UNPARALLELED PRECISION, SUPERIOR REPEATABILITY

GC Headspace Compendium

PerkinElmer®
For the Better

Better Precision Every Time

Headspace (HS) technology is an unsurpassed technique that is used by analytical labs to extract and concentrate complex samples that contain volatile organic compounds (VOCs) as well as semi-volatile chemicals. This technique eliminates the time-consuming steps and risk of human error associated with other GC sample preparation methods.

PerkinElmer's TurboMatrix™ HS samplers are engineered with our innovative pressure-based, time-based sampling, which allows samples to be introduced into the column without using a gas syringe or multiport valves. Instead, carrier gas pressures are precisely regulated to manage transfer, eliminating many of the sources of variability and contamination found in other systems.

In this reference guide you will find a mix of food, industrial, environmental and pharmaceutical applications that use TurboMatrix headspace technology integrated with our Clarus® GC platform. Learn how you can enable aggressive detection limits, excellent repeatability and deliver accurate, precise results – simply and more productively.

What's more, PerkinElmer has a full complement of the right, high quality consumables you need for your chromatography methods to enable reliable, reproducible results – every time.

TABLE OF CONTENTS

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

Meng Yuan

PerkinElmer, Inc.
Shelton, CT 06484 USA

Analysis of Butylated Hydroxytoluene in Food with Headspace Trap-GC/MS

Introduction

Butylated hydroxytoluene (BHT, 2,6-di-tert-butyl-4-methylphenol) is a common food additive. BHT is found in many types of food including butter, meats, cereals, chewing gum, baked goods, snack foods, dehydrated potatoes and beverages. It is used to preserve food odor, color and flavor. BHT is oxidized preferentially in fats or oils, protecting the foods from spoilage.

Concern exists that long-term human consumption of BHT may have potential health risks. It has undergone the additive application and review process required by the U.S. Food and Drug Administration (FDA); the committee concluded that no evidence in the available information on BHT demonstrates a hazard to the public when it is used at levels that are now current and in the manner now practiced. However, uncertainties exist requiring that additional studies should be conducted.[1] The chemical properties which make BHT an excellent preservative may also be implicated in health effects. The oxidative characteristics and metabolites of BHT may contribute to carcinogenicity. Some people may have difficulty metabolizing BHT, resulting in health and behavioral changes.

PerkinElmer®
For the Better

This application note will demonstrate a fast and easy analytical technique to determine the amount of BHT in foods. Headspace (HS) sample introduction is used because it provides a means to analyze food without any sample preparation. Headspace eliminates the need for solvents and other sample-preparation steps to reduce cost and complexity of extraction. In this application note, an adsorbent trap is used to concentrate the headspace sample and increase sensitivity, allowing for low-level detection or small sample sizes.

The analysis is carried out with gas chromatography mass spectrometry (GC/MS) – this will allow us to resolve the BHT from other volatile compounds in the food matrices and provide positive identification of the BHT with mass spectral data. Calibration of the system and analysis of food samples will be demonstrated.

Experimental

The instrumental platform for this application is the TurboMatrix™ HS Trap coupled to a Clarus® 680 GC/MS, both platforms from PerkinElmer. The transfer line of the HS was directly connected to the Elite™-17ms column with a universal butt connector. The samples are heated in a sealed vial at 80 ˚C for 30 minutes to drive the BHT from the food into the headspace. Using automated headspace technology, the gas is extracted from the vial, concentrated on an adsorbent trap (PerkinElmer® Air Toxics), and injected into the GC/MS system. Table 1 shows the detailed instrumental setup parameters for the HS Trap-GC/MS system.

Table 1. **Instrument Parameters.**

Sample Introduction	**PerkinElmer TurboMatrix HS-40 Trap**
Needle Temperature	90 ˚C
Transfer Line Temperature	110 ˚C
Oven Temperature	80 ˚C
Trap Low Temperature	40 ˚C
Trap High Temperature	280 ˚C
Dry Purge (Helium)	5 min
Trap Hold Time	6 min
Desorb Time	0.5 min
Thermostatting Time	30 min
Pressurization Time	1 min
Decay Time	2 min
Column Pressure	17 psi
Vial Pressure	35 psi
Desorb Pressure	10 psi
Universal Capillary Column Connector	Part No. N9302149
Transfer Line	Fused Silica 2 m x 320 µm

Gas Chromatograph	**PerkinElmer Clarus 680 GC**
Headspace Connector	Universal Connector
Inlet Temperature	150 ˚C
Oven Program Initial Temp	50 ˚C
Hold Time 1	1 min
Ramp 1	25 ˚C/min to 280 ˚C
Hold Time 2	1.8 min
Vacuum Compensation	On
Headspace Control	On
Column	Elite-17ms 30 m x 0.25 mm x 0.25 µm
Carrier Gas	Helium
Mass Spectrometer	**PerkinElmer Clarus 600 MS**
Mass Range	45-300 u
Solvent Delay Time	0.1 min
Scan Time	0.20 sec
InterScan Delay Time	0.02 sec
Transfer Line Temperature	240 °C
Source Temperature	200 °C
Multiplier	500 V

Calibration-Standards Preparation

A 10 ng/µL standard stock solution was prepared by diluting 0.1 mL of a 1000 µg/mL BHT standard to 10 mL with methanol. 1 ng/µL, 2 ng/µL and 5 ng/µL standard working solutions were prepared by diluting 0.1 mL, 0.2 mL and 0.5 mL of a 10 ng/µL BHT standard to 1 mL with methanol. 20 ng/µL, 50 ng/µL and 100 ng/µL standard working solution was prepared by diluting 0.02 mL, 0.05 mL and 0.1 mL of a 1000 µg/mL BHT standard to 1 mL with methanol.

The working curve was prepared by injecting 1 µL of each working standard solution into headspace vials. Working calibration standards at 1, 2, 5, 10, 20, 50, and 100 ng were prepared fresh each day.

One gram of each food sample purchased at local Shanghai markets were placed into the headspace vials. All headspace vials were sealed immediately and transferred to the headspace-trap vial tray.

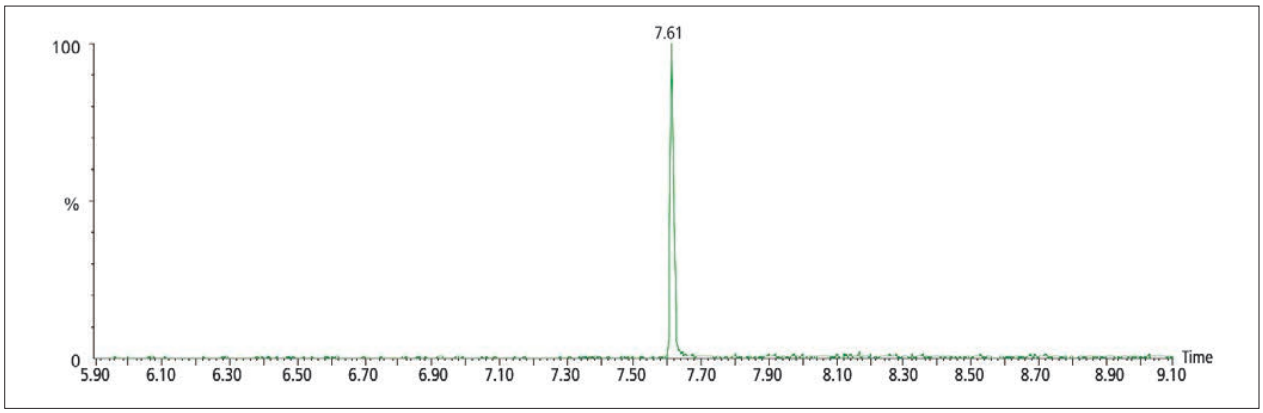


Figure 1. Example chromatogram of a 100 ng standard injection of BHT.

Results and Discussion

The instrument calibration included seven calibration levels in the working curve; the response of this calibration curve was linear (Table 2). Additionally, the method is precise throughout the calibration range, as demonstrated by the relative standard deviation of 3.2% at the calibration limit (1 ng, n=5) and 1.9% at 10 ng (n=5).

Figure 1 is an extracted ion chromatogram, of *m/z* 205, from the analysis of a 100 ng BHT standard. Figure 2 demonstrates the spectral data of BHT which matches exactly the fragmentation of BHT in the NIST® spectral library.

Following the calibration of the system, five food samples were analyzed: a cracker, powdered coffee creamer, instant noodles, sausage, and tea leaves. The BHT concentrations are quantified (Table 3). BHT concentration in the food samples analyzed here was below the quantitation limit of 1 ng/g. It can be seen in Figure 3 (Page 4) that the BHT peak is easily identified in the sample analysis. Each sample was analyzed in triplicate – the area reproducibility achieved (Table 3) demonstrates that the method remains very precise, even below the quantitation limit.

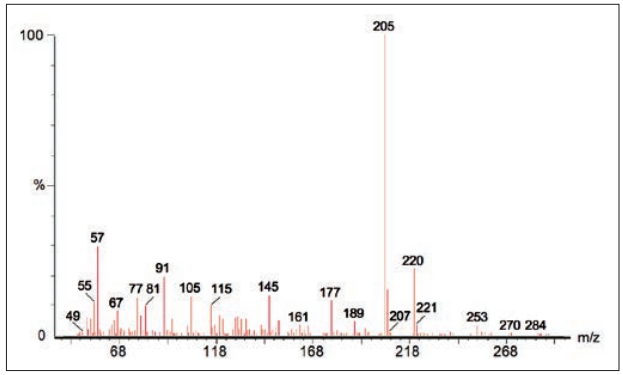


Figure 2. Background subtracted spectra from the analysis of a BHT standard.

***Table 2.* Calibration Table for BHT.**

Name	Retention Time (min)	Quantifier Ion	Qualifier Ion 1	Qualifier Ion 2	%RSD (n=5 at 1 ng)	%RSD (n=5 at 10 ng)	r^2
BHT	7.60	205	220	57	3.2	1.9	0.9980

***Table 3.* %RSD of BHT in Food Samples.**

Sample	BHT (ng/g) in 1 g of Sample	BHT (ng/g) in 1 g of Duplicate	BHT (ng/g) in 1 g of Triplicate	Values Mean (ng/g)	%RSD
Crackers	0.65	0.55	0.54	0.58	10.5
Coffee Creamer	0.66	0.73	0.69	0.69	5.1
Instant Noodles	0.67	0.67	0.70	0.68	2.5
Sausage	0.67	0.53	0.56	0.59	12.6
Tea Leaves	0.62	0.54	0.53	0.56	8.8

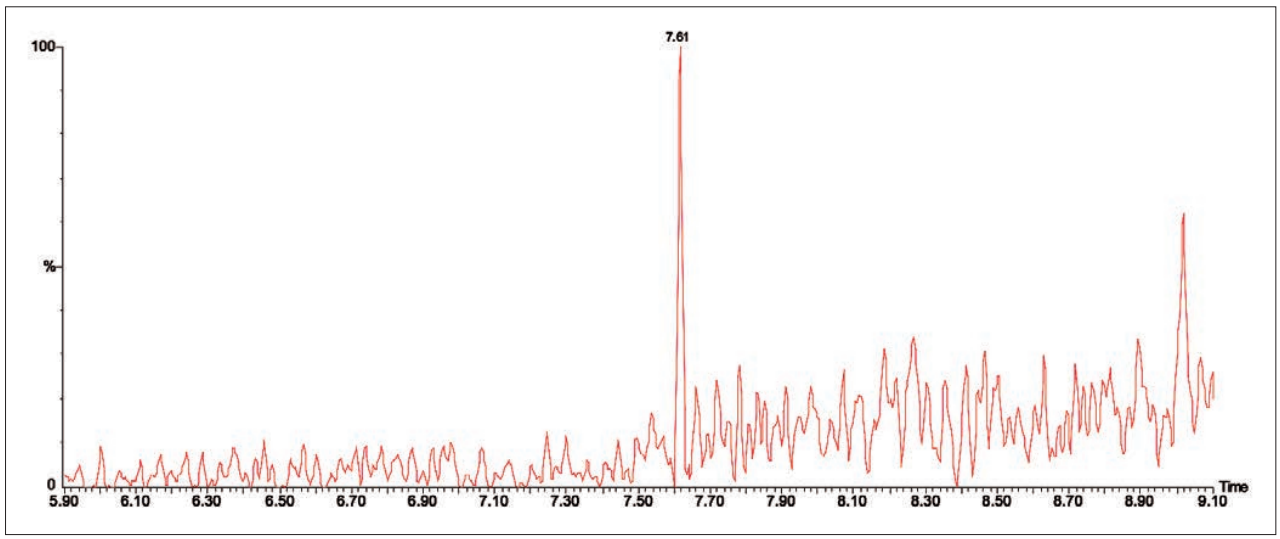


Figure 3. Resultant chromatogram from the analysis of instant noodles for BHT.

Conclusion

BHT is a common food additive used to prevent spoilage. Analysis of BHT is needed for both food quality and safety reasons. Food is often a complicated sample matrix which is time consuming to prepare and analyze. This method uses headspace technology to virtually eliminate sample preparation and reduce the cost and labor of the analysis. In addition to eliminating sample preparation, the method is both sensitive and precise as demonstrated by the analysis of standard reference materials and a variety of food samples. The throughput of the system is further improved by the Clarus 680 GC/MS with a fast-cooling GC oven, further improving throughput and productivity. The MS data provides positive confirmation of BHT in sample matrices.

References

1. Database of Select Committee on GRAS Substances (SCOGS) Reviews-Butylated Hydroxytoluene (BHT), available from http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=scogsListing&id=41

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

009138_01

APPLICATION NOTE

GC-Mass Spectrometry and Headspace Sampling

Author

Padmaja Prabhu

PerkinElmer, Inc.
Shelton, CT 06484 USA

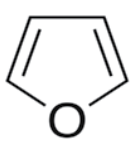
Determination of Furan in Food by Gas Chromatography-Mass Spectrometry and Headspace Sampling

Introduction

Furan is naturally occurring at low levels in many foods and drinks.[1] Furan consumption is of concern because it has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans, based on studies with laboratory animals. The U.S. FDA has recently published a report on the occurrence of furan in a large number of thermally processed foods, especially canned and jarred foods, including baby foods and infant formulas. The primary source of furan in food is considered to be thermal degradation of carbohydrates, such as glucose, lactose and fructose.

Of all the foods tested in various papers, coffee contained the largest amount of furans.[1] Furan is a colorless, volatile and lipophilic organic compound. It has a molecular weight of 68 and a low boiling point (31 °C). Due to its high volatility, furan levels in foods are easily determined, with high accuracy, by headspace methods.

This application note will demonstrate a rapid method for the identification and quantification of furan in food samples, using gas chromatography with headspace sampling and mass spectrometry. In addition to method optimization and standard analysis, we will analyze a number of food samples for furan. We chose to test coffee containing drinks, sauces, and canned foods, as previous studies demonstrated high levels of furan in these foods. The samples were randomly collected from the local market.



Synonyms: furfuran, oxole, tetrole, divinylene oxide, oxacyclopentadiene
Formula: C_4H_4O
MW: 68.07
MP: -85.6 ºC
BP: 31 ºC

Figure 1. Structure and physical properties of furan.

PerkinElmer®
For the Better

Experimental

The PerkinElmer® Clarus® 680 Gas Chromatograph, Clarus 600 C Mass Spectrometer and a TurboMatrix™ HS-40 system were used for this application. Table 1 presents the detailed operating parameters of the GC/MS and the HS system. The instrument interaction, data analysis and reporting was completed with the PerkinElmer TurboMass™ data system.

Table 1. **Detailed Instrument Conditions Used in the Determination of Furans.**

Instrument Details:	**Clarus 680 Gas Chromatograph**	
Analytical Column	PerkinElmer Elite™-624 N9316204 (60 meter, 0.32 mm i.d., 1.8 µm df)	
GC Column Flow	1.4 mL/min helium at constant flow mode	
GC Inlet Temperature	200 °C	
Split Ratio	2:1	
Oven Temperature Program	40 °C hold for 6.0 min, 20 °C/min to 110 °C and hold for 1.0 min, 70 °C/min to 250 °C and hold for 3.5 min; runtime is 20 min	
MS Parameters:	**Clarus 600 C Mass Spectrometer**	
MS Source Temperature	230 °C	
MS Interface Temperature	225 °C	
Scan Range	m/z 35-150	
Scan Time	2.5-25 min	
Multiplier	500 V	
Scans/Sec	5.56	
Headspace Parameters:	**TurboMatrix HS-40**	
Temperatures	Thermostatting Oven	60 °C
	Needle	100 °C
	Transfer Line	130 °C
Time	Injection	0.2 min
	Pressurization	0.5 min
	Withdrawal	0.2 min
	Equilibration	20 min
	Cycle	20 min
Options	Vial Vent	ON
	Shaker	ON
	Operation Mode	Constant
	Injection Mode	Time
	Hi Psi Injection	ON
PPC	Inject	35 psi
	Column/ Headspace Pressure	25 psi

Headspace is a perfect technique for sample introduction in furan analysis due to the ease of sample preparation and the limited interaction of the instrumentation with the sample matrix. Caution must be taken when setting the vial oven temperature; a high temperature can result in furan formation in the sample during analysis. To reduce this risk the method presented here uses a low incubation temperature.

Stock Solution: A stock solution of 1000 µg/mL of furan and furan-d_4 was used as the starting point for all standard solutions (SPEX CertiPrep®).

Standard Preparation:

10 µL of the stock furan solution was diluted to 10 mL in methanol to give a solution of 1 µg/mL. 20 µL of the stock furan-d_4 solution was diluted to 10 mL in methanol to give a solution of 2 µg/mL.

Calibration Curve: The volume of 1 µg/mL furan was diluted in water to achieve the final standard concentration presented in Table 2. 100 µL of furan-d_4 from 2 µg/mL stock was added to each headspace vial containing 10 mL of water resulting in an internal standard concentration of 0.02 µg/mL (20 ppb). 4 g of NaCl was added to each of the vials to decrease the miscibility of furan in water.

Preparation of Solutions:

Table 2. **Scheme Used for the Creation of a Five Level Calibration.**

Calibration Level No.	Concentration of Furan in ppb	Std Solution Added in µL	Final Vol. (mL)
1	1	10	10
2	2	20	10
3	10	100	10
4	20	200	10
5	40	400	10

*4 gm of NaCl was added to each of the headspace vials.

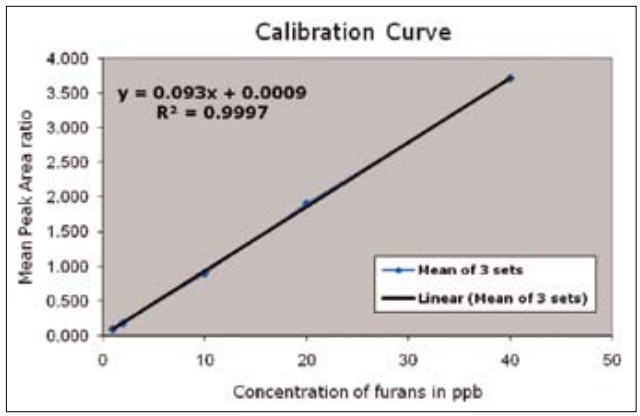


Figure 2. Calibration curve for furan.

Calibration: The MS was calibrated across the range of 1.0 to 40 ng/mL and each calibration point was run in triplicate to demonstrate the precision of the system. The average coefficient of determination for a line of linear regression was 0.9997 for furan. The calibration curve for furan is depicted in Figure 2.

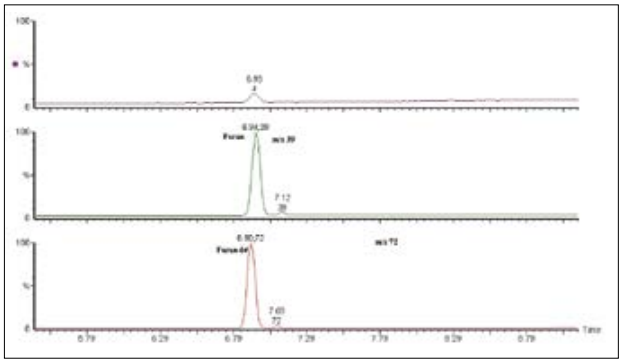


Figure 3. Example chromatogram of 40 ppb furan standard showing the total and extracted ion chromatograms as well as the extraction ion chromatogram for the furan-d_4 internal standard.

Also in Table 3 is the percent relative standard deviation (%RSD) for each calibration point (n=3). The precision of the system across the calibration range is excellent. The chromatograms and the spectrum from the analysis of standard material are shown in Figure 3.

Table 3. **% RSD's for Three Sets of Linearity Experiment.**

Sr. Number	Number of Levels	Mean Peak Area Average Relative Response (n=3)	%RSD
1	1	0.098	10.046
2	2	0.184	8.012
3	10	0.904	1.475
4	20	1.900	0.435
5	40	3.709	1.627

The precision of the method was measured at both 0.5 and 1 ppb. The detection limit of this method is approximately 0.5 ppb (Table 4).

Table 4. **RSD Values for Detection Limit and Quantification Level.**

Sr. No.	Conc. of Furan in ppb	Furan/IS Area Ratio	Conc. of Furan in ppb	Furan/IS Area Ratio
1	0.5	0.035	1	0.102
2	0.5	0.031	1	0.097
3	0.5	0.031	1	0.106
4	0.5	0.021	1	0.103
5	0.5	0.021	1	0.096
6	0.5	0.022	1	0.093
Mean		**0.03**		**0.1**
S.D.		**0.01**		**0.0**
%RSD		**23.75**		**4.78**

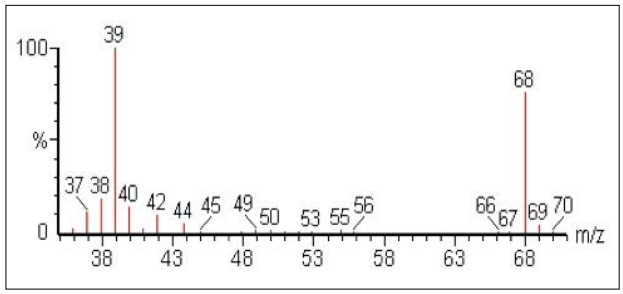


Figure 4. Full scan mass spectrum obtained experimentally for furan.

Table 5. **Method Validation Summary.**

Linearity:	1.0 ppb to 40 ppb of furan
RSD for Replicate Analysis:	for 1.0 ppb 4.78%
Detection Level:	0.5 ppb
Quantification Level:	1.0 ppb
Recovery Study:	at three levels for all the samples within 80-120%

Sample Preparation: Samples were collected from the local market. The samples included: coffee, milk, canned foods, sauces, peanut butter and apple juice (Table 6). All the samples were refrigerated before analysis. 10 mL of sample was transferred into a headspace vial; 4 g of NaCl was added to it. Milk and other viscous samples were diluted with water (1:2 or 1:4). The semi-solid samples were ground and 5 g of sample was added to headspace vials with 5 mL of saturated salt (NaCl) solution. Coffee powder was dissolved following directions on the package, and then treated like a non-viscous liquid sample.

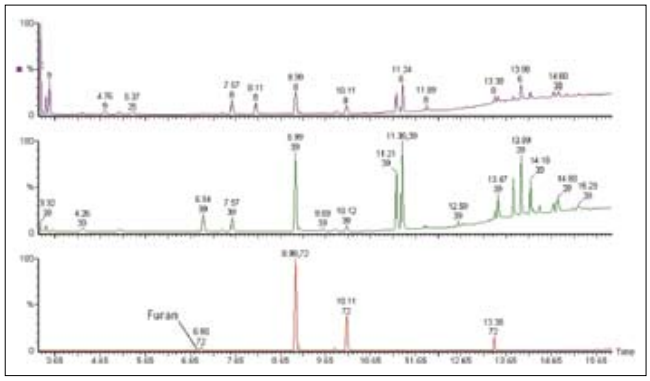


Figure 5. Experimental chromatogram from the analysis of espresso coffee with furan peak visible at 6.9 minutes.

Table 6. **Sample Analysis Results.**

Sample No.	Sample Details	Amt. of Furan Found in ppb
Sample 1	Lab Coffee	0.67
Sample 2	Chocolate Flavored Milk (AKCF)	1.67
Sample 3	Espresso Coffee	45.18
Sample 4	Coffee Flavored Milk (AKC)	10.87
Sample 5	Cocoa Flavored Milk (AKK)	1.76
Sample 6	Energy Drink (milk based) (NAEM)	13.21
Sample 7	Brewed Coffee	36.59
Sample 8	Filtered Coffee	253.99

Method Validation:

The recovery of the method was tested with the analysis of the brewed coffee sample spiked at three different levels: 2, 5, 10 µg/L. The measured amount was 2.03, 5.44, 9.54 µg/L demonstrating that the headspace technique is quantitative in its extraction of furan from an aqueous matrix.

Results

Eight samples of common beverages were analyzed using the HS-GC/MS method developed here. The samples were chosen because they had been shown to have detectable levels of furan in the literature. Of the samples analyzed, brewed coffee was demonstrated to have the highest levels of furan, at 250 µg/L. The remaining sample results are demonstrated in Table 6.

Conclusion

This application presents a method for the determination of furans in beverages using headspace sample introduction. Headspace GC is fast, reliable and can be used for the quantification of furans in common beverages. The internal standard calibration of furan across 1-40 µg/L responded linearly. Beverages were analyzed and the level of furan determined. The furan was identified by both the retention time and the MS fragmentation pattern. The method was validated at several levels and coffee matrix recovery values were between 95-101%.

References

1. Food Composition and Additives, Journal of AOAC International Vol. 88, No. 2, 2005.

2. Determination of Furans in Alcoholic Beverages.

3. Rapid and Improved Determination of Furan in Baby Foods and Infant Formulas by Headspace GC/MS.

4. Report on Carcinogens, Eleventh Edition.

5. Furans in food-Review, Food Research Institute, Bratislava, Slovak Republic.

6. Determination of Furans in Foods, CFSAN/ Office of Plant and Dairy Foods, May 7, 2004; updated June 2, 2005, and October 27, 2006.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

009368_01

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

A. Tipler, Senior Scientist

PerkinElmer, Inc.
Shelton, CT 06484 USA

The Determination of Low Levels of Benzene, Toluene, Ethylbenzene, Xylenes and Styrene in Olive Oil Using a TurboMatrix HS and a Clarus SQ 8 GC/MS

Introduction

Levels of benzene, toluene, ethylbenzene, xylenes and styrene (BTEXS) are a concern in olive oil. These compounds find their way into olive trees and hence into the olives and olive oil mainly as a result of emissions from vehicles, bonfires, and paints into ambient air near the orchards.

Various methods have been developed to detect and quantify these compounds down to levels of 5 ng/g (5 ppb w/w). This application note describes an easy to perform method using PerkinElmer® Clarus® SQ 8 GC/MS with a TurboMatrix™ 110 headspace sampler to achieve detection limits below 0.5 ng/g.

PerkinElmer®
For the Better

Method

The experimental conditions for this analysis are given in Tables 1 to 4.

Table 1. **GC Conditions.**

Gas Chromatograph	Clarus 680
Column	30 m x 0.25 mm x 1.0 µm Elite-Wax
Oven	35 °C for 1 min, then 10 °C/min to 130 °C
Injector	Programmable Split Splitless (PSS), 180 °C, Split OFF
Carrier Gas	Helium at 1.0 mL/min constant flow (7.2 psig initial pressure), HS Mode ON

Table 2. **HS Conditions.**

Headspace System	TurboMatrix 110 HS Trap in standard HS mode (trap port capped).
Vial Equilibration	90 °C for 20 minutes
Needle	130 °C
Transfer Line	140 °C, long, 0.150 mm i.d. fused silica (chosen to facilitate rapid conversion to HS trap operation for other applications)
Carrier Gas	Helium at 35 psig
Injection Time	0.15 min

Table 3. **MS Conditions.**

Mass Spectrometer	Clarus SQ 8 MS, Large Turbo Pump
Scan Range	35 to 350 Daltons
Electron Energy	70eV
Scan/Dwell Time	0.1 s
Interscan/Interchannel Delay	0.02 s
Source Temp	200 °C
Inlet Line Temp	200 °C
Multiplier	1400V

Table 4. **Sample Details.**

Sample	10.00 ±0.01 g of olive oil weighed directly into vial
Vial	Standard 22-mL vial with aluminum crimped cap with PTFE lined silicon septum

Calibration solutions

1 mL of each BTEXS component was added to a 100-mL volumetric flask and diluted to volume with methanol. 1 mL of this stock solution was further diluted to 100 mL with methanol to produce the working solution used to fortify 'clean' olive oil for calibration purposes. The w/v concentration of each analyte in each of these two solutions is given in Table 5.

Table 5. **BTEXS concentrations in calibration solutions.**

Component	Stock Solution (µg/µL)	Working Solution (ng/µL)
Benzene	8.77	87.7
Toluene	8.70	87.0
Ethylbenzene	8.67	86.7
p-Xylene	8.80	88.0
m-Xylene	8.64	86.4
o-Xylene	8.80	88.0
Styrene	9.06	90.6
Methanol	Balance	Balance

Experimental

Method Optimization

Figure 1 shows a total ion chromatogram (TIC) obtained from an empty vial into which 2 µL of the working mixture of the BTEXS components in methanol was injected and fully evaporated. The conditions given in Tables 1 to 3 were applied.

Excellent peak shape is apparent and a full baseline separation of all components has been achieved. Meta-xylene and para-xylene are easily separated on this highly polar chromatographic column. A solvent delay of 4.6 minutes eliminates the appearance of the methanol solvent peak in this chromatogram.

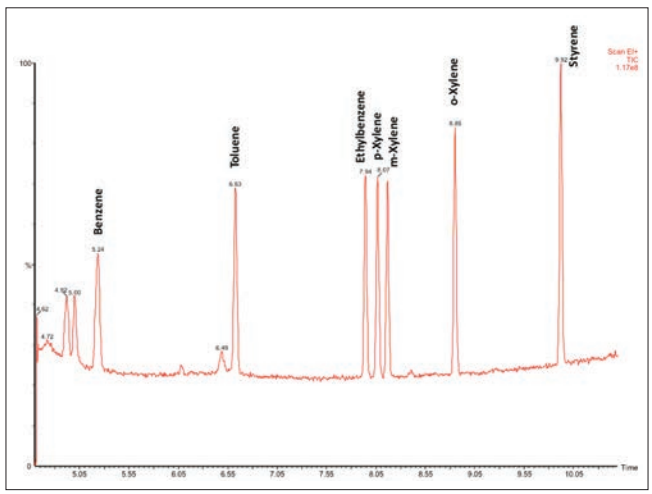


Figure 1. Chromatogram of 2 µL of working calibration solution added to an empty 22-mL HS vial.

Figure 2 shows a chromatogram (with the same scaling as Figure 1) run under the same analytical conditions of 2 µL working calibration mixture mixed into a 10 g sample of 'clean' olive oil. The analyte peaks are either close to the background noise level or are obscured by other components. The effective concentration of each analyte in the oil is approximately 17 ng/g (or ppb w/w). We need to see levels below 5 ng/g with this analysis and so it is clear that this will be a challenge with the method used to produce this chromatogram. The BTEXS compounds obviously have an affinity for the olive oil and so the partition coefficients are not favorable to the headspace phase – only a very small fraction of these will make it into the headspace.

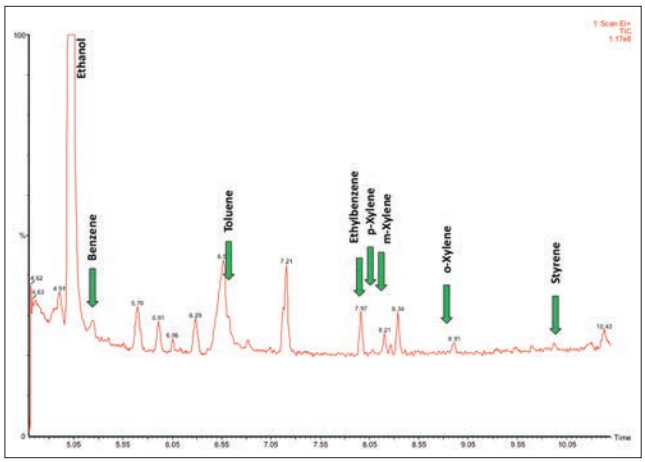


Figure 2. Chromatogram of 17 ng/g BTEXS in 10 g olive oil in a 22-mL HS vial with expected analyte retention times annotated.

By using the MS single-ion recording (SIR) mode of operation, the detector sensitivity and selectivity is significantly enhanced as shown in Figure 3. This chromatography was produced using the same chromatographic conditions as for Figure 2 but with the mixed single ion/full ion (SIFI) regime given in Figure 4 applied.

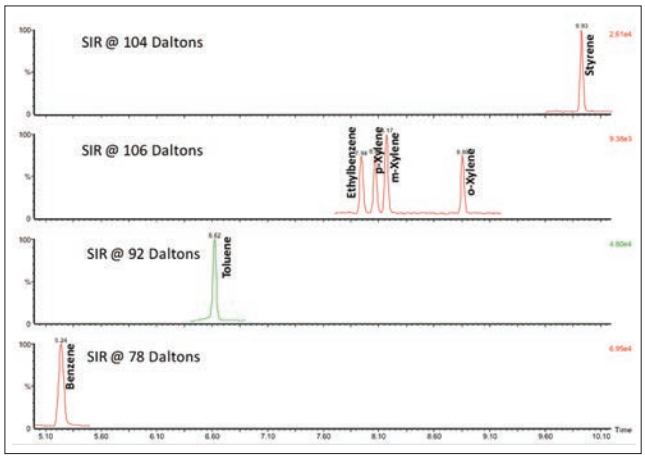


Figure 3. Chromatogram of 17 ng/g BTEXS in 10 g olive oil using SIFI settings given in Figure 4.

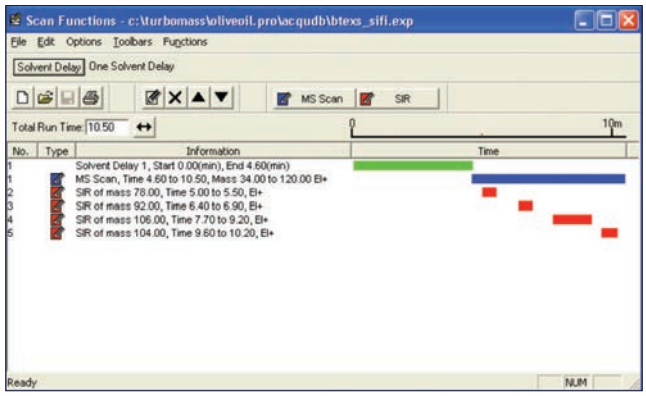


Figure 4. SIFI™ settings used to produce the chromatography shown in Figure 3.

Linearity

A series of calibration mixtures was prepared by adding volumes of the working solution to clean olive oil as listed in Table 6. Note – this is often referred to as "method of standard addition".

Table 6. **Calibration mixture preparation.**

Olive Oil (g)	Working Solution (µL)	Nominal Concentration (ng/g)
10.00	0	0
10.00	0.5	4.4
10.00	1.0	8.8
10.00	2.0	17.6
10.00	3.0	26.3
10.00	4.0	35.1
10.00	5.0	43.9
10.00	10.0	87.8

These mixtures were chromatographed using the conditions given in Tables 1 to 3. The analyte peak areas were obtained from the SIR traces. The clean olive oil was an off-the shelf product found to have low levels of BTEXS. The analyte peak areas found in this oil were subtracted from the calibration mixture responses, which were then used to prepare linear calibration profiles.

Figures 5 and 6 show calibration plots for the first and last eluting analytes, benzene and styrene, and Table 7 shows the least squares fit for each analyte. The linearity is excellent across this low concentration range especially for a complex sample matrix like olive oil.

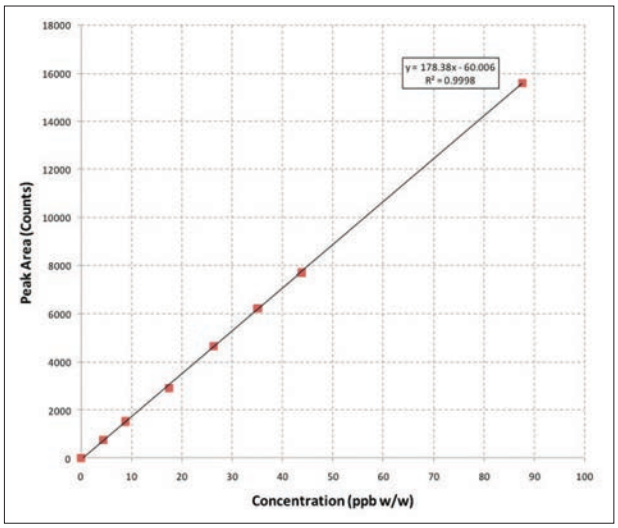


Figure 5. Calibration plot for benzene.

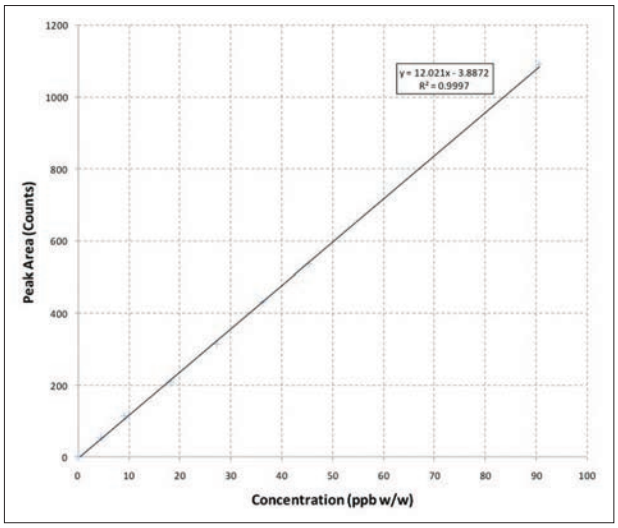


Figure 6. Calibration plot for styrene.

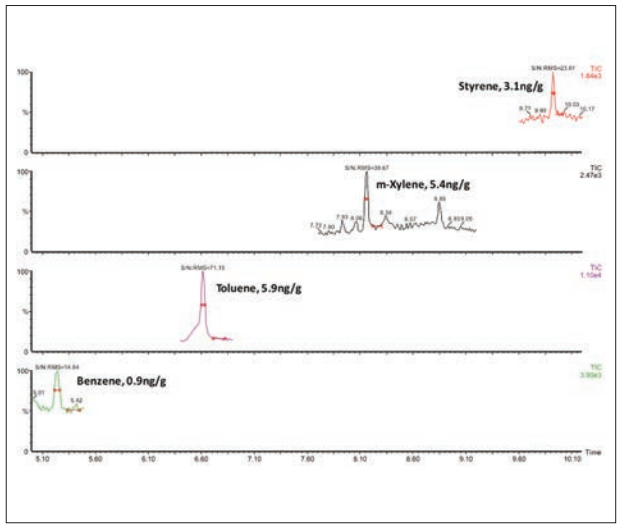


Figure 7. Chromatography of a sample containing low-levels of BTEXS with annotated signal to noise values.

Quantitative Precision

Ten samples of the clean olive oil were fortified with 5 µL of the working solution. Each was analyzed using the conditions given in Tables 1 to 3 and the amount of each analyte was determined using the calibration data from Table 7. The results are given in Table 8. An overall precision of 1.69 to 3.76% relative standard deviation is a very good result from this complex matrix.

Detection Limits

Figure 7 shows chromatography of a low-level sample. The calculated signal to noise ratios were used to predict the analytical detection limits shown in Table 9 based on a 2:1 ratio. These limits are over an order of magnitude below that of the 5ng/g requirement.

Table 7. **Least squares linear fit to calibration data.**

Statistic	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
Slope	178.38	51.465	10.07	11.568	10.708	8.4239	12.021
Intercept	-60.006	-1.6527	-5.6768	-6.7959	-1.1014	-6.7186	-3.8872
r^2	0.9998	0.9986	0.9995	0.9997	0.9998	0.9995	0.9997

Table 8. **Quantitative precision.**

Run #	Concentration in Spiked Sample (ng/g)						
	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
1	42.84	48.01	43.17	41.05	44.09	43.53	43.83
2	42.60	46.35	44.46	42.95	46.24	45.43	45.16
3	44.27	47.42	45.45	44.85	49.32	46.98	48.32
4	43.30	47.17	44.85	42.51	46.98	45.55	45.66
5	42.87	45.44	43.56	40.09	44.65	44.25	45.16
6	42.40	43.83	43.66	40.27	44.18	42.46	42.75
7	42.90	49.37	44.56	41.91	45.49	44.01	45.25
8	43.30	45.03	44.85	42.08	45.95	44.13	44.66
9	41.91	44.18	43.37	40.35	44.37	43.65	44.33
10	41.77	46.41	42.17	41.30	44.18	42.23	42.92
Mean	42.82	46.32	44.01	41.74	45.54	44.22	44.81
RSD%	1.69	3.76	2.25	3.51	3.66	3.26	3.53

Table 9. **Predicted limits of detection.**

Compound	Predicted Limit of Detection (ng/g)
Benzene	0.12
Toluene	0.16
Ethylbenzene	0.26*
p-Xylene	0.26*
m-Xylene	0.26
o-Xylene	0.26*
Styrene	0.26

** Peaks too small to quantify and so are based on value for m-Xylene.*

Sample Analysis

Seven different branded bottles of olive oil were purchased from a local supermarket and analyzed using this method. The results are given in Table 10. The determined concentrations are well within the range of this method.

Table 10. **Results from analysis of supermarket samples.**

Sample Source(s)	Concentration in Sample (ng/g)						
	Benzene	Toluene	Ethylbenzene	p-Xylene	m-Xylene	o-Xylene	Styrene
California	0.89	5.86	1.66	1.45	5.24	3.77	3.07
Italy, Greece, Spain, Tunisia	2.86	27.55	6.12	5.86	16.73	8.75	41.34
Italy, Spain, Greece, Tunisia	3.07	24.22	13.47	7.85	23.64	13.97	39.59
Italy, Spain, Tunisia, Turkey, Argentina	2.99	17.03	3.74	3.44	9.35	6.14	40.09
Spain, Argentina	2.43	34.99	7.22	7.42	18.97	10.65	126.11
Italy, Spain, Greece, Tunisia, Morocco, Syria, Turkey	4.09	35.71	19.13	17.10	59.31	28.10	61.05
Italy, Greece, Spain, Tunisia	1.25	2.79	ND	1.80	3.74	3.17	7.39

Conclusions

This method uses the new Clarus SQ 8 GC/MS to great effect. Sample preparation is extremely easy – 10 g of olive oil is weighed into a standard headspace vial and then sealed with a crimped cap. The analysis is fully automated and takes just 10.5 minutes for the chromatography and an additional 3.5 minutes for cool-down and equilibration between analyses.

Sub-ppb levels are possible using standard headspace sampling of light aromatics in a complex natural oil matrix without the need for vapor pre-concentration (for example with an HS Trap). Excellent quantitative performance has been demonstrated and the system is easily able to see low concentrations of these compounds in olive oil bought from a local supermarket.

PerkinElmer Accessories and Consumables for this application:

Item Description	Part No.
Elite Wax	N9316485
Injector Port Septa 6pk	N6101748
Ferrules	09920104
H/S Vials/Caps/Septa	N9303992
Marathon Filament	N6470012
Ergo Crimper	N6621037

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009750A_01

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

A. Tipler, Senior Scientist

PerkinElmer, Inc.
Shelton, CT 06484 USA

The Qualitative Characterization of Fruit Juice Flavor using a TurboMatrix HS Trap and a Clarus SQ 8 GC/MS

Introduction

The PerkinElmer® TurboMatrix™ Headspace Trap system coupled with a Clarus® SQ 8 GC/MS is a very convenient means of identifying low concentration volatile organic compounds (VOCs) in foodstuffs. In this application note, the VOCs in various fruit juices were investigated. Sample preparation simply involved dispensing a fixed volume of fruit juice into a sample vial and sealing it. The analysis was fully automated.

PerkinElmer®
For the Better

Method

The experimental conditions for this analysis are given in Tables 1 to 4.

Table 1. **GC Conditions.**	
Gas Chromatograph/ Mass Spectrometer	Clarus SQ 8
Column	60 m x 0.25 mm x 1.0 µm Elite-5MS
Oven	35 °C for 5 min, then 6 °C/min to 245°
Injector	Programmable Split Splitless (PSS), 180 °C, Split OFF
Carrier Gas	Helium at 2.0 mL/min (28.6 psig initial pressure), HS Mode ON

Table 2. **HS Trap Conditions.**	
Headspace System	TurboMatrix 110 HS Trap
Vial Equilibration	80 °C for 20 minutes
Needle	120 °C
Transfer Line	140 °C, long, 0.25 mm i.d. fused silica
Carrier Gas	Helium at 31 psig
Dry Purge	7 min
Trap	Air Toxics, 25 °C to 260 °C, hold for 7 min
Extraction Cycles	1 with 40 psig extraction pressure

Table 3. **MS Conditions.**	
Scan Range	35 to 350 Daltons
Scan Time	0.1 s
Interscan Delay	0.06 s
Source Temp	180 °C
Inlet Line Temp	200 °C
Multiplier	1700V

Table 4. **Sample Details.**
Sample 1 mL of each of the following fruit juices:
• Orange juice
• Grapefruit juice
• Apple juice
• Lemon juice
• Lime juice
• Cranberry juice
Vial Standard 22-mL vial with aluminum crimped cap with PTFE lined silicon septum

Results

The total ion chromatograms obtained from the six fruit juice samples are given in Figures 1 to 6. The component identities of the key peaks were established by performing mass spectral library searches. The results of these identifications are annotated in the following figures:

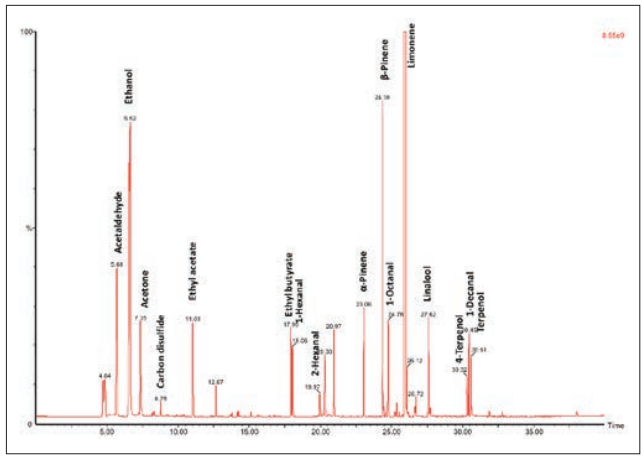


Figure 1. Full Total Ion Chromatogram obtained from orange juice.

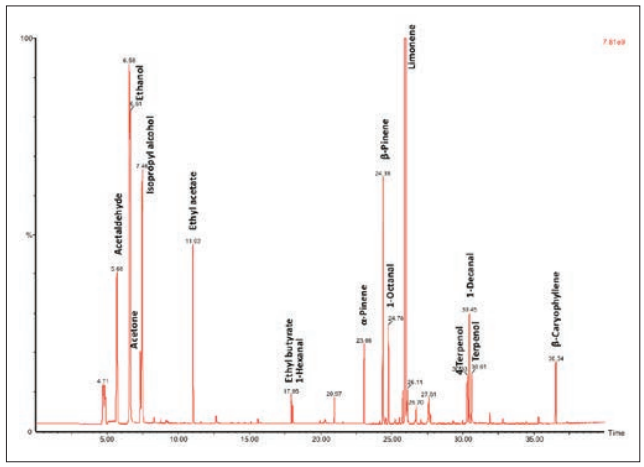


Figure 2. Full Total Ion Chromatogram obtained from grapefruit juice.

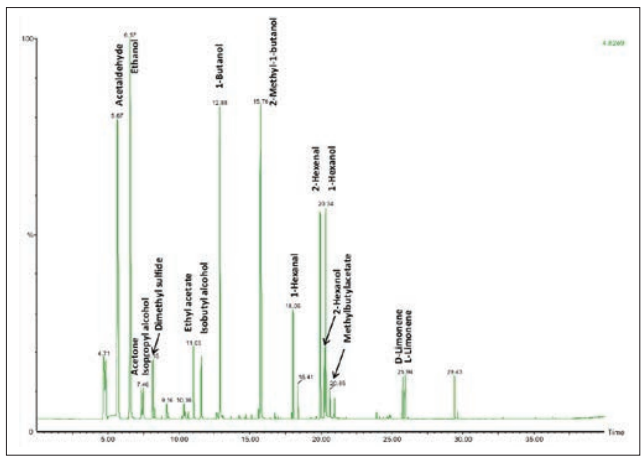


Figure 3. Full Total Ion Chromatogram obtained from apple juice.

Conclusions

This system provides a very simple and convenient way of characterizing the odor and flavor of natural products such as fruit juices. The use of GC/MS enables a very detailed aromatic profile of these fruit juices to be established. The use of a HS Trap system to perform the sample extraction enables low-level components to be visualized without compromising the system with injection of heavier, less volatile, unwanted sample material such as sugars and proteins.

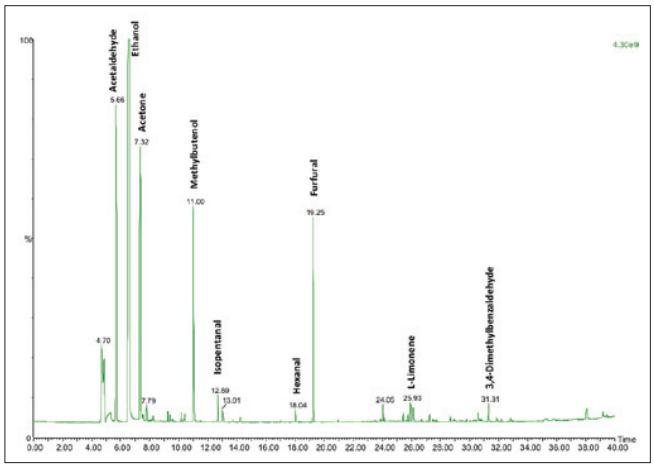


Figure 4. Full Total Ion Chromatogram obtained from lemon juice.

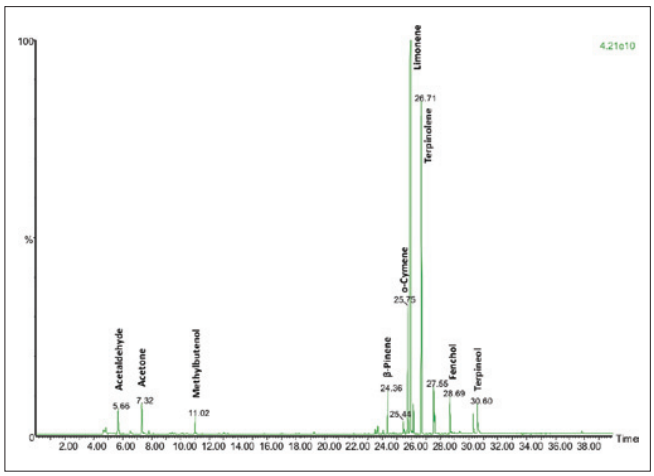


Figure 5. Full Total Ion Chromatogram obtained from lime juice.

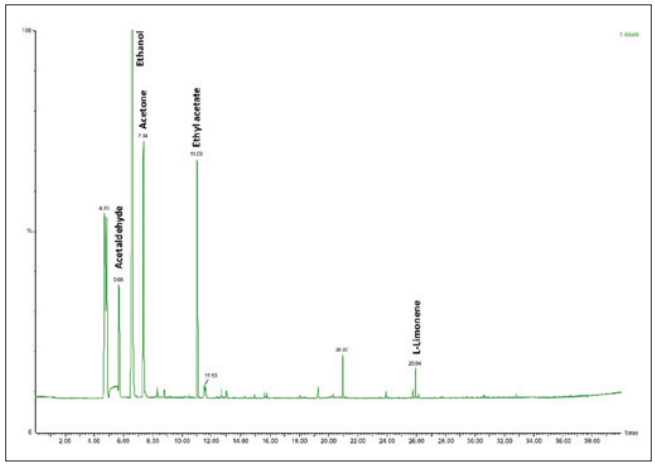


Figure 6. Full Total Ion Chromatogram obtained from raw cranberry juice.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009755A_01

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Authors

Lee Marotta
Sr. Field Application Scientist

Andrew Tipler
Senior Scientist

PerkinElmer, Inc.
Shelton, CT 06484 USA

Monitoring Volatile Organic Compounds in Beer Production Using the Clarus SQ 8 GC/MS and TurboMatrix Headspace Trap Systems

Introduction

Beer is a popular beverage produced by the fermentation of hopped malt extracted from barley and other grains. Although simple in concept, beer is a highly complex mixture of many compounds including sugars, proteins, alcohols, esters, acids, ketones, acids and terpenes. Flavor is an important quality of any beer and the chemical content of the beer is obviously responsible for that flavor. Aroma is an extremely important part of the flavor and so there is a strong interest by brewers in the volatile organic compounds (VOCs) in beer that affect its aroma.

Some VOCs have a positive effect on aroma (attributes) and some have a negative effect (defects). The ability to characterize these in beer products before, during and after fermentation would be an important tool in process control, quality assurance and product development.

This application note describes a system comprising a headspace trap sampler to extract and concentrate VOCs from a beer sample and deliver them to a gas chromatograph/ mass spectrometer (GC/MS) for separation, identification and quantification.

The purpose of our experiments is to demonstrate that attributes and defects can all be monitored using one detector and from a single injection with mass spectrometry (MS). The associated benefits include a quicker return on investment, enhanced productivity, more information from a single analysis, and less bench space requirements.

PerkinElmer®
For the Better

Instrumentation

In this analysis, we utilized a headspace trap system for sample introduction to characterize the flavor of beer. This technique ensures that non-volatile material in beer does not enter the analytical system, which can cause system contamination. The headspace trap extracts the volatile components from a large sample and focuses them onto an inline adsorbent trap. It also facilitates very easy sample preparation – a volume of beer is dispensed into a vial and sealed. The subsequent analysis is then fully automated.

A PerkinElmer® TurboMatrix™ Headspace Trap connected to a PerkinElmer Clarus® SQ 8 GC/MS was used for these experiments. Using a headspace trap instead of the classical headspace technique enables up to 100 times improved detection limits over classical headspace methods.

A slightly-polar 60 m x 0.25 mm x 1.0 µm Elite 5 (5% phenyl-silicone) column was used. This thick-film column provided sufficient retention to separate the early-eluting most volatile components and provided the dynamic range necessary to chromatograph both high level and low level components in the beer.

Experimental

Overview

Several experiments were performed that are key to the brewing industry:

- Quantitation of dimethyl sulfide (DMS), 2,3-butanedione (diacetyl), 2,3-pentandione and t-2-nonenal.
- Characterization of several types of beers
- Fermentation profiling
- Analysis of raw materials
- Aging studies

Analytical Method

The experimental conditions for this analysis are given in Tables 1 to 4.

Table 1. **HS Trap Conditions.**

Headspace System	TurboMatrix (40 or 110) HS Trap
Vial Equilibration	80 °C for 20 min
Needle	120 °C
Transfer Line	140 °C, long, 0.25 mm i.d. fused silica
Carrier Gas	Helium at 31 psig
Dry Purge	7 min
Trap	Air Toxics, 25 °C to 260 °C, hold for 7 min
Extraction Cycles	1 with 40 psig extraction pressure

Table 2. **GC Conditions.**

Gas Chromatograph/ Mass Spectrometer	Clarus SQ 8
Column	60 m x 0.25 mm x 1.0 µm Elite-5MS
Oven	35 °C for 5 min, then 6 °C/min to 245°
Injector	Programmable Split Splitless (PSS), 180 °C, Split OFF
Carrier Gas	Helium at 2.0 mL/min (28.6 psig initial pressure), HS Mode ON

Table 3. **MS Conditions.**

Scan Range	35 to 350 Daltons
Scan Time	0.1 s
Interscan Delay	0.06 s
Source Temp	180 °C
Inlet Line temp	200 °C
Multiplier	1700V

Table 4. **Sample Details.**

Sample preparation	5 mL of each sample was pipetted into a sample vial and sealed
Vial	Standard 22-mL vial with aluminum crimped cap with PTFE lined silicone septum

Calibration

A 10-point calibration was prepared for four target 'defect' compounds. The detection limit goal was 5.0 parts per billion (ng/mL). The standards were acquired in simultaneous Full Scan and Single Ion Monitoring acquisitions (SIFI). Examples of the chromatographic peaks and their signal to noise ratios at the 5.0 ppb level are given in Figures 1 to 4.

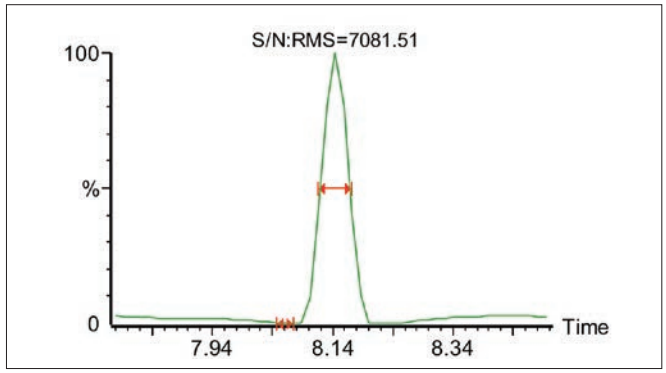


Figure 1. SIM chromatogram of dimethyl sulfide peak at 5.0 ppb.

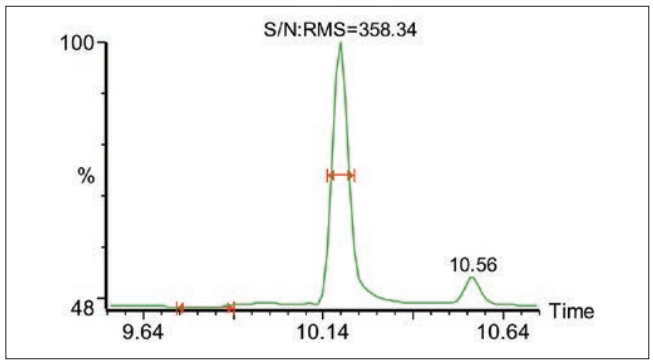


Figure 2. SIM chromatogram of 2,3-butanedione peak at 5.0 ppb.

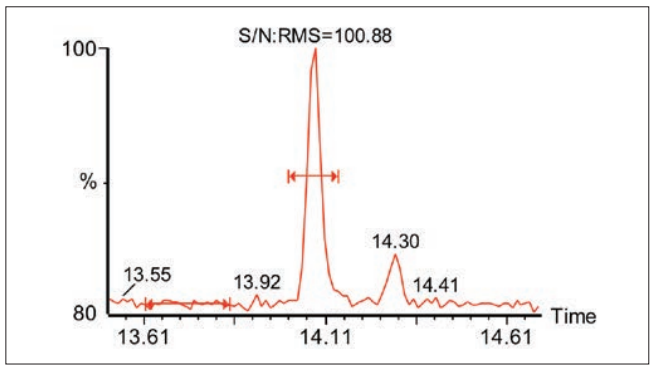


Figure 3. SIM chromatogram of 2,3-pentanedione peak at 5.0 ppb.

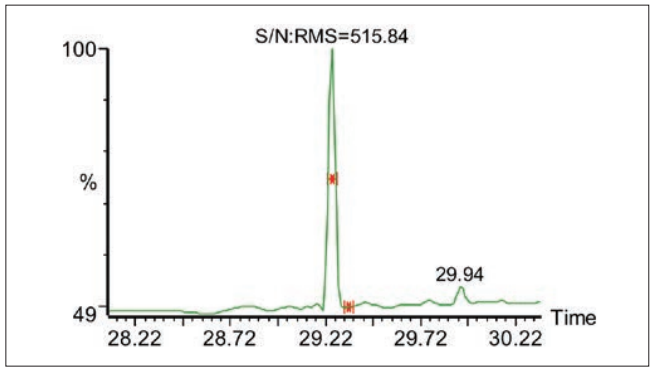


Figure 4. SIM chromatogram of t-2-nonenal peak at 5.0 ppb.

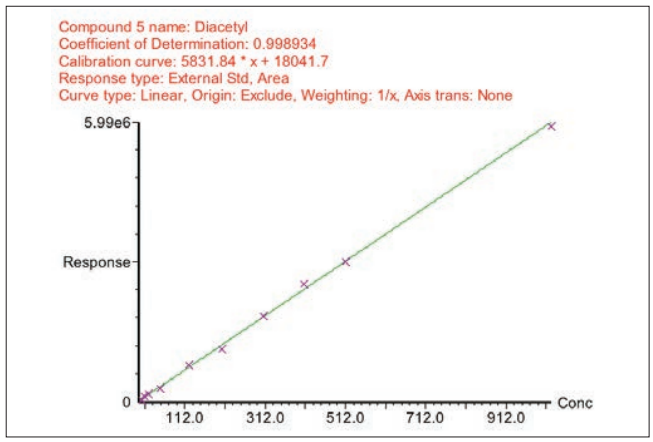


Figure 5. Calibration profile for 2,3-butanedione (diacetyl).

The calibration results are presented in Table 5. An example of one of the calibration plots is given in Figure 5. These data demonstrate a good linear response for these components in at low levels in a highly complex matrix.

Characterization of Beer

The MS detector enables the identification of components in beer. Figure 6 is an example of such characterization that was analyzed in our research center in Shelton, CT. Figure 7 is a comparison of the component identities and responses found in two competitive products.

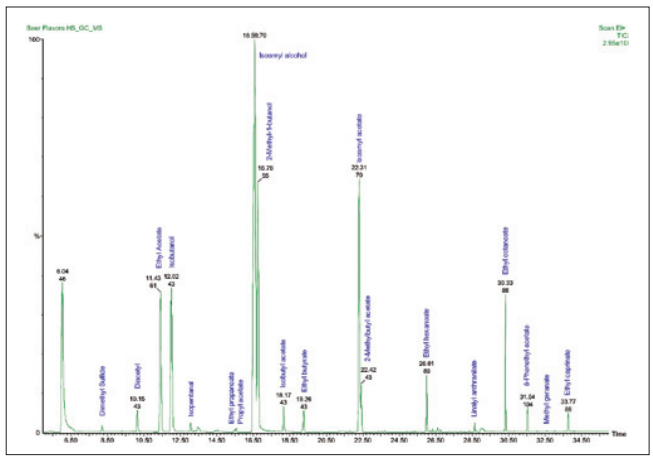


Figure 6. Typical chromatographic profile of volatile flavor compounds in an American pale ale.

***Table 5.* Calibration Data.**

Component Name	Signal to Noise Ratio Ratio at 5 ng/mL	r^2 over range 5 to 1000 ng/mL	Signal to Noise Ratio Ratio at 5 ng/mL	r^2 over range 5 to 1000 ng/mL
Dimethyl Sulfide	821 to 1	0.9934	7081 to 1	0.9945*
2,3-Butanedione	12 to 1	0.9989	358 to 1	0.9943
2,3-Pentanedione	20 to 1	0.9975	470 to 1	0.9983
t-2-Nonenal	19 to 1	0.9958	516 to 1	0.9960

*Reduced range due to overloading.

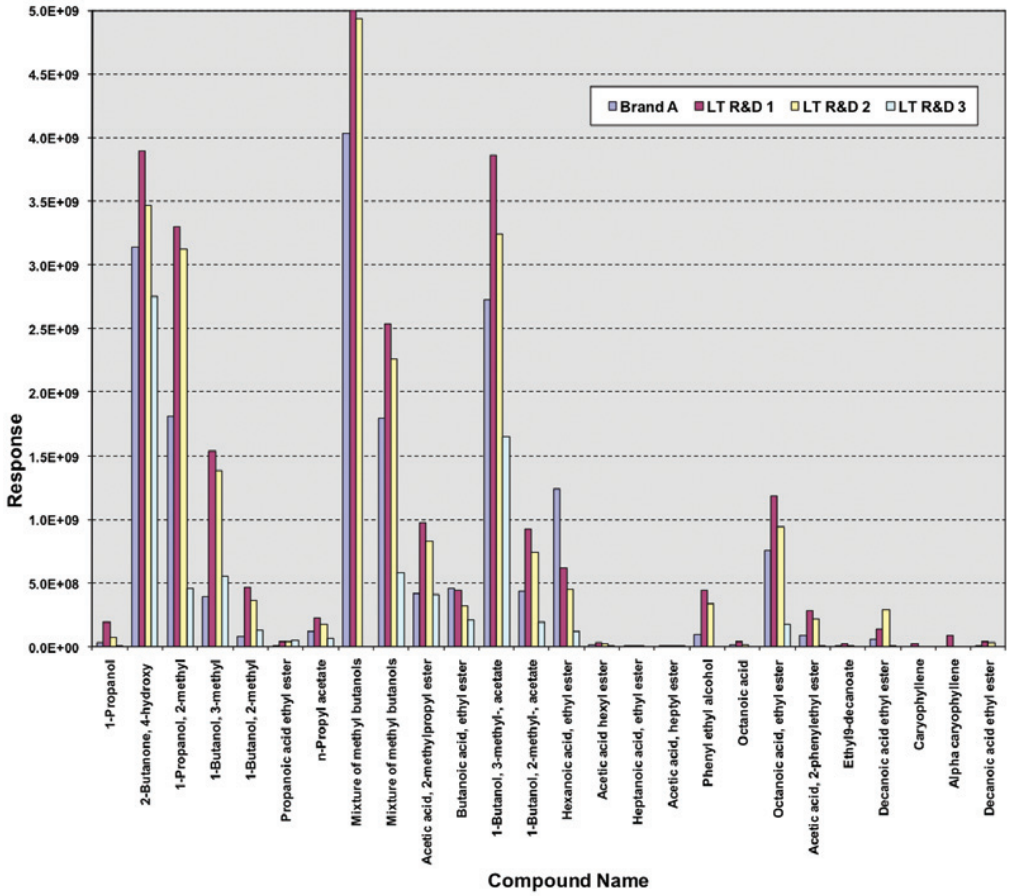


Figure 7. Comparison between two brands of beer (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

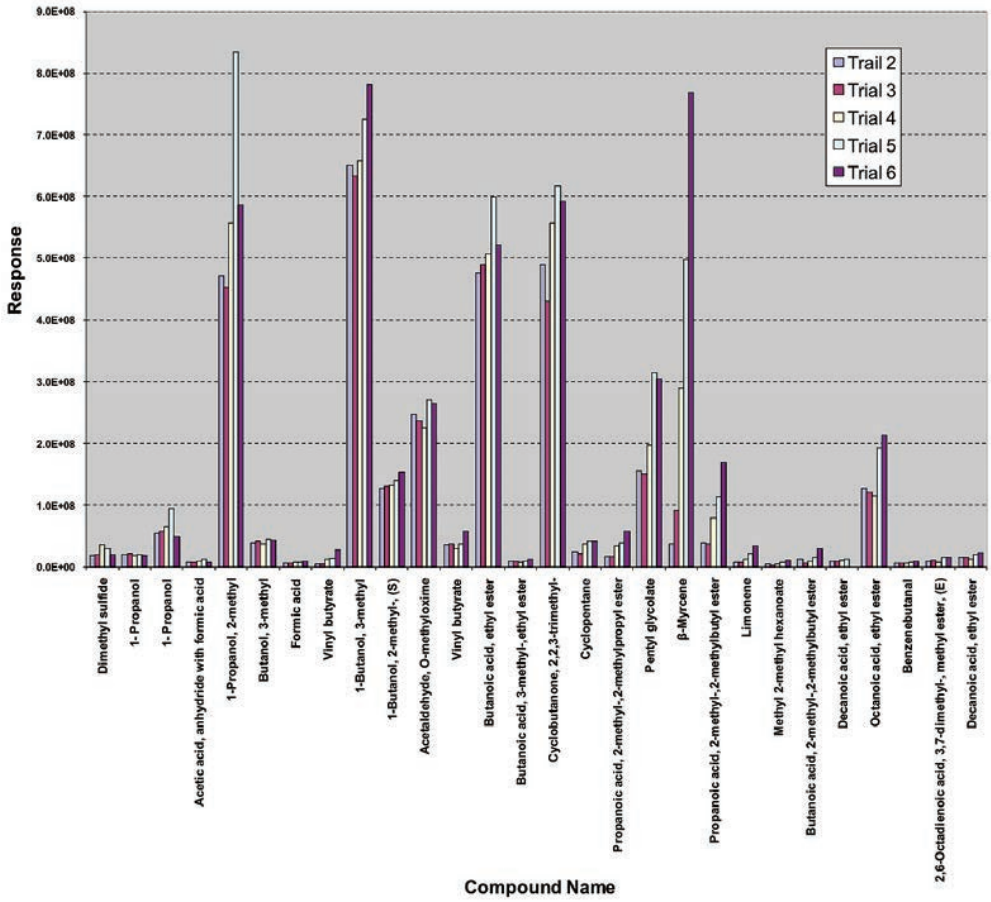


Figure 8. Comparison between different fermentations of the same beer type (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

Figure 8 shows the results of a research study comparing the flavor profiles of a beer from five different fermentations.

Fermentation Process

This analyzer provides the ability to obtain analytical results during the fermentation process.

An experimental batch of American pale ale was brewed and fermentation initiated. A sample was analyzed every eight hours starting with time zero and completing on day eight.

Specific gravity is often used as an indicator of the fermentation progress and is shown for this beer in Figure 9. The final gravity of 1.012 was achieved in about 100 hours.

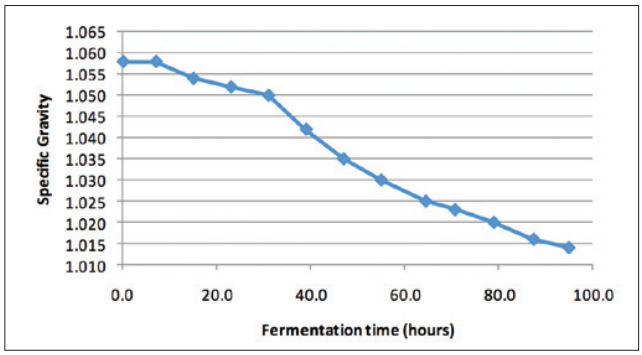


Figure 9. Specific gravity profile for the experimental beer during the fermentation process.

The concentrations of key components in the beer were checked during the fermentation process. The profiles of two key 'defects', 2,3-butanedione and dimethyl sulfide are shown in Figures 10 and 11 respectively. Trans-2-nonenal was not detected.

Analysis of Raw Materials

Figure 12 displays the results of a study comparing the components of different hops in order to understand and to improve the taste of beer.

Some beers use adjuncts to impact special flavors. The same system may be used to characterize these. Figure 13 displays the results of a comparison between orange peel from different suppliers for use in Belgian-style beers.

Aging Studies

Beer is a very complex matrix that ages over time due to chemical and biological activity so storage conditions are critical to its quality.

Exposure to air promotes the formation of aldehydes and other undesirable compounds that can impair the flavor of a good beer. The Clarus system is capable of monitoring such compounds. A compound of major concern is t-2-nonenal ('wet cardboard' flavor), which we monitored during the fermentation studies, yet was undetected.

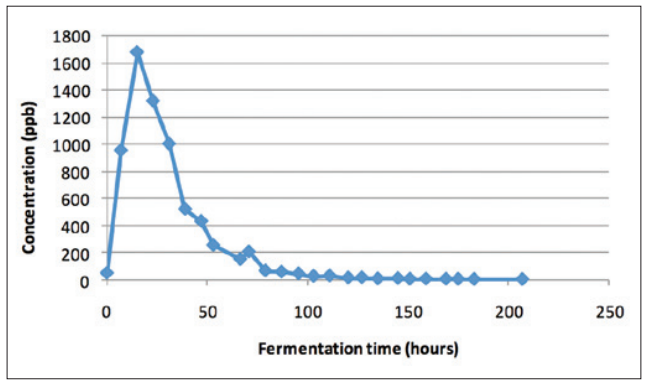


Figure 10. Concentration profile of 2,3-butanedione for the experimental beer during the fermentation process.

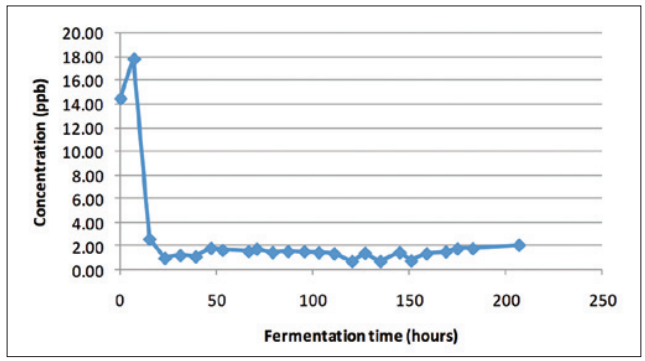


Figure 11. Concentration profile of dimethyl sulfide for the experimental beer during the fermentation process.

Another flavor concern is that bittering components (isohumolones) react to light and produce mercaptans and other volatile sulfur compounds giving a 'skunky' flavor to the beer. Figure 14 shows chromatograms of the same beer kept in the dark and also in bright sunlight. Major differences in the composition of the beer VOCs are apparent. Figure 15 identifies one of the sun-stuck components as an olefinic thiophene

Conclusion

The combination of the TurboMatrix HS Trap extraction technology with the state of the art Clarus SQ 8 GC/MS is a very powerful, yet easy to use tool for investigating many aspects of the beer production process. Virtually anything that is volatile and organic can be monitored in beer using a single column and applied conditions. The system may be deployed for checking raw materials, monitoring fermentation, quality control testing of a final product, product development, aging studies and trouble shooting.

Traditionally, this work would have been performed by skilled tasters, which of course continues to be an important part of any brewing process. The opportunity to compliment taste and olfactory determinations with hard objective analytical data can only enhance the art of making quality beer.

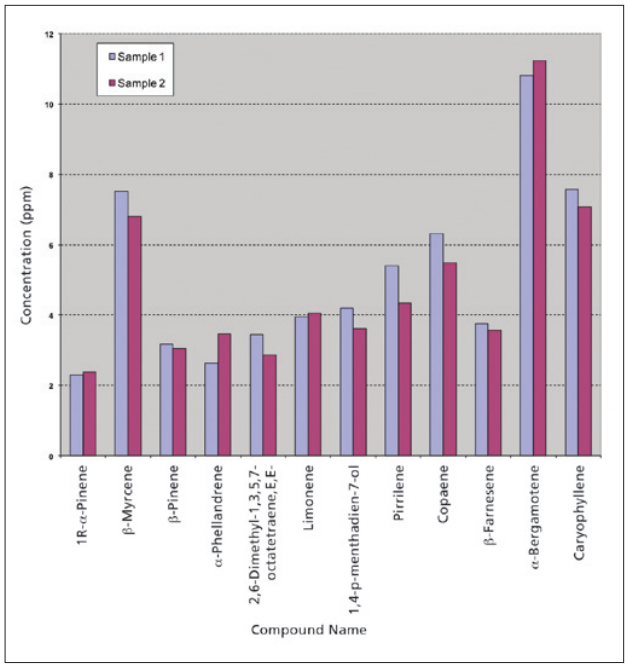


Figure 12. Hop VOC profiles (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

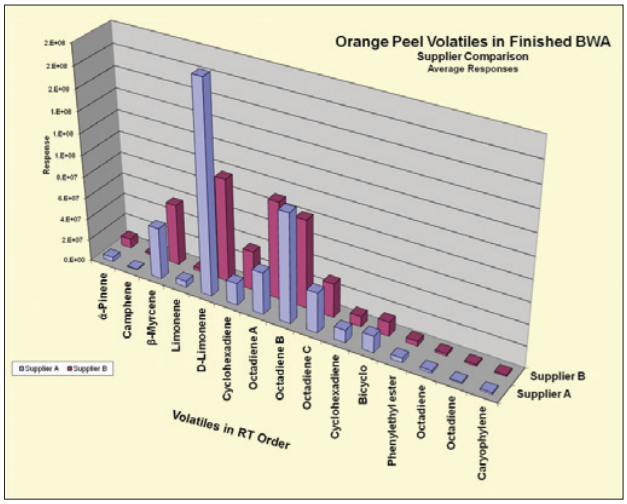


Figure 13. Flavor profiles in orange peel (data courtesy of the Long Trail Brewing Company, Bridgewater Corners, Vermont).

In this note, we have conducted many of the critical analyses relevant to beer brewing. We have shown good performance in determining levels of defects such as diacetyl and dimethyl sulfide. We have identified flavor components in the beer, hops and adjuncts.

All this is possible on a system that simply requires the beer to be sealed in a vial and placed on an autosampler tray. The system does the rest.

Acknowledgement

We thank Bill Yawney, the QC Manager, from Long Trail Brewing Company for allowing us to use some of his data in this application note. In addition, Bill provided us with valuable expert advice on the brewing process and the analysis of beer.

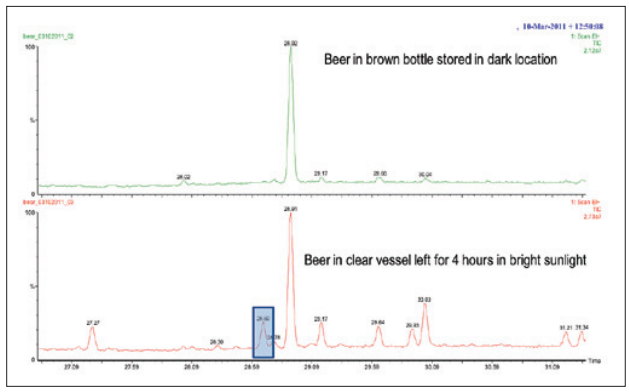


Figure 14. Effect of sunlight on beer volatiles.

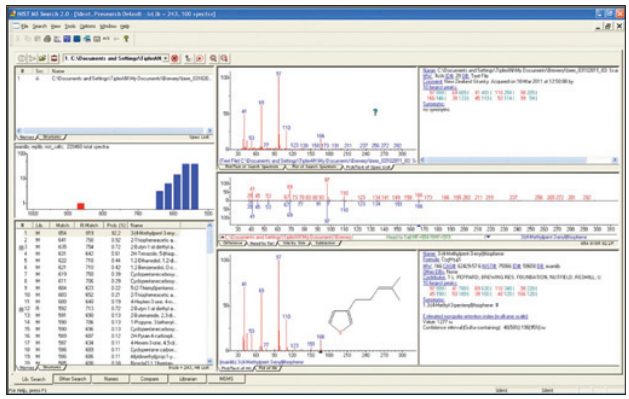


Figure 15. Library search on spectrum obtained from the peak highlighted in Figure 14.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009776_01

APPLICATION NOTE

Gas Chromatography

Author:

Mariacarola Salvi

PerkinElmer, Inc.
Monza, Italy

Determination of Residual Solvents in Flexible Packaging According to EN 13628-2:2004

Introduction

The reference standards for food contact materials are rapidly evolving in favor of increasing consumer protection.

The Commission Regulation (EC) No. 1935/2004 is the main reference legislation in the European community. This regulation establishes that any materials that come into contact with food must not release chemicals in quantities which could:

- Pose a danger to the health of consumers
- Result in an unacceptable change in the composition of food
- Change the organoleptic properties

Part 2 of the regulation focuses the attention of food contact material producers on the need to operate in terms of quality assurance. The Commission Regulation (EC No. 2023/2006) has made it mandatory to adopt a system of Good Manufacturing Practice (GMP); with GMP referring to the set of actions to ensure a consistently high quality both in production and control process. This requires not only a deep knowledge of the materials used but also of the entire production and control process.

PerkinElmer®
For the Better

Flexible Packaging

In case of printed flexible packaging, Commission Regulation (EC) No. 2023/2006 Annex I prohibits the printed side of the materials to come into contact with food. Verification by GMP is also required in order to prevent any "Set-off" (process transfer of substances, from the printed side of a film to the non-printed side, due to the fact that these materials are normally produced in coils) that could ultimately transfer these chemicals onto foods.

The solvents in the inks used to print flexible packaging may represent a possible source of food contamination and therefore must be controlled.

For the determination of residual solvents from printed materials, it is highly recommended that an analytical method such as the official UNI EN 13628-2:2004[1] is followed. If the application of a non-official method is adopted, it requires validation by the laboratory; a task that is often long, complex and expensive.

Experimental Instrumentation

The analysis was performed using a PerkinElmer Clarus® 580 gas chromatograph equipped with a capillary column injector and an FID detector coupled to an automatic TurboMatrix™ 40 Headspace sampler. The capillary column used was a PerkinElmer Velocity-1 (30 m, 0.32 mm, 3 um – P/N N9306329).

Figure 1. Clarus 580 GC and TurboMatrix 40 Headspace sampler.

Analytical Conditions

The instrument conditions are given below:

Table 1. Instrument Conditions.

HS Conditions:	
Thermostatting Temperature	110 °C
Needle Temperature	130 °C
Transfer line Temperature	150 °C
Thermostatting Time	20 min
Pressurization Time	3 min
Injection Time	0.06 min
Pressure	21 psi
Mode	Constant
GC Conditions:	
Carrier Gas	He 1.7 ml/min
Split Ratio	1:20
Injector Temperature	230 °C
Detector Temperature FID	280 °C
Ramp	50 °C for 5 min, ramp to 100 °C @ 5 °C/min, ramp to 250 °C @ 10 °C/min

Standard Preparation

Standards are prepared together as a stock mixture. Using the Total Vaporization Technique[2], different levels of the calibration curves were obtained analyzing increasing amounts of the standard mixture added to the vial prior to analysis.

Table 2. Calibration Amounts.

Solvent	Level 1 mg	Level 2 mg	Level 3 mg	Level 4 mg
Ethanol	0.0065	0.0130	0.0260	0.0390
Isopropanol	0.0064	0.0128	0.0256	0.0384
MEK	0.0066	0.0132	0.2640	0.0396
Ethyl Acetate	0.0074	0.0148	0.0296	0.0444
Isobutanol	0.0065	0.0130	0.0260	0.0390
Methoxy Propanol	0.0075	0.0150	0.0300	0.0450
Ethoxy Propanol	0.0073	0.0146	0.0292	0.0438
Toluene	0.0058	0.0116	0.0232	0.0348
Butyl Acetate	0.0073	0.0146	0.0292	0.0438
m-Xylene	0.0071	0.0142	0.0284	0.0426
o-Xylene	0.0073	0.0146	0.0292	0.0438

Analytical Results

The software runs the standards/sample, calibrates the instrument and automatically produces the report. In the real world, samples can widely vary in concentration, therefore it is paramount that a high level sample does not carryover and contaminate the following samples and give false high results. The inert flow path and post sampling needle purge ensures the lowest possible carryover, producing quality results day after day. Another important area to consider is that the instrument's natural background levels are as low as possible, thus enabling ultra-low level detection when needed for those difficult analyses.

The chromatogram in Fig. 2 was obtained from the standard mixture Level 3 (blue) as compared with a blank (red) that was obtained by the analysis of an empty vial. The blank is clean and void of extraneous peaks, thus simplifying the reporting of data.

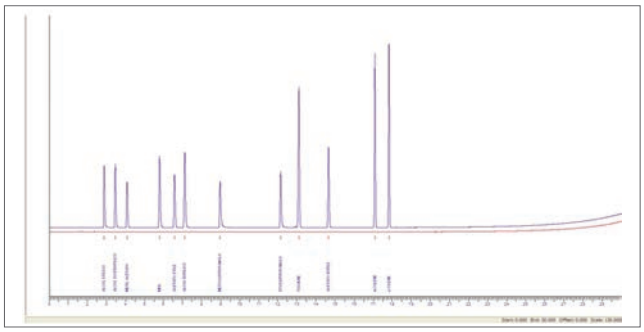


Figure 2. Chromatogram of Level 3 Standard.

Figures 3 and 4 represent the calibration curves for two example analytes: methyl acetate and toluene, both showing excellent linearity of the four calibration levels, thus enabling easy operation for the end user and improved accuracy of the results.

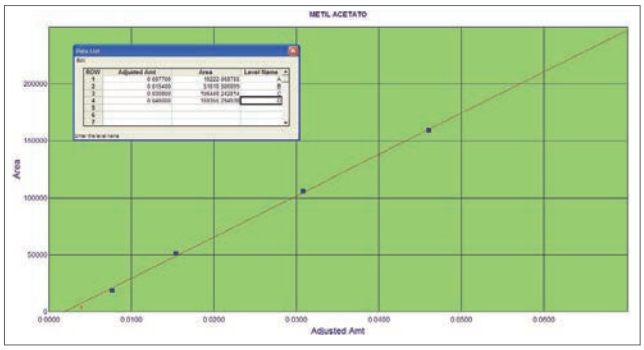


Figure 3. Methyl Acetate Calibration Curve.

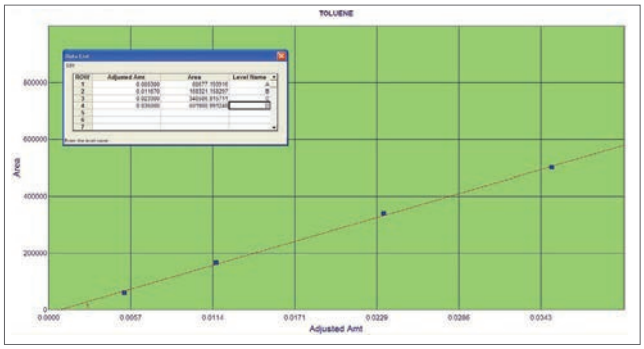


Figure 4. Toluene Calibration Curve.

Example of a Real Sample

A known Area (1 dm2) of the unknown sample is introduced into the vial and analyzed using the same analytical conditions as the standards above. The quantitative result obtained is then reported as the overall amount of solvents per m2 of material.

Figure 5 below shows the analysis of a real sample. For this sample the total content of solvent is found to be equal to 7.20 mg / m2.

Figure 5 also shows there is the presence of several unknown peaks, the one in the center is labeled "incognito." This is investigated further in the next section.

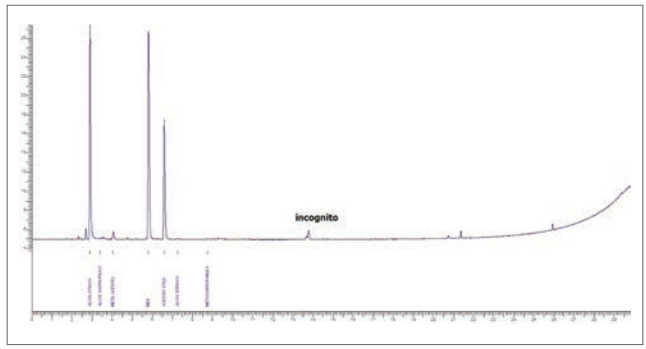


Figure 5. Chromatogram of Real Sample Showing Unknown Peak.

GC/MS

Although the standard UNI EN 13628-2:2004 requires the use of an FID detector, at times it may be necessary to identify an unknown solvent in a real sample, i.e. a solvent not included in the standard mixture. A mass spectrometer (MS) is a powerful detector for the determination of unknowns. We will use the same chromatographic system, vide supra, but coupled to a Clarus 560S MS. Figure 6 shows our target compound labeled as "incognito" at approximately five minutes into the chromatogram.

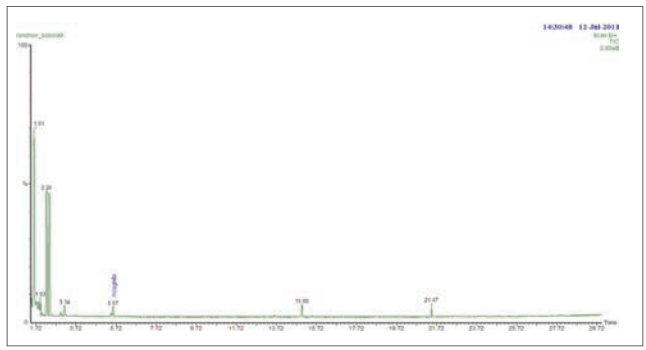


Figure 6. GC/MS Chromatogram of Sample Shown in Figure 5.

A mass spectrum of the unknown peak can easily be obtained by clicking on the peak. To assist in the identification of this unknown, the resulting mass spectrum was searched against a NIST mass spectra library that contains over 200,000 compounds. The NIST library software has selected the following solvent, 3-methyl heptane, as a possiblibility in Figure 7.

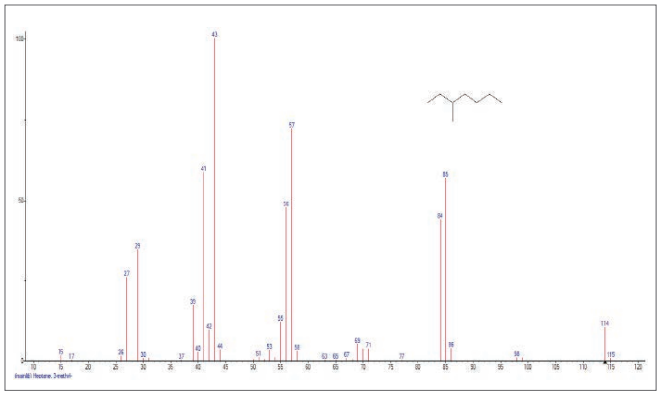


Figure 7. NIST Library Search Match of Peak Labeled "Incognito."

In order to verify and quantify this new solvent, it will be sufficient to have a small quantity of it added to the calibration mixture. Alternatively, in order to have a semi-quantitative result, you can compare the response factor to one of the other solvents inside the standard mixture.

Conclusion

The Clarus 580 GC and TurboMatrix HS system can easily and accurately quantify the amount of residual solvents according to the official method EN13628-2:2004.

References:

1. Uni En 13628-2:2004 Packaging - Flexible Packaging Material - Determination Of Residual Solvents By Static Headspace Gas Chromatography - Part 2: Industrial Methods.
2. Static Headspace-Gas Chromatography Theory and Practice by B. Kolb, L. Ettre, 1997 p. 142 Wiley-VCH.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009812_03 PKI

APPLICATION NOTE

Gas Chromatography

Authors:

Nathan Kuffel

Timothy Ruppel

PerkinElmer, Inc.
Shelton, CT

A Method for the Quantification of Ethanol Content in Consumable Fruit Juices by Headspace Injection

Introduction

The definition of an alcoholic beverage in the United States of America is a beverage that contains in excess of 0.5% ethanol by volume that is intended for consumption alone or when diluted. Production of alcohol has been long established in society with many styles that take advantage of the metabolism of sugars into ethanol. While the production of ethanol is desirable for alcoholic beverages, it is undesirable for other beverages which contain sugars that do not wish to be sold as an alcoholic beverage. Such sugar metabolism is naturally occurring and is well understood to happen in raw fruit as well as processed juice and can vary by type, variety and maturation in the growing season.

PerkinElmer®
For the Better

A new application has been developed in the accurate determination of ethanol content in samples of these products utilizing the PerkinElmer® TurboMatrix™ headspace (HS) autosampler for better reproducible results. Additionally, since ethanol is the only desired peak, this method allows for a quick run time giving users the opportunity to analyze high volume throughput samples multiple times. The main focus of this method is intended toward fruit juices and it is confirmed to give accurate results amongst a wide range of store bought juices. This application note outlines the principles and technology of this method in the analysis and quantification of ethanol in consumable juices.

Experimental

System

Gas Chromatograph	PerkinElmer Clarus® 580
Injector	Programmable Split Splitless (PSS)
Detector	FID
Electronic Pneumatics	PPC Carrier for PSS (Hydrogen), PPC FID Gases (Air & Hydrogen)
Column	30 m x 0.32 mmID x 1.8 µm Elite BAC-1 Advantage #N9315071
Headspace Apparatus	TurboMatrix
Data Analysis	Data processed on Waters® Empower® 3 software

Headspace Conditions

Temperature	Oven 60 °C, Needle 110 °C, Transfer Line 120 °C
Timing	Thermostat - 12 min, Pressurize – 1 min, Injection – 0.02 min, Withdraw – 0.3 min
Pressure	16 psig of Hydrogen Gas
Transfer Line Column	Split connection 2 m of 0.32 mmID fused silica, terminated in injector
Options	Operative Mode: Constant Inject Mode: Time

GC Conditions

GC Oven	45 °C Isothermal, Run Time: 2.50 min Equilibration Time: 0 min
Carrier Pressure	12 psig for 2.50 minutes, Split Flow 5.0 mL/min
FID	Range: x1 Attn: x-1 Temp: 250 °C Air: 450 mL/min H_2: 45 mL/min

Reagents

Off the shelf juices and deionized water are used for sample preparation. The internal standard solution used is t-butanol in deionized water.

Calibration

Ethanol standards with known amounts over the quantification range of 50 to 500 mg/dl ethanol v/v with an ISTD at a constant concentration. Vail and capped securely with headspace vial crimper.

Sample Preparation

A 50 µL volume of t-butanol is diluted in 250 mL of deionized water attached to the automatic dilutor. Precisely 75 µL of a juice sample and 750 µL deionized water/internal standard mixture are combined with an automatic dilutor into a standard autosampler vial. The vial is then securely sealed with a headspace vial crimper.

Table 1. Retention times of BAC compounds.

Compound	Retention Time (min)
Methanol	0.663
Acetaldehyde	0.697
Ethanol	0.806
Isopropanol	0.957
Acetone	1.033
t-Butanol	1.112
n-Propanol	1.268
Ethyl Acetate	1.924

Note: These compounds will not all be present in all fruit juices but are used to show the proper separation from ethanol in the case that an addition peak is present. It was also used to determine a reliable internal standard that would not co-elute with the ethanol. In this method t-butanol was used as the internal standard.

Results

It is necessary to have a good calibration curve and an internal standard for reference. Since ethanol has a very distinct and repeatable retention time, it allows for reliable integration of the area of the peak. The internal standard used is t-butanol, which elutes well after ethanol. The isothermal GC method allows for a minimum time between injections of 3.0 minutes, also referred to as PII (period from injection to injection). As expected the calibration produces an excellent quantitative linearity (0.997) and a high precision (1 % RSD) was seen at 500 ppm of ethanol.

Several commercial juices were analyzed for ethanol content with the results in Table 2.

Table 2. Ethanol content of selected store purchased juices.

Fruit Juice	Concentration of ethanol (mg/dl)
Orange juice A	56.5
Orange juice B	3.7
Mixed berry juice	57.0
Grape juice	236.2
Lemonade	13.2
Apple juice	86.4
Pomegranate juice	39.7

The results would indicate that the ethanol content in all cases is safely below the required limit at which a beverage is considered to be alcoholic. Also, the data suggests that the ethanol content is independent of the variety of fruit in the juice but further analysis at the point of manufacturer would be required to definitively make such a claim. It has been shown that the ethanol content that occurs naturally in different varieties of orange, for example, could be the cause of the different results between the two orange juices examined.

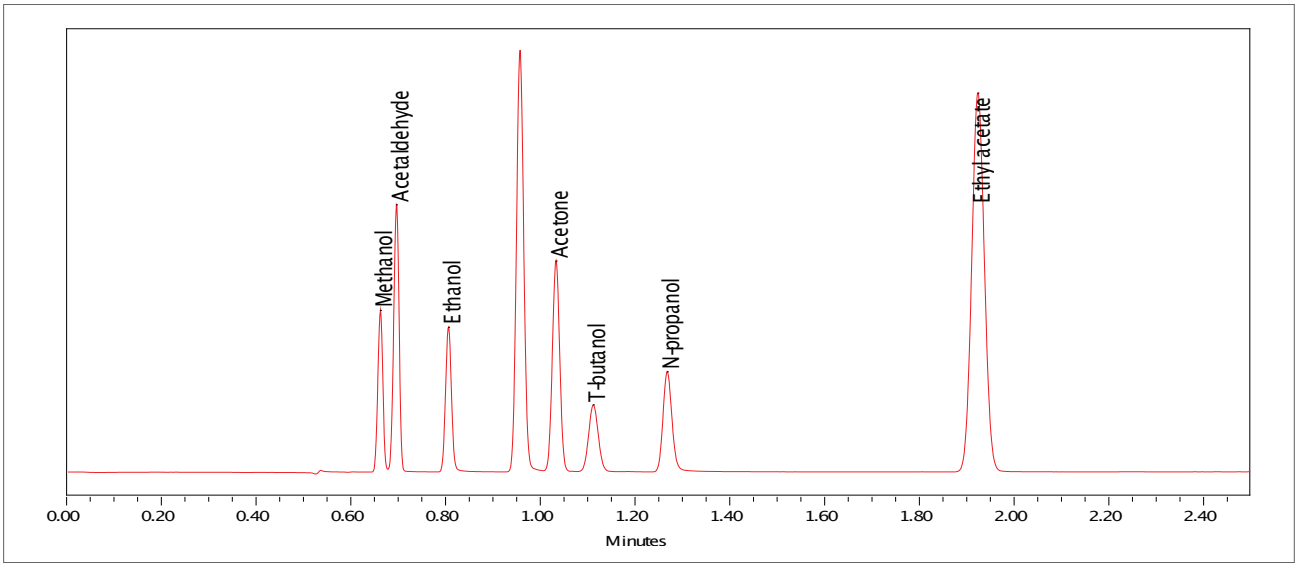


Figure 1. Chromatogram showing the elution order of the BAC mix that identified t-butanol as a suitable internal standard for the ethanol analysis.

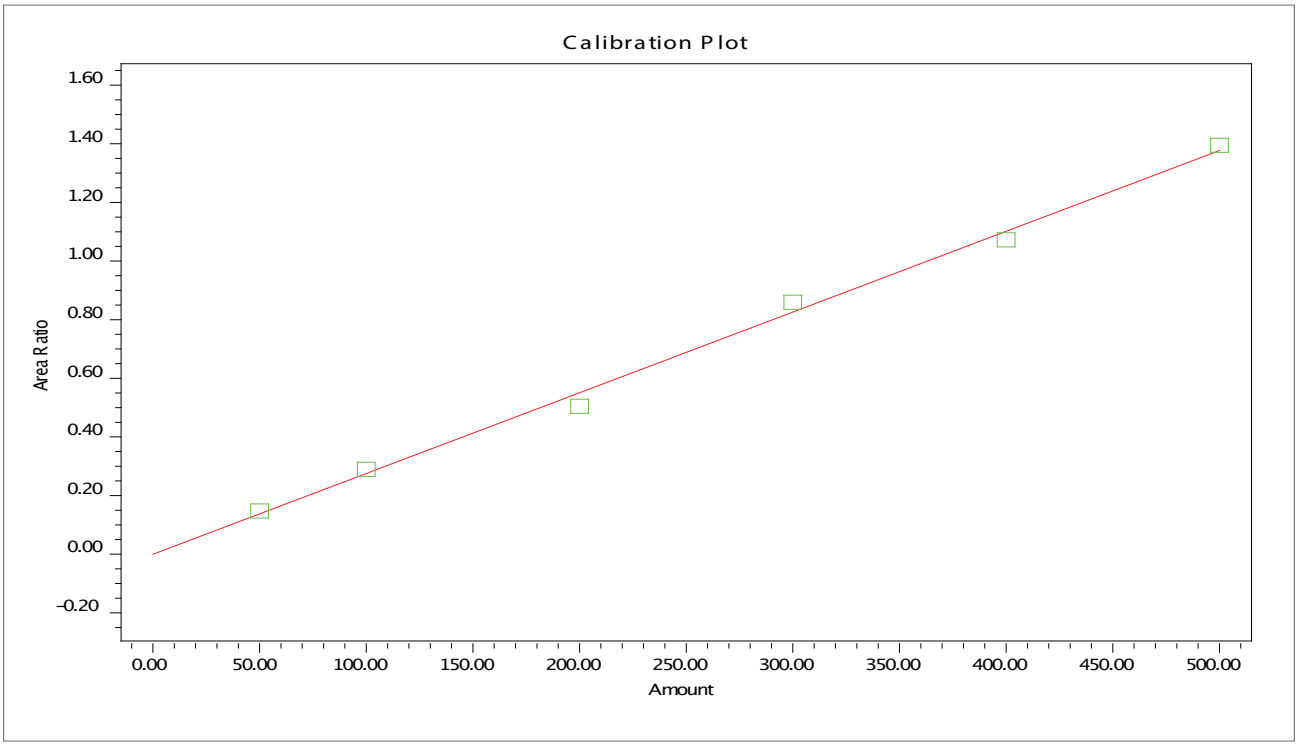


Figure 2. Calibration curve of ethanol used for the analysis of fruit juice.

Conclusion

This simplified method allows for favorable results in the quantification of ethanol content in consumable fruit juices. Additionally, the headspace introduction of the sample to the GC ensures that the amount of sample that gets on to the column is consistent. By decreasing the chance of errors in the preparation leads to concrete results that can be used as valid proof. These results are obtained from a TurboMatrix HS autosampler and the necessary time taken in sample preparation of each individual run due to the sensitivity of the headspace.

For Further Reading:

1. Timothy D. Ruppel; PerkinElmer Field Application Report, "Blood Alcohol Analysis Utilizing Headspace Autosampling and Dual-column GC Confirmation"
2. Timothy D. Ruppel; PerkinElmer Field Application Report, "Simulaneous and Rapid Separation of Blood-Alcohol Compounds and Commonly Abused Inhalants by Headspace-Gas Chromatography"
3. Paul L. Davis; Florida State horticultural Society, 1971, Pg 217- 222

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

012091_01 PKI

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Authors:

Kitsada Pitija, PhD

Tinnakorn Srisedkha, PhD

Chanchira Wiwatsamretkun

PerkinElmer Inc.
Bangkok, Thailand

Quantification of Rice Aroma, 2-Acetyl-1-Pyrroline (2-AP), Using TurboMatrix Headspace Trap Coupled with GC/NPD and GC/MS

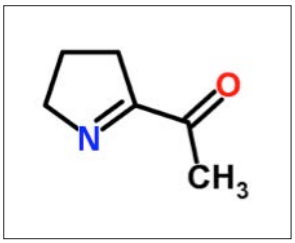


Figure 1. The structural of 2-acetyl-1-pyrroline (2AP).

Introduction

Rice is the most important staple food for a large part of the world's human population. Rice varieties with aroma quality, known as aromatic or fragrant rice, have earned a reputation and wide popularity. 2AP was firstly identified by R. Buttery and his co-workers[1] and it was suggested as one of the key characteristic compounds of aromatic rice. It is a five-membered N-heterocyclic ring compound and its structure is shown in the Figure 1.

In the past two decades, many techniques were reported for the extraction of 2AP in rice grains, such as purge and trap, steam distillation-solvent extraction, Likens-Nickerson simultaneous distillation-extraction, solvent extraction and solid phase microextraction. However, some of these methods are time-consuming, which require many steps for sample preparation and thus, are not appropriate to analyze large numbers of rice samples. The method employing headspace (HS) coupled with gas chromatography (GC) requires no sample preparation making it a rapid and efficient analysis technique[2-3].

PerkinElmer
For the Better

HS/GC is for applications involving the solvent-free extraction of volatile compounds. It is an unsurpassed technique, eliminating the time-consuming steps and risk of human error associated with other GC sample-preparation methods. This technique is engineered to deliver unparalleled precision, sensitivity and productivity in a broad range of specialized applications including forensics, food and beverage, pharmaceuticals, agricultural and environmental.

HS/GC coupled to a mass spectrometer (MS) detector provides a sensitive tool for identification of trace level volatile compounds in plant materials without involving complex extraction techniques. HS has the advantage of being a flexible, simple and a relatively economic extraction technique. TurboMatrix™ Headspace (HS) and Headspace Trap samplers are the clear choice for laboratories seeking outstanding throughput and precision.

Pressure-Balanced Technology

A PerkinElmer exclusive, pressure-balanced technology allows samples to be introduced into the column without using a gas syringe or multiport valves. Instead, carrier gas pressures are precisely regulated to manage transfer, eliminating many of the sources of variability and contamination found in other systems.

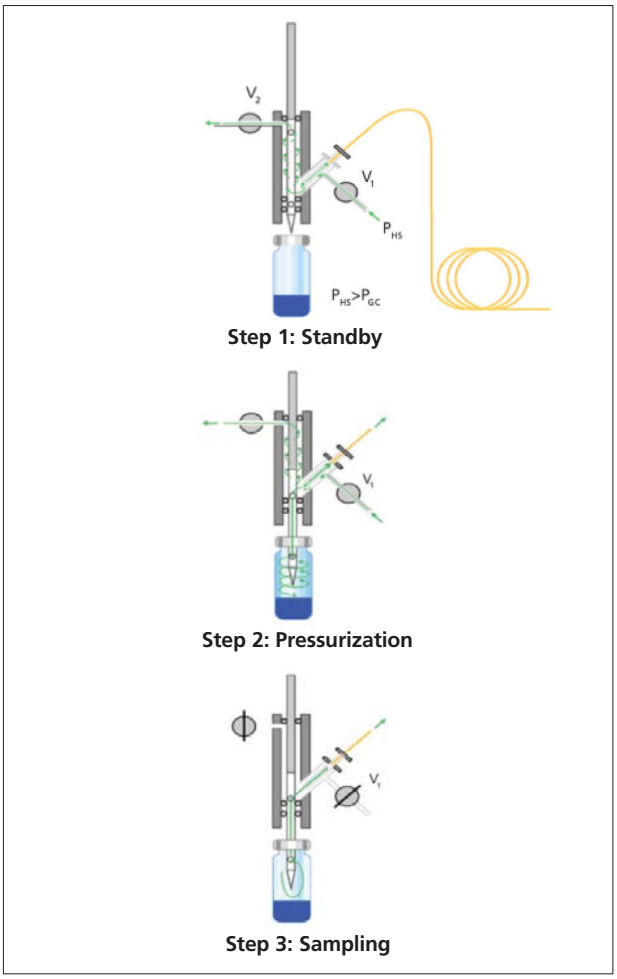


Figure 2. Pressure-balanced process.

Experimental

Chemicals and Reagents

All solvents used were analytical-reagent grade and purchased from the following sources: benzene from Merck (Darmstadt, Germany), benzyl alcohol from Fisher (Loughborough, UK), 2,4,6-trimethylpyridine (TMP), used as internal standards from Merck (Schuchardt, Germany) and 2-acetyl-1-pyrroline (2AP), from Toronto Research Chemicals (Ontario, Canada).

Sample Preparation

Rice grain samples were finely ground then the samples (0.50 g) were separately placed in headspace vial with 1.00 µl of 1000 ppm TMP as the internal standard. Headspace vials were sealed before analysis by HS-GC/NPD and GC/MS.

Calibration Procedure

Calibration standards (0.2- 10.00 µg/g) were generated by spiking varying amounts of 1000 µg/g of a standard AP in headspace vials containing 0.50 g of the non-aromatic rice (Supanburi rice). The internal standard solution, 1.00 µl of 1000 µg/g TMP in toluene, was added to each vial using the open vial sample introduction technique.

HS Trap GC/NPD and GC/MS Conditions

Static HS-GC analysis was carried out using an PerkinElmer Ltd, model SQ8 gas chromatograph-mass spectrometer equipped with PerkinElmer Ltd, model TurboMatrix 40 Trap Headspace Sampler (Figure 3). The optimum of HS-GCMS conditions are show in Table 1.

Results and Discussion

Separation and Identification of 2AP in Rice

The Turbomatrix 40 Trap headspace sampler and gas chromatography-mass spectrometric technique were developed for determination of 2AP, in rice grain samples. The chromatograms and mass spectrum of 2AP and TMP as internal standard obtained from HS trap and GC/MS were demonstrated in Figure 4 and Figure 5. The results showed that the good separation of the volatile compounds in rice samples added with TMP in Toluene were obtained from HS-GC/NPD. TMP and 2AP were eluted with the retention time at 5.05 and 5.47 minutes, respectively. Peak purity of these compounds were identified and confirmed by mass spectra data acquired from by HS-GC/MS analysis.

Figure 3. The Clarus 680 (GC/NPD) and SQ8T (GC/MS).

Table 1. Instrumental methodology.

TurboMatrix 40 Trap Headspace Sampler	
Thermostatting Temperature	120 °C
Needle Temperature	125 °C
Transfer Line Temperature	130 °C
Thermos Tatting Time	15 minutes
Pressurization Time	1.5 minutes
Trap and Dry Purge Time	2 minutes
Carrier Gas and Flow Rate	Helium, 15 psi
PerkinElmer Clarus 680 Gas Chromatograph	
Carrier gas and Flow Rate	Helium, 3 mL/min
Column	PerkinEler Elite-5MS (30 m × 320 µm ID×0.25 µm film thickness
Injection Temperature	200 °C
Temperature Programming	45-125 °C
Detector	NPD
Detector Temperature	250 °C
H_2 Flow Rate	4 mL/min
Air Flow Rate	80 mL/min
PerkinElmer Clarus SQ 8 T Single Quadrupole	
Mode	Electron impact (EI)
Inlet Line Temperature	200 °C
Source Temperature	200 °C
Mass Range	29-500 *m/z*
Software	TurboMass 6.1

Method Validation of 2AP

The method of TurboMatrix 40 trap headspace sampler and gas chromatography with mass spectrometer were developed for quantification of 2AP in fragrant rice using HS/GC. Calibration curve for 2AP analysis by headspace was generated by spiking known concentrations of the analyte into a non-fragrant rice variety (Supan Buri). The correlation between detector response was measured in terms of peak area ratios between 2AP and TMP. The response of 2AP standard was linear over a concentration range of 0.10 - 10.00 µg/g of rice samples using NPD detector with a correlation coefficient (r^2) 0.9924, Figure 6. The effective linear concentration ranges of the method were in the range of ranged 0.20-10.00 µg of 2AP/g of rice sample for HS-GC/NPD[4].

The percentage recovery of 2AP in the first headspace extraction step was 45.66%. Method validation performed for this developed SHS-GC/NPD method demonstrated the limits of detection (LOD) and limits of quantitation (LOQ) at 0.10 µg of 2AP and 0.0500 g of rice samples, respectively. The intraday and interday coefficients were 2.25% RSD (n=10) and 4.60% RSD (n=35), respectively.

Analysis of Rice Samples

These developed methods were applied to quantify the amount of 2AP in the rice samples. The concentration of 2AP found in all rice samples are shown in Table 2. It was observed that the amount of 2AP in rice sample were in the range 1.22-2.58 µg of 2AP/g of rice samples[5-6].

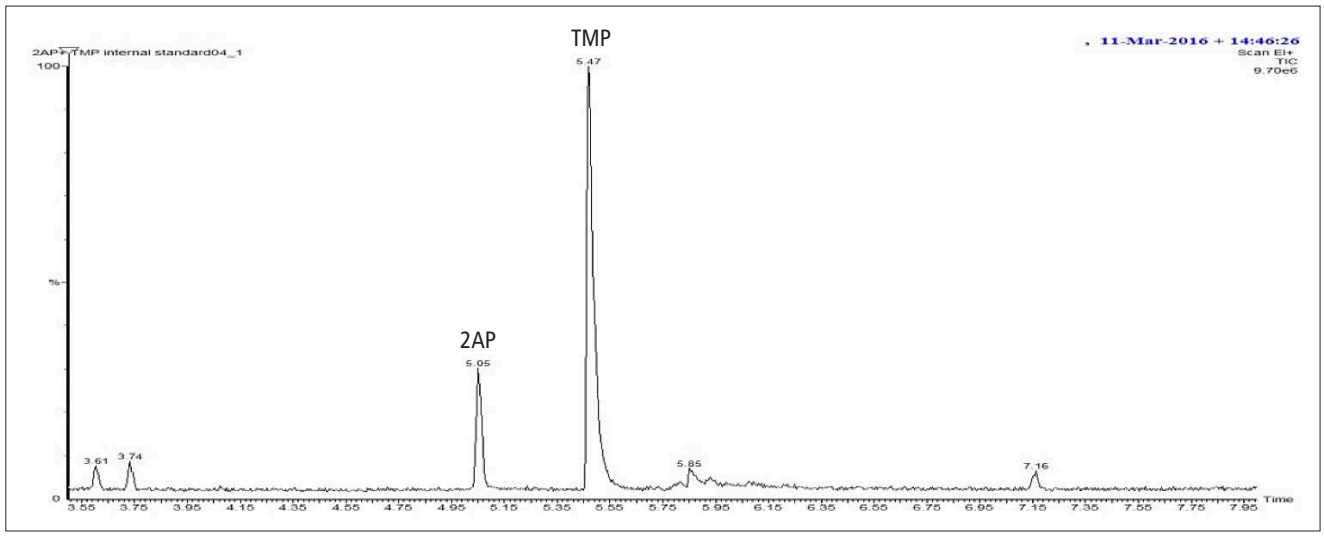


Figure 4. The chromatogram of 2AP and TMP standard obtained from HS trap and GC/MS.

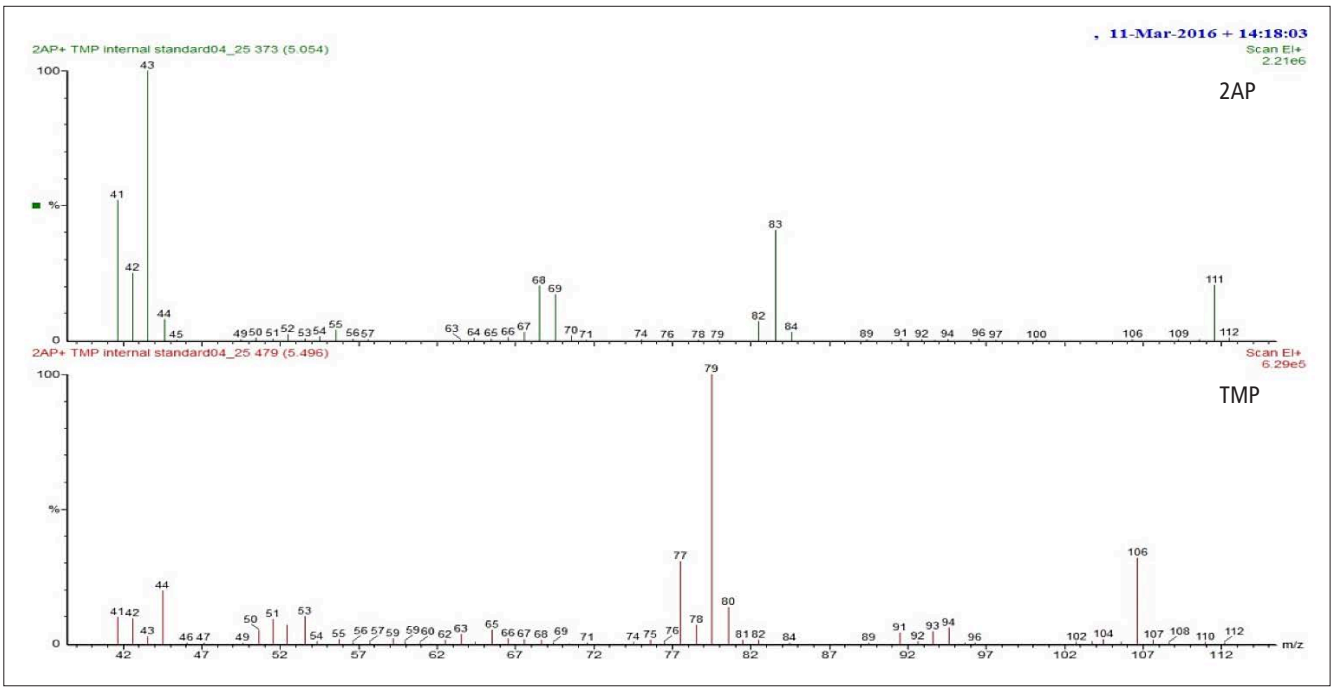


Figure 5. The mass spectrum of 2AP and TMP obtained from HS trap and GC/MS.

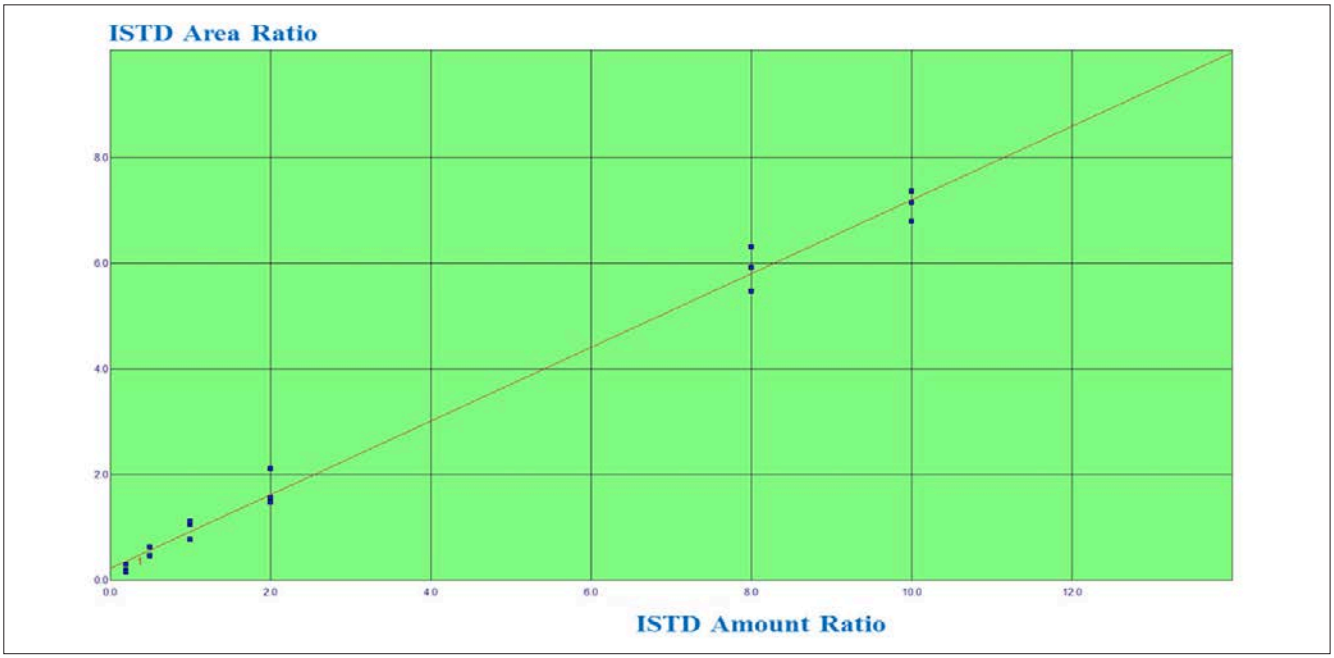


Figure 6. The calibration plot of a standard curve 2AP /TMP obtained from HS trap and GC/NPD concentration at 0.10-10.00 µg 2AP/g of rice sample.

Table 2. Quantification analysis of 2AP in Thai fragrance rice samples using HS-GC/NPD.

Rice Samples	Concentration of 2AP, µg/g, (mean ± SD)
KDML 105 (CR) Rice	2.53 ± 0.08
KDML 105 (Dech Udom) Rice	1.70 ± 0.10
Moo Rice	2.58 ± 0.06
Phakmai Rice	1.22 ± 0.05
Srisaket Rice	1.55 ± 0.01
Yasothron Rice	1.99 ± 0.01

Conclusions

The automated HS-GC/NPD technique was developed and applied for the analysis of various type of rice samples. This method was successful for the determination of a key aroma compound, 2AP, in rice sample with different varieties. The method described in this study is rapid, convenient and requires little sample preparation making it an ideal analysis tool for aroma analysis of rices.

Acknowledgement

The automated HS-GC/NPD technique was developed and The authors thank Assoc. Prof. Dr. Sugunya Mahatheeranont for her thoughtful advice and suggestions. We also would like to take this opportunity to thank Assoc. Prof. Dr. Aphichat Vanavichit for providing laboratory and facilities to make this study possible.

Acknowledgement

1. R. Buttery, J. Turnbaugh, and L. Ling, *J. Agric. Food Chem.,* 1988, 36, 1006-1009.
2. S. Wongpornchai, T. Sriseadka and S. Choonvisase, *J. Agric. Food Chem,* 2003, 51, 457-462.
3. B. Mutti and W. Grosch, *Nahrung,* 1999, 43, 302-306.
4. S. Mahatheeranont, S. Keawsa-ard and K. Dumri, *J. Agric. Food Chem,* 2001, 49, 773-779.
5. S. V. Mathure, K. V. Wakte and A. B. Nadaf, *Food Anal. Methods,* 2010.
6. T. Sriseadka, S. Wongpornchai and P. Kitsawatpaiboon, J. Agric. *Food Chem,* 2006, 54, 8183-8189.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

013275_01 PKI

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author:

Andrew Tipler

PerkinElmer, Inc.
Shelton, CT

Coffee Characterization Using Clarus SQ 8 GC/MS, TurboMatrix HS Trap and GC SNFR Olfactory Port

Introduction

Coffee is a very popular drink in most parts of the world and is one of the most traded agricultural commodities on the planet. The drinking of coffee, however, is a fairly recent activity. Although its origin may be attributed to Ethiopia a thousand years ago, its popularity as a beverage really started in the Middle East around the start of the 17th century.

Part of its popularity is due to the stimulating effect of its caffeine content (a cup of coffee may contain as much as 150 mg) and part is due to its rich complex taste. The taste of a cup of coffee depends on many factors – the coffee bean variety and horticulture and the way the beans are stored, roasted, ground and brewed. Even the water used to make the coffee can have an effect on its flavor.

PerkinElmer®
For the Better

For such a commercially significant product, it is important that there should be some means to characterize and control its taste at the various stages of production. This may achieved organoleptically (i.e. by smelling and tasting) or by using powerful analytical tools like gas chromatography mass spectrometry (GC/MS) to determine chemical composition.

Aroma plays a very important part in the taste of coffee. This application note presents a system for characterizing finished coffee aroma while simultaneously performing a chemical analysis on a mass spectrometer. Further data may be acquired using a flame ionization detector (FID) for chemometric processing to provide further insight into the individual character of each coffee sample. The results provide a powerful insight into both the chemical composition and the sensory perception of coffee aroma. Such a system can be used for quality control purposes, process and product development, storage studies, trouble-shooting and evaluating competitive products.

Instrumentation

In this analysis, a headspace trap system may be utilized for sample introduction to characterize the flavor of roasted coffee beans. This technique ensures that non-volatile material in the beans does not enter the analytical system, which can cause interference in the chromatography and potential system contamination. The headspace trap extracts the volatile components from a large sample and focuses them onto an inline adsorbent trap. It also facilitates very easy sample preparation – a weight of ground beans is dispensed into a vial and sealed. The subsequent analysis is then fully automated.

A PerkinElmer TurboMatrix™ Headspace Trap connected to a PerkinElmer Clarus® SQ 8 GC/MS with a flame ionization detector is used for these experiments. The MS provides the ability to identify each separated component and the FID is used to provide the quantitative data used in the chemometrics analysis. A schematic diagram of the GC system is given in Figure 1.

Using a headspace trap instead of the classical headspace technique enables up to 100 times improved detection limits over classical static headspace methods.

A polar 60 m x 0.25 mm x 1.0 µm Elite Wax column is used. This thick-film column provides sufficient chromatographic retention to separate the early-eluting most volatile components and provided the dynamic range necessary to chromatograph both high level and low level components in the coffee.

The column effluent is split between a PerkinElmer SNFR™ GC olfactory port, the MS detector and the FID. This splitting is performed using an S-Swafer™ in a standard active splitting configuration.

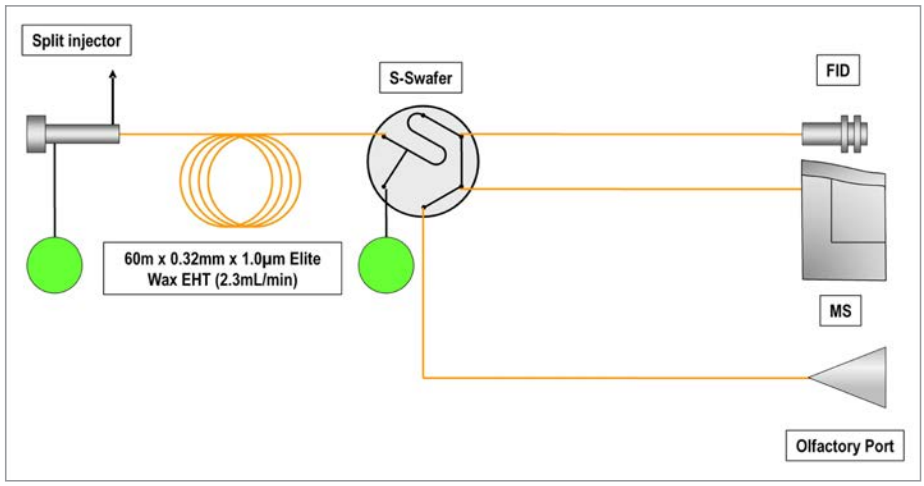


Figure 1. Schematic diagram of the GC system.

Experimental

Overview

Twenty seven varieties of pre-roasted and freshly roasted coffee beans from throughout the world were procured and examined in this work. These are listed in Table 1.

Table 1. Coffee samples examined.

1	Kona Cloud® Hawaiian coffee beans
2	Green Mountain® ground coffee (15 g packets)
3	Green Mountain® ground decaffeinated coffee (15 g packets)
4	Harar Longberry® Ethiopian coffee beans
5	Moka Harar® CP Select Ethiopian coffee beans
6	Kona Cloud® Hawaiian coffee beans medium roast
7	Kona Cloud® Hawaiian coffee beans dark roast
8	Other Kona coffee beans from Hawaii
9	Coffee beans from El Salvador
10	Coffee beans from Yemen
11	Coffee beans from Sidamo
12	Ground coffee from Trinidad
13	Ethiopian decaffeinated coffee beans
14	Guji Sueq'to Ethiopian coffee beans roasted before first crack
15	Guji Sueq'to Ethiopian coffee beans roasted just after first crack
16	Guji Sueq'to Ethiopian coffee beans roasted just before second crack
17	Guji Sueq'to Ethiopian coffee beans roasted just after second crack
18	Guji Sueq'to Ethiopian coffee beans roasted long after second crack
19	Guji Sueq'to Ethiopian coffee beans carbonized
20	Folgers® 5 g ground coffee bag
21	Folgers® 5 g ground decaffeinated coffee bag
22	Kona Cloud® freshly roasted beans
23	Trader Joe's® Cafe Pajoro beans (old)
24	Costa Rican El Trapiche beans bought at plantation
25	Costa Rican Britt® medium roasted beans
26	Costa Rican Britt® dark roasted beans
27	Barista® French roast ground coffee machine cartridge

Analytical Method

The experimental conditions for this analysis are given in Tables 2 to 8.

Table 2. HS trap conditions.

Headspace System	TurboMatrix 110 HS Trap
Vial Equilibration	80 °C for 20 minutes
Needle	120 °C
Transfer Line	140 °C, long, 0.25 mm i.d. deactivated fused silica
Carrier Gas	Helium at 25 psig
Dry Purge	7 min
Trap	Air Toxics, 25 °C to 260 °C, hold for 7 min
Extraction Cycles	1 with 40 psig extraction pressure

Table 3. GC conditions.

Gas Chromatograph/ Mass Spectrometer	Clarus 580 SQ 8
Column	60 m x 0.32 mm x 1.0 µm Elite-5MS connected directly to the HS Trap
Oven	40 °C for 1 min, then 5 °C/min to 200 °C for 5 min
Carrier Gas	Helium at 25 psig at injector and 13 psig at Swafer
Flame Ionization Detector	275 °C, range x1, attenuation x8

Table 4. MS conditions.

Scan Range	m/z 35 to 350
Scan Time	0.1 s
Interscan Delay	0.06 s
Source Temp	250 °C
Inlet Line temp	250 °C
Multiplier	1700V

Table 5. Olfactory port conditions.

Olfactory Port	PerkinElmer SNFR
Transfer Line	225 cm x 0.250 mm at 240 °C
Humidified Air	500 mL/min with jar set to 37 °C

Table 6. Chemometric.

Software	InfoMetrix Pirouette Version 4.0
Data	Collected using the flame ionization detector

Table 7. Swafer conditions.

Swafer	PerkinElmer S-Swafer in the S1 configuration
Settings	Developed using the Swafer Utility Software – see Figure 2.

Table 8. Sample details.

Sample Preparation	Beans were freshly ground and 1 g was weighed into a sample vial and sealed
Vial	Standard 22 mL vial with aluminum crimped cap with PTFE lined silicone septum

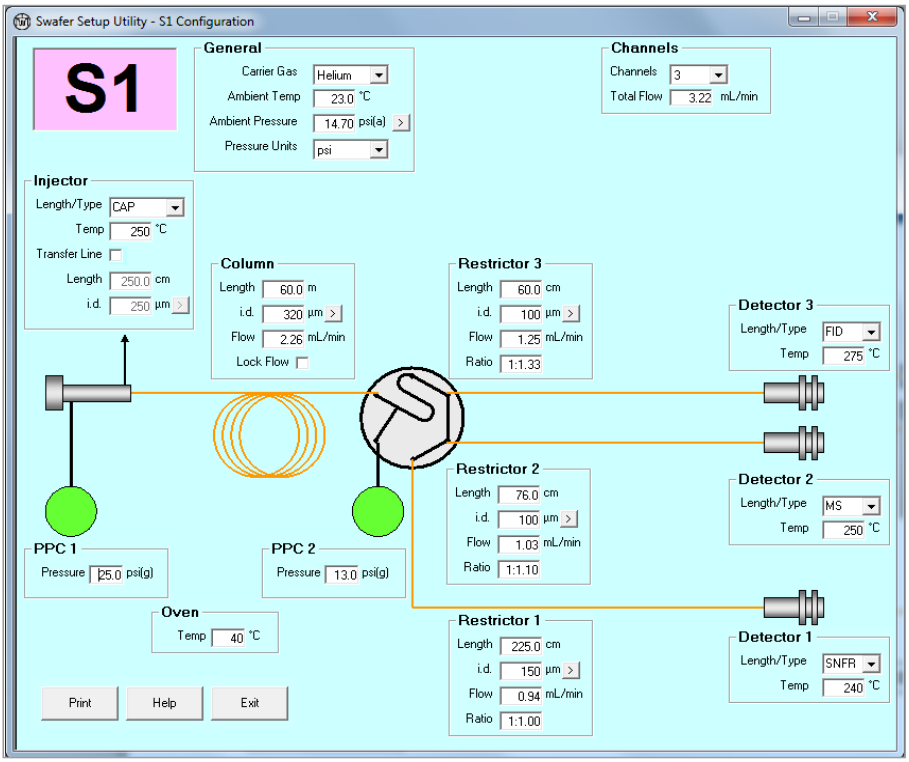


Figure 2. The S-Swafer in the S1 configuration for MS, FID and olfactory work.

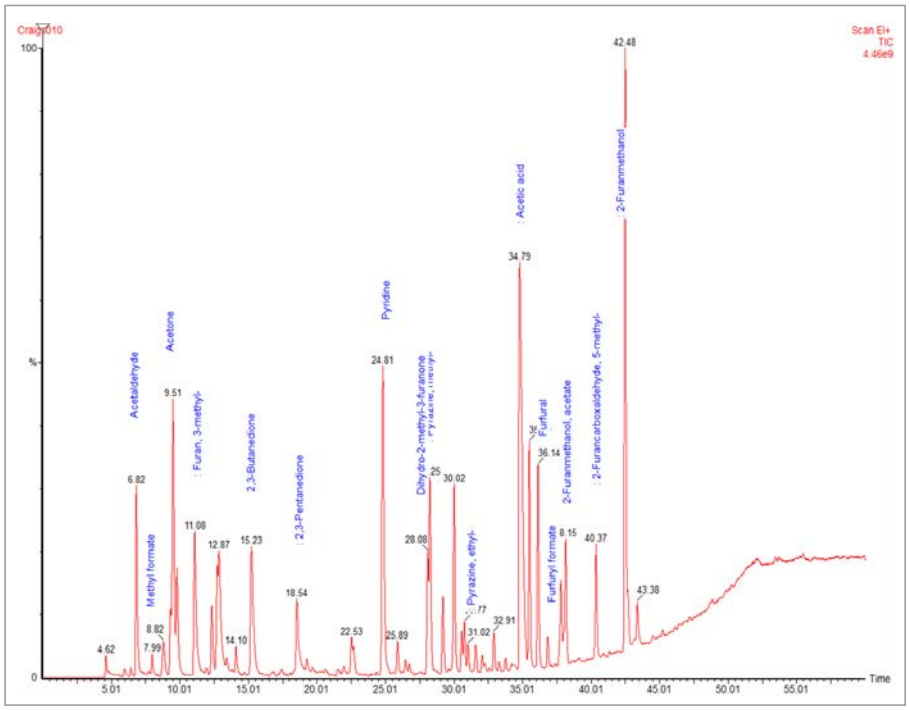


Figure 3. Typical chromatogram from 1 g coffee grains.

Results

Chromatography on the MS

Slow chromatographic times are preferred to enable the analyst to fully elucidate his or her sensory experience as the peaks elute. Faster chromatography is possible but then there is a risk that odors from adjacent peaks may start to overlap. Slower chromatography also gives the user more time to fully narrate and record their sensory perceptions.

Figure 3 shows a section from a chromatogram of coffee sample #3. The key components were identified using the library search capabilities of the TurboMass™ software supplied with the Clarus SQ 8 GC/MS.

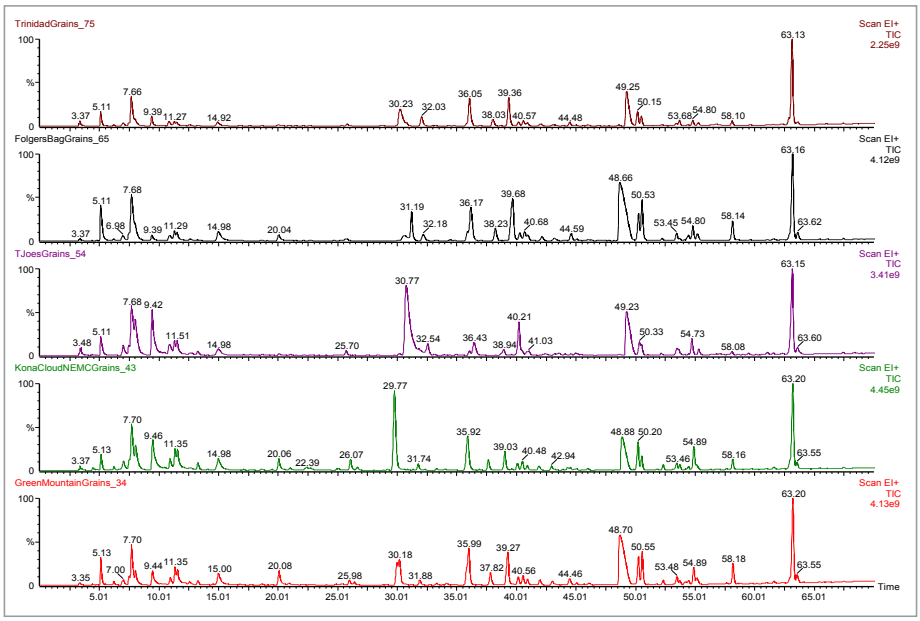


Figure 4. Chromatogram from five different types of ground coffee.

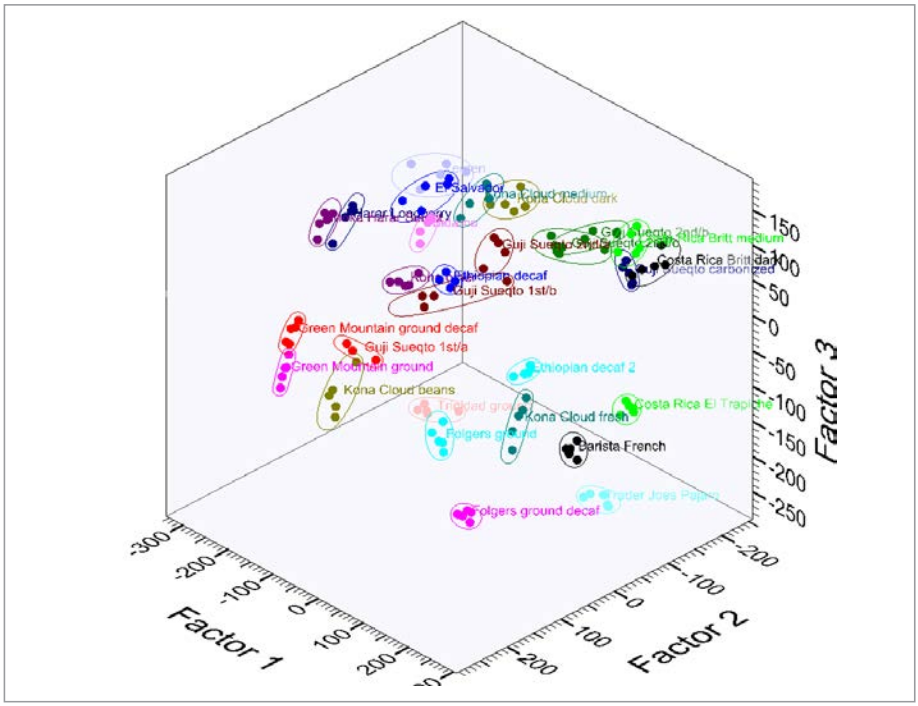


Figure 5. Principal component analysis loadings of first three factors for the 27 coffee samples examined.

Chemometrics

Visual analysis of the five chromatograms in Figure 4 shows the subtle differences between the different coffees. While it is possible to identify one or two peaks that differ between one or two chromatograms this is unfeasible in a production setting and a better, faster and more objective solution is required.

The InfoMetrix® Pirouette® software was used to perform a principal components analysis (PCA) on chromatographic data from replicate analysis of all 27 of the coffee bean samples collected using the flame ionization detector. The PerkinElmer FID data files were able to be read and processed by the Pirouette® software.

Figure 5 shows plots of the loadings for each coffee for the first three PCA factors. These factors can be regarded as a 'building blocks' that are common to each chromatogram but are present at different levels (or loadings). As can be seen in Figure 5 the three loadings for the replicate chromatograms for each sample are very similar giving rise to tight clustering in the plots. Each sample cluster is separated from the other sample clusters. In this way, subtle differences or patterns in the chromatography can be used to discriminate between different coffee types. This discrimination may be correlated with sensory perception; in which case, the PCA may be used to guide the user to specific chemical compounds or groups of compounds that are responsible for the aroma character of that coffee.

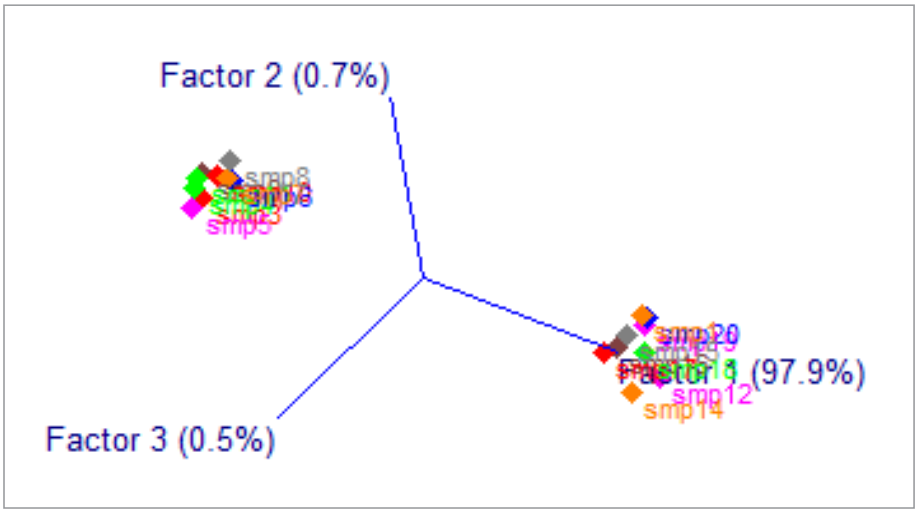


Figure 6. Detail from PCA map of two coffee sample chromatograms.

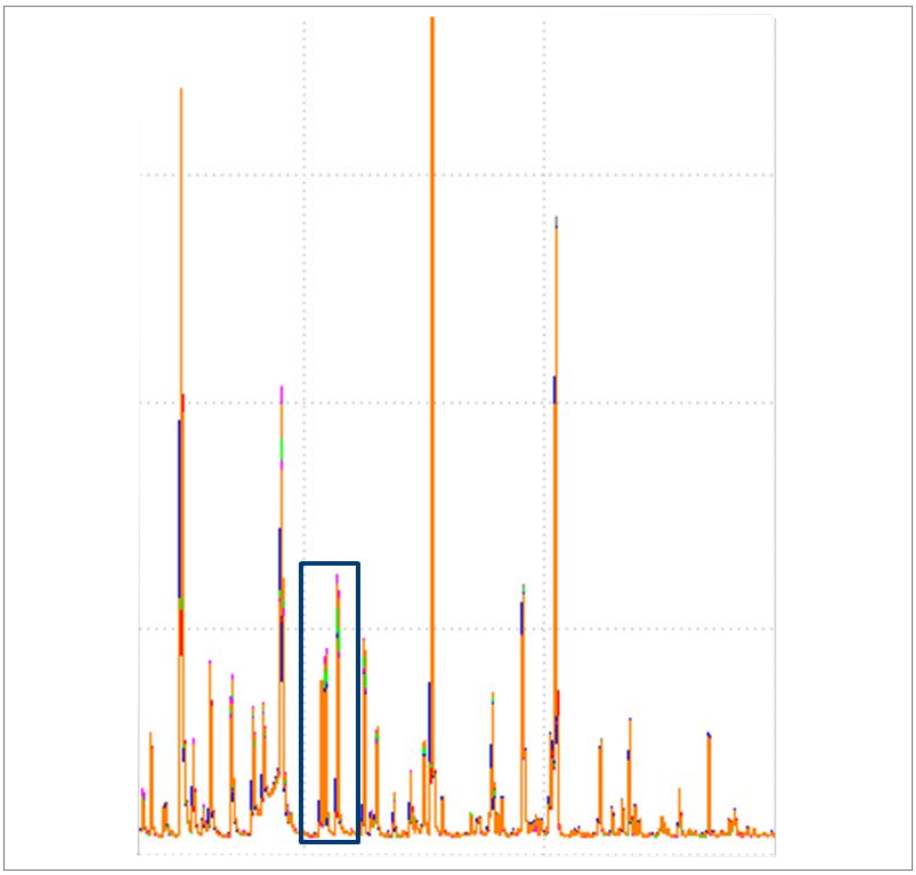


Figure 7. Chromatography of samples #2 and #22 overlaid.

For example, Figure 6 shows the PCA loadings for just two of the coffee samples. The replicate PCA results are tightly clustered for each sample type but well separated from the other sample type. Clearly there are differences in the chromatography between these two samples. Inspection of the PCA factors highlights an area in the chromatography where significant differentiation is apparent. This area is shown in Figures 6 and 7. In this instance, the difference is clear but there may be areas in the chromatography where the difference may be more subtle or may be because of a combination of peaks (patterns). This is where PCA would be a powerful tool to highlight such areas.

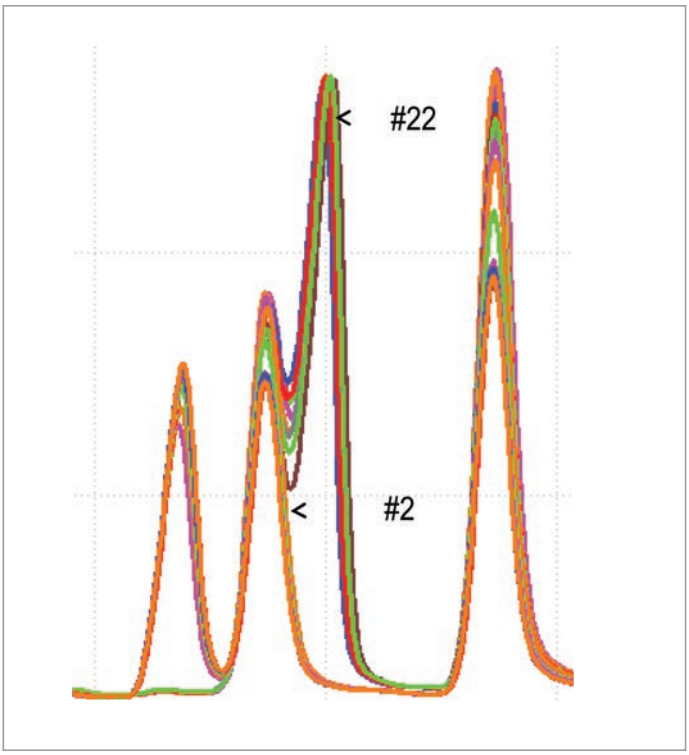


Figure 8. Detail from figure 7.

Olfactory Monitoring

Figure 9 shows an image of the SNFR system used for the olfactory monitoring. Figure 10 shows a photograph of Mr. Snow, a coffee expert, using the device to monitor the aroma of individual compounds. While the coffee aroma components are being monitored, the user is able to record his or her sensory perceptions by voice into the supplied microphone and by positioning a joystick to indicate the intensity of the aroma. This information may be accessed and reviewed when displaying the chromatogram after the run is complete.

Figure 9. The GC SNFR system.

Figure 10. Photograph of coffee expert monitoring coffee aroma compounds.

Conclusion

The combination of chromatographic, mass spectral, chemometric and olfactory data from a single analysis provides a very powerful insight into the aroma and taste of complex samples such as coffee. Users can quickly identify which compounds are largely responsible for the aroma of a given coffee and what are the key differences and similarities between different coffees. The system that produces all this data would be at home in both a development laboratory or in a QC environment.

Acknowledgements

The author would like to thank the following for their support, help, advice and donations of free coffee samples during this work:

Craig Sellman, Village Coffee Roastery, Scottsdale, Arizona

Gerry Nicholls, Founder of Objective Coffee Tasters Group of America

James Ameika, Owner of Kona Cloud® Coffee Plantation, Hawaii

Brian Rohrback, President of InfoMetrix®

Scott Ramos, InfoMetrix®, Chemometrician and Coffee Drinker

Miles Snow, Coffee Exert and Principal Scientist at PerkinElmer

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

011303_01

APPLICATION BRIEF

Gas Chromatography/ Mass Spectrometry

Authors:

David Scott

PerkinElmer, Inc.
Shelton, CT

Separation and Characterization of Indian and Australian Sandalwood Oils

Introduction

Sandalwood *(Santalum album L.)* is a desired wooden base note for many fragrances in perfume and other scented products including incense. The major component is alpha-santalol with distilled oil usually containing approximately 50%. The alpha-santalol is a weak odor compound with beta-santalol being the stronger odor compound more associated with sandalwood oil. Typically the tree is grown for 12 years before the total harvest of trunk, branch and root. There are many related species giving rise to counterfeiting and confusion among consumers.

The yield of the wood will change over time with a transition from beta- to alpha-santalol reported. There are more than 234 compounds[1] that have been identified in sandalwood extract to add to the overall experience with ongoing research still identifying others.[2] Not all compounds have an odor that contributes to the overall scent and it is possible to identify the tree source by the ratio of compounds present. There is also a synthetic santalone that is available and present in commercial fragrances. The addition of synthetic materials can be somewhat controversial as there is a school of thought that such synthetics take away the magic from a fragrance. The alternative viewpoint is that the synthetic material is not subject to natural disasters or simply weather impacting supply and there are fewer compounds present which may cause allergic reactions.

PerkinElmer®
For the Better

The application of Gas Chromatography-Olfactometry (GCO) and mass spectrometry are considered routine and are frequently the techniques of choice for analyzing extracts from these fragrant woods.

Experimental

Three oil samples were obtained from a commercial aromatic oil source. Separation was achieved with a 60 m wax column for the increased number of theoretical plates, though a shorter 30 m column could also be used for a faster separation. Post column, an S Swafer™ device was used to split the effluent between the SNFR™ olfactometry accessory for olfactometry and the Clarus® SQ8 MS for identification. Due to the sensitivity of the mass spectrometer, the samples were diluted with 1 drop added to 1 mL of methanol.

Two injection techniques were performed. A simple wet needle injection, and headspace (HS) trapping with the TurboMatrix™ HS trap.

The wet needle injection demonstrates the analysis of a distilled oil, and the TurboMatrix headspace (HS) trap describes the possible analysis of wood prior to distillation. No wood samples were available for this work however the excellent trapping efficiency of the TurboMatrix and 22 mL vial size suggests that a representative sample of wood chips could be analyzed prior to distillation.

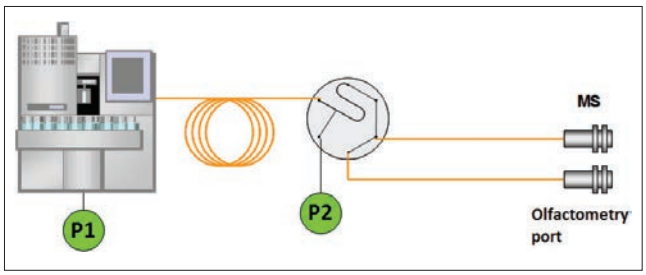


Figure 1. Schematic of the separation. For wet needle analysis the headspace system was removed and the capillary injector was used instead.

The Swafer configuration allows manipulation of the column flow rate and separation without impacting the active split between the mass spectrometer and olfactometry port. The split ratios and flows are a result of the selected transfer line geometries and carrier gas pressure. The addition of the second pressure source regulates the splitting and maintains the engineered configuration independent of the column head pressure. The independence from the column head pressure increases the options available to the chemist with respect to flow rate in the separation. The separation is critical from an identification standpoint and for optimum human interaction. It is all too easy for the human to be overwhelmed with multiple peaks and the odors rolling over one another making an accurate description troublesome. Therefore a 60 m x 0.32 id wax column was used starting at 40 °C and ramping 2 °C/min to 240 °C. The full instrument conditions are given in Tables 1 through 3.

Figure 2. PerkinElmer SNFR olfactometry accessory.

Table 1. TurboMatrix HS110 Trap.

Needle	210 °C
Oven	190 °C
Transfer Line	210 °C
Trap Low	30 °C
Trap High	350 °C
Vial Pressurization	1 min
Vial Desorb	3 min
Dry Purge	1 min
Trap Hold	5 min
Desorb	0.1 min
Thermostat	25 min
GC Cycle Time	140 min
Carrier Pressure	35 psi
Desorb Pressure	35 psi
Vial Pressure	40 psi

Table 2. GC Conditions.

Injectors	Both at 250 °C
Oven Program	40 °C (hold 2 min) and ramping 2 °C/min to 240 °C (hold 15 min)
Column	60 m x 0.32 id x 1.0 µm

Table 3. MS Conditions.

Transfer Line Temperature	200 °C
Source Temperature	180 °C
Mass Range	30 – 300 m/z
Scan Time	0.2 secs
Interscan Delay	0.1 secs
Ionization Mode	EI+
Run Time	108 min

Results

The compounds present in sandalwood oils are a direct result of the distillation/extraction of the oil from the wood.

A recent patent application[3] concerning the therapeutic uses of different sandalwood oils describes the different compositions of Santalum spicatum (West Australian) and Santalum album (East Indian) sandalwoods. Among the major differences are the higher alpha and beta santalol content in the Indian Sandalwood and the presence of several key components in Australian Sandalwood that are absent or significantly reduced in Indian Sandalwood oil.

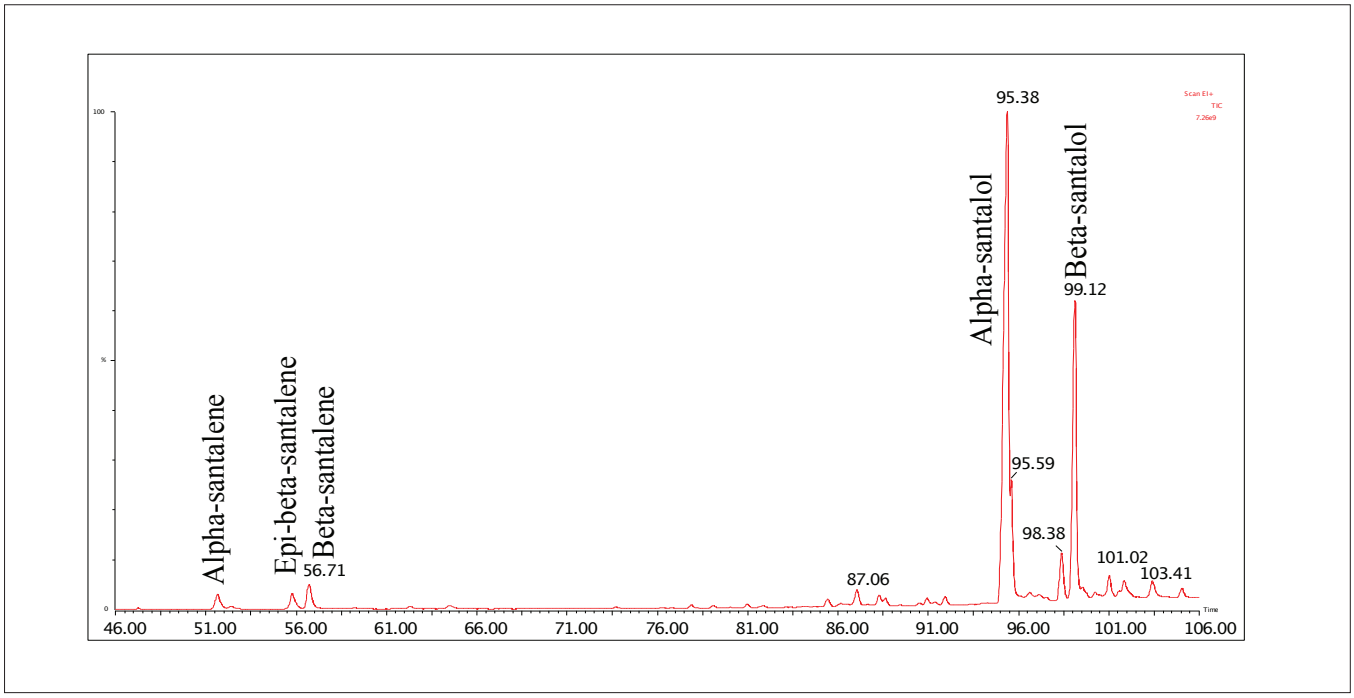


Figure 3. Chromatogram of Indian Sandalwood oil showing the santalene compounds and the large alpha- and beta-santalol content.

It is readily apparent that the Indian Sandalwood has significant alpha- and beta-santalol content but there are other compounds present in the Australian sample that are absent in the Indian oil. The Australian Sandalwood oil has less of the alpha- and beta-santalol but more significant is the increase in several other compounds that could be considered as indicative of Australian Sandalwood oil. The dendrolasin, (E)-nerolidol, the beta-bisabolol, apha-bisabolol and nuciferol are easily identified as distinct from the Indian Sandalwood.

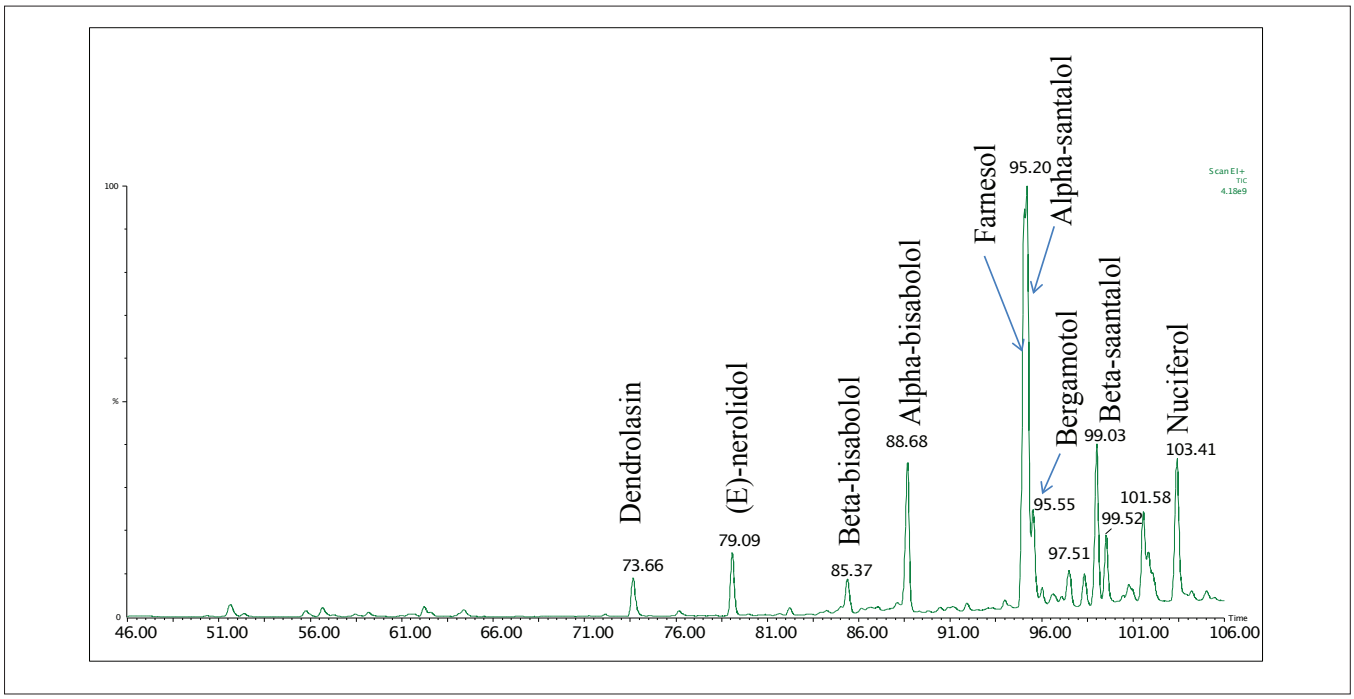


Figure 4. Chromatogram of Australian Sandalwood oil with the distinctive compounds indicated.

Analysis of the third oil sample gave strong indications that the sample was not an authentic sandalwood oil.

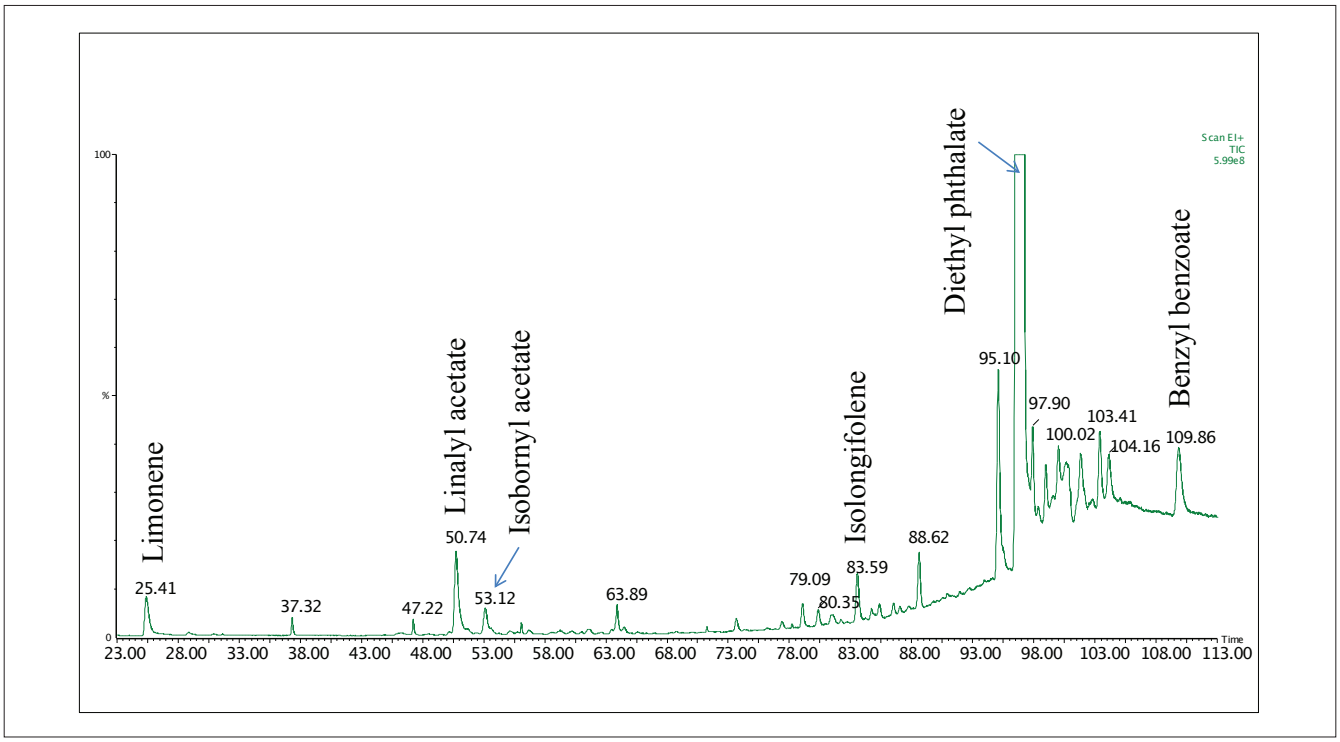


Figure 5. Chromatogram of Sandlewood oil showing the synthetic ester compounds and lack of alpha- and beta-santalol.

The presence of several synthetic esters and diethyl phthalate to fix them in the oil is strongly suggestive that the "sandlewood" sample is not an oil distilled from a species of Santalum. The isolongofolene and the benzyl benzoate are both woody tones that have been used to mimic the sandalwood experience. The limonene would appear to have been added to balance out the tones.

The common components are easily identified with a wet needle experiment and the TurboMatrix HS Trap was used to increase the sample volume without solvent column overloading. Four microliters of the sample were then injected into a headspace vial for a total evaporation experiment. The increased mass on column assists in the identification of smaller peaks and further highlights the differences in the two sandalwood species.

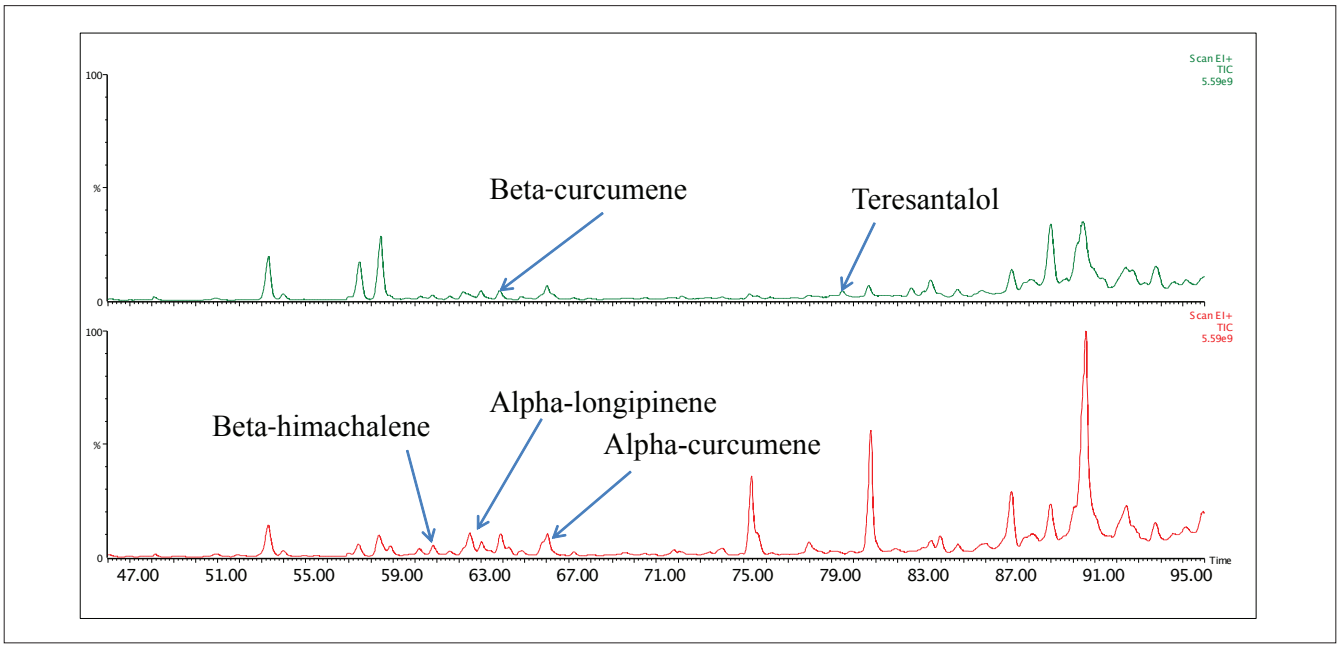


Figure 6. Chromatograms of Indian and Australian Sandalwood oil with TurboMatrix HS-Trap to elucidate small differences in the two samples.

Conclusion

The GCO system discussed successfully separated alpha- and beta-santalol in two commercially obtained sandalwood samples. Qualitative differences were identified demonstrating the capability of adulterant recognition. There is still active research in identifying new scents from botanical sources making GC-olfactometry an important tool in the quest for new sources of fragrance as well as preventing adulteration and counterfeiting.

References

1. N. Baldovini et al., *Flavour Fragr. J.* 2011, 26, 7–26
2. T. Hasagewa et al., *Flavour Fragr. J.* 2011, 26, 98–100
3. Ian Clements, Paul Castella, Corey Levenson, Application number PCT/US2011/026706, 2011.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

012021_01 PKI

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

Ruben Garnica

Andrew Tipler

PerkinElmer, Inc.
Shelton, CT 06484 USA

Analysis of Consumer Products by Headspace Trap GC/MS using the Clarus SQ 8

Fragrant soaps and detergents are a ubiquitous part of our modern society and add a certain romance to what would otherwise be mundane household chores. Great care and expense is spent in formulating the exact mixture of fragrant organic compounds to differentiate "apple blossom" or "crisp apple" from simple "apple" scent. At the other end of the spectrum are "fragrance-free" products – products that contain no fragrance producing organics, natural or otherwise. Unfortunately the term "fragrance-free" is unregulated and the actual composition of these products is left to the manufacturer's discretion. For both situations a comprehensive analytical technique is necessary to measure both composition and quality of any volatile organic compounds present. In this application brief we describe a quick and simple analytical technique using headspace trap gas chromatography/mass spectrometry (GC/MS) to determine the volatile fragrance compounds contained in various consumer products.

PerkinElmer®
For the Better

Method

The experimental conditions for this analysis are given in Tables 1 to 4. The vials used are the standard 22-mL vials with aluminum crimped caps with PTFE lined silicon septa. Liquid samples were placed directly into the sample vials. The two solid bar soap samples were thinly sliced into the sample vials to produce a maxima of surface area.

Table 1. **GC Conditions.**

Gas Chromatograph	**Clarus® 680**
Column	60 m x 0.25 mm x 1.0 µm Elite-5MS
Oven	35°C for 5 min, then 6 °C/min to 245 °C
Injector	Programmable Split Splitless (PSS), 180 °C, Split OFF
Carrier Gas	Helium at 2.0 mL/min (28.6 psig initial pressure), HS Mode ON

Table 2. **HS Trap Conditions.**

Headspace System	**TurboMatrix™ 110 HS Trap**
Vial Equilibration	80 °C for 20 min
Needle	120 °C
Transfer Line	140 °C, long, 0.25 mm i.d. fused silica
Carrier Gas	Helium at 31 psig
Dry Purge	7 min
Trap	CarboPack C, 25 °C to 260 °C, hold for 7 min
Extraction Cycles	1 @ 40 PSI

Table 3. **MS Conditions.**

Mass Spectrometer	**Clarus® SQ 8S**
Scan Range	35 to 350 Daltons
Scan Time	0.1 s
Interscan Delay	0.06 s
Source Temp	180 °C
Inlet Line temp	200 °C
Multiplier	1700V

Table 4. **Sample Details.**

Sample	**Sample Weight**
Liquid fabric softener	0.50 g
Liquid laundry detergent	0.50 g
Fruit scented dishwashing liquid	0.50 g
Fruit scented liquid hand soap	0.50 g
Fruit scented bar hand soap	0.50 g
"Fragrance-free" facial cleanser bar	0.50 g

Results

The total ion chromatogram obtained from the dishwashing liquid, fabric softener and laundry detergent samples are given in Figure 1. Compound identification is performed using the installed spectral search functionality in the TurboMass™ software in conjunction with the NIST® library and represents the highest probability result. Where possible the common (non-IUPAC) name is used. Compounds labeled with an asterisk (*) indicate a saturated signal and (fn) indicates compounds that likely serve a non-scent related function in the product. Both the fabric softener and laundry detergent, though not marketed directly as fragranced, contain a number of fragrant organic compounds that produce their characteristic sweet scent. The dishwashing liquid contains, in addition to the large limonene and ethyl methylbutyrate peaks, a number of additional trace fragrant compounds as illustrated in Figure 2. This complicated combination gives rise to its "apple blossom" scent. The total ion chromatogram obtained from the liquid hand soap sample, given in Figure 3, also presents a very refined combination of fragrant organic compounds. The combination of fruit scent combined with the smooth butter gives rise to its "white citrus" scent.

The total ion chromatograms from the two bar soap samples are given in Figure 4. The fruit scented bar soap clearly contains higher concentrations of fragrant organic compounds but the "fragrance-free" facial cleanser bar also contains these compounds. Figure 5 gives a zoom of the total ion chromatogram illustrating the low-level fragrant content of the facial cleanser bar. While the concentration is substantially less their presence is clearly detected. As discussed in the introduction, the use of the term "fragrance-free" is left to the description of the manufacturer and is not regulated and the consumer must be aware of these discrepancies.

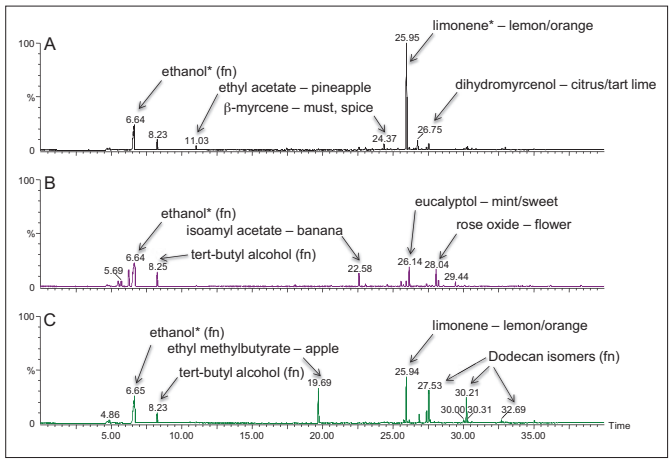


Figure 1. Full Total Ion Chromatograms obtained from (A) laundry detergent, (B) liquid fabric softener and (C) fruit scented dishwashing liquid with major fragrance compounds and scents labeled.

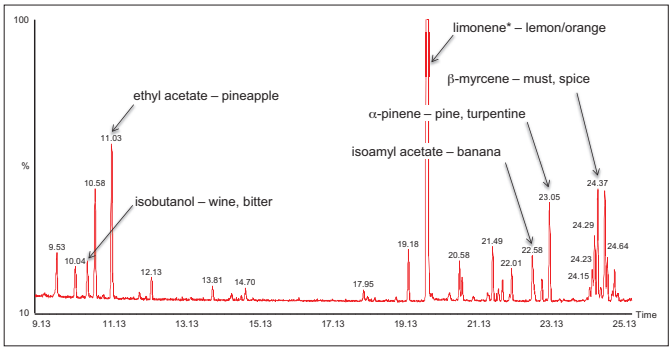


Figure 2. Zoom of Total Ion Chromatogram obtained from liquid hand soap.

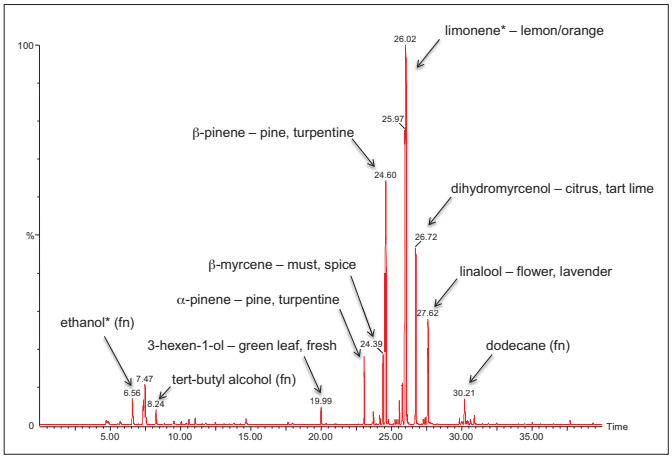


Figure 3. Full Total Ion Chromatogram obtained from fruit scent liquid hand soap.

Conclusions

In this application brief we outline a simple and comprehensive technique for the analysis of fragrance causing organic compounds in various consumer goods. The combination of GC/MS with HS Trap delivers enhanced sensitivity that provides for positive identification of individual components at low concentration levels. This technique has application in both formulation and regulation of these products and can help bring clarity to the issue of "fragrance-free" products.

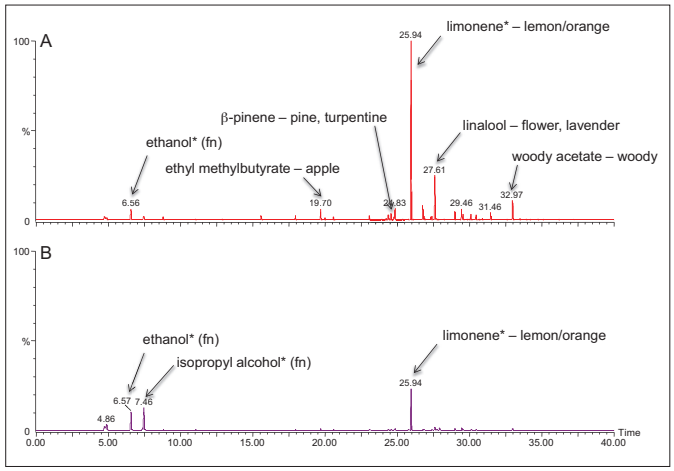


Figure 4. Full Total Ion Chromatograms obtained from (A) fruit scented bar soap and (B) "fragrance-free" facial cleanser bar.

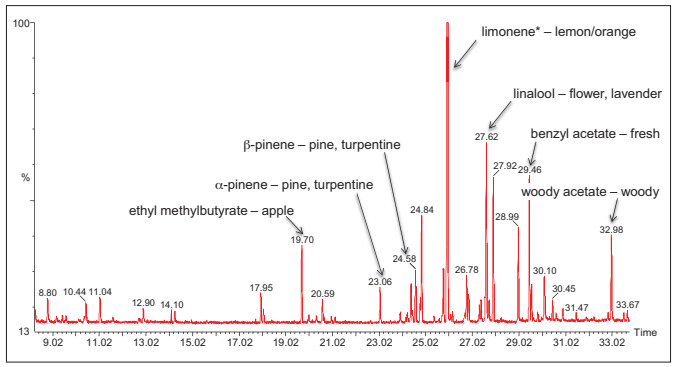


Figure 5. Zoom of Total Ion Chromatogram obtained from "fragrance-free" facial cleanser bar.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

009827_01

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

Ulrich Meier

PerkinElmer, Inc.
Ferdinand-Porsche-Ring 17
D-63110 Rodgau, Germany

Determination of Monomers in Polymers by Multiple Headspace Extraction – GC/MS

Abstract

Polymer production and quality control (QC) requires a variety of analytical testing – one common QC test is the analysis of residual monomers in the final polymer material. This analysis is well suited for headspace sampling because headspace eliminates the sample preparation. The sample is analyzed directly with no need to dissolve the polymer.

The analysis of solid samples with headspace does require compensation for the sample matrix, as calibration standards cannot be created with the same matrix interactions. Multiple headspace extraction (MHE) is a technique to exhaustively extract a sample and calculate the amount of analyte by comparison to an external standard.

This application note will discuss the testing of polymers for residual monomers by MHE-GC/MS. The polymers tested in this application are used for the manufacturing of corrective eyeglass lenses. This material is monitored for acrylic acids, such as methylmethacrylic acid methyl ester (MMA).

PerkinElmer®
For the Better

Introduction

Multiple headspace extraction is used because it is a technique to quantify samples in a solid or difficult matrix without matrix matching the calibration standards. The standard is analyzed, without matrix, in a total evaporation headspace with MHE, determining a response factor (Figure 1, Page 3). MHE is an option the user selects in the method which initiates a specific extraction process in the instrument.

In MHE, the HS method follows the standard extraction steps in the first round of extraction. The vial is equilibrated, pressurized, an injection is made, and the vial is vented. Following the first round of extraction, the MHE method differs from a standard HS method – rather than ejecting the vial after venting, the vial remains into the headspace oven, it is re-equilibrated, and the injection process repeated. The instrument can run this process unattended for the number of times, up to nine, that the operator selects in the method.

With this technique, the sample is extracted until nothing remains – this data is used to determine the total amount of analyte within the sample and the rate of extraction specific to the sample matrix. This data is input into a report template (Figure 2, Page 3) and used to calculate the amount of analyte in each sample of similar matrix.

Experimental

Headspace sample preparation is simple. A sample of known weight is placed into a headspace vial, then the vial is crimped and placed into the PerkinElmer® TurboMatrix™ HS-40 Headspace Sampler (HS). The headspace method was created with the parameters listed in Table 1. The conditions used for the PerkinElmer Clarus® 600 GC/MS system are presented in Tables 2 and 3. A PerkinElmer Elite™-5MS column achieved the necessary separation, while the Clarus 600 MS operated in full-scan mode, providing both qualitative and quantitative data.

Table 1. Headspace Conditions Used in Analysis of Monomers in Polymers.

Headspace Unit:	PerkinElmer TurboMatrix HS-40
Headspace Mode:	MHE
Oven Temperature:	180 °C
Needle Temperature:	185 °C
Transfer Line Temperature:	190 °C
Thermostat Time:	30 min
Vial Pressurization Time:	2 min
Withdraw Time:	0.2 min
Injection Time:	0.03 min
Column Pressure:	120 kPa
Injection Pressure:	160 kPa
Vial Pressure:	160 kPa
Vial Vent:	On
Transfer Line:	Fused Silica (0.32 mm)

Table 2. GC Conditions Used in Analysis of Monomers in Polymers.

Gas Chromatograph:	PerkinElmer Clarus 600 GC		
Analytical Column:	PerkinElmer Elite-5MS (30 m x 0.25 mm x 0.25 µm)		
Injection Port Type:	Programmable Split/Splitless		
Injector Temperature:	200 °C		
Injection Type:	HS-Control		
Carrier Gas Type:	Helium		
Carrier Gas Program:	80 kPa Constant		
Oven Program:	**Temp.**	**Hold Time**	**Rate**
	40 °C	4 min	5 °C/min
	160 °C	5 min	20 °C/min
	260 °C	2 min	End

Table 3. MS Conditions Used in Analysis of Monomers in Polymers.

Mass Spectrometer:	PerkinElmer Clarus 600 T MS
GC Inlet Line Temperature:	180 °C
Ion Source Temperature:	200 °C
Function Type:	Full Scan
Full-Scan Range:	*m/z* 45-350
Full-Scan Time:	0.35 sec
InterScan Delay:	0.05 sec
Solvent Delay:	0 min

Results

MHE is used because it is a technique to quantify samples in a solid or difficult matrix without matrix matching the calibration standards. The standard is analyzed, without matrix, in a total evaporation headspace with MHE, determining a response factor (Figure 1).

In this case, 1 µL of MMA was vaporized in the vial; the amount of 935 µg was calculated with the known density. The sample was exhaustively extracted over several MHE steps, in this case 5.

A sample, 0.688 g, of polymethyl methacrylate (PMMA) was placed into a headspace vial and analyzed by MHE. The total peak area of the analyte was calculated and the concentration of analyte in the sample determined by comparison to this response factor. The necessary calculations for MHE are completed using an Excel® macro available from PerkinElmer and pictured in Figure 2. The sample was determined to have 1726 µg/kg of MMA.

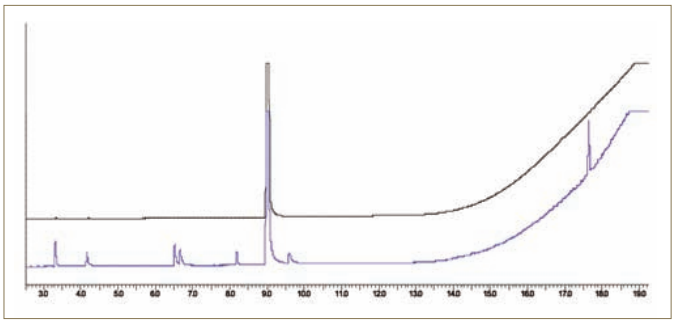


Figure 1. Chromatogram of a MMA calibration standard by total vaporization (top); and chromatogram of the MMA in a PMMA sample (bottom).

In this application, two samples of polycarbonate lenses were analyzed, both demonstrating MMA peaks. In routine analysis of the same polymer, in this case polycarbonate, the number of extraction steps can be reduced and the formula from a previous sample used. This is an acceptable practice, because the matrix will behave in a predictable fashion under specified experimental conditions. Reducing the number of extraction steps speeds up the analytical cycle time and continues to provide the accurate calibration of exhaustive extraction. Two samples of eye-glass lenses were analyzed (Figures 3 and 4) both containing MMA.

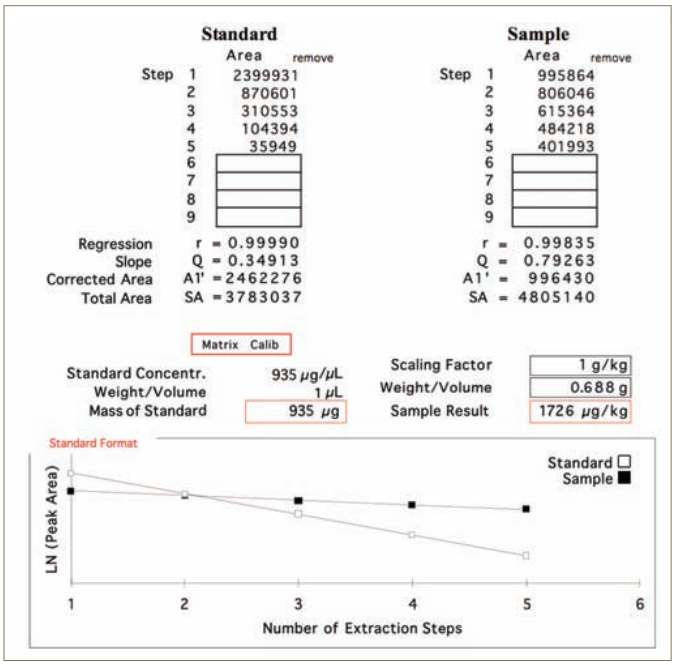


Figure 2. Example of the Excel®-based macro used to calculate concentration of MMA in MHE.

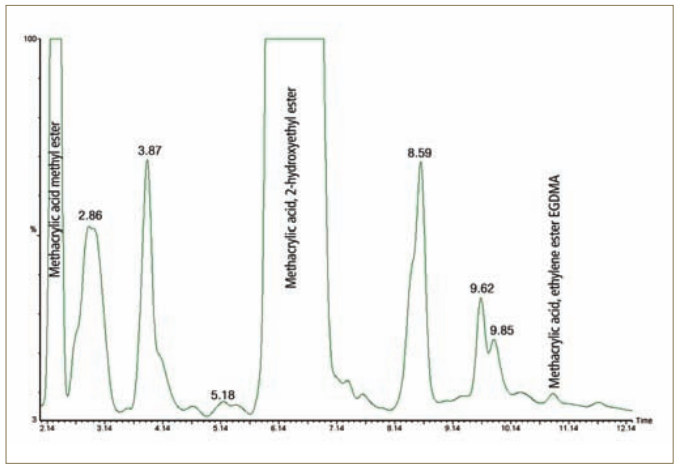


Figure 3. Chromatogram of Sample 1, demonstrating methacrylic acid methyl ester, methacrylic acid-2-hydroxyethyl ester, and methacrylic acid ethylene ester.

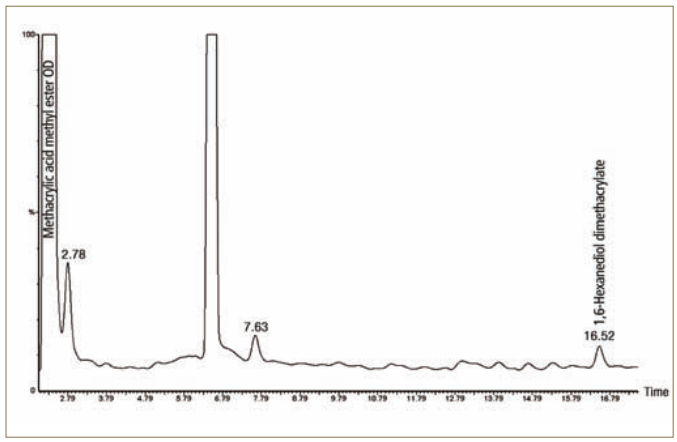


Figure 4. Chromatogram of Sample 2, demonstrating methacrylic acid methyl ester and 1,6-hexanediol dimethacrylate.

Conclusion

This paper has shown that multiple headspace extraction is a suitable and effective technique for the analysis of polymeric materials for residual monomers. The headspace sample introduction reduces sample preparation to an absolute minimum and solvent use is eliminated. Complicated matrix matching of calibration standards was replaced with an automated multiple headspace extraction technique. Additionally, mass spectral characteristics allowed for the identification of each analyte. Several samples were analyzed qualitatively and quantitatively here with a simple and effective headspace-GC/MS technique.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

008739_01

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

Meng Yuan

PerkinElmer, Inc.
Shelton, CT 06484 USA

Increasing Sensitivity in the Determination of Volatile Organics in Toys with Headspace Trap-GC/MS

Introduction

The safety of toys has gained publicity on a global scale, with numerous recalls and new regulations. One aspect of toys which needs to be considered under European Union regulations (EN-71) is the content of volatile organic compounds (VOCs). These compounds, such as benzene and toluene, are residual after the manufacture of various types of polymers, additives and coatings. VOCs are potentially hazardous to the health of children if present at high levels in toys. As a result, it is necessary to accurately determine the level of VOCs in toys to ensure safety.

This application note will present an approach developed to measure VOCs at low levels using headspace trap (HS Trap) sample introduction with gas chromatography/mass spectrometry (GC/MS). This technique is based on European standard method EN-71 Part 11,[1] which specifies details for the analysis of toy and toy-material extracts – included in this method are headspace-GC/MS parameters for VOC analysis. In this application note, the sensitivity of the method presented in EN-71 is improved with the use of headspace-trap instrumentation.

In addition to method optimization and calibration, a variety of toys are analyzed and the level of VOCs determined.

PerkinElmer®
For the Better

Experimental

VOCs in toys are identified and the amount is determined by HS Trap-GC/MS. Samples are heated in a sealed vial to 80 °C, allowing the volatile organics to migrate from the toy material into the headspace of the vial. The sample is equilibrated at this temperature for 40 minutes while this process occurs. Using the automated headspace trap technology of the PerkinElmer® TurboMatrix™ HS Trap, the headspace gas is extracted from the vial, concentrated on an adsorbent trap, and injected into a GC/MS system. In this application, a PerkinElmer Air Toxics trap was used.

The technique is very sensitive because the trap provides focusing before instrument introduction and remains clean because of limited sample contact. Table 1 shows the instrumental setup parameters for the HS Trap-GC/MS system.[2]

The headspace transfer line was passed through the GC injector port and connected to the GC column using a universal capillary-column connector.

Calibration-Standards Preparation

A 20 ng/µL standard stock solution was prepared by diluting 0.200 mL of a 1000 µg/mL VOC standard to 10.0 mL with methanol. From this, a working solution of 1 ng/µL was prepared by diluting 0.50 mL of the 20 ng/µL standard stock solution to 10 mL with methanol. Working calibration standards at 5, 10, 20, 50, and 100 ng/µL were prepared fresh each day.

An internal standard solution of toluene-d8 at 20 ng/µL was prepared by diluting 0.2 mL of a 1000 µg/mL toluene-d8 solution to 10 mL with methanol.

The working curve was prepared by injecting 5 µL and 10 µL of each working calibration standard and 1 µL, 2.5 µL and 5 µL of each standard-stock solution into headspace vials. Additionally, 5 µL of the 20 ng/µL internal standard solution was injected into each headspace vial. All headspace vials were sealed immediately and transferred to the headspace-trap vial tray.

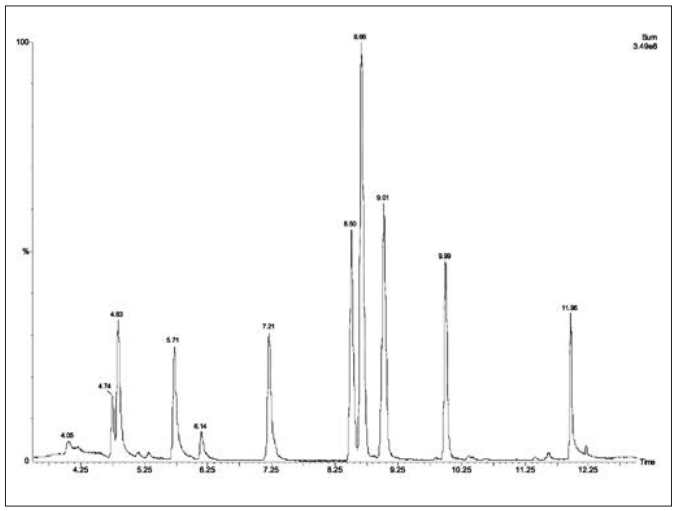


Figure 1. 50 ng injection of a reference standard for volatiles analysis by EN-71 with HS Trap.

Table 1. **Instrument Parameters.**

Sample Introduction	**PerkinElmer TurboMatrix HS-40 Trap**
Needle Temp	90 °C
Transfer Line Temp	120 °C
Oven Temp	80 °C
Trap Low Temp	45 °C
Trap High Temp	280 °C
Dry Purge (Helium)	5 min
Trap Hold Time	6 min
Desorb Time	0.5 min
Thermostatting Time	40 min
Pressurization Time	1 min
Decay Time	2 min
Column Pressure	15 psi
Vial Pressure	35 psi
Desorb Pressure	10 psi
Transfer Line	Fused Silica 2 m x 320 µm (Part No. N9301357)

Gas Chromatograph	**PerkinElmer Clarus® 600 GC**
Headspace Connector	Universal Connector (Part No. N9302149)
Oven Program Initial Temp	50 °C
Hold Time 1	1 min
Ramp 1	15 °C/min to 210 °C
Hold Time 2	5.33 min
Vacuum Compensation	On
Column	Elite™ Volatiles 30 m x 0.25 mm x 1.4 µm (Part No. N9316388)
Carrier Gas	Helium
Mass Spectrometer	**PerkinElmer Clarus 600 MS**
Mass Range	45-220 u
Solvent Delay Time	0.1 min
Scan Time	0.20 sec
InterScan Delay Time	0.05 sec
Transfer Line Temp	200 °C
Source Temp	200 °C
Multiplier	400 V

Results

Five calibration levels are recommended for method EN-71 Part 11. The standard deviation of response should be below 15 %RSD (relative standard deviation). Table 2 shows %RSD data of a 50 ng standard. All compounds meet the specified criteria of RSD less than 15%. Figure 1 is an example chromatogram of a 50 ng standard injection.

Toluene-d8 was used as an internal standard. Peak-area ratio was used to calculate amounts of VOC.

The peak-area ratio for the component in the sample was calculated by dividing the peak area of the component (target ion) by the peak area (target ion) of the internal standard toluene-d8 (IS):

$$\text{peak-area ratio} = \frac{\text{peak area of the component ion}}{\text{peak area of the IS ion}}$$

Amounts of VOC (concentration in ng) were calculated by plotting the peak area ratio in the following calibration functions:

$$\text{conc(x) in ng} = \frac{\text{y(peak-area ratio)} - b^{y=ax+b}}{a}$$

Method detection limits (MDL) were calculated to give an indication of the measurement capability. The quantification limit is generally 10x above the MDL. The method detection limits were calculated using the following equation:

$$\text{MDL} = t_{(n-1,\ a\ =\ .99)}\ x\ s$$

An empty vial was analyzed to determine the baseline and seven samples were prepared at 5 ng. Each individual MDL was obtained by multiplying the standard deviation by the 99% t-statistic. Table 2 also shows the list of calculated MDLs.

Following the calibration of the system, 4 toy samples (toy ball, noise putty, modeling compound, crab) obtained from the local market were analyzed. The resultant chromatogram for the analysis of the toy-ball sample is pictured in Figure 2. The sample preparation with headspace analysis is very simple.

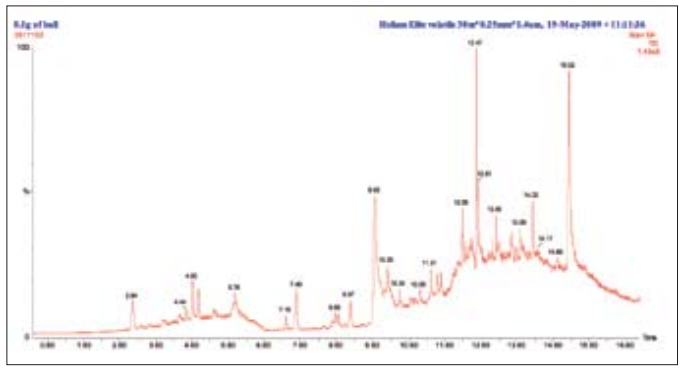


Figure 2. Total ion chromatogram of 0.1 g of toy-ball sample.

Table 2. **Calibration Table for 12 Volatiles.**

Name	Retention Time	Quantifier Ion	Qualifier Ion 1	Qualifier Ion 2	%RSD	r^2	MDL (µg/g)
Dichloromethane	4.041	49	86	84	9.51	0.9962	0.002
Benzene	5.703	78	77	52	4.41	0.9939	0.002
Trichloroethylene	6.128	130	132	95	4.31	0.9970	0.003
Tolune-d8	7.134	98	100	–	Internal Standard		–
Toluene	7.192	91	92	65	2.62	0.9995	0.002
Ethylbenzene	8.487	91	106	51	5.45	0.9993	0.002
m,p-Xylene	8.649	91	106	105	6.42	0.9992	0.002
Cyclohexanone	8.972	55	98		6.76	0.9979	0.003
o-Xylene	8.997	91	106	105	5.94	0.9979	0.002
1,3,5-Trimethylbenzene	9.973	105	120	119	4.82	0.9983	0.002
Nitrobenzene	11.595	77	123	51	4.91	0.9956	0.010
Isophorone	11.958	82	138	54	8.42	0.9959	0.009

Table 3. **VOC Content (µg/g) for Four Toy Samples.**

	Sample	(µg/g)
Ball	Dichloromethane	68.0
	Benzene	11.1
	Toluene	26.5
	Ethylbenzene	10.5
	m,p-Xylene	10.4
	Cyclohexanone	94.3
	o-Xylene	13.2
	1,3,5-Trimethylbenzene	15.4
	Isophorone	15.1
Noise Putty		
	Benzene	26.1
	Toluene	19.4
	Cyclohexanone	84.5
	Nitrobenzene	9.5
Modeling Compound		
	Benzene	33.4
	Ethylbenzene	71.6
	m,p-Xylene	20.3
	o-Xylene	16.0
Crab	Benzene	20.1
	Toluene	62.8
	Ethylbenzene	16.4
	m,p-Xylene	11.9
	Cyclohexanone	303.0
	Isophorone	67.7

A known amount of the toy was cut into small (1 mm x 1 mm) pieces with a razor blade and placed into the headspace vial, internal standard was added and the vial was capped. Detectable solvents were seen in each sample (Table 3) – however, the levels determined in this application were below regulatory limits.

Discussion

Headspace trap is an additional sample-handling technology to improve upon the sensitivity of static headspace. In this article, HS Trap demonstrated high sensitivity and linearity across the range of 25–500 ng.

The HS Trap uses heat to extract compounds out of the toys into the headspace, offering three advantages: easy sample preparation, high sensitivity, and no cross-contamination of samples. After the analytes are extracted, the trap is dry-purged to eliminate the moisture. Then the trap is heated and carrier gas transfers a narrow band of the desorbed analytes into the GC/MS system.

Table 4 compares the guideline of EN-71 for selected compounds with the MDL achieved using this method. The method developed provides sufficient capability to measure with confidence at the concentrations lower than regulatory level.

Table 4. **MDL Guideline of EN-71 and MDL in this Method.**

	MDL in EN-71 (ng)	MDL in this Method (ng)
Dichloromethane	10	2.2
Benzene	30	1.7
Trichloroethylene	20	3.1
Toluene	20	2.2
Ethylbenzene	40	1.5
m,p-Xylene	30	1.8
Cyclohexanone	30	2.8
o-Xylene	20	1.9
1,3,5-Trimethylbenzene	10	2.0
Nitrobenzene	60	10.2
Isophorone	40	9.6

Conclusion

This application note shows that the Clarus 600 GC/MS system with TurboMatrix HS Trap meets and exceeds the requirements for method EN-71 Part 11, including minimum detection limits and calibration requirements. The calibration of the system was demonstrated across the range of 25–500 ng, with a linear response. Toy samples were analyzed and the VOC content was determined. Advantages of the headspace-trap technology for this application include ease of use, high sensitivity, ease of disposable sample vials, and no cross-contamination of samples. Plus, the novel GC oven design of the Clarus 600 GC improves separation and decreases run time.

References

1. EN-71 Part 11 "Safety of toys - Part 11: Organic chemical compounds – Methods of analysis" 2004-07-01

2. Yuan, Meng "Measuring Volatile Organic Compounds by Headspace Trap GC-MS in the Beijing Food Laboratory", American Laboratory, On-Line Edition, April 2009

3. Yuan, Meng "Reliably Detect 86 of Pesticides in a Single Run Down to 10pg with GC/MS in the Beijing Food Lab", PerkinElmer Field Application Report, available from www.perkinelmer.com

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

008957_01

APPLICATION NOTE

Gas Chromatography

Authors:

Timothy D. Ruppel

Gerald Hall

PerkinElmer, Inc.
Shelton, CT

Determination of Ethylene Glycol in Used Engine Oil by Headspace-Gas Chromatography

Introduction

The presence of ethylene glycol in used motor oil is an indication of antifreeze coolant leakage into the crankcase of an internal combustion engine, thus predicting engine-wear problems. Several options for the determination of glycols currently exist, including colorimetric tests which are easy to perform, but subjective in interpretation and not particularly sensitive, fast or cost effective. Gas chromatography (GC) can also be used for analysis, but the ethylene glycol is difficult to detect and quantify due to its low molecular weight, low volatility and high polarity. Ethylene glycol chromatographic peak shape is often difficult to control and carryover can be a problem.

PerkinElmer®
For the Better

Injecting used engine oil directly into a gas chromatograph for the determination of ethylene glycol introduces high molecular-weight oil and non-volatile components into the injector and the column. Consequently, the chromatography is very long, the column lifetime is shortened and the sample throughput is low, since high boiling components from the oil matrix must elute before the next injection. ASTM Method D4291-98 specifies diluting the oil sample with hexane, extracting the glycol into water and analysis by GC. This is a very labor intensive sample preparation procedure and an unforgiving chromatographic method, whereby water and the polar analyte are injected on-column.

An alternative to ASTM Method D4291-98 is investigated here, which involves a very simple in-situ derivatization technique that allows the glycols to be made more volatile and less polar. Headspace (HS) extraction is used to isolate the glycols from the complex sample matrix and inject into a gas chromatograph for rapid separation and quantification without the oil matrix. The result is a rapid, high-throughput method capable of analyzing hundreds of samples per day for ethylene glycol and propylene glycol in motor oil.

Experimental

The system used for this work and the chromatographic conditions are shown in Table 1.

Standards Calibration

Prepare glycol standards over the quantification range of 0.01% to 0.2% w/w in motor oil.

Sample Preparation

Add 100 µL of the sample oil into a 22 mL headspace vial. Add 5 mg of derivatizing reagent (PerkinElmer Part Number N9301741). Seal the vial for headspace analysis. A positive displacement pipette is used to accurately dispense oil samples due to viscosity. Vials can be preinoculated with the derivatizing reagent for faster sample preparation.

Results

The derivatization goes to completion quickly and easily under the heated headspace conditions. The headspace extraction removes the volatile components from the sample matrix for a very clean injection into the chromatographic column. The high molecular weight motor oil, soot and other non-volatiles are never introduced into the column. Peak retention is optimized to resolve ethylene glycol from early eluting derivatization by-products. The isothermal GC method allows for a three minute time or less between injections as shown in Figure 1. This is a 10-fold increase in throughput when compared with current ASTM methods.

Excellent quantitative linearity (0.997), shown in Figure 2 and precision (3% RSD) were demonstrated over the range of 0.01% to 0.2% ethylene glycol. System maintenance consists of headspace o-ring seal replacement after roughly 2000 injections.

Table 1. Experimental conditions using Clarus® 580 GC and TurboMatrix™ HS driven by Waters® Empower® 3 Chromatography Data Software (CDS).

Headspace Sampler	**PerkinElmer TurboMatrix™ HS-40 or HS-110**		
Temperatures (°C)	Sample oven: 120	Needle: 150	Transfer line: 160
Timing (min)	Thermostat: 18	Inject: 0.01	Withdraw: 0.5
	GC cycle time: 3	Period from injection to injection: 3	
Pressure	40 psig Helium	Pressurize: 1.0 min	Transfer line: 320 µm deactivated fused silica
Vials	22 mL headspace vials, PTFE-lined silicone septa		
Gas Chromatograph	PerkinElmer Clarus® 580 GC		
Injector	Split/Splitless with PPC	180 °C 25 psig	Split: 50 mL/min
Detector	FID with PPC	Range: x1 Attn: x32	Temperature: 250 °C
	Air: 450 mL/min	H2: 45 mL/min	
Column	15 m x 0.32 mm ID x 0.25 µm Elite-5	100 °C for 2 min (isothermal)	Equilibration Time: 0 min
Software	Empower® 3 CDS		

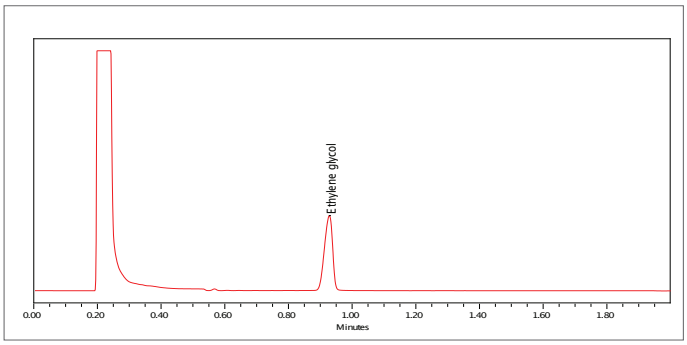


Figure 1. Chromatogram shows elution of ethylene glycol.

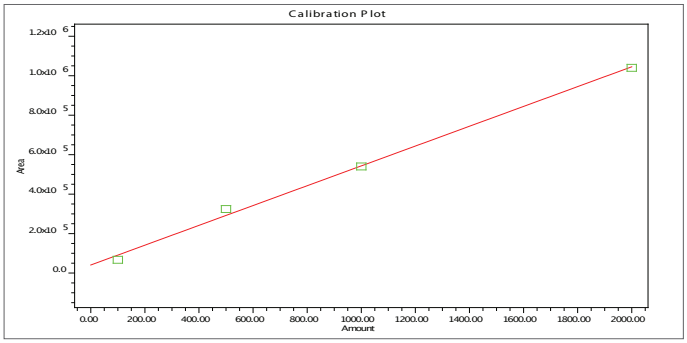


Figure 2. Calibration curve of ethylene glycol.

Conclusion

A practical method has been developed and tested which will allow for high throughput testing of ethylene glycol as a diluent in used motor oil. Up to 400 samples per day can be analyzed using this method, which provides results directly comparable to established methods. The headspace injection of used motor oil means less sample preparation, high throughput and less human error. Cost analysis (without labor and initial startup costs) has been calculated to be less than $0.70 US per sample.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

012090_01 PKI

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

Lee Marotta

PerkinElmer, Inc.
Shelton, CT 06484 USA

The Determination of Low Level Benzene, Toluene, Ethyl Benzene, and Xylenes (BTEX) in Drinking Water by Headspace Trap GC/MS

Introduction

BTEX is a grouping of structurally similar volatile organic compounds including benzene, toluene, ethyl benzene and the three xylene isomers. These compounds are known pollutants and are typically found near petroleum production and storage sites. BTEX are regulated toxic compounds while benzene is also an EPA target carcinogen. The investigation of these compounds, especially in drinking water at low levels, is critical to protect public health. This application note focuses on exceeding the current EPA detection limit requirement for BTEX while meeting and/or exceeding all other criteria in EPA method 524.2 for these analytes.

Instrumentation

A PerkinElmer® TurboMatrix™ Headspace (HS) sample handling system was used to volatilize and concentrate BTEX in water samples. To enhance detection limits, an inline trap was employed, which focused these analytes prior to injection onto the analytical column. A PerkinElmer Clarus® SQ 8S Gas Chromatograph Mass Spectrometer (GC/MS) configured with the standard capacity turbo molecular pump was the analytical system used.

PerkinElmer®
For the Better

The GC provided rapid cool down to shorten the period between injections (more samples analyzed in a "clock"). Using the temperature programmable low volume inlet improved peak efficiency by reducing "dead" volume and resulted in enhanced resolution, faster chromatography and improved detection limits. The PerkinElmer SQ 8S MS operating in full scan mode was used for this analysis, providing up to 20 times improved detection limits for this application.

Experiment and Results

The experimental conditions are presented in Tables 1 – 3. An Elite 624 column (20 m x 0.18 mm x 1.0 µm) was used in this application, which is also the column of choice for several laboratories analyzing volatile organic compounds by HS Trap. The narrow bore, shorter, efficient column aided in enhancing peak efficiency for shorter analysis time and signal-to-noise performance. Analyte equilibrium was empirically determined to be eight minutes.

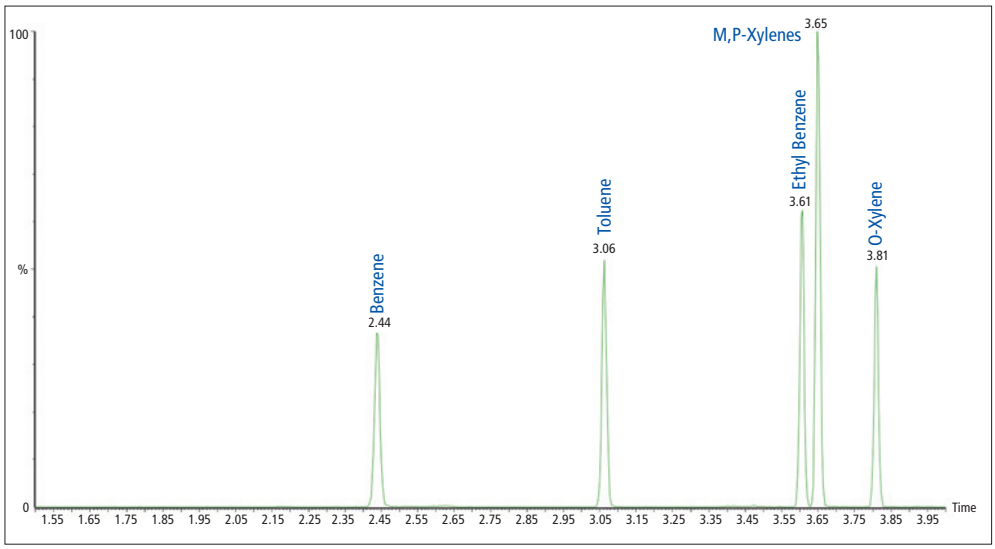


Figure 1. An example mass at 4.0 ppb acquiring in full scan.

Figure 1 presents a sample chromatogram recorded at 4.0 parts per billion (ppb) acquiring in full scan. Analytical results are displayed in Table 4 and include the 12-point calibration curve results, signal-to-noise recorded for the 0.02 ppb standard and precision measurements performed using 1.0 ppb standards.

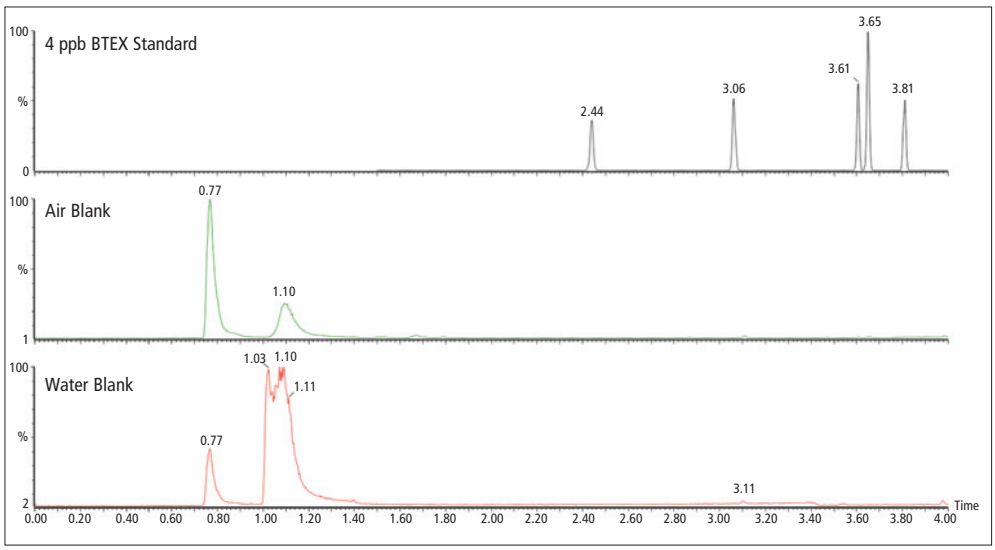


Figure 2. Water blank is the bottom chromatogram; air blank is the middle chromatogram; 4.0 ppb standard demonstrating separation from water is the top chromatogram.

Figure 2 illustrates the excellent water management ability of the HS Trap system. In these experiments, a two minute dry purge completely isolated the water from the target analytes. A three minute dry purge reduced water levels to baseline intensity, however a quicker analytical method was the goal of this application so a longer dry purge was avoided.

***Table 1.* Headspace Trap Conditions.**

Headspace System	TurboMatrix HS Trap
Sample Temperature	80 ˚C
Needle Temperature	110 ˚C
Transfer Line Temperature	120 ˚C
Trap Low/Trap High	35 ˚C to 260 ˚C
Equilibration Time	8 min
Dry Purge	2.0 min
Trap Hold	2.5 min
Trap Material	Carbopack B & X
Outlet Split	n/a
All HS Pressures	23.3 psi

***Table 2.* Gas Chromatograph Conditions.**

GC/MS	Clarus SQ 8S
Column	Elite 624-20 m x 0.18 mm x 1.0 µm
Oven	40 ˚C for 0.5 min, then 35 ˚C/min to 185 ˚C
Injector (PSS)	Temp Programmable Split/Splitless at 180 ˚C
Inlet Configuration	HS Mode turned ON
Carrier Program (He)	1 mL/min for 0.4 min, then 0.7 mL/min
Split Flow from GC	n/a

***Table 3.* Mass Spectrometer Conditions.**

Ionization Mode	Electron Impact
Acquisition	Full Scan
Mass Range	35 to 350 amu
Filament Delay	1.5 min
Scan Speed	0.15 sec
Interscan Delay	0.04 sec
Run Time	4 min
Ion Source Temperature	200 ˚C
Transfer Line Temperature	200 ˚C

***Table 4.* Analytical Results.**

	s/n at 0.02 ppb	Linearity, r^2 0.02 to 60 ppb	Precision at 1 ppb (n=7)
Benzene	370 to 1	0.9996	2.85%
Toluene	550 to 1	0.9994	2.76%
Ethyl Benzene	578 to 1	0.9993	2.53%
m,p-Xylenes*	670 to 1	0.9997	1.07%
o-Xylene	240 to 1	0.9994	3.86%

**The amounts are double for meta and para xylenes since they co-elute.*

Conclusion

In this application note the analysis of BTEX in water samples by HS Trap GC/MS using the Clarus SQ 8S Mass Spectrometer was performed. An analytical technique with a short cycle time and excellent performance is described. The analysis of BTEX using a mass spectrometer such as the Clarus SQ 8S not only allows the benefit of added sensitivity (therefore lower detection limits) but also additional analyte confirmation, which provides molecular level identification thus limiting false positives. Water management in the system was exceptional, and water from the matrix could be fully eliminated with an additional minute of dry purge time. With the sample preparation and mechanical advantages HS trap delivers versus purge and trap systems, greater uptime using this approach can be expected as well. This solution provides contract laboratories with compelling reasons and benefits for investing in this system, such as longer intervals between system maintenance, operator ease of use, fast cycle times (72 samples analyzed within a 12 hour clock), instrument and method robustness and optimal analytical performance.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

010004_01

APPLICATION NOTE

Gas Chromatography

Authors

Lee Marotta

Dennis Yates

PerkinElmer, Inc.
Shelton, CT USA

Methane, Ethylene, and Ethane in Water by Headspace-Gas Chromatography (HS-GC) with Flame Ionization Detection (FID)

Introduction

The rapid development of natural gas from unconventional sources in North America has created an energy "gold rush" not seen in contemporary times. The advent of horizontal drilling technologies and hydraulic fracturing has made this production economical and presents an energy source of sufficient magnitude that could last 100 years.

The technology presents a number of environmental challenges as the wells are drilled vertically through aquifers on their way to the deep shale deposits thousands of feet under the surface, and then turned horizontally and drilled another several thousand feet through the shale deposit. Herein lies the challenge: in the process of drilling the wells and preparing them for production (including "fracking" to optimize production), opportunities arise for contamination of the clean drinking water aquifers with methane and other low molecular weight organics (e.g., propane and ethane). Correctly drilled and cemented well bores should not be an issue, but any errors in engineering could result in contamination.

It is also possible that methane already exists at a low concentration in the aquifer from diffusion of the gas occurring naturally. There is a need (by property owner and lease holder) to confirm the level of gas in the aquifer before and during drilling, and also after the well is placed into production.

PerkinElmer®
For the Better

Traditionally, methane in water is determined using U.S. Environmental Protection Agency (EPA) method RSK 175 (RSKSOP175, 2004) or an alternative (Vandegrift, 1998). PerkinElmer's TurboMatrix™ HS and Clarus® 680 GC combination offers a simple, economical and reliable measurement technique to determine methane and other target gases in water. This application note summarizes the experimental approach and subsequent results to confirm the viability of the method.

Instrumentation

A PerkinElmer® TurboMatrix Headspace (HS) connected to a PerkinElmer Clarus 680 Gas Chromatograph (GC) with dual flame ionization detectors (FID) were used in these experiments.

Since detection is performed using an FID, the technique of column confirmation may be employed to confirm identity of components. An Elite-Q PLOT column with dimensions 30 m x 0.32 mm (PerkinElmer Part No. N9316359) was used for quantitation and the Elite-U PLOT column with dimensions 30 m x 0.32 mm was used for confirmation. These columns were directly connected to the deactivated fuse silica headspace transfer line via a "Y" connector.

Experimental Conditions

A stock standard was used for these experiments (Supelco® Part No. 23437). This stock standard contained methane, ethylene, acetylene and ethane in approximately one molar percent concentration in nitrogen for each component.

The headspace and GC operating conditions are displayed in Table 1.

To validate the method, the following experiments were performed:

1. **Background:** Blank air and water were investigated for interferences. Since methane may be present in ambient air, four (4) 22 mL Headspace Crimp Vials (PerkinElmer Part No. N9306079) containing 15 mL of the deionized (DI) water, used in preparing standards, were investigated to determine the concentration of the methane in the blank samples.
2. **Calibration:** A five-point calibration curve was created establishing method linearity and reporting limits. Five (5) headspace vials were prepared with 15 mL of DI water then capped using PTFE silicone septa. A 2 µL, 5 µL, 10 µL, 20 µL and 50 µL volume of the stock standard was inserted through the septum (PerkinElmer Part No. N9303992) into the water of five of the vials, respectively, attaining concentrations as described in Table 3.
3. **Accuracy:** Four (4) water samples were prepared as quality controls from 5 to 50 ppb to confirm method accuracy.
4. **Precision:** Five (5) 40 ppb standards were prepared from the stock standard, and analyzed for precision.

Table 1. **Headspace and GC Conditions.**

HS Conditions	
Sample Temperature:	90 °C
Equilibration Time:	10 min
Needle Temperature:	110 °C
Transfer Line Temperature:	120 °C
Inject Time:	0.06 min
Withdrawal Time:	0.4 min
Pressurization Time:	1.0 min
HS Mode:	Constant
HS Pressure:	20 psi
GC Conditions	
Oven Temperature	
Initial Temperature:	40 °C
Initial Hold:	4.5 min
Ramp:	40 °C/min
Final Temperature:	205 °C
Final Hold:	1 min
Detector (FID)	
Detector Temperature:	240 °C
Air Flow:	400 mL/min
Hydrogen Flow:	40 mL/min
Range:	1
Attenuation:	-6 (or 1)

Note: The columns are directly connected to the HS transfer line; therefore, inlet parameters are not applicable.

Results

Figure 1 demonstrates separation of the four gases in the stock standard on the Elite-Q PLOT column. The concentration of the standard represented in Figure 1 is 10 parts per billion (ppb). Since acetylene is not a target analyte of this application, and acetylene is not found in samples, it is recommended that a standard mix be used not containing this analyte to avoid integration challenges between ethylene and acetylene.

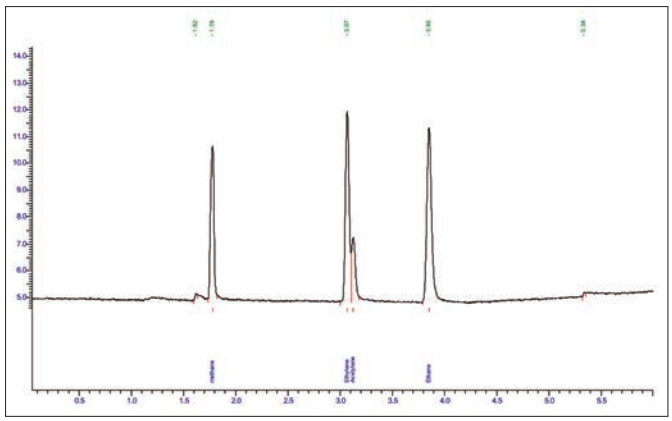


Figure 1. 10 ppb standard (Q PLOT).

Figure 2 is a chromatogram of a water blank (15 mL volume). To compensate for the methane present in ambient air, this point was incorporated on the calibration curve to subtract for the presence of methane in air. Since the headspace vials are sampled in air, this air is trapped in the vial. The concentration of methane in air is below the reporting limit. Table 2 tabulates the precision of methane in the blank.

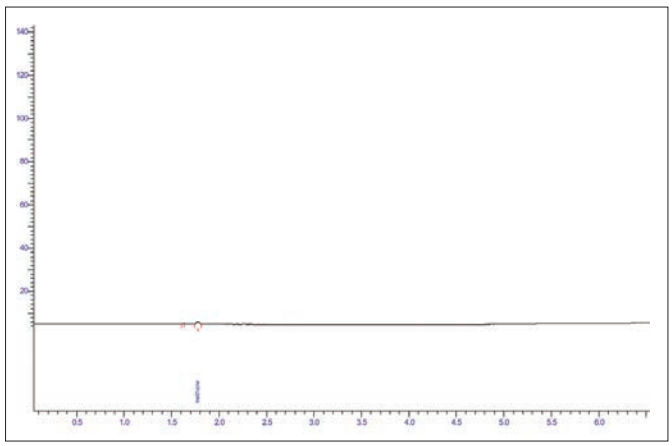


Figure 2. Chromatogram of blank (15 mL water).

Figure 3 graphically demonstrates the results of the external standard calibration curve of each component. The linearity achieved was excellent with a correlation coefficient (r^2) of 0.9996 and better. Table 3 contains the concentrations of the standards used to prepare these curves.

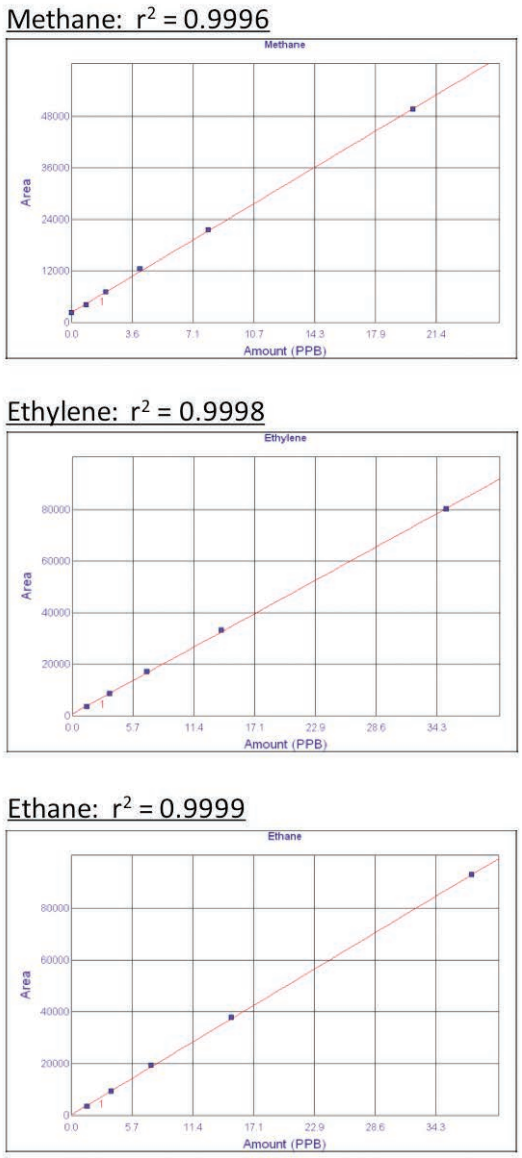


Figure 3. Calibration curves.

Table 2. **Repeatability of Four Blanks for Methane.**

Sample Name	Area (Methane)
15 mL Water Blank	2093.5
15 mL Water Blank	2163.7
15 mL Water Blank	2337.4
15 mL Water Blank	2124.3
Average	**2179.7**
%RSD	**5%**

Table 3. **Standard concentrations in Parts Per Billion (ppb) or µg/L.**

Level No.	Methane	Ethylene	Ethane
1	0.80	1.40	1.50
2	2.00	3.50	3.75
3	4.00	7.00	7.50
4	8.00	14.00	15.00
5	20.00	35.00	37.50

Table 4. **Results from four (4) Quality Control Samples.**

Methane			Ethylene			Ethane		
Actual Amt.	Calc. Amt.	%Dev	Actual Amt.	Calc. Amt.	%Dev	Actual Amt.	Calc. Amt.	%Dev
2.00	2.05	2.50	3.50	3.43	-2.00	3.75	3.59	-4.27
10.00	10.72	7.20	17.50	18.68	6.74	18.75	19.91	6.19
14.00	15.19	8.50	24.50	26.40	7.76	26.25	28.43	8.30
20.00	20.69	3.45	35.00	36.44	4.11	37.50	39.14	4.37

Table 5. **Repeatability of Peak Area Calculations Using Level 4 Concentration (Refer to Table 3).**

Conc. Level	Methane Area	Ethylene Area	Ethane Area
4	43180	70067	80441
4	44330	70199	81390
4	43421	67911	79164
4	44331	71017	82016
4	42184	66722	76234
Average	**43489**	**69183**	**79849**
% RSD	**2.1**	**2.6**	**2.9**

Table 4 tabulates the results of the quality control study. These controls were processed using the five-point calibration for each component.

Table 5 represents the results of the precision study at Level 4.

Discussion

In this experiment, the blank was used as a point on the calibration curve to correct for the presence of methane in ambient air (subtracting the blank), which improves accuracy for the low level methane amount and allows for very easy sample preparation essentially filling the vial with a known amount of water and capping it.

The reporting limit of 1 ppb methane in water was achieved. The lowest point of the curve prepared for this application for methane was 0.8 ppb, and 1st order is maintained through this point.

The recoveries obtained in this experiment from four (4) quality control samples are from 90% to 98%. This accuracy is excellent and incorporates errors due to method and operator. Since these gaseous standards and quality control samples are prepared manually with a gas-tight syringe human error is a contributory factor; therefore, the accuracy is exceptional.

Instrument and method repeatability (precision) is 2.1% for methane which is an acceptable repeatability for this application.

Conclusions

Examining the results of these experiments, the PerkinElmer TurboMatrix HS and PerkinElmer Clarus 680 GC provide a viable solution determining methane and other low molecular weight hydrocarbons in water delivering accuracy, precision and ease of use.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

010316A_01

APPLICATION NOTE

Gas Chromatography/
Mass Spectrometry

Author

Padmaja Prabhu

PerkinElmer, Inc.
Shelton, CT USA

Detection and Quantification of Formaldehyde by Derivatization with Pentafluorobenzylhydroxyl Amine in Pharmaceutical Excipients by Static Headspace GC/MS

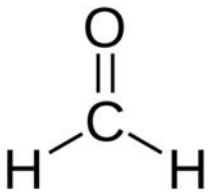


Figure 1. Structure and properties of formaldehyde.

Introduction

Although considered pharmacologically inert, pharmaceutical excipients have been shown to interact with active drug substances to affect the safety and efficacy of drug products.[1] Therefore, there is an increasing awareness of the necessity to understanding interactions between excipients and the active pharmaceutical ingredient (API) in finished dosage forms. One of the areas of major concern is the potential chemical interaction between impurities in the excipient with the drug molecules, leading to formation of reaction products.[2] Even trace amounts of reactive impurities can cause significant drug stability problems as the quantity of excipients in a formulation often far exceeds that of an API on a weight and molar basis. Trace amounts of reaction products can then easily exceed 0.2% qualification thresholds for a degradation in many drug products.[1] Formaldehyde present in excipients has been implicated in the degradation of several drug products where it can form adducts with primary and/or secondary amine groups.[2] It has also been reported that formaldehyde can induce cross-linking in gelatin capsules causing an adverse effect on in-vitro dissolution rates of drugs. Because of the extremely high reactivity of aldehydes, a timely evaluation of their presence in excipients during formulation design is essential to avoid unexpected drug stability problems in later stages of product development.

PerkinElmer®
For the Better

The work presented here describes the development of a systematic approach for the detection and quantification of formaldehyde in excipients to provide additional insight to formulation development. Specifically, a static headspace GC (SHS-GC) method based on PFBHA derivatization and Electron Impact ionization (EI) Mass Spectrometry (MS) employing both scan and selected-ion monitoring (SIM) simultaneously was developed and employed to screen excipients for the presence of formaldehyde.

Formaldehyde (Figure 1) is a colorless gas with a characteristic pungent odor. It is a volatile organic compound having molecular weight of 30 amu and low boiling point (-21 °C). Because of its high volatility, formaldehyde levels in various matrices can be easily determined by headspace methods with a fair degree of accuracy.

Experimental

The PerkinElmer® Clarus® 680 Gas Chromatograph, Clarus Mass Spectrometer and a TurboMatrix™ Headspace 40 system were used for this application. Tables 1, 2 and 3 present the detailed operating parameters of the GC/MS and the headspace system. The instrument interaction, data analysis and reporting was completed with the PerkinElmer TurboMass™ data system.

Stock solution: 50 mg of 40% formalin was weighed into a 100 mL flask and diluted to volume with bottled water to make 200 µg/mL mixture of formaldehyde.

Solution A: 5 mL of the stock solution was diluted to 100 mL with bottled water to give a concentration of 10 µg/mL.

Solution B: 5 mL of the mixture A was diluted to 100 mL with bottled water to give a concentration of 0.5 µg/mL.

Internal standard solution (I.S.): 50 mg of cyclohexanone was weighed into a 50 mL flask and diluted to volume with bottled water to make 1000 µg/mL. 10 mL of this solution was diluted to 250 mL with water and finally 20 mL of this was diluted to 1000 mL with water. This I.S. solution was used for dilution of calibration curve standards and preparation of solution.

PFBHA solution: 100 mg of PFBHA was dissolved in bottled water and 1 mL of this was added to each of the HS vials for derivatizing formaldehyde in samples and standard.

Calibration curve: Varying volumes of 0.5 µg/mL formaldehyde (solution B) was diluted in water to achieve the final standard concentration presented in Table 4. The solutions were made up to the final volume with I.S. solution. 1 mL of PFBHA solution was added to each of the vials for derivatization. 1 g of NaCl was added to each of the vials to decrease the miscibility of formaldehyde in water and enhance equilibration in the headspace.

Table 1. **Details of GC method.**

Instrument Details	**Clarus 680 Gas Chromatograph**
Analytical column	PerkinElmer Elite -5 MS (30 meter, 0.25 mm i.d., 0.25 µm df)
GC column flow	1.2 mL/min helium at constant flow mode
GC inlet temperature	190 °C
Split ratio	5:1
Oven temperature	50 °C hold for 3.0 min, 7 °C/min to 150 °C and hold for 5.0 min, 40 °C/min to 280 °C and hold for 5.0 min runtime is 20 min.

Table 2. **Details of HS method.**

Instrument Details	**TurboMatrix HS-40**	
Temperatures	Thermostatting	60 °C
	Needle	100 °C
	Transfer line	130 °C
Time	Injection	0.2 min
	Pressurization	0.5 min
	Withdrawal	0.2 min
	Equilibration	20 min
	Cycle	20 min
Options	Vial vent	ON
	Shaker	ON
	Operation mode	Constant
	Injection mode	Time
	Hi psi injection	ON
PPC	Inject	25 psi
	Column/headspace pressure	25 psi

Table 3. **Details of MS conditions.**

Instrument Details	**Clarus Mass Spectrometer**
Source temperature	200 °C
Interface temperature	200 °C
Scan range	m/z 40-380
SIM mode: ions monitored	178, 181, 197
Scan time	7.5-30 min
Multiplier	550

Table 4. **Scheme used for the creation of a five level calibration.**

Calibration level #	Concentration of formaldehyde in ppb	Standard solution added in mL	Final vol. (mL)
1	10	2 (from solution B)	100
2	20	4 (from solution B)	100
3	30	0.6 (from solution A)	100
4	40	0.8 (from solution A)	100
5	50	1.0 (from solution A)	100

*1 g of NaCl was added to each of the headspace vials.

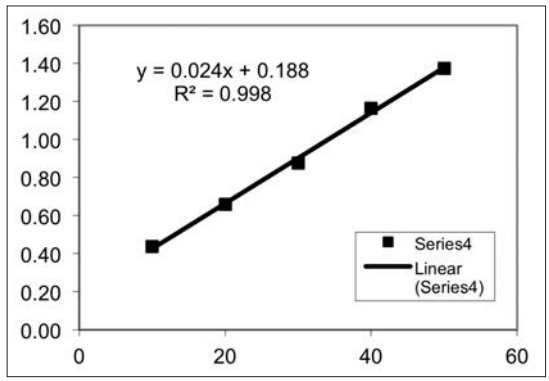


Figure 2. Calibration curve for formaldehyde.

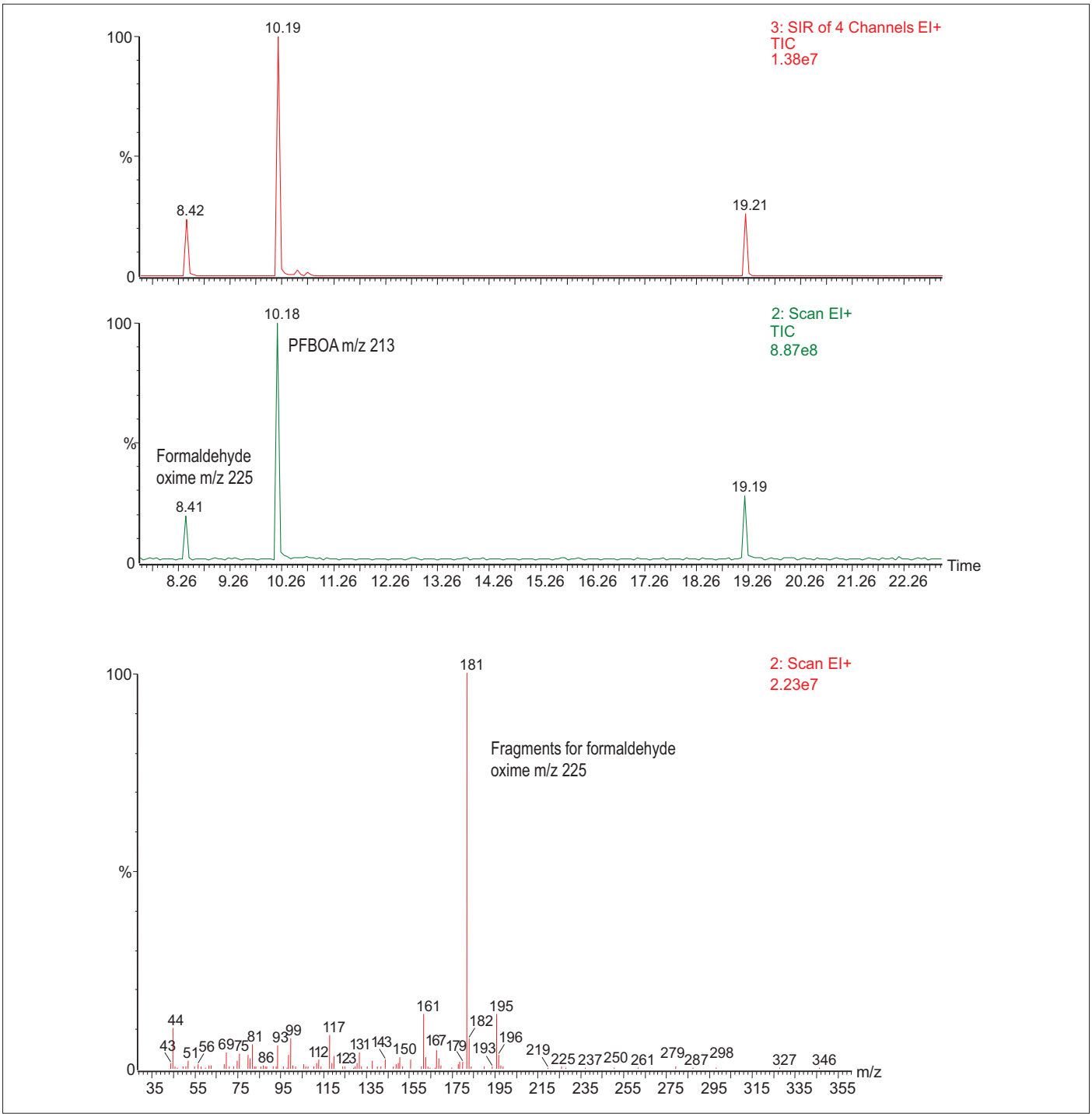


Figure 3. Example chromatogram and MS spectrum for formaldehyde in standard.

Calibration: The MS was calibrated across the range of 10.0 to 50 ppb of formaldehyde, each calibration point was run in triplicate to demonstrate the precision of the system. The calibration curve for formaldehyde is depicted in Figure 2. The average coefficient of determination for a line of linear regression was 0.998 for formaldehyde. Precision of the system across the calibration range is excellent. The percent relative standard deviation is shown in Table 5. Chromatograms and the spectrum from the analysis of standard material are shown in Figure 3. The peak at retention time 8.41 is that of formaldehyde oxime and the unreacted PFBOA elutes at 10.82 min.

Table 5. **% RSDs for three sets of linearity experiment.**

Concentration of formaldehyde in ppb	Mean peak area ratio average relative response (n=3)	%RSD
10	0.44	9.23
20	0.66	4.42
30	0.88	6.29
40	1.17	10.76
50	1.37	9.77

The precision of the method was measured at 5 ppb. The loss of precision at 5 ppb indicates the detection limit of this method to be approximately 5 ppb RSD 19.30%.

Sample preparation

Plasdone, pregelatinised starch, povidone, polyethlene glycol (PEG-400), microcrystalline cellulose and lactose samples were obtained from one of the local pharmaceutical firms (Getz Pharma®, India). The headspace sample preparation is relatively very easy. A weighed sample (0.05 g to 0.2 g) was placed in a headspace vial and 10 mL of I.S. solution was added to each of the vials; 1 g of NaCl and 1 mL of PFBOA solution was added to each of the vials. The vials were incubated at the headspace conditions and analyzed. All the samples were freshly prepared and analyzed immediately.

Method validation

The recovery of the method was tested with the analysis of the excipient sample spiked with formaldehyde at three different levels: 10, 20, 30 ppb (See Table 6). The recovery values are as shown in the results table and indicate the headspace technique is quantitative in its extraction of formaldehyde from an aqueous matrix.

Table 6. **Summary of method validation experiment**

Linearity:	10.0 ppb to 50 ppb of formaldehyde
RSD for replicate analysis:	for 10.0 ppb is 9.23%
Detection level:	5.0 ppb
Quantification level:	10.0 ppb
Recovery study:	at three levels for all the samples 80-120%

Results and Discussion

Six samples of common excipients were analyzed using the SHS-GC/MS method developed here. These samples were chosen because they had been shown to have detectable levels of aldehydes in the literature. Of the samples analyzed, PEG-400 demonstrated to have the highest levels of formaldehyde at 3.5 ppm. Table 7 presents the results of the pharmaceutical sample study. The typical chromatogram and the spectrum from the analysis of samples material is shown in Figure 4.

Table 7. **Results of pharmaceutical study.**

Sample number	Sample details	Amt. of formaldehyde found in ppm	Recovery
1	Plasdone	2.2	81.37-108.66
2	Pregelatinised starch	1.2	91.33-112.86
3	Povidone	0.5	92.43-95.34
4	PEG-400	3.5	76.63-111.15
5	Microcrystalline cellulose (MCC)	0.3	93.28-104.46
6	Lactose monohydrate	0.5	99.16-104.46

Prior to the selection of an analytical technique for the determination of low-molecular-weight formaldehyde in excipients, a target level of method sensitivity (detection limit) with regard to excipient reactivity was considered. As the exact correlation between the aldehyde content and their reactivity with the pharmaceutical product is not known, and is a case-by-case relationship in pharmaceutical formulations, a worst case scenario was assumed to determine the desired limit of detection. It is not uncommon that the weight ratio of excipients to API in formulations exceeds 100:1.[1] For a small molecule API with a molecular weight of 500, the presence of aldehydes at 1 µg/g in excipients may result in a level of formaldehyde adduct as high as 0.2%, the lowest qualification threshold specified in the ICH guideline.[3] Therefore, 1 µg/g was thought to be reasonable as a threshold for the total content of low-molecular-weight aldehydes in excipients.[1]

One of the primary advantages of PFBHA derivatization is that it can be done in aqueous solution, desirable for headspace analysis as water generally provides very clean backgrounds.

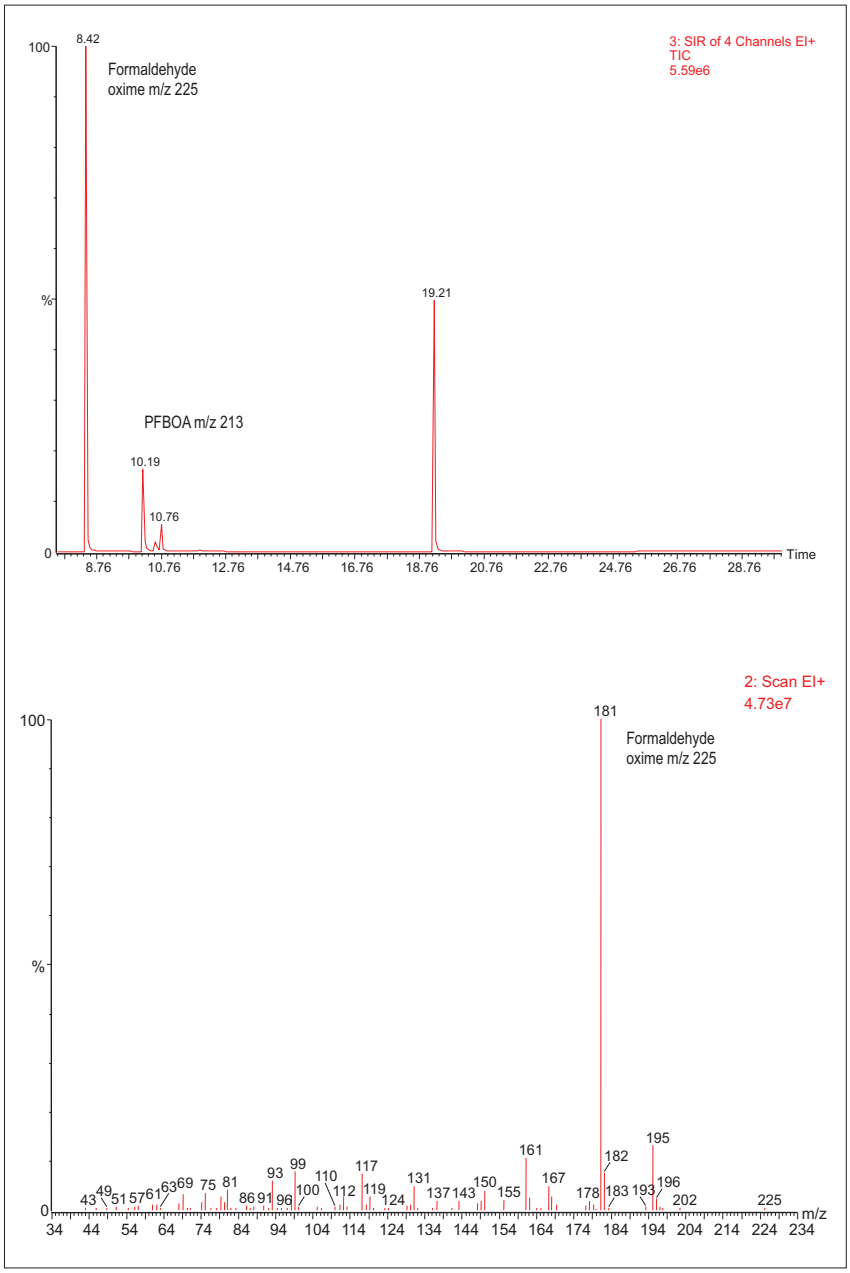


Figure 4. Chromatogram and spectrum of formaldehyde peak in sample.

Conclusion

This application note presents a simple and effective method for the determination of formaldehyde in pharmaceutical excipients using SHS-GC/MS. The method is fast, reliable and can be used for the quantification of low-molecular-weight aldehydes in most excipients commonly used in pharmaceutical products. Excellent quantification and linear instrument response was reported across a 1 to 50 ppb concentration range of formaldehyde. The method was validated using several samples obtained from a local pharmaceutical company and observed recovery values were all between 80-120%. By combining GC with MS, formaldehyde oxime was identifiable not only through retention time matching but by the resulting mass spectrum, which was confirmed by library search.

References

1. Excipient Toxicity and Safety, M.L. Weiner, L.A. Kotkoskie, Marcel Dekker, New York, 1999.

2. Journal of Chromatography, Volume A 1104, Issues 1-2, Pages 1-384 (February 2006), Detection and Quantification of Low-molecular Weight Aldehydes in Pharmaceutical Excipients by Headspace Gas Chromatography, Zhong Li, Laura K. Jacobus, W. Peter Wuelfing, Mary Golden, Gregory P. Martin and Robert A. Reed.

3. ICH Harmonized Tripartite Guidelines, Q3B, Impurities in New Drug Products.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

010014B_01

APPLICATION NOTE

Gas Chromatography

Author

Padmaja Prabhu

PerkinElmer, Inc.
Shelton, CT 06484 USA

Pressure-Balanced Headspace for the Determination of Class I, II and III Residual Solvents in Pharmaceuticals by USP Chapter <467> Methodology

Introduction

Residual solvents are used in the manufacture of active pharmaceutical ingredients (APIs), excipients, or in preparation of drug products and are not removed during the purification processes. Residual solvents are one of the three main impurities in pharmaceutical materials; the other two are organic and inorganic impurities. Solvents have a number of uses in the pharmaceutical manufacturing process, may sometimes be critical in the synthesis and can determine characteristics like crystal form, purity and solubility. Residual solvents do not provide any therapeutic benefit and should be removed to the extent possible, fulfilling quality-based requirements as per International Conference on Harmonization (ICH) guidelines – this is one of the standards to control the quality and the purity of the pharmaceutical substances, excipients, or drug products.

PerkinElmer®
For the Better

Both the ICH and the United States Pharmacopoeia (USP) have guidelines for limiting the amounts of solvents used in pharmaceuticals. The ICH lists three classes of solvents based on their toxicity to humans and environmental health. Until 2008, the USP limited and tested for only chloroform, dioxane, methylene chloride and trichloroethylene. In harmonization with the ICH, the USP has changed the general chapter <467>, which became effective July 1st, 2008. The chapter now includes a comprehensive listing of the Class I, II and III solvents and their control limits, with procedures for identification, confirmation and quantification (Procedure A, B and C, respectively). This chapter is applicable to all the articles that use or produce residual solvents and to all the manufacturers who produce official excipients, APIs and drug products.

USP chapter <467> suggests analysis of residual solvents using a gas chromatograph (GC) equipped with a flame ionization detector (FID) and an automated headspace sampler (HS). The new chapter employs three testing procedures which are used to screen and identify (Procedure A), confirm (Procedure B) and quantitatively determine (Procedure C) the residual solvents in the sample. When the user has information about the specific solvents utilized during the manufacturing of the article, only Procedure C needs to be performed. If the solvents used are unknown, all three procedures are needed for identification and quantitation. If only Class III solvents are used in the manufacture of an article, an alternative loss-on-drying method is permitted, however, if Class II and III solvents are also present, it is advisable to analyze by chromatographic techniques.

This paper will demonstrate the analysis of all three classes of residual solvents by pressure-balanced headspace sample introduction and GC-FID analysis. In addition to a discussion of the instrumental technique, the choice of the diluent will also be studied; two diluents will be used throughout.

Experimental

A PerkinElmer® Clarus® 600 GC equipped with FID detector and a PerkinElmer TurboMatrix™ HS-40 Headspace Sampler is the instrumental platform for this application. The TurboMatrix HS is a pressure-balanced headspace sampler; the basis of sample collection in this system is a calculation of sample volume, allowing gas at a known flow rate to enter the analytical column for a specific time.

When compared to other headspace technology, the pressure-balanced sampling of the TurboMatrix HS provides superior precision and inertness as a result of the simple, inert sample path. This technology does not require gas-sampling valves or other moving parts, reducing the sample contact with hot metal loops and the maintenance associated with moving parts. The TurboMatrix HS-40 includes a multi-position vial oven with overlapped vial thermostatting capability. Overlapped thermostatting automatically optimizes the use of the multi-position oven – this allows the next sample to inject as soon as the GC oven becomes ready, providing unparalleled sample throughput. Complete headspace parameters are described in Table 1.

Table 1. Detailed Headspace Analytical Conditions.

Headspace Unit:	PerkinElmer TurboMatrix HS-40
Headspace Mode:	Constant
Needle Temperature:	105 °C
Transfer Line Temperature:	110 °C
Oven Temperature:	80 °C
Thermostat Time:	20 min
Vial Pressurization Time:	2.0 min
Withdraw Time:	0.1 min
Injection Time:	0.12 min
Column Pressure:	48 psig
Injection Pressure:	48 psig
Vial Pressure:	48 psig
Vial Vent:	On
Transfer Line:	Fused Silica (0.53 mm)

Table 2. Detailed Gas Chromatographic Analytical Conditions.						
Gas Chromatograph	PerkinElmer Clarus 600 GC with FID					
Analytical Column (G43)	PerkinElmer Elite-624 (30 m x 0.53 mm i.d. x 3.0 µm df)					
Analytical Column (G16)	PerkinElmer Elite-Wax (30 m x 0.32 mm i.d. x 0.5 µm df)					
Injection Port Type	Programmable Split/Splitless					
Injector Temperature (or Program)	200 °C					
Injection Type	HS-Control					
Injector Temperature	140 °C					
Carrier Gas Type	Helium					
Flow Rate (G43)	3.0 mL/min					
Flow Rate (G16)	1.0 mL/min					
Split Ratio	1:5					
FID Temperature	250 °C					
	Class I and III			**Class II**		
Oven Temperature Program (G43)	**Temperature**	**Hold Time**	**Rate**	**Temperature**	**Hold Time**	**Rate**
	40 °C	20 min	10 °C/min	40 °C	17 min	40 °C/min
	240 °C	10 min	End	240 °C	2 min	End
Oven Temperature Program (G16)	40 °C	20 min	6 °C/min	50 °C	19 min	40 °C/min
	165 °C	1 min	25 °C/min	220 °C	1 min	End
	220 °C	2 min	End			

Building upon the throughput of the TurboMatrix HS, the Clarus 600 GC features a best-in-class oven with high-speed cooling, resulting in a shorter period between the end of one run and the beginning of the next. This becomes especially useful in methods when the initial oven temperature is close to ambient. Complete gas chromatographic conditions are presented in Table 2.

Discussion

In this application note, a comprehensive list of solvents is analyzed, with a method optimized for chromatographic resolution and run time. The analysis of each solvent is performed on both the G16 and G43 phases to provide complete resolution of all solvents included in chapter <467>. In addition to separation on multiple phases, two diluents are used in each class of solvents. The diluent choice is an important variable in method development. The material and analyte solubility, boiling point, as well as the solvents used in manufacture, need to be considered. The response for each analyte changes with the diluent used, thus care should be exercised when selecting the diluent so that sensitivity and resolution can be optimized. Some solvents, typically non-polar, show very good response with water as a diluent, while the others, typically polar, in organic diluents.

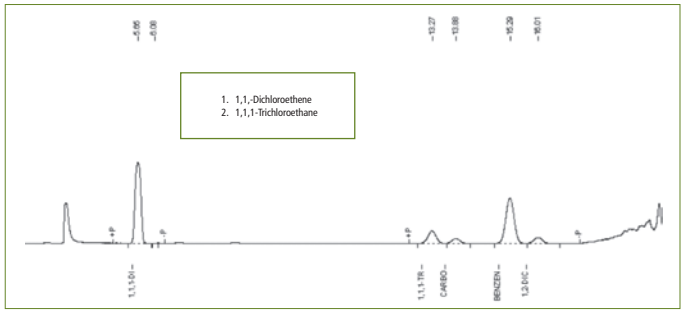


Figure 1. The analysis of Class I solvents in water using a G43 phase.

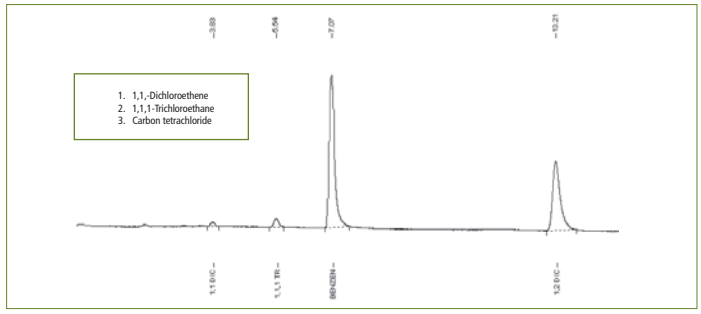


Figure 2. The analysis of Class I solvents in N,N-Dimethylacetamide using a G16 phase.

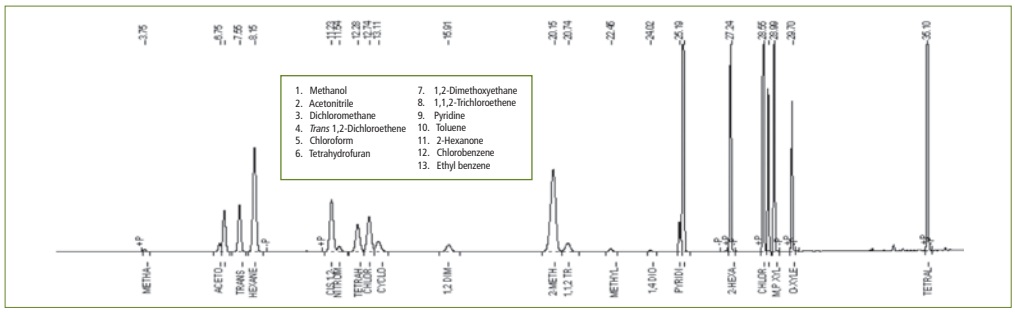


Figure 3. The analysis of Class II in 1,3-Dimethyl-2-Imidazolidinone using a G43 phase.

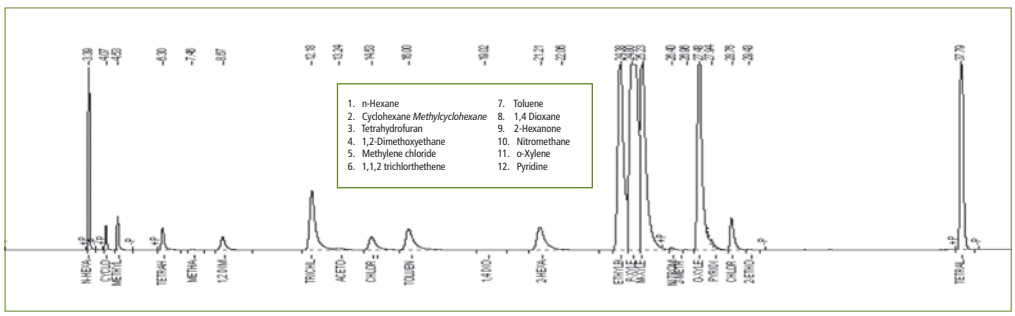


Figure 4. The analysis of Class II in solvents in water using a G16 phase.

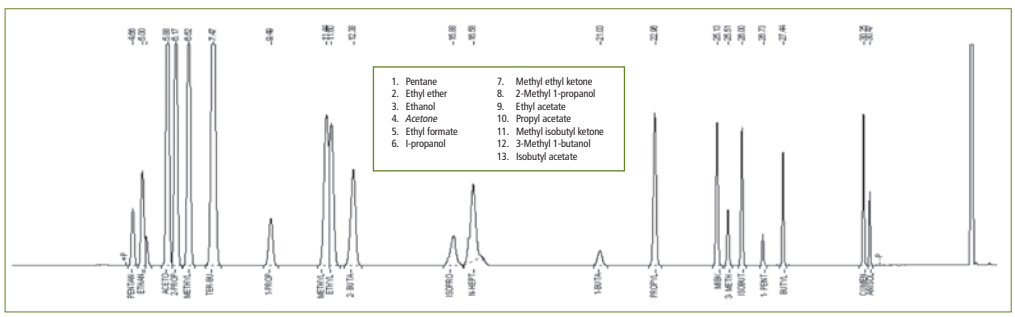


Figure 5. The analysis of Class III solvents in N-Methyl-2-Pyrrolidone using a G43 phase.

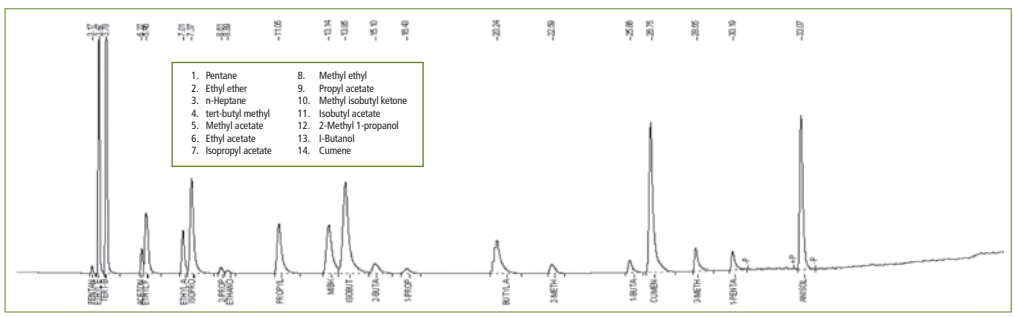


Figure 6. The analysis of Class III solvents in water using a G16 phase.

Procedure A & B – Identification and Confirmation of Materials for Solvents

Procedure A is used to identify the residual solvents in a pharmaceutical sample. In this, all solvents were initially analyzed using the G43 column and associated GC conditions. Multiple diluents are used in Figures 1, 3 and 5.

The residual solvents were confirmed using Procedure B on a G16 column. The elution order is different between the G43 and G16 phases, allowing confirmation of the analyte identification by retention time on 2 orthogonal column phases. In addition, several co-eluting compounds on Procedure A are now resolved, while other compounds now co-elute. Figures 2, 4 and 6 demonstrate the results of the analysis with a G16 phase.

Procedure C – Quantification

After identification and confirmation of residual solvents in pharmaceutical materials by Procedures A and B, the analytes are quantified by the procedure which provides the optimal separation of solvents present in the sample. The exact analytical procedure chosen for quantification is based on the optimal separation conditions for the analytes of interest.

Conclusion

The revised chapter <467> aligns the USP methodology for the analysis of residual solvents with that set by the International Conference on Harmonization. In this paper, we have presented a comprehensive analysis for the identification, confirmation and quantitation of Class I, II, and III solvents. The full suite of analytes is separated while maintaining an efficient analysis.

The overlapping thermostatting of the TurboMatrix HS assured that the system was ready to inject as soon as the GC achieved its starting conditions. Furthermore, the fast-cooling capability of the Clarus 600 GC oven was used to reduce the injection-to-injection time of this application, increasing productivity.

The full list of typically-analyzed solvents was presented with two different diluents, on both the G43 and G16 phases. The choice of diluent is based on both the solubility of the material under test and the boiling point of the least-volatile solvent expected. A combination of column selectivities provided the separation for all of the solvents in Class I, II, and III.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

008723_01

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

013318_01 PKI