use of the SPME Arrow was acknowledged for increasing sensitivity. Signal response was compared with extraction via an SPME fiber or an SPME Arrow (1.10 mm), both with the same DVB/carbon WR/PDMS SPME phase. Figure 3 displays the increase of the response with the use of the SPME Arrow (1.10 mm) compared to the SPME fiber. Figures 4 and 5 focus on the response increase of the two main target compounds in smoke impact analysis, guaiacol and 4-methylguaiacol, respectively. With the use of the SPME Arrow (1.10 mm; part number 5191-5861), the response for guaiacol was four times higher than with the SPME fiber (part number 5191-5874). The SPME Arrow response for 4-methylguaiacol was seven times higher than with the SPME fiber.

Additional information regarding this method can be found in Agilent application note *Analysis of Free Volatile Phenols in Smoke-Impacted Wines by SPME*.[2]

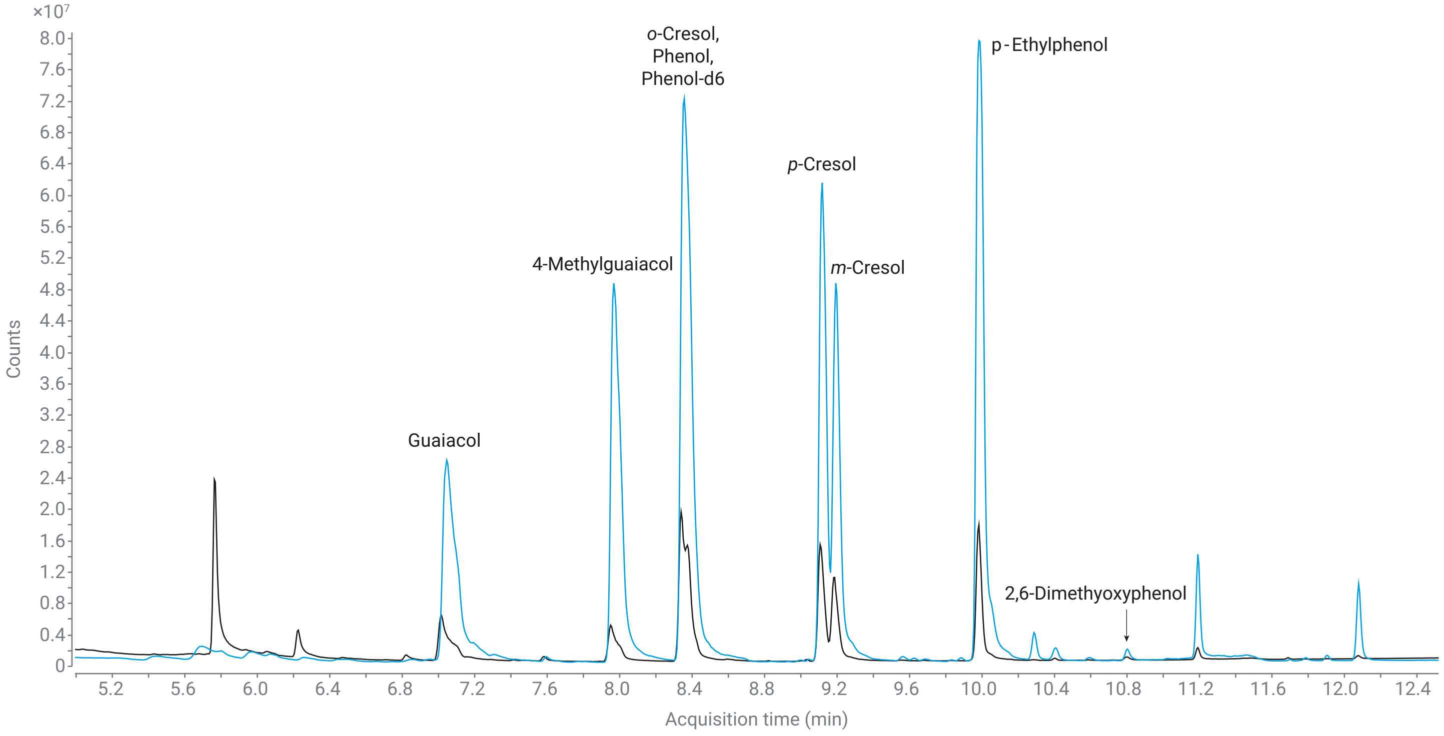

Figure 3. TIC scan of smoke impact compounds at 50 ppb extracted with the Agilent SPME fiber, DVB/C-WR/PDMS/10 (p/n 5191-5874, black trace) and the Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861, blue trace).

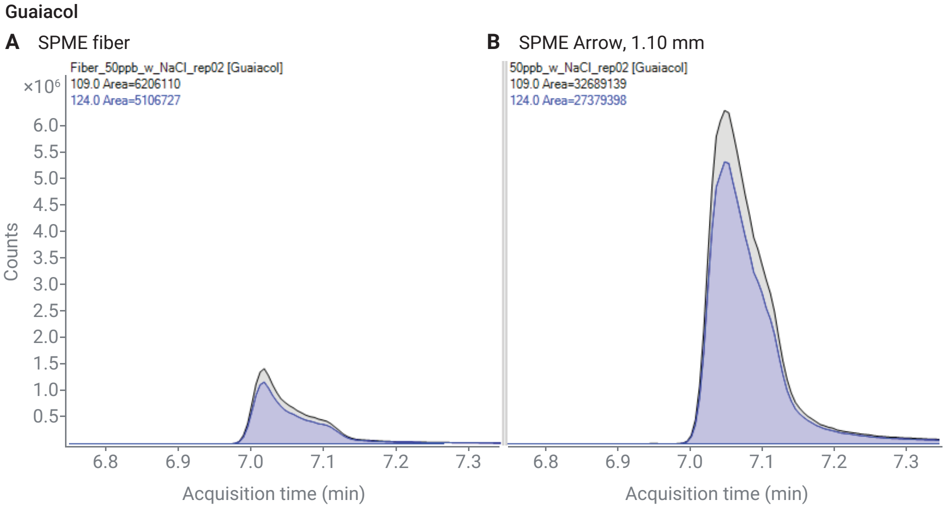

Figure 4. SIM traces of guaiacol at 50 ppb extracted with the A) Agilent SPME fiber, DVB/C-WR/PDMS/10 (p/n 5191-5874) and B) Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

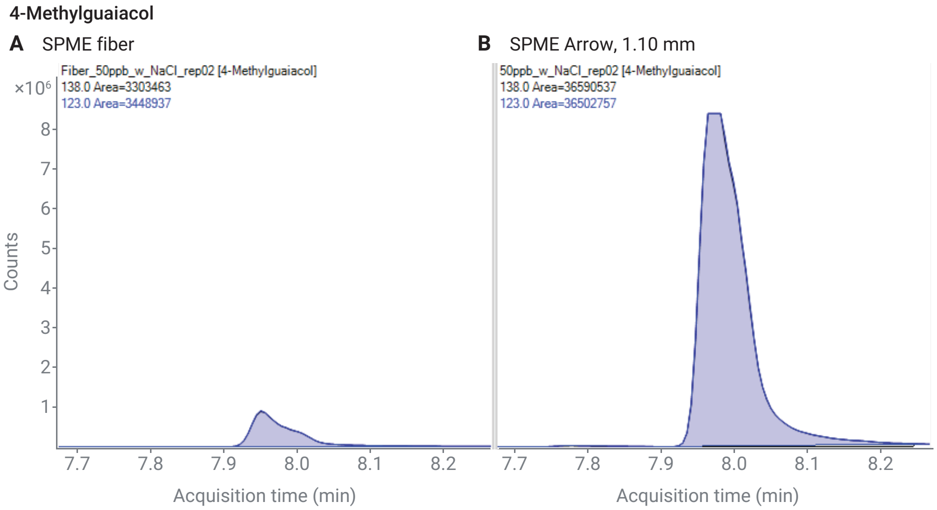

Figure 5. SIM traces of 4-methylguaiacol at 50 ppb extracted with the A) Agilent SPME fiber, DVB/C-WR/PDMS/10 (p/n 5191-5874) and B) Agilent SPME Arrow, DVB/carbon WR/PDMS, 1.10 mm, 120 µm (p/n 5191-5861).

Conclusion

In this application, the Agilent DVB/carbon WR/PDMS SPME Arrow (1.1 mm) demonstrates the significant benefit in extraction efficiency due to its larger sorption phase volume.

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Application Note

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Volatile Organic Compounds Analysis in Soils and Sediments Using the Agilent 8697 Headspace Sampler

Author

Jie Zhang
Agilent Technologies
(Shanghai) Co., Ltd, China

Abstract

This application note describes volatile organic compounds analysis in soil and sediments using the Agilent 8697 headspace sampler, 8860 GC, and 5977B GC/MSD system. The system performance in terms of repeatability, linearity, limit of detection, limit of quantitation, and method recovery rate were evaluated with good results. The area repeatability was in the range of 1.0 to 4.3%; the LOD and LOQ in the quartz sand blank was from 0.51 to 1.21 µg/kg and from 1.7 to 4.1 µg/kg, respectively. The recovery rate for the soil samples at spiked concentrations of 50 and 125 µg/kg was 78.2 to 125.9% and 71.7 to 108.7%. The linearity across the tested concentration range is excellent, with the R^2 of all components better than 0.996. The test results met or exceeded the requirements of Chinese standard HJ 642-2013.

Introduction

Volatile organic compounds (VOCs) are widely used in many industries as solvents or chemical intermediates. VOCs leaked or emitted into industry waste pollute the soil and sediments that the wastewater flows through. Considering that many VOCs have adverse environmental effects and soil remediation is costly, decisions regarding the significance of contamination and cleanup must be based on accurate VOC measurement. In China, the soil pollution prevention law was passed by the National People's Congress on 31 August 2018 as the first comprehensive framework law for addressing soil pollution, creating new obligations and potential liabilities for land-use-rights holders. The law requires the land-use-rights holder to investigate soil conditions upon the government's identification of soil pollution risk.

Headspace and purge-and-trap methods are used for VOCs contamination measurement in soil and sediments, with the method used dependent on the sample concentration. The static headspace method features easy operation and good repeatability. It allows use of an autosampler and minimizes carryover.

Chinese standard HJ 642-2013 is an environmental protection standard for determination of VOCs in soil and sediments by headspace GC/MS. It gives guidelines for handling of VOCs in soil and sediments when using a headspace method.

The 8697 headspace sampler is a platform that can introduce VOCs in the soil and sediments to a GC or GC/MSD platform for analysis as required by HJ 642-2013 standard. The 8697 headspace sampler has smart features that are developed to improve user experience and expand its diagnostic capability. The 8697 headspace sampler connects to Agilent smart GCs, including the 8860, 8890, and Intuvo 9000 GC with integrated communication. Users can access the browser interface of the smart GC or workstation to configure or set the headspace parameters. Compared to previous headspace products, the 8697 headspace sampler has more automated diagnostic functions, which can be executed from the browser interface of the GC. With the help of this user-initiated diagnosis process, it is easy to know whether the 8697 headspace sampler is in good health and ready for sample analysis. Clear guidance on the browser interface, in text or in image formats, makes the headspace maintenance or troubleshooting more straightforward. Besides the smart maintenance and diagnosis features, the 8697 headspace sampler provides 48-vial capacity and 12-position air bath vial oven for precise temperature control of every sample throughout its equilibration time, which meet the routine sample throughput requirement in most commercial testing labs.

In this application note, VOCs in soil were analyzed on the 8697 headspace/8860 GC/5977B GC/MSD platform by following Chinese standard HJ 642-2013. The linearity, repeatability, LOD, and LOQ for the targeted 36 VOCs were evaluated to show the system's excellent performance for VOCs analysis.

Experiment

Chemicals and standards

Stock solution: A mixture of 36 volatile organic compounds (VOCs) in methanol at 1,000 mg/L; internal calibration standard of fluorobenzene, chlorobenzene-d_5, and 1,2-dichlorobenzene-d_4 in methanol at 2,000 mg/L; and 2,000 mg/L surrogate standard of toluene-d_8 and 4-bromofluorobenzene in methanol were used.

Matrix modifier: 500 mL of organic-free water was adjusted to pH ≤2 with phosphoric acid and saturated with analytical-grade sodium chloride.

Working solution: The VOCs and surrogate stock solutions were mixed and diluted by methanol to 10 mg/L as the working solution. The IS stock solution was diluted to 50 mg/L by methanol for later use.

Calibration standards and soil sample preparation

The quartz sand was weighed at 2 g. The sand and 5 mL matrix modifier were added to a 20 mL headspace vial. Aliquots of VOCs/surrogates and IS working solutions were spiked into the modifier solution quickly, and then the vials were sealed immediately and shaken to well mix the standard. The final calibration standards are prepared approximately at 4, 10, 20, 50, and 100 µg/L, and the internal standards were spiked with the concentration of 50 µg/L.

The soil sample was weighed at 2 g in a 20 mL sample vial. Matrix modifier of 5 mL was added then spiked with IS to 50 µg/L. The vial was then quickly sealed and shaken to mix well.

Instrumentation and analytical conditions

An 8860 GC was equipped with a split/splitless inlet. The 8697 headspace sampler was used to extract and transfer the VOCs in the sample to the GC. The gas in the headspace of sample vials went through the 8697 transfer line and entered the split/splitless inlet before separation on an analytical column. The sample was then analyzed using a 5977B GC/MSD configured with an inert extraction ion source.

Agilent MassHunter Acquisition software version 10.0 was used for data collection. MassHunter Qualitative Analysis version B.08.00 and MassHunter Quantitative Analysis version B.08.00 were used for peak identification and quantitation.

The analytical conditions are listed in Table 1.

Results and discussion

According to HJ 642-2013, MSD performance should be checked daily to ensure MS data validity and reliability. The MSD was tuned, and the tune result was verified by analysis of the headspace gas in a 20 mL vial with 5 µL of 100 µg/mL BFB standard, according to the HJ 642-2013 requirements for mass spectrum quality. Table 2 shows the tune evaluation result.

Table 1. Analytical conditions of the Agilent 8697 headspace sampler/8860 GC/5977B GC/MSD system.

Parameters	Setpoints
Inlet Temperature	250 °C
Liner	4 mm id Ultra Inert inlet liner, split (p/n 5190-2295), glass wool removed
Column Flow	Constant flow, 1.2 mL/min
Split Ratio	10:1
Oven Program	40 °C (2 min), 8 °C/min to 90 °C (4 min), then 6 °C/min to 200 °C (10 min)
Column	Agilent J&W DB-624 GC column, 60 m × 0.25 mm, 1.40 µm (p/n 122-1364)
MSD Transfer Line	230 °C
MS Source	280 °C
MS Quad	150 °C
Gain Factor	1
Drawout Plate	6 mm
8697 Loop Size	1 mL
Vial Pressurization Gas	He
HS Loop Temperature	100 °C
HS Oven Temperature	80 °C
HS Transfer Line Temperature	110 °C
Vial Equilibration Time	50 min
Vial Size	20 mL, PTFE/silicone septa (p/n 8010-0413)
Vial Shaking	Level 7, 136 shakes/min with acceleration of 530 cm/S^2
Vial Fill Mode	Default
Vial Fill Pressure	15 psi
Loop Fill Mode	Custom
Loop Ramp Rate	20 psi/min
Loop Final Pressure	9 psi
Loop Equilibration Time	0.1 min
Carrier Control Mode	GC carrier control
Vent After Extraction	On

Table 2. MSD Etune result conformity assessment.

Target Mass	Rel to Mass	Lower Limit %	Upper Limit %	Rel. Abn %	Raw Abn	Pass/Fail
95	95	100	100	100	96,889	Pass
96	95	5	9	7.3	7,109	Pass
173	174	--	2	0	0	Pass
174	95	50	--	64.3	62,325	Pass
175	174	5	9	7.4	4,612	Pass
176	174	95	105	96.3	60,018	Pass
177	176	5	10	6.6	3,981	Pass

The data of the calibration standards were acquired in selected ion monitoring (SIM) mode. The total ion chromatogram (TIC) in Figure 1 showed the separation and detection for the 20 µg/L standard.

The instrument repeatability was verified based on the analyte absolute response. System linearity performance was verified based on analytes' quantitated concentrations instead of absolute area response, because HJ 642-2013 uses ISTD method for quantitation. Six vials of 20 µg/L calibrants were run for repeatability assessment. The response RSD% of the 38 VOCs (i.e., 36 analytes and 2 surrogates) are in the range of 1.0 to 4.3% (Figure 2), which demonstrated excellent sampling and detection precision. Instrument linearity were evaluated from 4 to 100 µg/L in 5 mL of matrix modifier with 2 g of quartz sand as control matrix, corresponding to 10 to 250 µg/kg analytes in real samples. All targeted components showed good linearity, with correlation coefficients (R^2) of regression formula better than 0.996. The calibration curves of four representative compounds eluting at the early, middle, and late part of the chromatogram are shown in Figure 3. The linearity results were achieved on the 6 mm MSD drawout plate. When using a 3 mm drawout plate, there were several compounds with R^2 less than 0.99, which did not meet the HJ standard requirement.

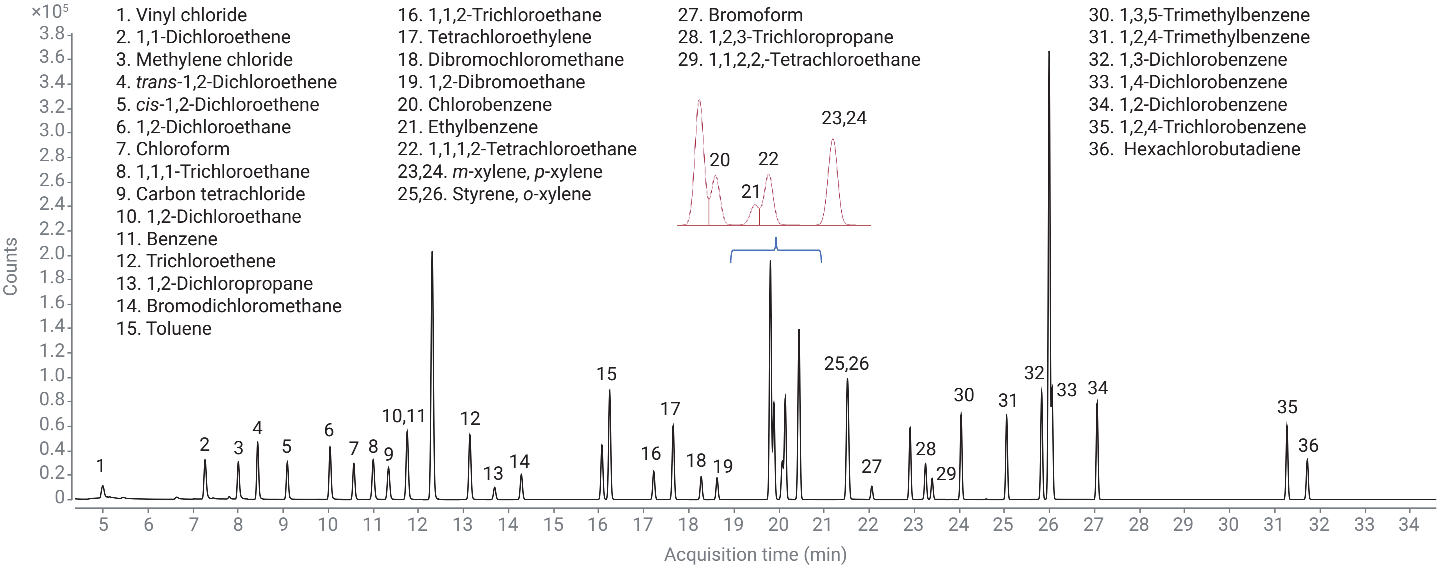

Figure 1. TIC SIM of 20 µg/L VOCs standard in 5 mL matrix modifier.

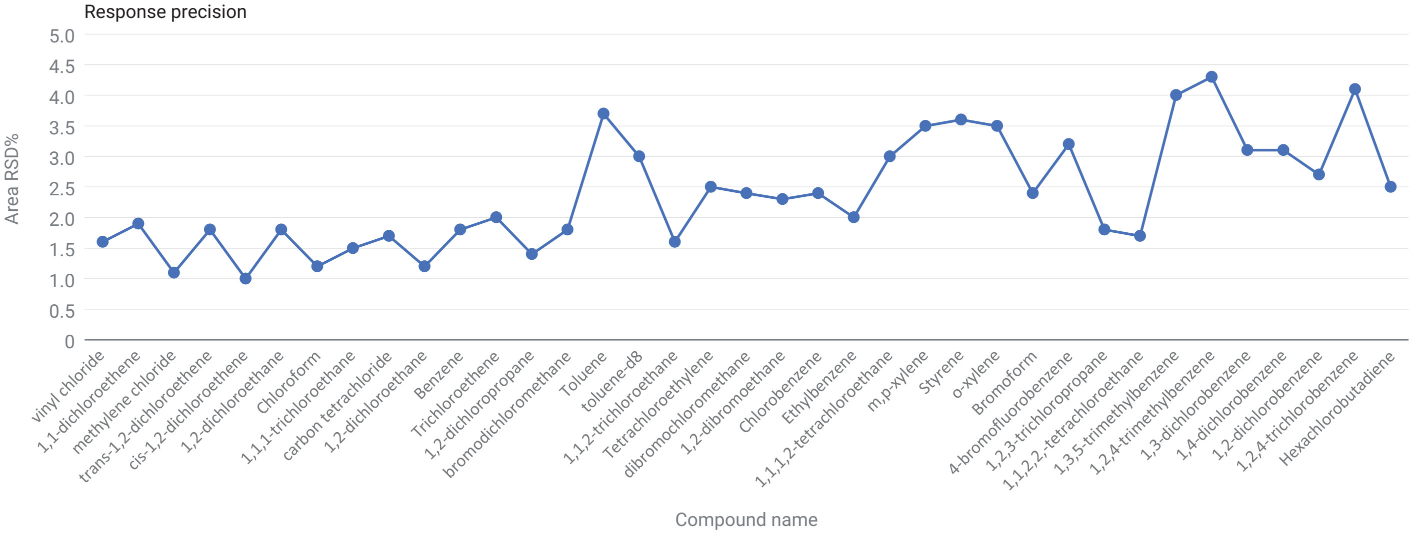

Figure 2. Area precision of six vials of 20 µg/L calibration standards in 5 mL matrix modifier.

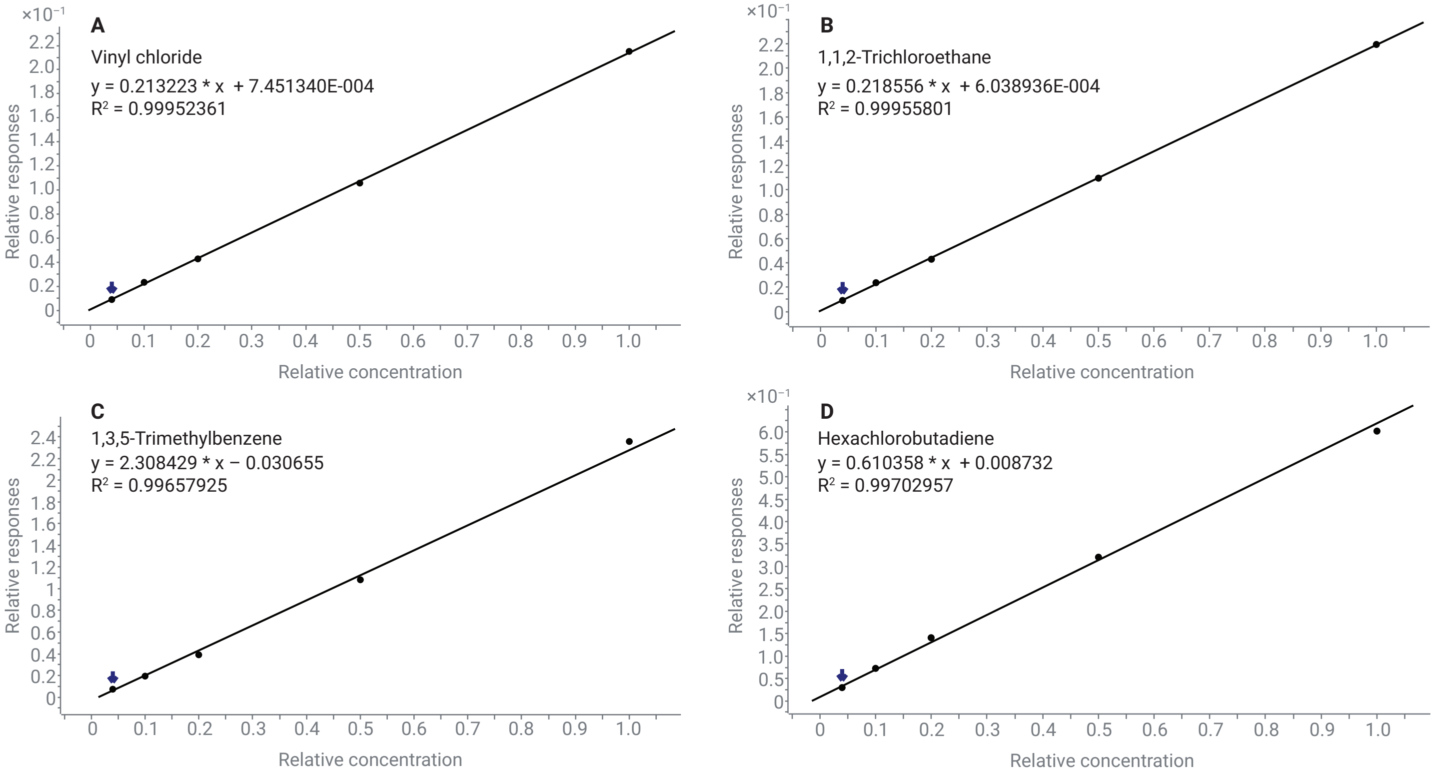

Figure 3. Calibration curves for representative compounds: (A) Vinyl chloride with $R^2 = 0.9995$; (B) 1,1,2-trichloroethane with $R^2 = 0.9995$; (C) 1,3,5-trimethylbenzene with $R^2 = 0.9965$; (D) hexachlorobutadiene with $R^2 = 0.9970$.

The method recovery performance was tested on 2 g of real soil samples spiked with 20 and 50 µL 10 mg/L calibration standards (corresponding to 50 and 125 µg/kg VOCs in soil sample). The soil sample without spiking was tested as blank, and the difference between the spiked sample and the blank was used for recovery rate calculation. The chromatograms of one soil blank and two spiked soil samples are shown in Figure 4. The surrogate's recovery rate was:

- 103.5% for toluene-d_8 and 109.7% for 4-bromofluorobenzene at 50 µg/kg
- 93.6% for toluene-d_8, and 95.9% for 4-bromofluorobenzene at 125 µg/kg

This met the standard requirements on surrogate recovery rate in real sample. The recovery rates of 36 target VOCs were between 78.2 to 125.9% at 50 µg/kg and between 71.7 to 108.7% at 125 µg/kg (Figure 5). The recovery rates demonstrated equivalent performance to the reference results in HJ 642-2013 standard.

The instrument detection limits (IDL) for 36 targeted VOCs and two surrogates were calculated based on quantitation precision of 4 µg/L standards in eight vials. The translated method LOD and LOQ (in the unit of µg/kg) in blank quartz sand based on IDL were shown in Table 3 (Appendix) as a reference of the LOD and LOQ in real soil or sediments sample. The LOD and LOQ for VOCs in quartz sand was between 0.51 to 1.21 µg/kg and 1.7 to 4.1 µg/kg, good enough to detect the targeted VOC compounds at the single-digit µg/kg level as specified by the HJ 642-2013 method.

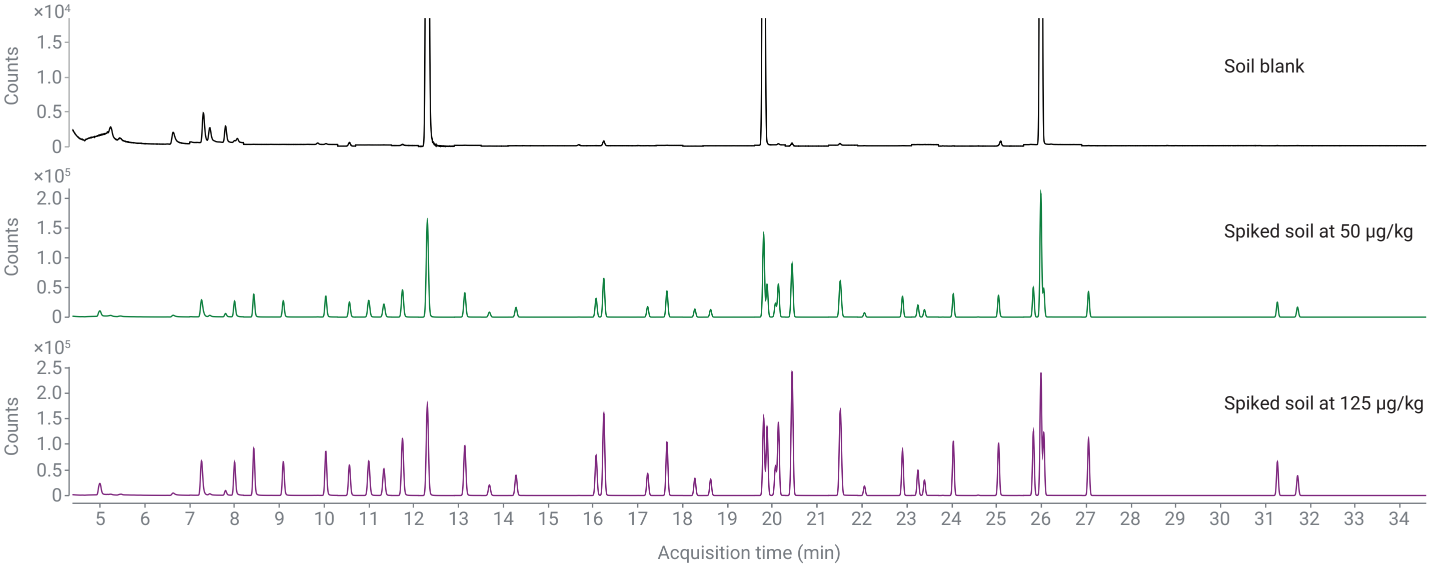

Figure 4. TICs of soil blank and spiked soil samples.

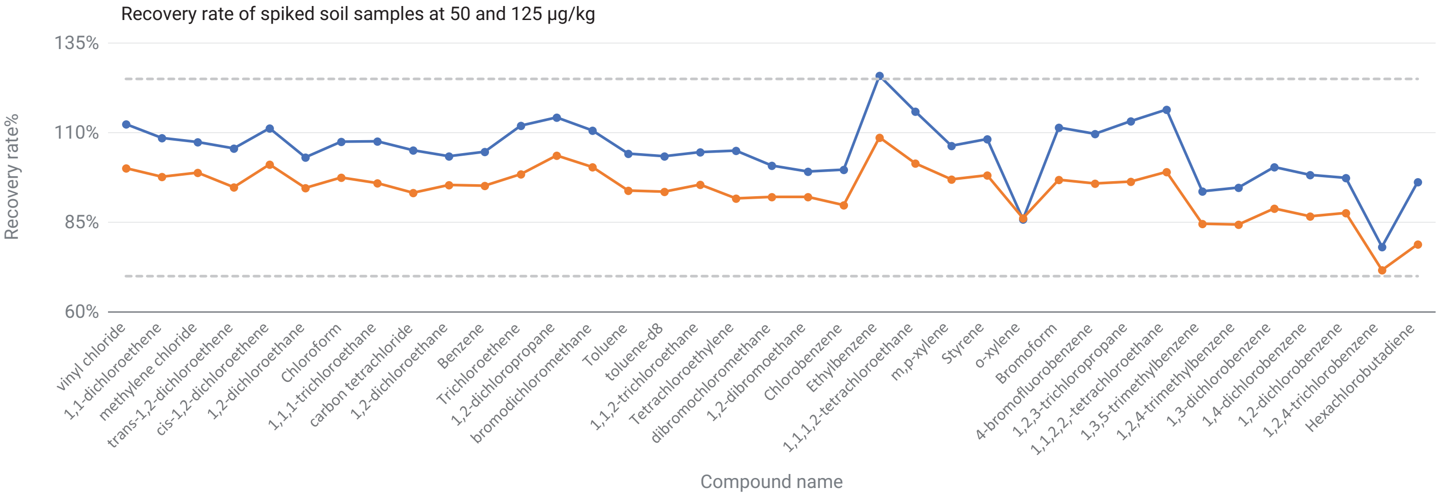

Figure 5. Recovery rate of spiked soil samples.

Conclusion

This application note demonstrated that the 8697 headspace sampler is an excellent means of introducing VOCs trapped in soils and sediments to GC for identification and quantitation. The combination of the 8697 headspace sampler with the 8860 GC and 5977B GC/MSD system delivered good repeatability, which was demonstrated in response precision from 1.0 to 4.3% for 38 VOCs. The calibration curves of targeted compounds in the tested concentration range (4 to 100 µg/L) showed satisfactory linearity, with all correlation coefficient better than 0.996. The LOD and LOQ achieved on the described system showed equivalent performance as demonstrated in the HJ 642-2013 standard. The recovery rate of spiked soil samples at 50 and 125 µg/kg was 78.2 to 125.9% and 71.7 to 108.7%, demonstrating that the 8697 headspace sampler coupled with the 8860 smart GC can provide reliable and accurate VOCs analysis in real-world soil and sediments samples.

Reference

1. Chinese Environmental Protection Industry Standard HJ 642-2013: Soil and Sediment–Determination of Volatile Organic Compounds–Headspace-Gas Chromatography/Mass Method. Chinese Ministry of Environmental Protection (published on 21 January 2013).

Appendix

Table 3. Instrument linearity, LOD, area precision, and recovery rate at applied operation conditions.

Name	RT/min	CF Formula	CF R^2	Response RSD%	LOD (µg/kg)	LOQ (µg/kg)	Recovery Rate	
							50 µg/kg	125 µg/kg
Vinyl chloride	4.997	y = 0.213223 * x + 7.451340E-004	0.9995	1.6	1.22	4.05	112.4%	100.1%
1,1-Dichloroethene	7.271	y = 0.280056 * x + 0.002079	0.9994	1.9	0.62	2.06	108.5%	97.7%
Methylene chloride	8.004	y = 0.251349 * x + 0.002364	0.9992	1.1	0.73	2.43	107.4%	98.9%
trans-1,2-Dichloroethene	8.435	y = 0.287348 * x + 0.002651	0.9991	1.8	0.83	2.77	105.6%	94.8%
cis-1,2-Dichloroethene	9.093	y = 0.397743 * x + 0.003192	0.9994	1	0.62	2.05	111.3%	101.2%
1,2-Dichloroethane	10.040	y = 0.297696 * x + 0.002091	0.9994	1.8	0.75	2.48	103.1%	94.6%
Chloroform	10.566	y = 0.369739 * x + 0.005035	0.9992	1.2	0.60	1.99	107.5%	97.5%
1,1,1-Trichloroethane	10.999	y = 0.329690 * x + 0.002463	0.9995	1.5	0.62	2.06	107.6%	96.0%
Carbon tetrachloride	11.336	y = 0.318289 * x + 0.002059	0.9996	1.7	0.57	1.89	105.1%	93.3%
1,2-Dichloroethane	11.746	y = 0.193792 * x + 0.001507	0.9993	1.2	0.74	2.45	103.4%	95.4%
Benzene	11.750	y = 1.051625 * x + 0.006656	0.9996	1.8	0.72	2.41	104.7%	95.2%
Trichloroethene	13.141	y = 0.360773 * x + 0.002391	0.9996	2	0.65	2.15	112.0%	98.5%
1,2-Dichloropropane	13.689	y = 0.291754 * x + 8.914385E-004	0.9998	1.4	0.65	2.16	114.3%	103.7%
Bromodichloromethane	14.281	y = 0.338244 * x + 0.001356	0.9997	1.8	0.65	2.15	110.6%	100.4%
Toluene	16.069	y = 1.164353 * x + 0.001734	0.9997	3.7	0.85	2.85	104.2%	93.9%
Toluene-d8	16.243	y = 0.883187 * x - 1.193050E-004	0.9995	3	0.83	2.76	103.5%	93.6%
1,1,2-Trichloroethane	17.217	y = 0.218556 * x + 6.038936E-004	0.9996	1.6	0.67	2.24	104.6%	95.5%
Tetrachloroethylene	17.652	y = 0.384694 * x + 0.002042	0.9997	2.5	0.68	2.26	105.0%	91.7%
Dibromochloromethane	18.272	y = 0.287628 * x + 6.739824E-004	0.9992	2.4	0.71	2.38	100.8%	92.1%
1,2-Dibromoethane	18.622	y = 0.236576 * x + 2.992586E-004	0.9991	2.3	0.82	2.74	99.2%	92.1%
Chlorobenzene	19.884	y = 1.062315 * x + 0.003659	0.9997	2.4	0.68	2.28	99.7%	89.8%
Ethylbenzene	20.071	y = 0.587171 * x + 0.002820	0.9995	2	0.61	2.04	125.9%	108.7%
1,1,1,2-Tetrachloroethane	20.135	y = 2.915519 * x − 0.001669	0.9997	3	0.62	2.06	115.9%	101.5%
m,p-Xylene	20.442	y = 2.580795 * x − 0.023563	0.9983	3.5	0.70	2.34	106.4%	97.0%
Styrene	21.510	y = 1.204945 * x − 0.009297	0.9989	3.6	0.69	2.29	108.2%	98.1%
o-Xylene	21.537	y = 1.637392 * x − 0.019908	0.9974	3.5	0.79	2.64	85.9%	86.2%
Bromoform	22.055	y = 0.350684 * x + 1.159518E-005	0.9990	2.4	0.78	2.61	111.5%	96.9%
4-Bromofluorobenzene	22.902	y = 0.892720 * x + 0.002945	0.9998	3.2	0.75	2.49	109.7%	95.9%
1,2,3-Trichloropropane	23.249	y = 0.801680 * x + 0.002143	0.9994	1.8	0.75	2.48	113.2%	96.4%
1,1,2,2,-Tetrachloroethane	23.397	y = 0.617940 * x + 0.001429	0.9995	1.7	0.74	2.48	116.5%	99.1%
1,3,5-Trimethylbenzene	24.037	y = 2.308429 * x − 0.030655	0.9966	4	0.72	2.41	93.7%	84.6%
1,2,4-Trimethylbenzene	25.046	y = 2.284967 * x − 0.033058	0.9963	4.3	0.82	2.74	94.7%	84.4%
1,3-Dichlorobenzene	25.823	y = 1.737283 * x + 0.007524	0.9997	3.1	0.68	2.26	100.4%	88.9%
1,4-Dichlorobenzene	26.054	y = 1.710684 * x + 0.009649	0.9997	3.1	0.73	2.45	98.2%	86.7%
1,2-Dichlorobenzene	27.052	y = 1.626089 * x + 0.010158	0.9995	2.7	0.64	2.12	97.4%	87.6%
1,2,4-Trichlorobenzene	31.265	y = 1.080196 * x + 0.006445	0.9996	4.1	0.95	3.17	78.2%	71.7%
Hexachlorobutadiene	31.715	y = 0.610358 * x + 0.008732	0.9995	2.5	0.51	1.69	96.3%	78.9%

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Application Note

An Alternate Testing Protocol for EPA 1613B using Agilent Triple Quadrupole GC/MS

Determination of 2,3,7,8-substituted tetra- through octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans

Authors

Coreen Hamilton and
Xinhui Xie,
SGS AXYS Analytical Services
Ltd.

Tarun Anumol,
Anastasia Andrianova, and
Dale Walker,
Agilent Technologies, Inc.

Abstract

This study provides data used to create an alternate testing protocol for the U.S. Environmental Protection Agency (EPA) to use for Agilent 7010B Triple Quadrupole GC/MS analysis of tetra- through octa-dioxins and furans that is equivalent to EPA Method 1613B. EPA Method 1613B is used for the determination of the 17 toxic tetra- through octa-chlorinated Dibenzo-*p*-Dioxins and Dibenzofurans (CDDs/CDFs) in aqueous, solid, and tissue matrices by isotope dilution gas chromatography/high-resolution mass spectrometry (GC/HRMS) using magnetic sector instruments. Traditionally used for dioxins analysis because of their high sensitivity, GC/HRMS instruments are expensive to maintain, require a highly specialized skill set to operate, and are being phased out by manufacturers. However, current GC/MS/MS (GC/TQ) technology provides many of the specificity and sensitivity advantages of HRMS for the analysis of regulated dioxins and furans, without the cost and complexity, and with added versatility and robustness. This application note describes a method developed in collaboration with SGS AXYS Analytical Services Ltd., SGS AXYS Method 16130, that uses the Agilent 7890B gas chromatograph coupled with an Agilent 7010B Triple Quadrupole GC/MS. Performance factors investigated included sensitivity, linearity, method detection limits (MDLs), recovery, and results compared to reference material. The GC/TQ results met the QA/QC and performance specifications described in Method 1613B for the analysis of polychlorinated dioxins and furans (PCDDs/PCDFs) in environmental matrices. Overall, the GC/TQ method produced accurate data for real-world sample matrices, offering a lower cost, more efficient alternative to GC/HRMS.

Introduction

Dioxins are pollutants of concern due to the adverse effects of trace-level chronic exposure, persistence in the environment, and bio-accumulation in the food chain.[1] For this reason, they are monitored by environmental agencies worldwide. The U.S. Environmental Protection Agency (EPA) has promulgated Method 1613B for the determination of the 17 toxic 2,3,7,8-substituted tetra- through octa-chlorinated CDDs/CDFs in aqueous, solid, and tissue matrices by isotope dilution gas chromatography/high-resolution mass spectrometry (GC/HRMS) using magnetic sector instruments. As originally written, Method 1613B requires a high mass resolution of ≥10,000, which can only be achieved using GC/HRMS. Traditionally, magnetic sector MS instruments have been used for this analysis due to lack of better alternatives. However, magnetic sector MS instruments are expensive to maintain and require a highly specialized skill set to operate. In addition, with suppliers discontinuing or phasing out manufacture of magnetic sector GC/HRMS instruments, an alternate technique that provides data of the same quality, with easier and more robust operation, is required.

MS/MS technology offers many of the specificity and sensitivity advantages of HRMS methods without the need for high mass resolution, or the cost and complexity of HRMS instruments. Approval of a method that uses GC/MS/MS (GC/TQ) for determination of dioxins and furans has the potential to lower laboratory costs. Developed in collaboration with SGS AXYS ANALYTICAL SERVICES LTD, this application note describes a GC/TQ method using an Agilent 7890B gas chromatograph coupled with an Agilent 7010B Triple Quadrupole GC/MS that meets the QA/QC and performance specifications in Method 1613B for the analysis of polychlorinated dioxins and furans (PCDDs/PCDFs) in environmental matrices. The method--SGS AXYS Method 16130--is approved by the US EPA as an alternate testing protocol for analyzing the Dioxins in EPA 1613B. Performance factors investigated in this application note included sensitivity, linearity, method detection limits (MDLs), recovery, and results for reference materials.

The EPA has reviewed the SGS AXYS Method 16130 using the 7010B Triple Quadrupole GC/MS and supporting validation data submitted by SGS AXYS, and has determined that it meets requirements as an alternate testing protocol for measurement of 2,3,7,8-substituted tetra- through octa-chlorinated dibenzo-p-dioxins and dibenzofurans (PCDDs/PCDFs) in wastewater with performance similar to the methods listed in 40 CFR Part 136. Though the EPA has not yet promulgated the method or published it in the CFR at the time of this publication, on a facility-by-facility basis laboratories may seek approval from their regional authority to use the method in measuring PCDDs/PCDFs in wastewater in per the Clean Water Act (CWA) program.

Experimental

Sample preparation and extraction

Sample cleanup is required to maintain the MS instrument in good condition, and to avoid mass fluctuations and changes in ionization efficiency due to background matrix. For this application note, analyses were performed using real-world sample extracts from four matrices (aqueous, solids, biosolids, and tissues) that had been archived at SGS AXYS Analytical Services after preparation and extraction per EPA Method 1613B.[1] In this procedure, stable isotope-labeled analogs of 15 of the 2,3,7,8-substituted CDDs/CDFs are spiked prior to extraction. After extraction, $^{37}Cl_4$-labeled 2,3,7,8-TCDD is added to each extract to measure the efficiency of the cleanup process. After cleanup, the extract is concentrated to near dryness. Immediately prior to injection into the GC for GC/TQ analysis, internal standards were added to each extract.

GC/TQ analysis and instrumentation

GC/TQ analysis was carried out with a 7890B gas chromatograph coupled with a 7010B Triple Quadrupole GC/MS. The 7890B gas chromatograph was equipped with a 60 meter Agilent DB-5 column (part number 122-5061). All GC/HRMS data used for comparison were also collected using a DB-5 column operated under similar conditions. The 7010B Triple quadrupole GC/MS was operated in the MRM mode and equipped with a high-efficiency EI source (HES). The GC/TQ parameters are provided in Table 1.

The GC/TQ system was tuned to Agilent specifications using perfluorotributylamine (PFTBA) and the default HES tune. The method stipulates that the system is ready to operate as long as the vendor-specified tune criteria are met. Method 1613B requires a mass resolution check every 12 hours. The analogous parameter when using MS/MS is a mass calibration and tuning check. Every 12 hours the mass calibration was monitored by measuring the amount of peak drift from the expected masses for PFTBA. If the peak apex had shifted more than 0.3 amu from the expected value, then the instrument was recalibrated.

The need for lock mass monitoring of the GC/HRMS system for Method 1613B was replaced by use of a stability reference compound in the GC/TQ method. A small but constant amount of PFTBA, the reference compound used for tuning and mass calibration, was introduced and the

MRM transition 414.0 → 264.0 was monitored throughout the run. Any changes in the ionization efficiency and ion transmission can be observed as a change in the reference compound signal intensity.

Two transitions were monitored for each of the native PCDD/PCDF analytes and their corresponding ^{13}C-labeled analogues. Two masses from the molecular ion cluster were used as the transition precursors, each with its own product ion (loss of neutral $CO^{35}Cl$). MRM delivers a unique product ion that can be monitored and quantified in a complicated matrix, providing the selectivity needed for PCDD/PCDF analysis. The triple-stage selection process for ions reaching the detector results in low noise and thus a high signal-to-noise ratio (S/N) and good sensitivity and selectivity for analytes. The primary and secondary transitions for each analyte and labeled compound are listed in Table 2. Agilent MassHunter software was used for data acquisition, analysis, and reporting.

Table 1. GC/TQ parameters.

Parameter	Value
Gas Chromatograph	
Model	Agilent 7890B gas chromatograph
Column	Agilent DB-5, 60 m × 0.25 mm, 0.1 µm (p/n 122-5061)
Column Pneumatics	Constant flow, He carrier gas
Injector Mode	Splitless
Injector Liner	Inlet liner, splitless, double taper, deactivated (p/n 5181-3315)
Injection Volume	1.0 µL
Injector Temperature	290 °C
Flow Rate	0.93 mL/min
Temperature Program	90 °C for 2 min, 22 °C/min to 200 °C, 1 °C/min to 215 °C, hold 10 min, 5.2 °C/min to 300 °C, hold 2.7 min
Total Run Time	51.05 min
Equilibration Time	0.1 min
Mass Spectrometer	
Model	Agilent 7010B Triple Quadrupole GC/MS
Ionization Mode	EI, 70 eV
Acquisition Mode	MRM
Filament Current	100 µA
Collision Gas	N_2 at 1.5 mL/min
Quench Gas	He at 2.25 mL/min
GC Interface Temperature	290 °C
Ion Source Temperature	290 °C
Quadrupole 1 Temperature	150 °C
Quadrupole 2 Temperature	150 °C

Table 2. MRM transitions.

Analytes	Primary MRM Transition (*m/z*)	Collision Energy (CE)	Secondary MRM Transition (*m/z*)	CE	Surrogate
2,3,7,8-TCDD	319.9 → 256.9	24	321.9 → 258.9	24	$^{13}C_{12}$-2,3,7,8-TCDD
1,3,6,8-TCDD	319.9 → 256.9	24	321.9 → 258.9	24	$^{13}C_{12}$-2,3,7,8-TCDD
1,3,7,9-TCDD	319.9 → 256.9	24	321.9 → 258.9	24	$^{13}C_{12}$-2,3,7,8-TCDD
1,2,3,7,8-PeCDD	355.9 → 292.9	25	353.9 → 290.9	25	$^{13}C_{12}$-1,2,3,7,8-PeCDD
1,2,3,4,7,8-HxCDD	389.8 → 326.9	25	391.8 → 328.9	25	$^{13}C_{12}$-1,2,3,4,7,8-HxCDD
1,2,3,6,7,8-HxCDD	389.8 → 326.9	25	391.8 → 328.9	25	$^{13}C_{12}$-1,2,3,6,7,8-HxCDD
1,2,3,7,8,9-HxCDD	389.8 → 3269	25	391.8 → 328.9	25	Mean of $^{13}C_{12}$-1,2,3,6,7,8/1,2,3,4,7,8-HxCDD
1,2,3,4,6,7,8-HpCDD	423.8 → 360.8	25	425.8 → 362.8	25	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD
OCDD	457.7 → 394.8	26	459.7 → 396.8	26	$^{13}C_{12}$-OCDD
2,3,7,8-TCDF	303.9 → 240.9	33	305.9 → 242.9	33	$^{13}C_{12}$-2,3,7,8 -TCDF
1,2,7,8-TCDF	303.9 → 240.9	33	305.9 → 242.9	33	$^{13}C_{12}$-2,3,7,8 -TCDF
1,2,3,7,8-PeCDF	339.9 → 276.9	35	337.9 → 274.9	35	$^{13}C_{12}$-1,2,3,7,8-PeCDF
2,3,4,7,8-PeCDF	339.9 → 276.9	35	337.9 → 274.9	35	$^{13}C_{12}$-2,3,4,7,8-PeCDF
1,2,3,4,7,8-HxCDF	373.8 → 310.9	35	375.8 → 312.9	35	$^{13}C_{12}$-1,2,3,4,7,8-HxCDF
1,2,3,6,7,8-HxCDF	373.8 → 310.9	35	375.8 → 312.9	35	$^{13}C_{12}$-1,2,3,6,7,8-HxCDF
2,3,4,6,7,8-HxCDF	373.8 → 310.9	35	375.8 → 312.9	35	$^{13}C_{12}$-2,3,4,6,7,8-HxCDF
1,2,3,7,8,9-HxCDF	373.8 → 310.9	35	375.8 → 312.9	35	$^{13}C_{12}$-1,2,3,7,8,9-HxCDF

Table 2. MRM transitions (continued).

Analytes	Primary MRM Transition (*m/z*)	Collision Energy (CE)	Secondary MRM Transition (*m/z*)	CE	Surrogate
1,2,3,4,6,7,8-HpCDF	407.8 → 344.8	36	409.8 → 346.8	36	$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF
1,2,3,4,7,8,9-HpCDF	407.8 → 344.8	36	409.8 → 346.8	36	$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF
OCDF	441.7 → 378.8	35	443.7 → 380.8	35	$^{13}C_{12}$-OCDD
Cleanup Standard					
$^{37}Cl_4$-2,3,7,8-TCDD	327.9 → 262.9	33	-		$^{13}C_{12}$-1,2,3,4-TCDD
Labeled Surrogates					**Recovery Calculated Using**
$^{13}C_{12}$-2,3,7,8-TCDD	331.9 → 268.0	24	333.9 → 270.0	24	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDD	367.9 → 303.9	25	365.9 → 301.9	25	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDD	401.9 → 337.9	25	403.9 → 339.9	25	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,6,7,8-HxCDD	401.9 → 337.9	25	403.9 → 339.9	25	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDD	435.8 → 371.9	25	437.8 → 373.9	25	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-OCDD	469.8 → 405.8	26	471.8 → 407.8	26	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-2,3,7,8 -TCDF	315.9 → 252.0	33	317.9 → 254.0	33	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,7,8-PeCDF	351.9 → 287.9	35	349.9 → 285.9	35	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-2,3,4,7,8-PeCDF	351.9 → 287.9	35	349.9 → 285.9	35	$^{13}C_{12}$-1,2,3,4-TCDD
$^{13}C_{12}$-1,2,3,4,7,8-HxCDF	385.9 → 321.9	35	387.9 → 323.9	35	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,6,7,8-HxCDF	385.9 → 321.9	35	387.9 → 323.9	35	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,7,8,9-HxCDF	385.9 → 321.9	35	387.9> 323.9	35	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-2,3,4,6,7,8-HxCDF	385.9 → 321.9	35	387.9 → 323.9	35	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,6,7,8-HpCDF	419.8 → 355.9	36	421.8 → 357.9	36	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
$^{13}C_{12}$-1,2,3,4,7,8,9-HpCDF	419.8 → 355.9	36	421.8 → 357.9	36	$^{13}C_{12}$-1,2,3,7,8,9-HxCDD
Recovery Standards					
$^{13}C_{12}$-1,2,3,4-TCDD	331.9 → 268.0	24	333.9 → 270.0	24	
$^{13}C_{12}$-1,2,3,7,8,9-HxCDD	401.9 → 337.9	25	403.9 → 339.9	25	
Cl-DPE Transitions					
Descriptor			**Type**	**Substance**	
1	375.8 → 305.9	30	M+2	HxCDPE	
2	409.8 → 339.9	25	M+2	HpCDPE	
3	445.8 → 373.8	30	M+4	OCDPE	
4	479.7 → 407.8	30	M+4	NCDPE	
5	513.7 → 443.7	30	M+4	DCDPE	

As with the GC/HRMS Method 1613B, individual PCDD/PCDFs were identified by comparing the GC retention time and MRM transition product ion ratio (primary/secondary transition, Table 3), with the corresponding retention time of the authentic standard and the theoretical transition product ion ratio. Though not used here, Agilent's-patented retention time locking (RTL) technology could be used for this application. RTL provides the same retention times on one Agilent GC/MS system to those on another like system with the same nominal column. It also enables a single GC to have the same retention time after the column is trimmed for maintenance.

Shown in Table 3, the QC limits (±15% of theoretical) of Method 1613B were applied to the MS/MS data. The non-2,3,7,8 substituted isomers and congeners were identified when retention times and ion-abundance ratios were within predefined limits. Isomer specificity for 2,3,7,8-TCDD and 2,3,7,8-TCDF was achieved using GC columns that resolve these isomers from the other tetra-isomers.

Table 3. Theoretical product ion ratios and ratio QC limits.

Species Monitored	MRM Transition Precursor *m/z* (Primary/Secondary)	MRM Transition Product* Ion Theoretical Ratio‡	QC Limit**	
			Lower	Upper
Cl_4CDD†	(M+2)/M	0.96	0.82	1.10
Cl_4CDF	(M+2)/M	0.96	0.82	1.10
Cl_5CDD	M/(M+2)	0.78	0.66	0.90
Cl_5CDF	M/(M+2)	0.78	0.66	0.90
Cl_6CDD	(M+4)/(M+2)	0.64	0.54	0.74
Cl_6CDF	(M+4)/(M+2)	0.64	0.54	0.74
Cl_7CDD	(M+4)/(M+2)	0.80	0.68	0.92
Cl_7CDF	(M+4)/(M+2)	0.80	0.68	0.92
Cl_8CDD	(M+4)/(M+2)	0.96	0.82	1.10
Cl_8CDF	(M+4)/(M+2)	0.96	0.82	1.10

* Product ions are due to loss of [$CO_{35}Cl$].

** QC limits represent ±15% windows around the theoretical MRM transition product ion ratios.

† Does not apply to $^{37}Cl_4$-2,3,7,8-TCDD (cleanup standard).

‡ Transition product ion ratios are calculated as secondary ion/primary ion.

Method evaluation samples analyzed

Calibration was performed using a six-point calibration series of solutions covering the working concentration range. The operational range was 0.1 to 200 ng/mL for 2,3,7,8-TCDD and 2,3,7,8-TCDF; 1 to 2,000 ng/mL for OCDD and OCDF; and 0.5 to 1,000 ng/mL for all other dioxins and furans in the method. In addition to target (native) PCDDs/PCDFs, the calibration solutions also contained a suite of labeled surrogates (at 100 ng/mL except for $^{13}C_{12}$-OCDD at 200 ng/mL) and recovery standards ($^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9-HxCDD at 100 ng/mL). Following the procedure in Method 1613B, at least three initial calibrations were used to determine linearity of the GC/TQ instrument response.

Three method detection level (MDL) experiments were run (one each of spiked aqueous, solids, and tissues), per 40 CFR 136.3, Appendix B, Revision 2, on the GC/TQ instrument and compared to the Method 1613B minimum required levels (MRLs).

Extracts of nine real-world samples each from four matrices (aqueous, solids, biosolids, and tissues) were run by GC/TQ and compared to GC/HRMS results for PCDDs/PCDFs previously obtained for the same extracts. The samples were selected to be representative of different wastewater producers and environmental situations.

Four replicates of each of spiked reference (clean) materials (reagent water, Ottawa sand, and vegetable oil) were analyzed to produce an Initial Performance and Recovery (IPR) dataset to determine method recovery. Results were compared to Method 1613B recovery specifications.

A solids standard reference material (NIST 1944) and a tissue certified reference material (EDF 2525) were analyzed to determine the accuracy of the GC/TQ method. No aqueous reference samples were available. Results were compared to the certified values. In addition to the NIST and EDF samples, tissue and sediment/soil proficiency testing samples provided by Sigma-Aldrich RTC were also analyzed by GC/HRMS and GC/TQ.

Batch QC (blanks and ongoing precision and recovery samples) accompanying each of the extracts were also run. Blanks from method detection limit (MDL), recovery, and sample batches were run and compared to Method 1613B criteria.

Results and discussion

Chromatography performance and sensitivity

The GC/TQ analysis provided good chromatographic separation and detection of the target PCDDs/PCDFs as shown for TCDFs and TCDDs in Figure 1A, and for HxCDDs in Figure 1B. Method 1613B calls for calculation of the percent valley between the GC peaks that elute most closely to the 2,3,7,8-TCDD and TCDF isomers. The height of the valley between the isomers most closely eluting to the 2,3,7,8-TCDD labeled “x” in Figure 2 does not exceed 25% of the 2,3,7,8-TCDD peak height “y.” This parameter can be set as an outlier in the MassHunter Quantitative Analysis method as shown in Figure 3A. If the valley exceeds 25%, the analytical conditions need to be adjusted or the analysis repeated using a different GC column. Figure 3B demonstrates that the front and rear valley height/peak height resolution values were 20.4 and 7.8, respectively, and did not exceed the 25% threshold.

The 7010B Triple Quadrupole GC/MS system showed good sensitivity and S/N for PCDD/PCDFs. The GC/TQ system also provided very good reproducibility at low-level spikes, allowing for low-level quantitation, which is critically important because the EPA lowest Concentration Minimum Reporting Level (LCMRL) takes into account both sensitivity and reproducibility in its calculations. The system provided at least 10:1 S/N requirements for all compounds at the calibration standard level 1 (CS1)-level as required by EPA, and generally exceeded that with requirement with low RSDs.

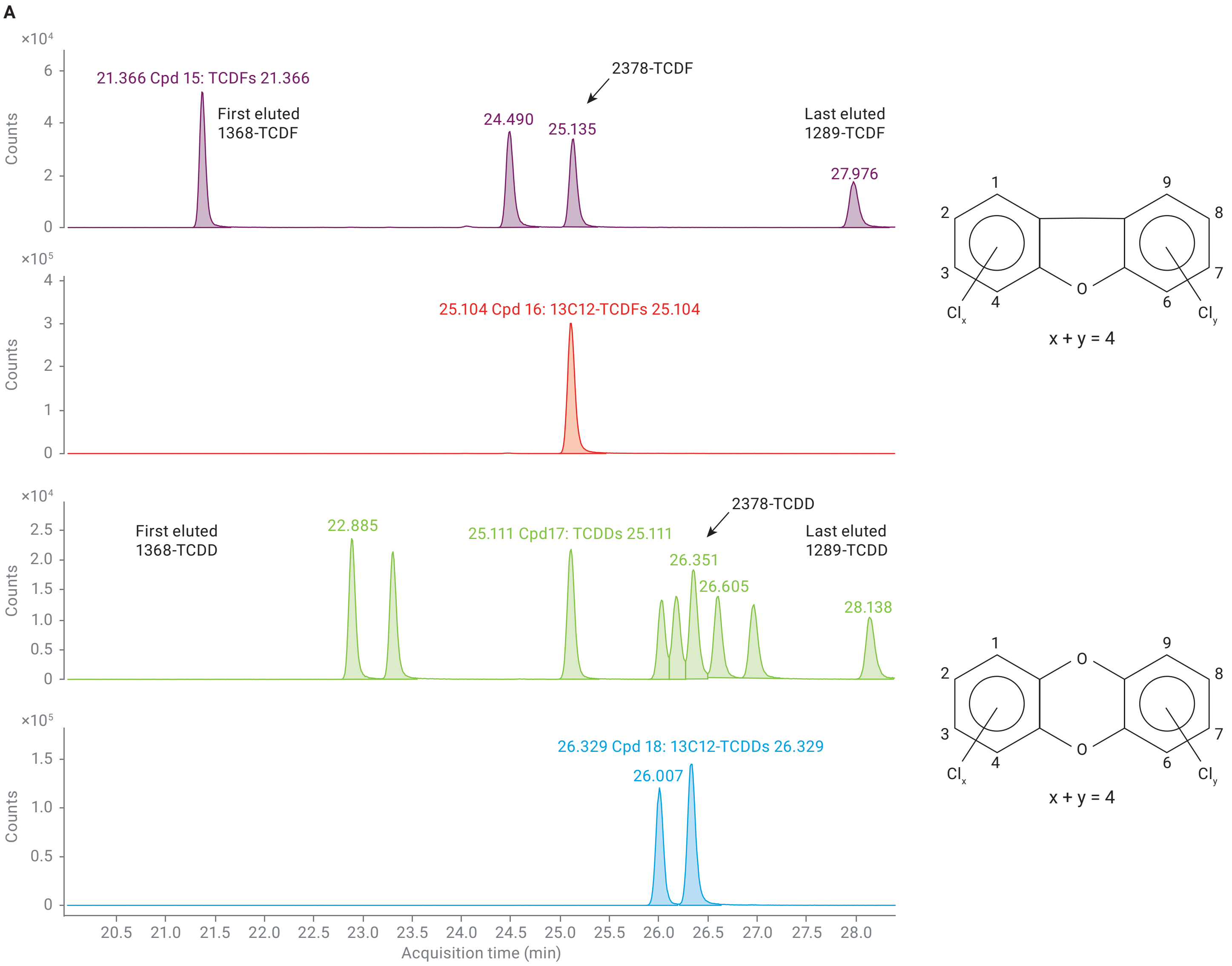


Figure 1A. MRM chromatograms for tetrachlorinated dibenzofurans (TCDFs), labeled TCDF ISTD, tetrachlorinated dibenzodioxins (TCDDs), and labeled TCDD ISTD.

B

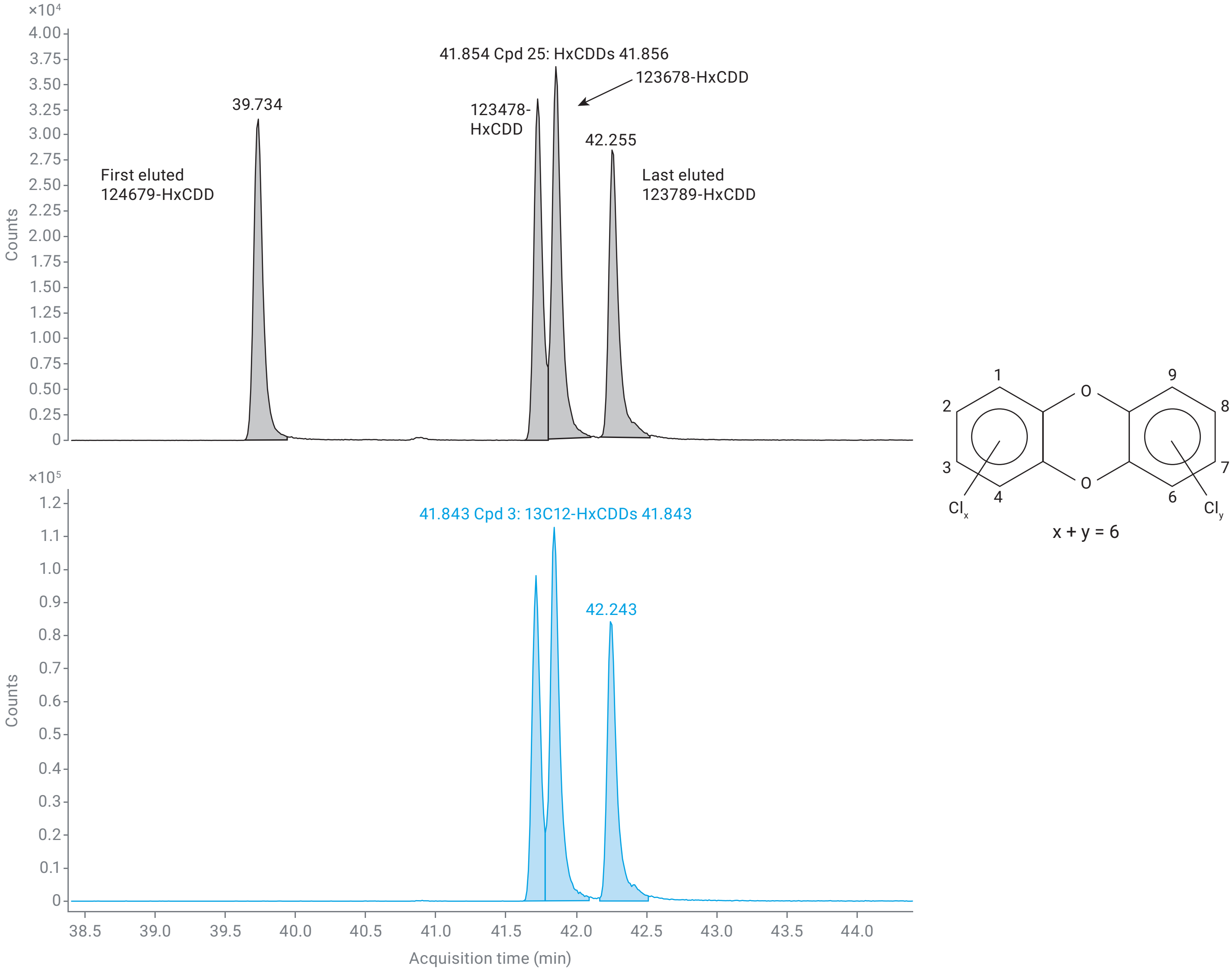


Figure 1B. Hexachlorinated dibenzodioxins HxCDDs and the corresponding ISTD.

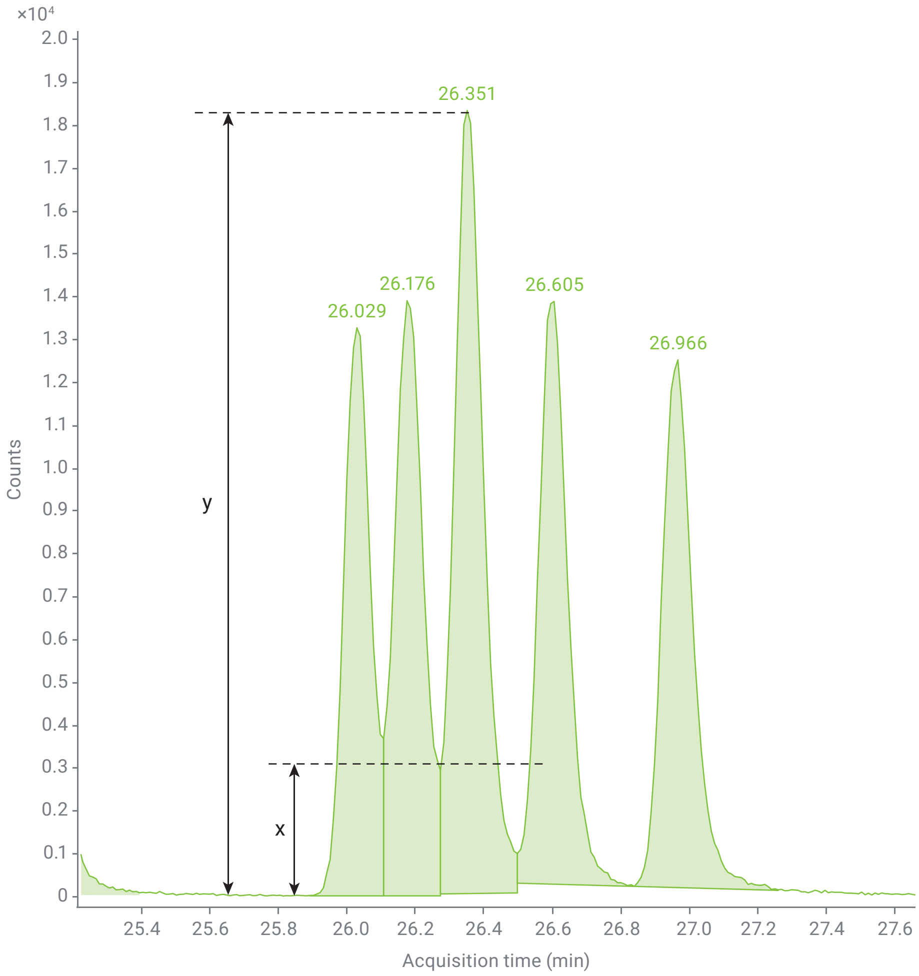
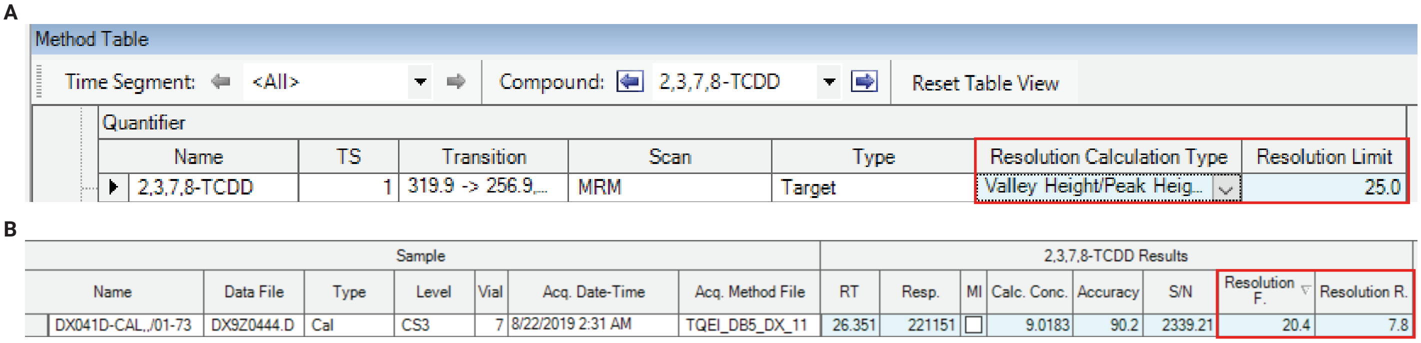


Figure 2. 2,3,7,8-TCDD and its close eluters.



Quantifier

Name	TS	Transition	Scan	Type	Resolution Calculation Type	Resolution Limit
2,3,7,8-TCDD	1	319.9 -> 256.9,...	MRM	Target	Valley Height/Peak Heig...	25.0

Sample							2,3,7,8-TCDD Results							
Name	Data File	Type	Level	Vial	Acq. Date-Time	Acq. Method File	RT	Resp.	MI	Calc. Conc.	Accuracy	S/N	Resolution F.	Resolution R.
DX041D-CAL,,/01-73	DX9Z0444.D	Cal	CS3	7	8/22/2019 2:31 AM	TQEI_DB5_DX_11	26.351	221151	☐	9.0183	90.2	2339.21	20.4	7.8

Figure 3. (A) Method setup for resolution check in MassHunter Quantitative Analysis; (B) front and rear valley height/peak height resolution calculated for 2,3,7,8-TCDD and its closest eluting isomers.

The 7010B triple quadrupole GC/MS is equipped with a high-efficiency EI source that produces up to 20 times more ions and maximizes ion transfer into the quadrupole mass analyzer, allowing significantly more sensitivity while still maintaining robustness.

Linearity, MDLs, total PCDD/PCDF

The GC/TQ system showed good linearity over the Method 1613B calibration range and met Method 1613B specifications. Linearity values expressed in terms of % RSDs of response factors for the target analytes across the calibration range were less than 20% and ranged from 2.2 to 15.4%. The 20% RSD limit does not apply to the labeled compounds, which are quantified by internal standard, not by isotope dilution. The %RSD of the PCDD/PCDF response factors for the five sets (days) of initial calibrations for the GC/TQ system are shown in Table 4. The results underscored the excellent dynamic range of the 7010B triple quadrupole GC/MS system.

The GC/TQ MDL results for the aqueous (1 L), solid (10 g), and tissue (10 g) samples are shown in Table 5. The results obtained using the 7010B triple quadrupole GC/MS system far surpassed Method 1613B MRLs.

Total PCDD and PCDF concentrations from the real-world sample extracts were reported by MassHunter software for each level of chlorination by summing the concentration of the individual peaks meeting quantification criteria (peak shape, S/N, and product ion ratio) in the appropriate retention time window. Figure 4 shows the comparison of the total PCDD and PCDF concentrations determined using GC/HRMS and GC/TQ. The results for the two technologies were comparable.

Table 4. %RSDs of the PCDD/PCDF response factors for the five days of initial calibrations.

Date Acquired	19-AUG-19	21-AUG-19	06-JAN-20	07-JAN-20	08-JAN-20
Data File ID	DX9Z0415-A1	DX9Z0444-A1	DX9Z0830-A1	DX9Z0837-A1	DX9Z0853-A1
Name	RRF %RSD	RRF %RSD	RRF %RSD	RRF %RSD	RRF %RSD
2,3,7,8-TCDF	4.0	3.0	4.4	2.7	2.4
1,2,3,7,8-PeCDF	3.7	2.8	3.4	2.9	2.7
2,3,4,7,8-PeCDF	3.8	3.5	4.1	3.9	4.3
1,2,3,4,7,8-HxCDF	3.1	4.5	4.4	2.3	5.6
1,2,3,6,7,8-HxCDF	3.0	3.5	5.3	3.6	8.1
2,3,4,6,7,8-HxCDF	3.0	3.9	6.2	4.5	1.3
1,2,3,7,8,9-HxCDF	4.6	5.4	6.7	2.7	6.0
1,2,3,4,6,7,8-HpCDF	3.2	4.3	3.7	4.8	4.3
1,2,3,4,7,8,9-HpCDF	4.6	4.7	4.6	5.8	4.0
OCDF	7.1	10.2	9.0	7.0	6.3
2,3,7,8-TCDD	2.9	4.8	6.3	5.6	7.3
1,2,3,7,8-PeCDD	4.6	4.6	2.2	2.3	3.9
1,2,3,4,7,8-HxCDD	4.3	4.0	2.3	2.3	3.1
1,2,3,6,7,8-HxCDD	5.4	5.3	5.2	2.6	5.3
1,2,3,7,8,9-HxCDD	5.3	3.4	6.8	3.6	4.7
1,2,3,4,6,7,8-HpCDD	2.6	3.9	8.4	4.3	4.9
OCDD	3.6	3.6	5.7	4.5	4.8
^{13}C-2,3,7,8-TCDF	6.1	5.4	6.9	8.0	7.8
^{13}C-1,2,3,7,8-PeCDF	15.2	17.6	21.7	22.4	23.5
^{13}C-2,3,4,7,8-PeCDF	17.5	19.9	25.0	26.3	26.1
^{13}C-1,2,3,4,7,8-HxCDF	3.1	3.0	4.5	4.4	2.5
^{13}C-1,2,3,6,7,8-HxCDF	2.2	4.8	5.8	3.7	1.7
^{13}C-2,3,4,6,7,8-HxCDF	2.4	2.5	4.4	4.6	1.8
^{13}C-1,2,3,7,8,9-HxCDF	4.1	3.6	2.9	4.1	3.3
^{13}C-1,2,3,4,6,7,8-HpCDF	3.4	3.8	4.4	8.3	3.0
^{13}C-1,2,3,4,7,8,9-HpCDF	4.3	3.2	3.4	10.2	5.0
^{13}C-2,3,7,8-TCDD	8.4	10.3	11.5	13.2	13.2
^{13}C-1,2,3,7,8-PeCDD	16.7	19.7	24.7	25.9	25.3
^{13}C-1,2,3,4,7,8-HxCDD	2.1	3.2	3.8	3.3	2.4
^{13}C-1,2,3,6,7,8-HxCDD	2.8	3.4	2.8	4.0	3.2
^{13}C-1,2,3,4,6,7,8-HpCDD	4.8	5.3	4.3	9.0	5.5
^{13}C-OCDD	7.5	5.6	7.0	9.0	6.9
^{13}C-1,2,3,4-TCDD	17.6	8.6	15.0	11.4	13.6
^{13}C-1,2,3,7,8,9-HxCDD	36.2	31.6	38.3	38.4	27.5
^{37}Cl-2,3,7,8-TCDD	9.7	11.9	11.4	15.8	14.3

Table 5. GC/TQ MDL results with comparison to Method 1613B MRLs.

Compound	Aqueous	Solid	Tissue
	MDL and (MRL) in pg/L	MDL and (MRL) in pg/g	MDL and (MRL) in pg/g
2,3,7,8-TCDD	1.1 (10)	0.029 (1)	0.057 (0.5)
1,2,3,7,8-PeCDD	1.39 (50)	0.037 (5)	0.051 (2.5)
1,2,3,4,7,8-HxCDD	1.05 (50)	0.042 (5)	0.061 (2.5)
1,2,3,6,7,8-HxCDD	1.08 (50)	0.045 (5)	0.033 (2.5)
1,2,3,7,8,9-HxCDD	1.78 (50)	0.064 (5)	0.067 (2.5)
1,2,3,4,6,7,8-HpCDD	1.19 (50)	0.070 (5)	0.032 (2.5)
OCDD	9.4 (100)	0.311 (10)	0.085 (5)
2,3,7,8-TCDF	0.56 (10)	0.60 (1)	0.056 (0.5)
1,2,3,7,8-PeCDF	1.0 (50)	0.037 (5)	0.046 (2.5)
2,3,4,7,8-PeCDF	1.25 (50)	0.039 (5)	0.033 (2.5)
1,2,3,4,7,8-HxCDF	0.89 (50)	0.032 (5)	0.029 (2.5)
1,2,3,6,7,8-HxCDF	1.11 (50)	0.031 (5)	0.046 (2.5)
1,2,3,7,8,9-HxCDF	1.22 (50)	0.048 (5)	0.084 (2.5)
2,3,4,6,7,8-HxCDF	1.26 (50)	0.026 (5)	0.034 (2.5)
1,2,3,4,6,7,8-HpCDF	0.92 (50)	0.255 (5)	0.064 (2.5)
1,2,3,4,7,8,9-HpCDF	1.35 (50)	0.028 (5)	0.043 (2.5)
OCDF	2.81 (100)	0.365 (10)	0.113 (5)

Recoveries

Three sets of spiked clean matrix one each of aqueous (1 L), solids (10 g) and tissues (10 g) were run and the mean percent recovery (n = 4) and percent RSD calculated (Figure 6). Results were compared and determined to conform to Method 1613B IPR specifications.

Proficiency, SRM, and CRM results

The evaluation report from Sigma-Aldrich RTC, Inc. concluded that both GC/HRMS and GC/TQ results obtained from the proficiency tests were acceptable and met study criteria and with an overall score of 100%. These results indicate the accuracy of PCDD/PCDF data from the 7010B Triple Quadrupole GC/MS analysis of the environmental matrices. The results of the GC/TQ analysis of the solids SRM (NIST 1944) and tissue CRM (EDF 2525) also demonstrated the accuracy of the GC/TQ method.

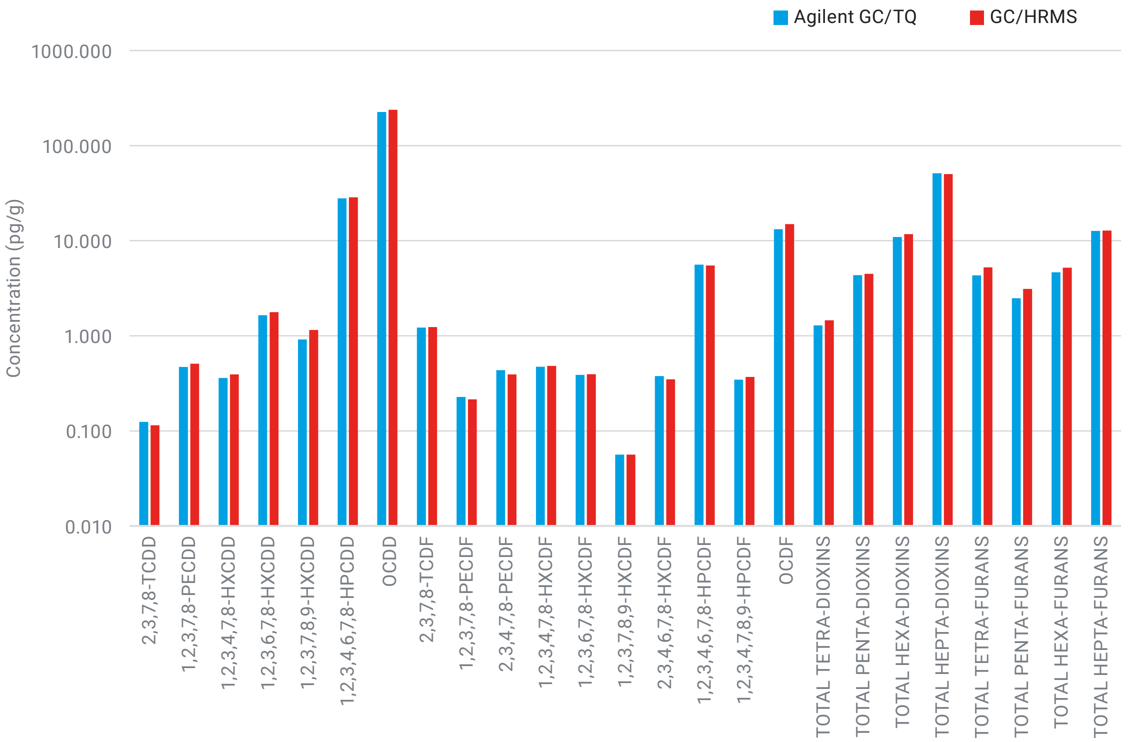


Figure 4. Comparison of total PCDD/PCDF for a real-world biosolids sample determined by GC/TQ (blue bars) and GC/HRMS (red bars).

Table 6. Fortified concentration, mean percent recovery (n = 4), and percent RSD for spiked clean matrix.

	Aqueous			Solids			Tissues		
	Total Conc. (pg/L)	Mean % Recovery	RSD (%)	Total Conc. (pg/L)	Mean % Recovery	RSD (%)	Total Conc. (pg/g)	Mean % Recovery	RSD (%)
2,3,7,8-TCDD	200	99	2	20	102	2	20	102	1
1,2,3,7,8-PECDD	1,000	98	2	100	99	2	100	100	1
1,2,3,4,7,8-HXCDD	1,000	97	2	100	99	1	100	99	1
1,2,3,6,7,8-HXCDD	1,000	96	3	100	98	3	100	98	2
1,2,3,7,8,9-HXCDD	1,000	103	4	100	109	3	100	118	12
1,2,3,4,6,7,8-HPCDD	1,000	98	2	100	100	2	100	98	1
OCDD	2,000	98	2	200	100	2	200	99	1
2,3,7,8-TCDF	200	99	2	20	101	2	20	101	1
1,2,3,7,8-PECDF	1,000	97	2	100	100	2	100	100	1
2,3,4,7,8-PECDF	1,000	97	2	100	99	2	100	99	1
1,2,3,4,7,8-HXCDF	1,000	95	2	100	98	1	100	97	1
1,2,3,6,7,8-HXCDF	1,000	98	4	100	102	2	100	98	2
1,2,3,7,8,9-HXCDF	1,000	102	3	100	103	2	100	102	1
2,3,4,6,7,8-HXCDF	1,000	97	3	100	99	2	100	98	1
1,2,3,4,6,7,8-HPCDF	1,000	107	3	100	108	2	100	109	6
1,2,3,4,7,8,9-HPCDF	1,000	98	3	100	100	2	100	100	1
OCDF	2,000	92	2	200	97	2	200	94	3
^{13}C-2,3,7,8-TCDD	2,000	70	8	200	58	12	200	73	4
^{13}C-1,2,3,7,8-PECDD	2,000	74	9	200	62	15	200	78	5
^{13}C-1,2,3,4,7,8-HXCDD	2,000	81	4	200	64	10	200	71	9
^{13}C-1,2,3,6,7,8-HXCDD	2,000	79	5	200	61	9	200	70	9
^{13}C-1,2,3,4,6,7,8- HPCDD	2,000	87	5	200	69	12	200	74	9
^{13}C-OCDD	4,000	76	5	400	60	14	400	63	9
^{13}C-2,3,7,8-TCDF	2,000	67	7	200	53	11	200	65	3
^{13}C-1,2,3,7,8-PECDF	2,000	68	9	200	57	14	200	71	5
^{13}C-2,3,4,7,8-PECDF	2,000	69	9	200	57	15	200	74	4
^{13}C-1,2,3,4,7,8-HXCDF	2,000	77	5	200	63	9	200	66	10
^{13}C-1,2,3,6,7,8-HXCDF	2,000	78	6	200	61	9	200	68	8
^{13}C-1,2,3,7,8,9-HXCDF	2,000	75	4	200	60	12	200	73	8
^{13}C-2,3,4,6,7,8-HXCDF	2,000	79	5	200	62	10	200	70	9
^{13}C-1,2,3,4,6,7,8- HPCDF	2,000	77	6	200	62	9	200	66	9
^{13}C-1,2,3,4,7,8,9- HPCDF	2,000	83	5	200	67	12	200	71	12
^{37}Cl-2,3,7,8-TCDD	200	73	6	20	69	7	20	79	3

Note about potential interferences

In this study, the analysis of 36 real-world samples of four sample matrices showed no interferences, and chromatography and quantified results for GC/TQ were equivalent to GC/HRMS. In addition, concentrated standards of PAH, alkylated PAH and chlorinated pesticides showed no response when analyzed by the GC/TQ method. However, because there is incomplete chromatographic separation of the chlorinated diphenyl ethers (CDPEs) from PCDFs, a characteristic *m/z* for each chlorinated diphenyl ether must be monitored. If detected at the retention time of any PCDFs, additional cleanup must be performed per Method 1613B. The GC/HRMS requirement to monitor CDPEs and perform additional cleanup when detected remains when using the GC/TQ method.

In addition, although there are small mass differences (about 6 amu) between some PCBs and some PCDD/PCDFs at the same level of chlorination, the DB-5 column provides complete chromatographic separation of these compounds. However as with GC/HRMS, interferences from fragments of higher homolog PCBs are possible. It is recommended that extract cleanup procedures include a step to remove PCBs from sample extracts.

Conclusion

GC/TQ technology provides many of the specificity and sensitivity advantages of HRMS for the analysis of regulated dioxins and furans without the cost and complexity of HRMS instruments, with added versatility and robustness. Approval of GC/TQ technology for determination of dioxins and furans as an alternative testing protocol to Method 1316B has the potential to significantly lower laboratory costs and increase operational efficiency.

This application note described and evaluated the GC/TQ SGS AXYS Method 16130 using the Agilent 7890B gas chromatograph coupled with an Agilent 7010B Triple Quadrupole GC/MS. The method will eventually be added into the Federal Register. The results obtained were determined to meet the QA/QC and performance specifications in Method 1613B for the analysis of PCDDs/PCDFs in environmental matrices. Performance factors investigated included sensitivity, linearity, MDLs, recovery, and results compared to reference material. The results of the performance tests demonstrated that GC/TQ using 7010B Triple Quadrupole GC/MS provides data of the same quality for real world samples representing complex matrices.

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Accelerated Determination of Microplastics in Environmental Samples Using Thermal Extraction Desorption-Gas Chromatography/Mass Spectrometry (TED-GC/MS)

Authors

Ulrike Braun, Paul Eisentraut,
Korinna Altmann,
Maria Kittner, and
Erik Dümichen
Bundesanstalt für
Materialforschung und
-prüfung (BAM),
Berlin, Germany

Kurt Thaxton and
Eike Kleine-Benne
GERSTEL Inc.

Tarun Anumol
Agilent Technologies, Inc.

Abstract

There is growing interest in quantifying microplastics in environmental samples. This application note presents a thermal extraction desorption-gas chromatography/mass spectrometry (TED-GC/MS) method that is well suited to automation and increased sample throughput. The method is also able to detect all particle sizes in the sample as long as the limit of detection (LOD) is reached and allows analysis of larger samples of 15 to 25 mg or more. Samples were decomposed by thermogravimetric analysis (TGA), and the gaseous decomposition products were trapped on a solid-phase sorbent, followed by thermal desorption-gas chromatography/mass spectrometry (TD-GC/MS) using an Agilent 5977B GC/MSD coupled to an Agilent 7890B GC. Target microplastic particle (MP) polymers were identified in environmental samples including surface water, finished compost, house dust, and drinking water. Quantification of MP polymers in environmental samples provided LODs of 0.06 to 2.2 µg, allowing the detection of MPs in trace amounts with sample weights of up to 1 g. Method repeatability was adequate for reliable quantification with RSDs of approximately 6 to 12%.

Introduction

Microplastic particles (MPs) are defined as any solid plastic particles insoluble in water with any dimension between 1 µm and 1,000 µm (ISO/TC 21960). Consisting mainly of synthetic polymers, MPs are observed nearly everywhere in the environment. Though, the systematic occurrence of MPs in environmental samples has not yet been sufficiently investigated, it is necessary to fully understand their relevant sources and entry pathways into the food and environmental ecosystem and to initiate sustainable prevention strategies. MPs are mainly formed in the environment by fragmentation of macroplastics, and remain for an uncertain length of time. This persistence increases the likelihood that MPs will be ingested and accumulated in organisms, the risks of which are unclear and the subject of controversy. As a result, there is increased interest in determining MPs in environmental samples such as soils, water, air, and organic matter.

MP analysis commonly relies on spectroscopic imaging techniques, such as infrared (IR) or Raman microscopy that provide particle numbers, and polymer chemical identity, geometry, and size distributions.[1] The disadvantage of these techniques is that they are often time-consuming and require extensive sample preparation. Small particles (<10 µm) cannot directly be analyzed by spectroscopic imaging, but are expected to be the relevant fraction for toxicology. Quantification of MPs in environmental samples using traditional GC/MS or LC/MS methods has been challenging, because of their very high molecular weights and poor solubility in most solvents.

Thermo-analytical methods offer an alternative. Complementary to imaging techniques, they provide information on mass content that can be essential for regulatory and monitoring purposes. As temperature is increased, pyrolytic decomposition of polymers occurs, forming smaller molecules that can be analyzed using spectroscopic techniques such as IR spectrometry and mass spectrometry. In addition, the thermal decomposition products formed can generate structural information about the polymer analyzed. However using such evolved gas analysis tools for environmental samples produces unspecific signals, which cannot be unambiguously related to specific MP type. Pressurized liquid extraction in combination with pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) can provide reliable determination of MPs in environmental samples, however large quantities of solvents, some of which are not environmentally friendly, must be used and the additional extraction step carries the risk of particle loss or contamination.[2,3]

Another approach, TED-GC/MS, is better suited to automation and increased sample throughput without an additional sample preparation step, including reproducible automated analysis of polymer decomposition processes.[4-6] A further advantage of TED-GC/MS is its ability to detect all particle sizes in the sample as long as the limit of detection is reached. In this approach, the sample is decomposed by TGA and the gaseous decomposition products are trapped on a solid-phase sorbent, followed by TD-GC/MS. MP samples can be very heterogeneous. By using a TGA furnace with a relatively large oven, TED-GC/MS allows analysis of larger samples of 15 to 25 mg or more, resulting in a more representative sample and eliminating the need to take multiple samples. This is in contrast to Py-GC/MS, where sample sizes are typically ten times less and thus require running ten times the samples to achieve the same degree of representation, while also potentially not covering a large enough sample volume to achieve homogeneity.

An instrument system developed by the Bundesanstalt für Materialforschung und -prüfung (BAM) in cooperation with GERSTEL GmbH & Co. KG adds a heated coupling device that enables a sample robot to transfer thermal desorption tubes from the pyrolysis gas flow of the TGA furnace to the TD-GC/MS system for automated analyses.[4] This application note describes use of a 5977B GC/MSD coupled to a 7890B GC as part a complete automated TED-GC/MS system, which is exclusively available through GERSTEL and Agilent. Further details about the experimental setup and methodology are provided in the publications by Dümichen *et al.*,[4] Altmann *et al.*,[5] and Eisentraut *et al.*[6]

Experimental

TED-GC/MS instrumentation

TGA heats the sample in an inert atmosphere, during which measurements of mass versus temperature can be made. The technique is convenient, reliable, and robust for analysis of sample weights up to 1 g and temperatures up to 1,000 °C. TGA was performed using a thermogravimetric furnace equipped with an autosampler (TGA2, METTLER TOLEDO) and 150 µL alumina crucibles. Dümichen *et al* determined that a TGA purge gas flow rate of 30 mL/min N_2 is the best compromise between analyte response and contamination, and that a heating rate of 10 °C/min provides the best average response.[4] The TGA parameters are provided in Table 1.

Connected directly behind the TGA furnace as an extension of the exhaust, the coupling device enables the vertical sample transfer required by the sample robot. The coupling was maintained at its maximum temperature of 240 °C and required two independent 3 mL/min N_2 flows to avoid contamination. Separating the TGA from the GC/MSD minimizes or eliminates contamination and overloading of the GC/MS from the relatively large amounts of natural organic matter or MPs that are decomposed during TGA, which is a concern with traditional Py-GC/MS.

Because nonpolar or semipolar compounds are the most important compounds released during polymer decomposition in MP analysis, poly(dimethylsiloxane) (PDMS) (Sorb-Star, ENVEA GmbH) was used as solid-phase sorbent agent to capture the components purged from the TGA furnace for subsequent TD-GC/MS analysis. Designed specifically for chemical analysis, the cylindrical PDMS adsorption bar was 2 mm by 20 mm (diameter by length). If desired, GERSTEL's PDMS Twister devices may be used for similar results.

A Thermal Desorption Unit (TDU 2, GERSTEL) performed the thermal extraction step. It was mounted on top of the coupling device, allowing the TD tube to be automatically transferred by the sample robot based on the time program. Another TDU attached to the GC/MS performed the thermal desorption step.

The GC/MS system was a 7890B GC coupled to a 5977B GC/MSD. The 7890B GC was equipped with cryo-injection (CIS 4, GERSTEL). The analytes were cryo-focused in the cooling trap (CIS) at –100 °C using liquid nitrogen. When the thermal desorption process was completed, the trapped analytes were quickly reheated, separated, and analyzed by GC/MS. The TD-GC/MS parameters are presented in Table 2.

Table 1. TGA parameters for TED-GC/MS.

Parameter	Value
Model	METTLER TOLEDO large furnace TGA 2
Sample Mass	20 mg (standard conditions; but up to 1,000 mg possible)
Purge Gas	30 mL/min N_2
Heating Rate	10 °C/min
Total Exhaust Flow	56 mL/min
Heating Range	25 to 600 °C

Table 2. TD-GC/MS parameters for TED-GC/MS.

Parameter	Value
Thermal Desorption	
Model	GERSTEL TDU 2
Mode	Splitless
Total Flow	24 mL/min He
Temperature Program	50 to 200 °C at 40 °C/min Hold at 200 °C for 5 min
Cryofocus And Injection	
Model	GERSTEL CIS 4
Mode	Solvent Vent
Temperature Program	-100 to 270 °C at 12 °C/s Hold at 270 °C for 3 min
Gas Chromatograph	
Model	Agilent 7890B gas chromatograph
Column	Agilent HP-5MS, 30 m × 250 µm, 0.25 µm
Flow Rate	1 mL/min He
Temperature Program	Hold at 40 °C for 1 min, 40 to 300 °C at 5 °C/min, hold at 300 °C for 3 min
Mass Spectrometer	
Model	Agilent 5977B GC/MSD
Interface Temperature	300 °C
Ion Source Temperature	230 °C
Quadrupole Temperature	150 °C
Ionization Mode	EI, 70 eV
Mode	Scan, 35 to 350 *m/z* (under specific circumstances 25 to 450 *m/z* for PE)

The complete TED-GC/MS instrument setup is shown in Figure 1. Water filtrate, air filter samples, neat polymers, soil, compost, and other materials filled into heat-resistant conventional or smart filter crucibles can be measured directly without additional sample preparation. In this approach, the total TED-GC/MS measurement time is approximately two hours and 30 minutes: one hour for TGA, one hour for GC/MS analysis, and about 30 minutes for GC cool down and TGA device bake out. After the thermal extraction step and during the GC/MS run, the TGA furnace can be cleaned by heating to 1,000 °C under oxidizing atmosphere. A new sample measurement can then be started after completion of the GC/MS measurement. Complete automation allows measurement of up to ten samples per day, including blank runs between measurements. In general, a sample mass of 20 mg is sufficient, but if needed, masses up to 1 g can be investigated.

Agilent MassHunter GC/MS Acquisition Software revision B.07.04.2260 was used for data acquisition. Agilent Enhanced ChemStation software revision F.01.03.2357 was used for data analysis.

Figure 1. Complete TED-GC/MS instrument setup with the TGA furnace (right) and coupling device (top). The TD-GC/MS system based on the Agilent 5977B GC/MSD (left), and sample robot. The patented smart filter crucible is shown bottom right. Photos courtesy of BAM.

Samples

TED-GC/MS allows routine determination of the polymers commonly used in manufacturing, and thus of environmental concern, including those in tire materials. Polymers determined in this study were polyethylene (PE), polypropylene (PP), polystyrene (PS), poly(ethylene terephthalate) (PET), polyamide (PA), poly(methyl methacrylate) (PMMA), and styrene-butadiene rubber (SBR), which is associated with tire materials. Several different types of samples were analyzed to demonstrate method applicability to MP detection:

- Fractions of filtered surface water
- Finished compost
- House dust
- Drinking water in PET bottles

The technique is versatile and allows other experiments, such as the analysis of a PE/PP blend with unknown relative weight mass[5] and the determination of adsorbed pollutants on activated carbon from wastewater treatment filters that were analyzed per Dittmann *et al.*[7]

Polymer identification

The polymers in the MP samples were identified via detection of the characteristic fragment ions associated with their decomposition products and retention times (t_R) (Table 3). Polystyrene deuterated on the benzene ring (d_5PS) was used as an internal standard to provide a relative reference value for measurement quality control. One decomposition product of the internal standard is the deuterated dimer of styrene d_{10}-(2,4-diphenyl-1-buten) with the characteristic fragment ions *m/z* 96 and 218, which can be distinguished from the decomposition product of PS (2,4-diphenyl-1-butene) at *m/z* 91 and 208 using MS, but also by chromatographic separation.

Table 3. Specific polymer markers and characteristic fragment ions.

Polymer	Polymer Marker (Decomposition Product)	t_R (min)	Characteristic Fragment Ion(s) (*m/z*)
PE	1,14-Pentadecadiene	23.4	55 or 81
PP	2,4,6,8-Tetramethyl-10-undecene	18.8	111
PS	2,4-Diphenyl-1-butene	28.7	91 or 208
PET	Ethyl benzoate	15.3	105
SBR	Cyclohexenylbenzene	19.6	104
PA 6	Caprolactam	18.0	113
PMMA	Methyl methacrylate	3.7	69 or 100

Polymer quantification

Quantification of polymers is typically performed using standard addition. By rerunning samples after adding polymer standards in known amounts (available from BAM), the change in peak response was used to determine polymer mass contents.

A faster alternative is the use of response factors obtained from one-point calibration experiments or external calibration where polymers in various levels are added to suitable plastic-free matrices. It was determined that for samples from the same or similar origin, a standard addition for a single, carefully selected sample was suitable for quantification of the entire set of samples.

Results and discussion

Identification and quantification

Excellent separation and detection of the polymer decomposition markers of PE, PP, PS, PET, PA, acrylates, and SBR by TED-GC/MS using the 7890 GC and 5977B GC/MSD was obtained (Figure 2 total ion chromatogram, TIC). Polymer identification was achieved by analysis of the extracted ion chromatograms using the characteristic ions.

The results for the samples quantified using standard addition are presented in Figure 3. The total polymer content found in house dust and compost was 21 µg/mg and 13 µg/mg, respectively. However, the polymer compositions of the samples differed. In house dust, the fiber polymers PET, PA, and PP were found in the highest amounts, while the compost mainly included PE and PP, which are polymers commonly used for packaging.

In the fractions of filtered surface water, PE and SBR were dominant, but small amounts of PP and PS were also found. A component of tires, SBR is mainly identified in the fractions <100 µm. A total of 73 µg/L of target polymers was found in the surface water.

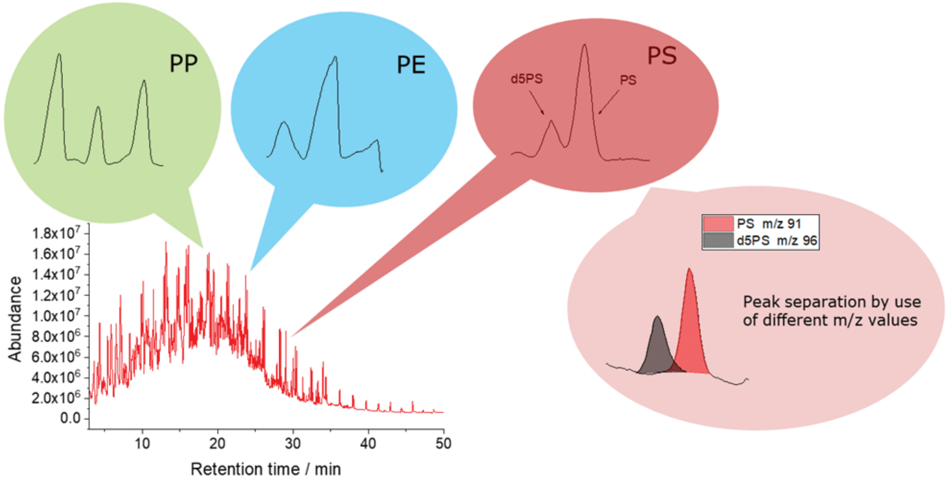

Figure 2. Total ion chromatogram (TIC) from the TED-GC/MS analysis of an environmental sample. The insets show extracted ion chromatograms that were used to identify the polymers based on their characteristic ions.

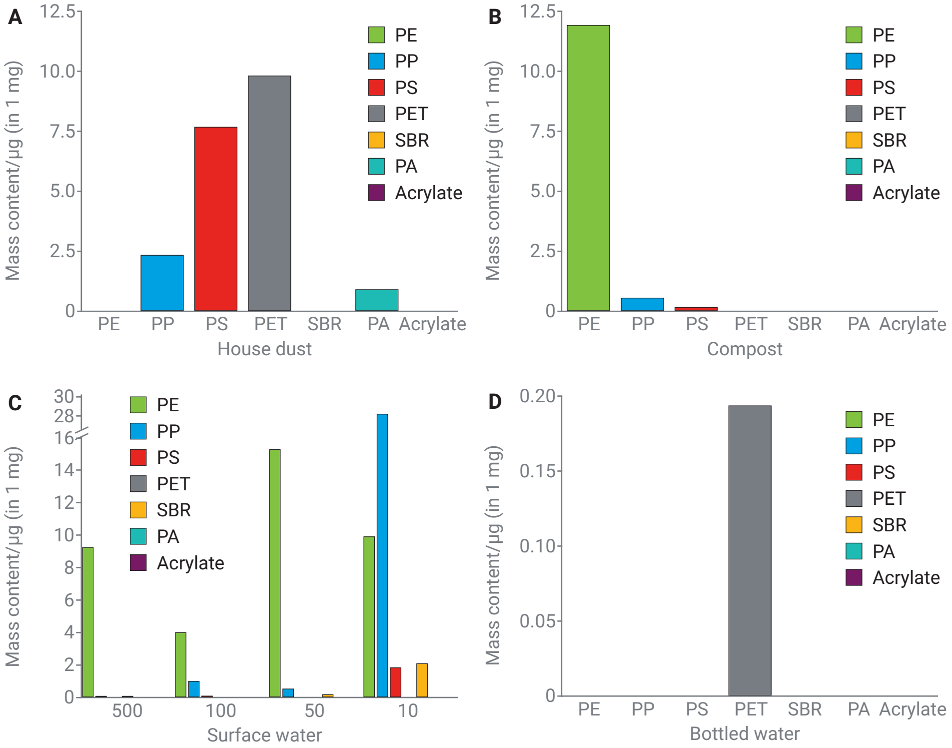

Figure 3. MP polymer content found in house dust, finshed compost, surface water, and bottled water by TED-GC/MS. In the surface water analysis chart, the bars show the MP content of the different particle fractions in µm resulting from the sampling.

Because bottled water typically contains lower levels of MP, a filter crucible with 5 µm pore size was used. Up to 10 L of bottled or tap water were poured directly onto the filter and filtered using vacuum filtration with subsequent drying and analysis of the filter contents. A total of 0.193 µg/L of PET was found in the bottled water.

Limit of detection (LOD)

The LOD values for eight polymers were calculated on the basis of a quintuple signal-to-noise ratio. The LOD values were determined for the neat polymers without environmental matrix and for the respective polymer in fluvial suspended particulate matter (SPM). Six conventional standard polymers (PE, PP, PS, PET, SBR, and PMMA) were analyzed. Also analzyed were two biodegradable polymers, polylactide (PLA) and polybutylene adipate terephthalate (PBAT) with the specific decomposition products 3,6-dimethyl-1,4-dioxan-2,5-dione (t_R = 13.9 minutes, *m/z* = 56), and terephthalic acid dibut-3-enyl-ester (t_R = 34.7 minutes, *m/z* = 203).

Figure 4 presents the LODs of the neat polymers and in the presence of an environmental matrix. Method sensitivity for the various neat polymers differed as shown by their LOD values: high (LOD <0.2 µg; e.g. PP, PS, SBR, and PMMA), medium (LOD <0.5 µg; e.g. PET, PBAT, and PLA) and low (LOD >2.0 µg; e.g. PE). The addition of an environmental matrix, in this case SPM, resulted in increased LODs for all polymers analyzed. In conclusion, the matrix-induced increases in LOD were polymer-specific and probably influenced by their respective thermal decomposition mechanisms.

Repeatability

The repeatability of the TED-GC/MS method using the 5977B GC/MSD was determined by measuring specific thermal decomposition products of PP (Table 4) in seven separate runs. The mass fragments with the highest intensities were chosen for integration, and the integration values were normalized to the sample masses. The results showed good repeatability for reliable quantitation (Figure 5). Three of the degradation products had a Relative Standard Deviation (RSD) of approximately 6% (Table 4). Repeatability results could be improved using internal standards.

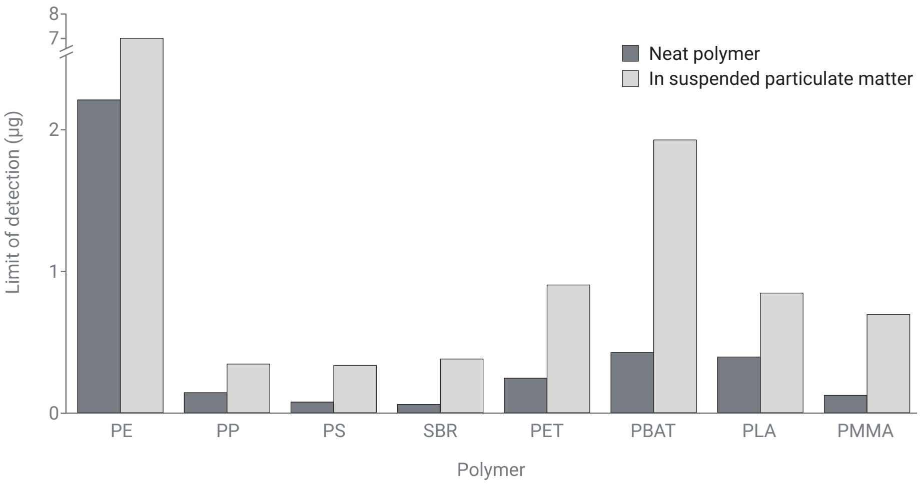

Figure 4. LOD values for the neat polymers compared to the LOD of the polymer in suspended particulate matter.

Table 4. Polymer products monitored in the repeatability experiment with %RSD.

Polymer	Name	Formula	t_R (min)	RSD (%)
PP1	2,4-Dimethyl-1-heptene	C_9H_{18}	5.84	11.98
PP2	2,4,6-Trimethyl-1-nonene	$C_{12}H_{24}$	12.50	5.17
PP2*	2,4,6-Trimethyl-1-nonene	$C_{12}H_{24}$	12.62	6.85
PP3	2,4,6,8-Tetramethyl-10-undecene	$C_{15}H_{30}$	18.97	5.89
PP3*	2,4,6,8-Tetramethyl-10-undecene	$C_{15}H_{30}$	19.17	12.46
PP3**	2,4,6,8-Tetramethyl-10-undecene	$C_{15}H_{30}$	19.42	8.11

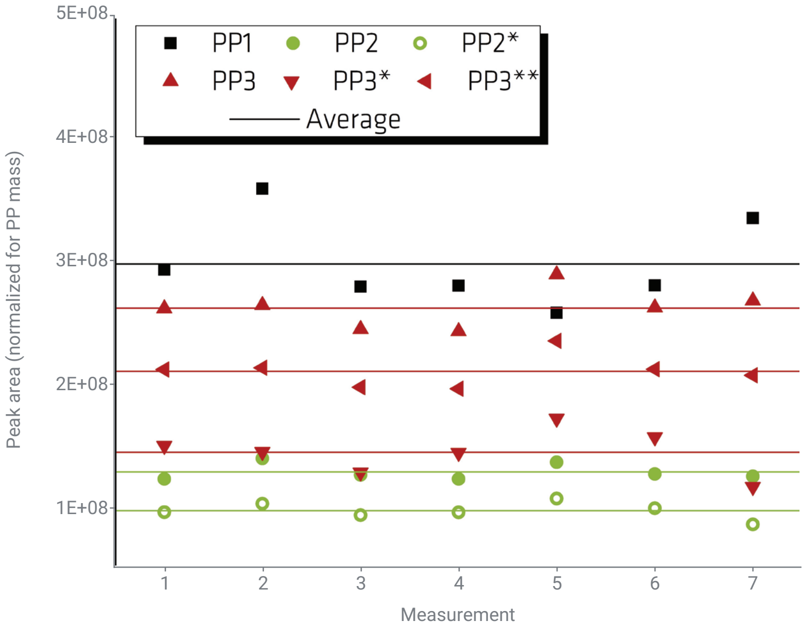

Figure 5. Integration results for the repeatability determination normalized to the sample mass for six selected decomposition product peaks. The average values are shown as a straight line.

Conclusion

Compared to other thermo-analytical approaches to MP measurement, TED-GC/MS using a thermobalance with the 7890B GC and 5977B MSD is well suited for reproducible automated analysis of polymers via their specific decomposition products. Target MP polymers were identified in a variety of environmental samples including surface water, finished compost, house dust, and drinking water. Quantification of MP polymers in environmental samples was performed by standard addition. The specific LODs of 0.06 to 2.2 µg for the different polymer types allow the detection of MPs in the trace amounts with sample weights of up to 1 g. Method repeatability was adequate for reliable quantification with RSDs of approximately 6 to 12%.

TED-GC/MS offers reproducible quantitation in addition to automation. Due to the limited capacity of the sorbent and the low desorption temperatures, the method is robust and minimizes concerns about contamination of the sensitive GC/MS equipment. The results are expressed in terms of mass contents, which enables use of the method for determination of legal limit values for regulation and monitoring. TED-GC/MS analysis is also promising for application to other scientific fields, such as the analysis of polymer blends with unknown relative weight mass and the determination of adsorbed pollutants on activated carbon from wastewater treatment filters.

Though it is possible to use Py-GC/MS for MPs determination, TED-GC/MS offers automation, simplified sample preparation, reproducible quantitation, and the ability to analyze sample weights up to 1 g by weighing samples into crucibles. Analyzing the same amount of sample by Py-GC/MS would require running multifold samples, and consequently much more analysis and data-evaluation time. The ability to analyze larger sample amounts is also important due to the heterogeneous nature of MP samples. In addition, with the limited capacity of the sorbent and low desorption temperatures, overloading the GC/MS is less likely, making the method robust and without contamination concerns for long-term usage. The fast measurements provided by TED-GC/MS provide a new path to more widespread monitoring of MP pollution.

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