Combi PAL SPME Manual

Supplement to the Combi PAL System Users Manual

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Introduction

The Combi PAL SPME option offers the analyst several features that will enhance the utility of this exciting sample preparation technique. These include:

1. Choice of 2,10 or 20-mL vials

2. Large number of samples (Standard configuration is two trays with 98 2-mL vials per tray or 32 10/20-mL vials per tray. (Up to two additional trays can be added, if necessary.)

3. Shaking and heating the sample during the extraction process

4. Constant heating time for each sample

5. The fiber can be automatically conditioned before a series of runs or after the desorption step in each run with an optional heating accessory.

6. User-selectable sampling and injection depths

7. Automatic method development by using sequential methods with different parameters (incrementally increasing extraction times or extraction temperatures, for example).

This manual covers the operation of the Combi PAL in the SPME mode with the basic software that is installed in the Combi PAL itself; additional features are possible with the optional Cycle Composer software, where a PC controls the Combi PAL.

Note!   Prior to reading this manual the reader should first read the "Combi PAL System User Manual" and become familiar with the general operation of the Combi PAL including defining the position of objects and building methods and jobs.

Procedure for SPME Sampling with the Combi PAL

Preparation

It is assumed here that chromatographic conditions have been optimized for the analytes—i.e. that an appropriate column, temperature program and detector have been selected.

Injector Insert

The injector insert is important in assuring good results when a SPME fiber is desorbed. A straight unpacked insert with an inner diameter between 0.75 to 0.80 mm should be used. An insert of smaller diameter will not allow the fiber sheath to penetrate the injector. Larger inserts (2-4 mm id) will result in broadening of early-eluting peaks.

Varian sells SPME inserts for the 1093 injector (SPI) and for the 1078/1079 injectors.

Injector Septum

The SPME fiber assembly includes a septum-piercing protective needle (**Figure 1**), which is a blunt, hollow 23-24-gauge[1] tube.

fiber support rod

protective needle
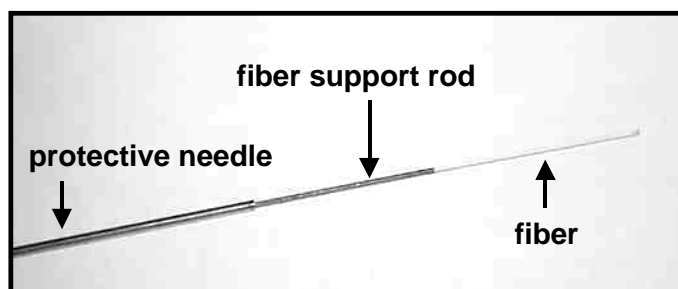
fiber

Figure 1. Detail of the fiber assembly

In comparison, liquid injection into a GC is usually accomplished with a tapered 26-gauge[2] needle. Therefore, sample introduction with a SPME fiber is more likely to result in septum failure. A septumless injector seal, such as the Merlin Microseal™ (**Figure 2**), is highly recommended.
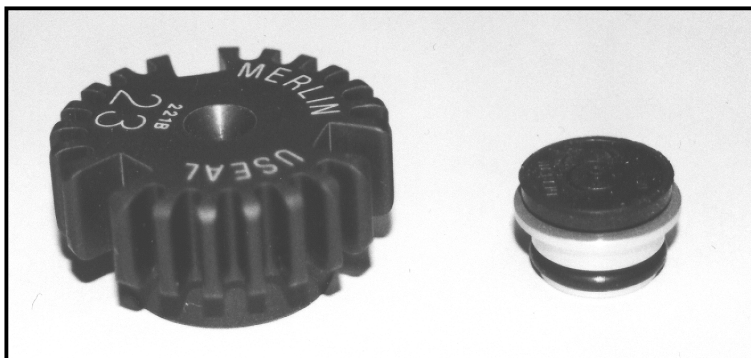
Figure 2. The Merlin Microseal™ can be installed in a GC injector in place of a septum. The device contains a "duckbill" that allows a needle to enter the injector without leaking.

This is available from Varian for the 1078/1079 injector and from other vendors for non-Varian injectors.

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[1] Originally, Supelco used 24-gauge tubing in manufacturing SPME fiber assemblies, but 23-gauge tubing was required for the Merlin Microseal™. Both gauges are now available.

[2] The higher the gauge number, the narrower, the outer diameter.

It is possible to use a conventional GC septum with SPME. To minimize septum failure, the following procedure is recommended:

1. Install a new septum.
2. Puncture the septum with a SPME protective needle (**Figure 1**) three or four times.
3. Remove and inspect the new septum. Pull off and discard any loose particles of septum material.
4. Reinstall the septum.

The user should monitor the head pressure on the column as the protective needle enters and leaves the injector to verify the integrity of the seal. A subtle leak will be indicated by shifts in retention time, no peaks or poor area count precision and/or the presence of air in a mass spectrometer.

Injector Temperature

Although, temperature-programmable injectors have become popular for minimizing decomposition of labile compounds and for eliminating discrimination based on volatility, SPME fibers are generally desorbed under hot, isothermal conditions. Rapid desorption from the fiber is necessary for sharp peaks without sample carryover. Injector temperature is normally 10-20°C below the temperature limit of the fiber and/or the GC column (usually 200° to 280°C).

Sample Vials

Many SPME applications will require heating of the sample. For these applications, only vials recommended for the Combi PAL should be used. These vials are 10-mL and 20-mL with magnetic crimp-top caps and an 8-mm opening[3]. Two-mL vials with magnetic caps are also available—however, these are not recommended as the holes in the caps are small and fiber breakage is possible.

Special adapters are required for the agitator when using 10-mL and 2-mL vials. The adapters for the 10-mL vials are shipped with the instrument.

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[3] For SPME sampling in the tray (without heating or agitation), 2,10 or 20-mL vials without magnetic caps may be used.

Setting up the Combi PAL for SPME

Refer to the Combi PAL system User Manual for installation of the Combi PAL and for setting the **x y z** parameters of the agitator, tray holders, trays and GC injectors.

"FiberExp" position

In order for the SPME cycle to operate correctly, it is necessary for the injection unit of the Combi PAL to be positioned next to the agitator just before the extraction. This position has been designated as "FiberExp".
From the "Job Queue" page, enter the following sequence:

```
Menu
   ↳ Setup
        ↳ Objects
             ↳ Vials
                  ↳ FiberExp
```
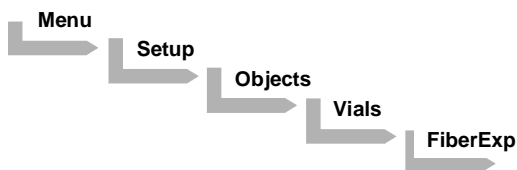
Assuming the agitator is installed on the right side, set the **x**, **y** and **z** parameters so that the right edge of the injection unit is resting on the left rear edge of the agitator (**Figure 3**). If the agitator is on the left side, then the left edge of the injector unit should rest on the right rear edge of the agitator. Press "F4/Home".

Figure 3. Injection unit in the "FiberExp" position with the right edge just touching the left rear edge of the agitator.
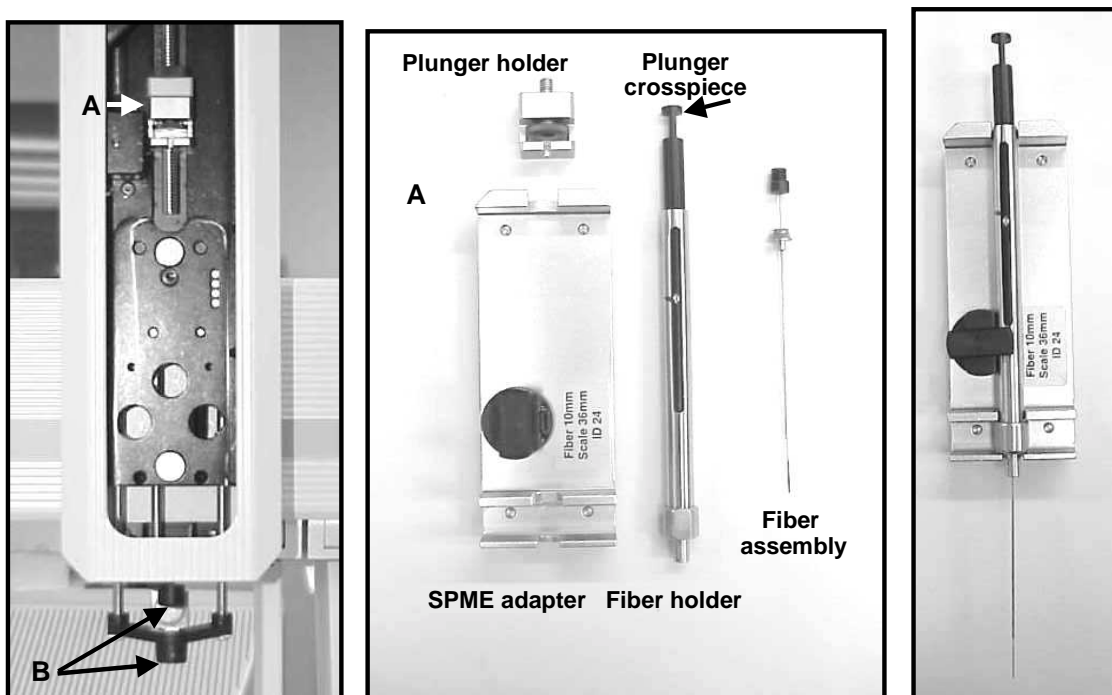
Figure 4. Left: injector unit with plunger holder installed (A). The arrows marked "B" are pointing to the upper and lower needle guides; **Center:** unassembled parts; **Right:** fiber holder and fiber assembly installed in the SPME adapter.

Installation of the SPME adapter

1. Press "F1/Menu" and then F1/Chang Syr. The injection unit will move to a position that will facilitate installation of the SPME adapter.
2. If the injection unit is directly over a sample tray, the "Chang Syr" position should be changed.
3. Press "Continue" and then:

Utilities
  Syringe

4. Press "F3/Set Pos" and set the **x y z** positions to a location where there is a clear space under the fiber. Then Press "F4/Home" and repeat Step 1.
5. Install the plunger holder into the injection unit (**Figure 4 left**).
6. Install a fiber assembly in the fiber holder and place it in the SPME adapter (**Figure 4 right**). Pull up the plunger so that the fiber is completely withdrawn into the protective needle.
7. Place the SPME adapter, partially into the injection unit. In order to do this, bend the top of the SPME adapter foreward slightly (**Figure 5A)** and thread the protective needle carefully through the upper and lower needle guides at the bottom of the injection unit.
8. Push the plunger down so that approximately 1.5 to 2 cm of the fiber and fiber support rod are exposed
9. Place the plunger crosspiece into the plunger holder. Allow the syringe adapter to "click" into place by magnetic force, against the syringe carrier.
10. Tighten the plunger retaining screw against the plunger crosspiece (**Figure 5B**) and press "Continue".
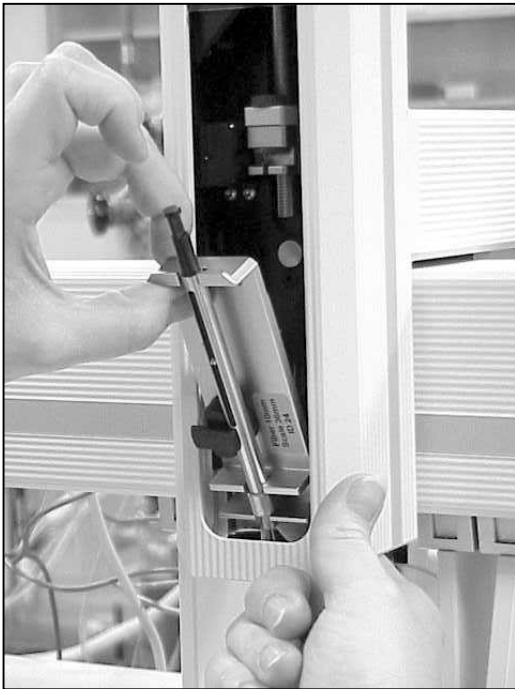
Figures 5A and 5B. Installation of the fiber adapter in the Combi PAL injection unit

Note!   Reverse the above procedure to remove the fiber. Be sure to pull up the plunger of the fiber holder so that the unprotected fiber is not pulled through the upper and lower needle guides.

Standby position of the fiber

In this step, the end of the fiber is set so that it is just barely withdrawn into the protective needle. This will minimize coring when penetrating vial or injector septa.
From the "Job Queue" page, enter the following sequence:

Scroll through the various parameters until you reach "Standby Pos" and press enter. With your thumb, push up the lower needle guides until the end of the protective needle is visible. If the fiber is not exposed, turn the dial counterclockwise until you can see the fiber. Then turn the dial clockwise slowly, until the end of the fiber is flush with the end of the hollow tube. Turn the dial clockwise an additional 0.1-mm.

Enter the value and press "F4/Home".

This procedure should be repeated whenever a fiber is installed.

Position of the fiber in the GC injector

If you are using a Varian 1078/1079 injector with or without a Merlin Microseal, the default parameter in the software is set correctly for fiber desorption. For other injectors, the procedure is:
1. Access the method "Test SPME" (see the Combi PAL System user manual)

2. Build a job list with one vial and the method "Test SPME "

3. Press "F4/Start"

4. While the fiber is desorbing in the injector, mark the fiber holder to record the position of the small metal circle (see **Figure 6A**) during the desorption step.

5. After the cycle is completed, remove the fiber holder from the Combi PAL.

6. Line up a septum nut, septum and injector insert (**Figure 6B).**

7. Push the fiber needle through the septum nut and septum into the insert. Push the plunger down to the mark on the fiber holder.

8. When the fiber is at the proper depth in the insert, measure the distance in mm from the top of the injector nut to the end of the exposed fiber. This is the injector penetration depth.

Use this value in "Test SPME" and other SPME methods.
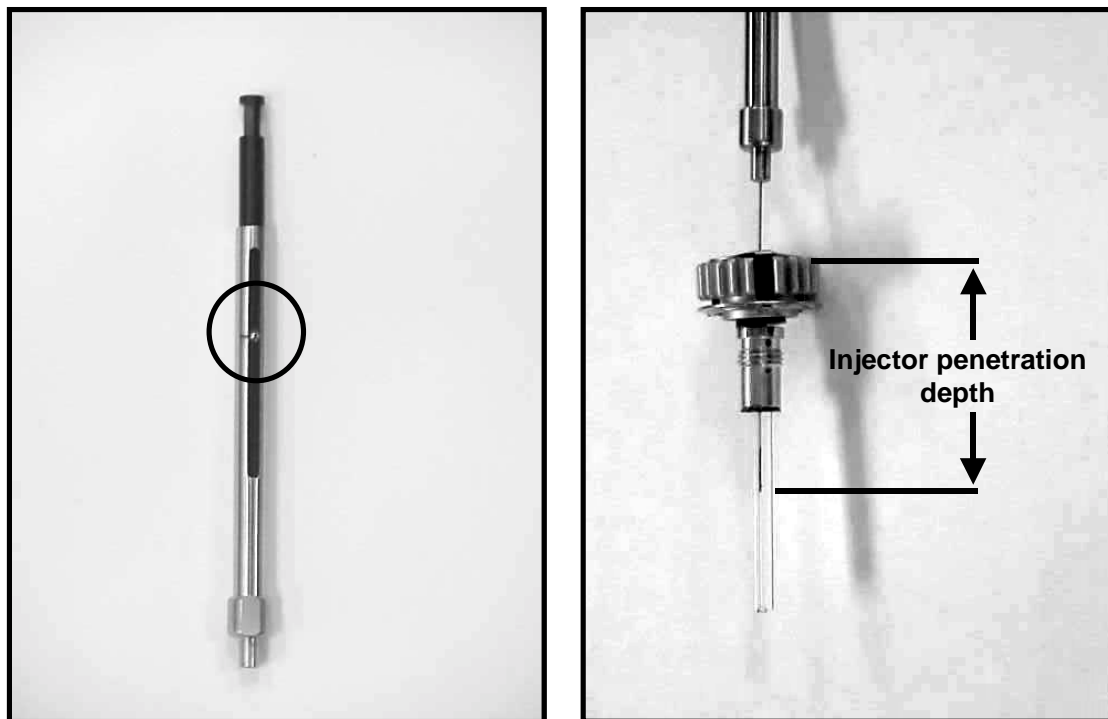
Figures 6A and 6B. 6A shows the mark in the center of the fiber holder that was made during the desorption. 6B shows the injector parts lined up so that the injector penetration depth can be observed.

Note! You might want to make the first injection without a fiber assembly installed in the fiber holder.

After you have set the injection position and made an injection with a fiber installed, verify that the fiber is intact after the injection. Press: "F1/Menu" and then "F1/Change Syr" to view the fiber.

<u>Fiber depth in the sampling vial</u>

The default parameter for "Vial Penetr" in the "TestSPME" method is 22 mm. This is the minimum depth that the fiber can be set to penetrate the vial.

Labels on figure:
- Protective needle
- Seal
- Magnetic steelcap
- Fiber support rod
- SPME fiber
- Vial Penetr. (22.0mm - 31.0mm)
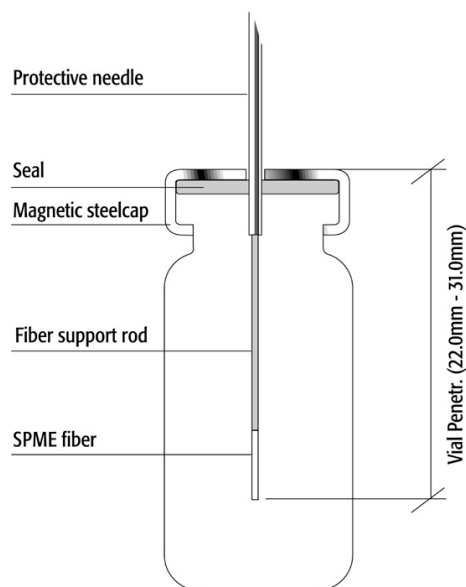
Figure 7. Showing the "Vial Penetr" parameter

If the liquid phase is to be sampled, the depth of the fiber in the vial and/or the amount of liquid in the vial should be adjusted so that the fiber rod is above the meniscus of the liquid phase. Sample volumes for various vials are suggested in the table.

Vial Volume	Volume of Sample (mL)	
	Headspace sampling	Liquid sampling
2-mL	0.6	1.3
10-mL	6.0	9.0
20-mL	15.0	18.0

Optional bakeout of the fiber after injection

With some fibers, a high temperature is necessary to desorb the analytes completely. Often the GC injector cannot be set to a high enough temperature because a column with a low temperature limit is installed. With the Combi PAL the user can bake the fiber after desorption in a separate bakeout station (**Figure 8**). This is an optional piece of hardware.
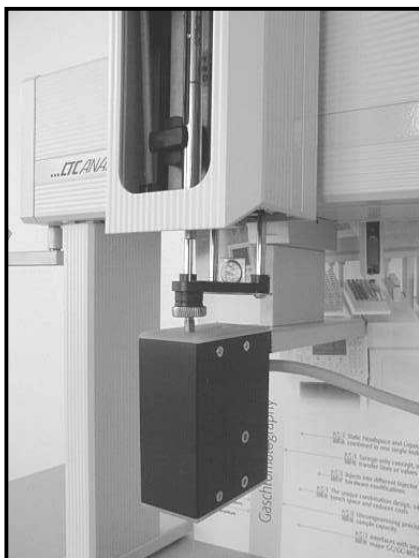
Figure 8. Optional bakeout station. The baking occurs with a flow of inert purge gas.

To enable this feature, install the bakeout station. Then define the position of the bakeout station ("NdlHeater") as follows:

Menu
> **Utilities**
> > **Injector**
> > > **NdlHeatr**
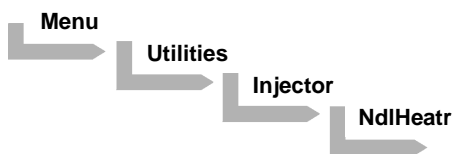
Set the **x y z** parameters.

The temperature can be set in increments of 5°C, from 30° to 350°C.
To set the temperature, Press:

Menu
> **Setup**
> > **Objects**
> > > **Injectors**
> > > > **NdlHeatr**
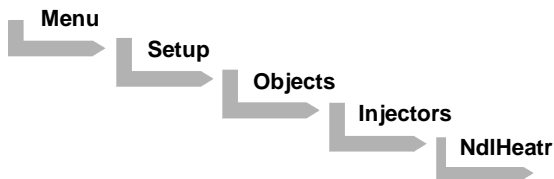
Building a SPME method

See the "Combi PAL System User Manual" for details on how to build a method.
The parameters in the SPME method are discussed below:

PARAMETER	VALUE	COMMENTS
Cycle	SPME	
Syringe	Fiber	
Pre Inc Time	00:00:00-23:59:59	Allows the sample to be preheated prior to insertion of the fiber.
Incubat Temp	30.0°C –200°C or OFF	"OFF" for sampling in the tray at ambient temperature without agitation. A maximum of 80°C is suggested.
Agi Speed	100 – 750 rpm	These are the speeds for the _pre-incubation_ period only. Agitation speed during the extraction is fixed to protect the fiber.
Agi On Time	0s - 99s	Set "0s" to turn off agitation during pre-incubation and extraction.
Agi Off Time	0s - 99s	
Vial Penetr	22.0 – 31.0 mm	Distance from top of vial septum to end of fiber **(Figure 7)**
Extract Time	00:00:10-23:59:59	Sampling time in liquid or headspace
Desorb to	None - Waste 2	Normally an injector such as "Front" or "Rear" is entered here.
Inj Penetr	44.0mm – 67.0mm	Distance from top of injector nut to end of fiber **(Figure 6B)**
Desorb Time	00:00:10-23:59:59	Time in injector
Fiber Bakeout	00:00:00-23:59:59	For baking out the fiber after desorption in the optional bakeout station.
GC-Runtime	00:00:30-23:59:59	Enter the complete GC cycle time including cool-down and re-equilibration to coordinate the Combi PAL and GC cycles.

After the incubation temperature is determined, it is convenient to set the standby temperature of the agitator to this temperature. Enter the following sequence from the "Job Queue" page.

Menu
→ **Utilities**
→ **Tray**
→ **Agitator**
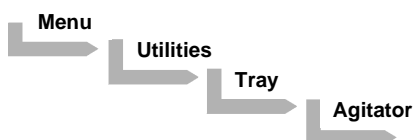
Scroll down to "Standby Temp" and set the temperature.

Note!   To sample in the tray, "Agi on time" must be set to "0" and "Incubat Temp" must be set to "off"

Running the SPME test sample

The **SPME Sensitivity Test Sample** is composed of 1 ng/μL each of nitrobenzene and nitrotoluene in water (1% methanol has been added to stabilize the sample). These compounds were selected because they exhibit a good response with many GC detectors, including the flame ionization detector, the electron capture detector, the thermionic selective detector, and the ion trap detector.

To run the SPME Sensitivity Test Sample, use the following conditions:

Column: Nearly any capillary column can be used to separate the components in the test sample.

For a non-polar fused silica column, the following conditions are suggested: 50°C for 1 minute; then 20° C/minute to 150°C; hold for 2 minutes, carrier gas flow appropriate for the column.

Injector: 200°C isothermal.

Detector: Settings depend on the detector used.

Suggested SPME conditions: 100-μm PDMS fiber, conditioned according to the instructions in the package. Place the entire sample in a vial. Extract 10 minutes and desorb one minute. Heating and agitation are not necessary.

A representative SPME test sample chromatogram is shown (**Figure 9**).

GC CONDITIONS
Injector: 200°C
Column: 4m x 0.53μm fused silica,
coated with 1-micron methyl silicone,
50°C/min, hold 1 min
20°C to 110°C, hold 2 min
Detector: 240°C, FID, range 10,
attenuation 128

1. Nitrobenzene
2. Nitrotoluene
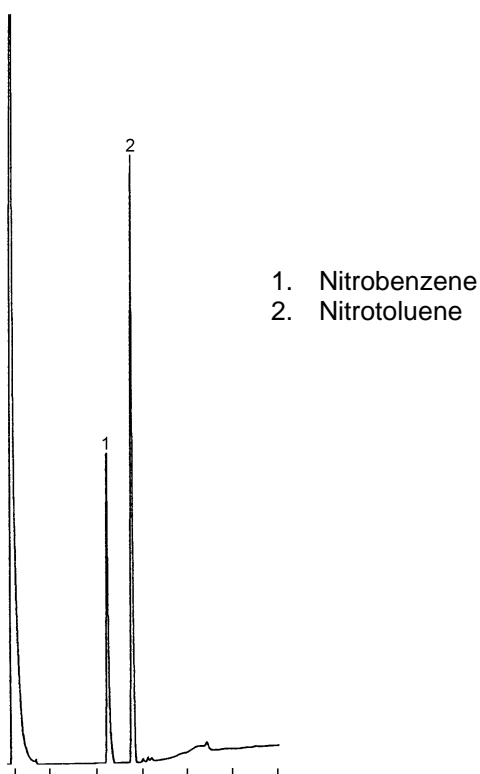
Figure 9. Chromatogram of the SPME test sample.

Troubleshooting

Symptom	Possible Cause	Recommended Action
Fiber breaks in injector	Improper depth in injector	Verify (see above) that the bottom of the SPME fiber syringe is not less than 5 mm into the insert
Fiber breaks in injector	Septum corings or other particles are in the injector	Replace insert. If septum particles are present, consider using a seal such as the Merlin Microseal to eliminate the septum.
Poor precision	Vials are leaking.	Verify that the cap cannot be turned after sealing. Reduce the extraction temperature to see if the precision improves. Temperatures > 80°C are not recommended.
Poor precision	Poor sample handling.	See the Advantage Note on SPME method development in the "SPME Application and Advantage Note" section of this manual.
Sample carryover	Fiber is not fully desorbed	Increase desorption time and or/temperature or bake out the fiber after each injection.
Sample carryover	Fiber support rod is submerged in liquid sample	Reduce fiber penetration depth in vial or reduce amount of sample in the vial.
Extraneous peaks in blanks	Contamination is in the GC	Verify that the GC is clean by making a run without injecting.
Extraneous peaks in blanks	Contamination is in the sample vial septa	Sample an empty vial without a septum installed. Sample an empty vial with a septum installed. If the contamination is from the septum, bake the septum in a laboratory oven at 150°C overnight. This will minimize extraneous peaks.

Supplies

Item	Varian part number
Combi PAL SPME kit	03-925903-91
SPME insert for 1078/9 injectors[4]	03-925330-00
SPME insert for 1093 (SPI) injectors	03-918832-05
Test sample, SPME	03-918967-00
Merlin Microseal™ SPME Kit (23 Gauge) for Varian 1078/1079 injectors	03-926099-01
Merlin Microseal™ SPME Replacement seal (23 Gauge)	03-926099-02
Replacement O-ring	27-402426-00
10-mL vials pk/100	MLA201000
20 mL vials pk/100	MLA202100
Seals for 10/20-mL vials (8-mm holes) with septa pk/100	MLA200050ML
Seals for wash and waste vials (fit 10-mL vials above) pk/20	MLAL1000023
Caps for wash and waste vials (fit 10-mL vials above) pk/10	MLAL1000118
23-gauge SPME fibers[5] for Merlin Microseal™	
SPME fiber, PDMS Auto Merlin 100µm pk/3	SU57341U
SPME fiber, Carboxen/PDMS Auto Merlin 75µm pk/3	SU57343U
SPME fiber, PDMS/DVB Auto Merlin 65µm pk/3	SU57345U
SPME fiber, Carbowax/DVB (StableFlex) Auto Merlin 70µm pk/3	SU57339U
24-gauge SPME fibers for conventional GC septa	
SPME fiber, PDMS Auto 100µm pk/3	03-918963-02
SPME fiber, PDMS Auto 30µm pk/3	03-918963-10
SPME fiber, PDMS Auto 7µm pk/3	03-918963-03
SPME fiber, Polyacrylate, Auto 85µm pk/3	03-918963-06
SPME fiber, Carbowax/DVB Auto 65µm pk/3	03-918963-12
SPME fiber, Carbowax/DVB (StableFlex) Auto 70µm pk/3	SU57338U
SPME fiber, DVB/PDMS Auto 65µm pk/3	03-918963-14
SPME fiber, DVB/PDMS (StableFlex) Auto 70µm pk/3	SU57327U
SPME fiber, Carboxen/PDMS Auto 75µm pk/3	03-918963-16
SPME fiber, Carboxen/PDMS (StableFlex) Auto 85µm pk/3	SU57335U
SPME fiber, Carboxen/DVB/PDMS (StableFlex) Auto 80µm pk/3	SU57329U
SPME fiber, Carboxen/DVB/PDMS (StableFlex, 2cm) Auto 80µm pk/3	SU57348U

[4] Inserts for non-Varian injectors can be ordered from Supelco

[5] Other SPME phases with a 23-gauge protective needle must be ordered from Supelco

SPME References

Useful Web pages:

Supelco	http://www.sigma-aldrich.com/SAWS.nsf/Pages/Supelco?EditDocument
Varian	http://www.varianinc.com/csb/gcnotes/spmeindex.html
University of Texas (SPME bibliography)	http://www.cm.utexas.edu/~brodbelt/spme_refs.html

Books

1. Pawliszyn, J*., Solid Phase Microextraction: Theory and Practice*, Wiley-VCH, Inc, New York, 1997

2. Wercinski, S. ed, *Solid Phase Microextraction: a Practical Guide*, Marcel Dekker, New York, in press

Journal Articles and Book Chapters

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Ai, Jui	1997	Headspace Solid Phase Microextraction. Dynamics and Quantitative Analysis before Reaching a Partition Equilibrium	Anal. Chem., Vol. 69 (16), PP 3260-3266
Ameno, K.; Fuke, C.; Ameno, S.; Kinoshita, H.; Ijiri, I.	1996	Application Of A Solid-Phase Microextraction Technique For The Detection Of Urinary Methamphetamine And Amphetamine By Gas Chromatography	Can. Soc. Forensic Sci., 29(2), 43-48
Arthur, C. L., Buchholz, K. D., Potter, D. W., Zhang, Z., Pawliszyn, J.	1993	Theoretical And Practical Aspects Of Solid Phase Microextraction With Thermal Desorption Using Coated Fused Silica Fibers	Natl. Meet. Am. Chem. Soc., Div. Environ. Chem vol.33 (1), pp.424-427
Arthur, C. L., Potter, D. W., Buchholz, K. D., Motlagh, S., Pawliszyn, J.	1992	Solid-Phase Microextraction For The Direct Analysis Of Water: Theory And Practice	LC-GC, VOL.10 (9), PP.656, 658, 660-1
Arthur, C. L., Buchholz, K. D. Potter, D. W., Motlagh, S., Killam, l., Pawliszyn, J.	1993	Practical And Theoretical Aspects Of Solid Phase Microextraction For The Direct Analysis Of Groundwater	PROC. WATER QUAL. TECHNOL. CONF., PT.2, PP.1315-33
Arthur, C. L., Killam, l. M., Motlagh, S., Lim, M., Potter, D. W., Pawliszyn, J.	1992	Analysis Of Substituted Benzene Compounds In Groundwater Using Solid-Phase Microextraction	ENVIRON. SCI. TECHNOL., VOL.26 (5), PP.979-983
Arthur, C. L., Pawliszyn, J.	1990	Solid Phase Microextraction With Thermal Desorption Using Fused Silica Optical Fibers	ANAL. CHEM.,VOL.62 (19), PP.2145-8
Arthur, C.L., Killam, L. M., Buchholz, K. D., Pawliszyn, J., Berg, J. R.	1992	Automation And Optimization Of Solid-Phase Microextraction	ANAL. CHEM. VOL. 64 (17), PP.1960-6
Barshick, S.-A., Griest, W. H.	1998	Trace Analysis of Explosives in Seawater Using Solid-Phase Microextraction and Gas Chromatography/Ion Trap Mass Spectrometry	Anal. Chem., 70, pp 3015-3020
Bartelt, R.J.	1997	Calibration of a Commercial Solid Phase Microextraction Device for Measuring Headspace Concentrations of Organic Volatiles	Anal. Chem. Vol. 69, pp364-372
Berg, J. R.	1993	Practical Use Of Automated Solid Phase Microextraction	AM. LAB. (SHELTON, CONN.) VOL.25 (17), PP.18, 20, 22-4
Boyd-Boland, A. A.; PAWLISZYN, J. B.	1996	Solid-Phase Microextraction Coupled With High-Performance Liquid Chromatography For The Determination Of Alkylphenol Ethoxylate Surfactants In Water	ANAL. CHEM. 68 (9) PP.1521-9
Boyd-Boland, A.A., Chai, M., Luo, Y., Zhang, A., Yang, M., Pawliszyn, J., Gorecki, T.	1994	New Solvent-Free Sample Preparation Techniques based on Fiber and Polymer Technologies	Environ. Sci. Technol., 28 (13), pp 569 A-574A

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Boyd-Boland, A.A., Magdic, S., Pawliszyn, J.	1996	Simultaneous Determination of 60 Pesticides in Water Using Solid-phase Microextraction and Gas Chromatography-Mass Spectrometry	Analyst, 121, pp 929-938.
Brand, G.	1994	Evaluation Of Solid Phase Microextraction (Spme) Technology For Applicability to Drinking Water Analysis	Proc. Water Qual. Technol. Conf. , Part 1PP. 273-83
Buchholz, K. D., Pawliszyn, J.	1993	Determination Of Phenols By Solid-Phase Microextraction and Gas Chromatographic Analysis	ENVIRON.SCI. TECHNOL VOL.27 (13), PP.2844-2848
Buchholz, K.D., Pawliszyn, J.	1994	Optimization Of Solid-Phase Microextraction Conditions For Determination Of Phenols	ANAL. CHEM., VOL.66 (1), PP.160-7
Chai, M., Arthur, C. L., Pawliszyn, J., Belardi, R. P., Pratt, K. F.	1993	Determination Of Volatile Chlorinated Hydrocarbons In Air And Water With Solid-Phase Microextraction	ANALYST (CAMBRIDGE, U. K.), VOL.118 (12), PP.1501-5
Chai, M., Pawliszyn, J.	1995	Analysis Of Environmental Air Samples By Solid-Phase Microextraction And Gas Chromatography-Ion Trap Mass Spectrometry	ENVIRON. SCI. TECHNOLVOL.29 (3), PP.693-701
Chen, J.I, Pawliszyn, J.B.	1995	Solid Phase Microextraction Coupled To High-Performance Liquid Chromatography	ANAL. CHEM. VOL.67 (15), PP.2530-3
Daimon, H., Pawliszyn, J.	1996	High Temperature Water Extraction Combined With Solid Phase Microextraction	ANAL. COMMUN. 33 (12) PP. 421-424
De la Calle Garcia, D., Magnaghi, S., Reichenbacher, M., Danzer, K.	1996	Systematic Optimization of the Analysis of Wine Bouquet Components by Solid Phase Microextraction	J. High Resol. Chromatogr., Vol. 19, pp257-262.
Dean, J J., Tomlinson, W. R., Makovskaya, V.,Cumming, R.,Hetheridge, M.,Comber, M.	1996	Solid-Phase Microextraction As A Method For Estimating the Octanol-Water Partition Coefficient	ANAL. CHEM. 68 (1) PP.130-3
Eisert, R., Levsen, K.	1995	Determination Of Pesticides In Aqueous Samples By Solid-Phase Microextraction In-Line Coupled To Gas Chromatography-Mass Spectrometry	J. Am. Soc. Mass Spectrom., 1995, VOL. 6 (1), PP 1119-30
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SPME Advantage and Application Notes

The following data represent typical results. For further information, contact your local Varian office.

SPME advantages

Method Development Tips for Automated SPME

(Replaces GC Advantage Note 11)

Zelda Penton
Varian Chromatography Systems

Key Words: *SPME, Method Development*

Introduction
Automated solid phase microextraction (SPME) can yield detection limits in the ppb range or better for organic compounds in water or solids. Linearity is excellent, and relative standard deviations are often better than 3%.
The purpose of this note is to help the novice become familiar with the SPME technique. Experience has shown that sample preparation is the key to good results with SPME; therefore, techniques for working with volatile samples will also be discussed.

Some guidelines to help the user get started are given below. These suggestions are discussed in greater detail in the following sections.

Guidelines for Getting Started
1. A new fiber should be conditioned, following the manufacturer's recommendations. A blank run should be made after conditioning, to verify that there are no extraneous peaks.
2. If the fiber has been properly conditioned and a blank sample shows extraneous peaks, these are usually due to siloxanes from the AutoSampler vial septa (Figure 1). These peaks may be a problem with trace analysis, especially with an FID or a MS. To minimize these peaks, bake the septa in a GC oven at 150º C overnight, and store in a clean container (not plastic).
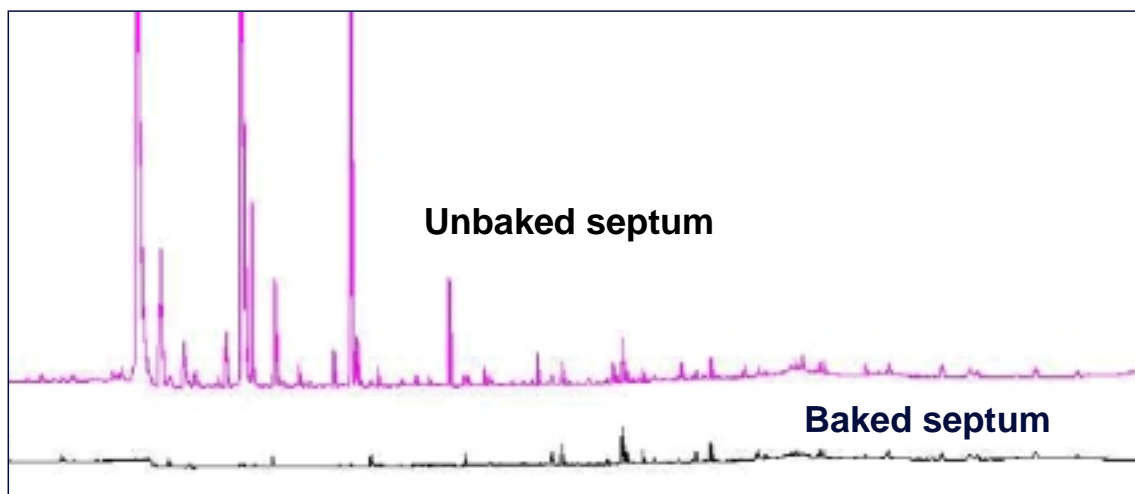

Figure 1. SPME chromatogram (100 µm PDMS fiber) of an empty vial with a baked and unbaked septum. FID detector

3. The injector insert should have an internal diameter of 0.75-0.80 mm.
4. If possible, use a Merlin Microseal™ or other seal to avoid using a septum in the injector. Note that the Merlin Microseal™ requires SPME fibers with 23-gauge needles.
5. For quantitative work, the fiber should be changed after approximately 100 runs.
6. With water-soluble analytes, saturating the sample with salt (usually sodium chloride or sodium sulfate) can enhance sensitivity
7. Standards and samples are normally prepared and diluted in storage containers and then transferred to AutoSampler vials for analysis. Prepare samples and standards carefully so that volatiles are not lost:
    a) After preparation, water samples containing volatiles should completely fill the storage container without any headspace.
    b) Store samples in the refrigerator. Chill the AutoSampler vials before adding the sample.
    c) Transfer samples to the AutoSampler vials with a pipette of sufficient capacity to deliver the entire sample in one step. The outer diameter of the pipette should be small enough to allow the pipette to easily fit into the AutoSampler vial.
8. When sampling the liquid phase, verify that only the fiber (not the support rod) is submerged in the liquid phase.
9. A reasonable extraction time is 15 minutes followed by 1-3 minutes desorption; however, these conditions should be optimized for each analysis. It is not necessary to achieve equilibrium if the total analysis time will be prolonged. For many samples, RSD's under 5% can be obtained prior to reaching equilibrium.
10. Shaking (Figure 2) the sample during extraction, is beneficial for SPME extraction of semivolatiles but has little effect on volatiles.

11. Heating the sample during extraction is very useful for sampling semivolatiles in the headspace over dirty samples.

1. α-HCH
2. γ-HCH
3. β-HCH
4. Heptachlor
5. δ-HCH
6. Aldrin
7. Heptachlor epoxide
8. Endosulfan I
9. 4,4'-DDE
10. Dieldrin
11. Endrin
12. 4,4'-DDD
13. Endosulfan II
14. 4,4'-DDT
15. Endrin aldehyde
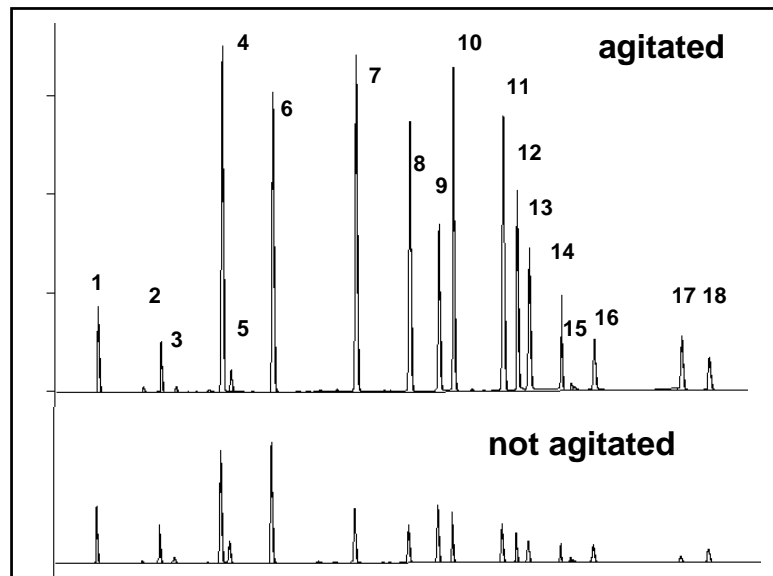16. Endosulfan sulfate
17. Methoxychlor
18. Endrin ketone

Figure 2. Increased extraction of pesticides from water (2 ppb) with shaking. Sampling from the liquid phase at ambient temperature.

Frequently Asked Questions

How Long Does a Fiber Last?
The fiber life will vary with experimental conditions but typically, there is no evident deterioration in chromatography after 100 runs when desorbing into an injector heated to 220ºC. This is true even when immersing the fiber into water that is saturated with salt and is at pH 2. One sign of an aging fiber is deterioration of precision. This might also be due to the aging of the septum. When using a conventional GC septum, it is best to change the septum when changing the fiber.

Is it necessary to use a septumless injector seal?
Users sometimes express concerns that the protective needle on the SPME fiber assembly might core the injector septum. This is a real concern and a septumless seal as the Merlin Microseal™ is highly recommended. However, it is possible to get acceptable results with a conventional injector septum. Figure 3 shows no change of retention time after 46 runs, indicating that the septum is intact. Again, the practice at Varian is to change the septum and the fiber at the same time after about 100 runs according to the following procedure:

1. Install a septum
2. Puncture the septum several times with the protective needle of the SPME fiber assembly.
3. Remove and inspect the new septum. Remove any loose particles of septum material.
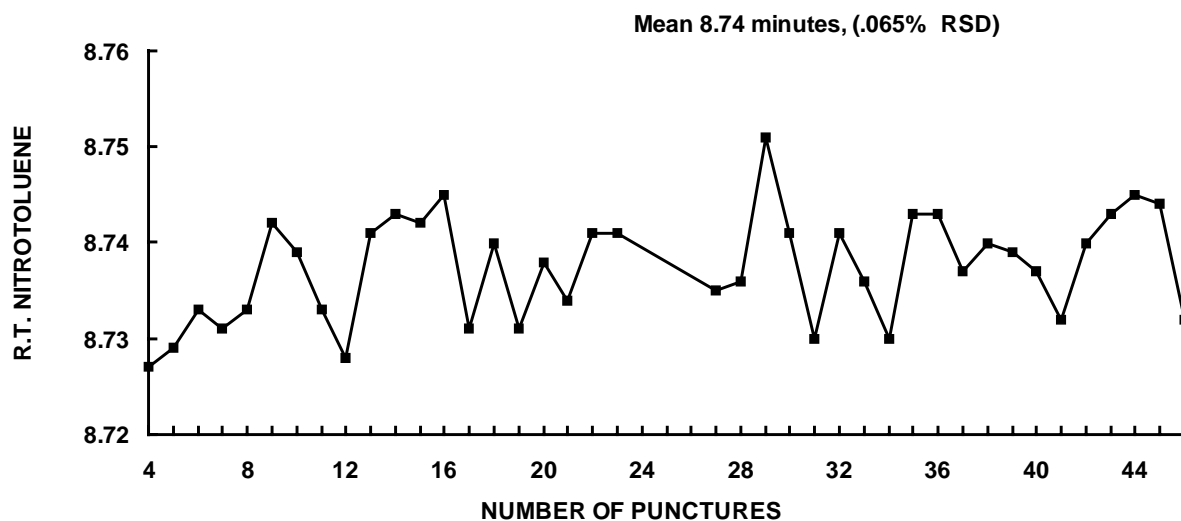4. Reinstall the septum.

Figure 3. Demonstrating the integrity of the injector septum after 46 SPME injections. Note that the scale on the Y axis is 0.04 minutes. A Thermogreen™ LB-2 (Supelco) septum was used in this study.

What volume of sample should be added to the AutoSampler vials?
The AutoSampler vial should not be filled to the top. It was observed when sampling volatiles, that equilibrium was attained faster when headspace was present, even when liquid was being sampled. Furthermore, immersion of the metal fiber-support rod in the liquid sample may result in the adsorption and/or breakdown of analytes.

Headspace   **Liquid**   **Do not fill lthe vial!**
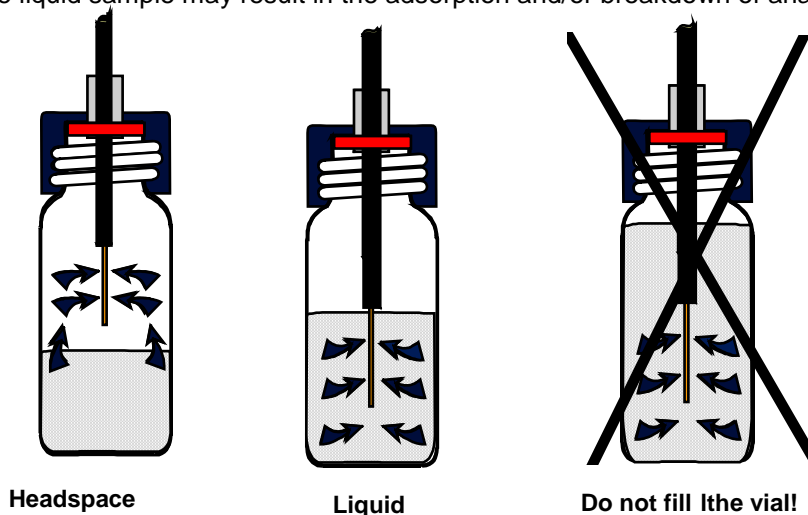
A final reason not to fill the vial, is the possibility of carryover if liquid sample enters the fiber needle.

What are the recommended extraction and desorption times?
Extraction time varies inversely with the volatility of the analyte and also depends upon the relative volumes of the phases in the vial. Satisfactory precision can often be obtained without achieving equilibrium. This is convenient if the GC cycle time is relatively short and prolonged sampling times would greatly lengthen the total analysis time. A reasonable sampling time is fifteen minutes, but extraction times may be longer if the GC cycle time permits.
At least two minutes is recommended to desorb all traces of the analytes to minimize carryover. The injector temperature should normally be at least 200ºC but should not be higher than the temperature limit of the analytical column or the SPME fiber. If carryover is present, a longer desorption time and/or higher injector temperature should be used.

Is cryofocusing necessary?
Injector: After initial studies, it was concluded that injecting into a hot injector gives the best results, even when the sample contains very volatile analytes such as vinyl chloride.

Column: Cryogenic focusing may be useful to improve the peak shapes of very volatile compounds if the column is not very retentive. SPME sampling does not require special GC conditions. Peaks tend to be sharper with SPME than with samples introduced using conventional static headspace.

Is the fiber easily saturated and do compounds tend to be displaced in mixtures?
This depends on many factors including sample size, the affinity of the fiber for the components of a particular sample, and the fiber thickness. Table 1 shows that benzene gave the same response whether it was the only organic compound in water or in a test mixture containing several other compounds. In another experiment, there was a linear response to benzene up to concentration ranges greater than 300 ppm. Nevertheless, it is important in developing and validating a method, to do recovery and linearity studies. Diluting the sample to a lower concentration will minimize displacement effects.

Conc. (ppm)	MeCl$_2$	CHCl$_3$	Benzene	TCE	Dioxane	Toluene	Xylene	TMB*
1	4281	3567	175183	51579	1203	308884	427923	637614
2	8471	6322	337571	101351	2165	617885	872322	1251551
4	17894	12478	704235	207027	4192	1233313	1713901	2331268
1			154458					
2			328038					
4			674635					

Table 1. FID area counts for benzene alone in water at (bottom 3 rows) and in a mixture with several other organic compounds in water (top 3 rows). All of the compounds were at the concentration shown at left except chloroform which was at half the concentration shown. These data were obtained by SPME fiber sampling of the liquid phase with a 100-µm PDMS fiber.
*1,2,4-trimethylbenzene

Is it better to sample the liquid or the headspace?
Theoretically, the response should be the same if the volumes sampled are similar. This has been found to be the case for the compounds listed in Table 1. Practically, for compounds of very low volatility, the extraction time from the headspace is long and liquid sampling is preferable.

Does the fiber pick up contaminants in the air that will interfere with the analysis?
After a fiber that has been conditioned, the first run each day should be a blank. Ghost peaks often appear from AutoSampler vial septa (see above). If this occurs, the user should bake the septa in a lab oven at 150ºC before use.

In order to minimize the presence of extraneous peaks, the SPME software parameters should be set so that the GC is ready for the sample to be injected immediately after extraction. See the manual for a detailed explanation.

What are the benefits of heating during SPME extraction?

The effect of heating depends on both the compound and the fiber but generally, volatile compounds show an increased response upon heating to 40-45°C. Above these temperatures, the response goes down due to migration of analytes out of the fiber. Compounds of lower volatility show a higher optimum temperature (Figure 4)
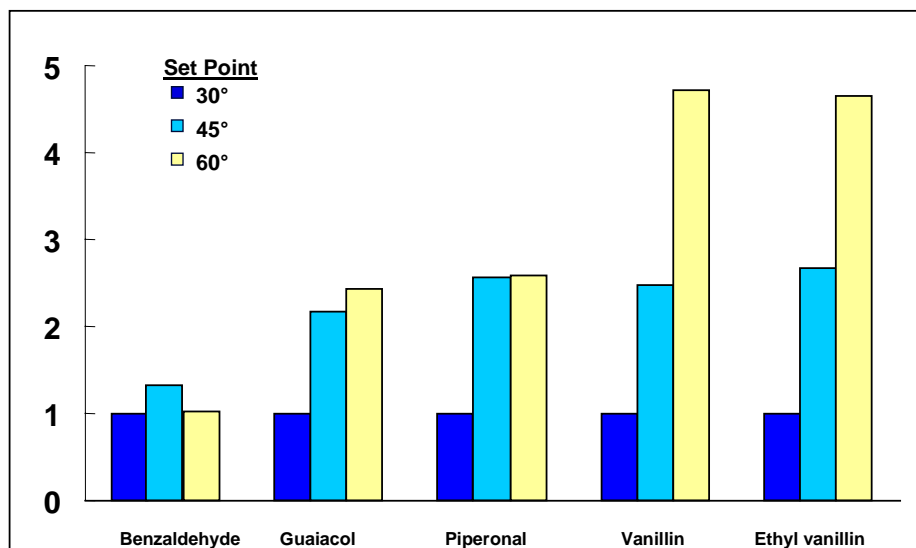

Figure 4. Variation of response for various flavor compounds after SPME sampling of the headspace over flavored coffee at 30°, 45° and 60°C. Fiber: 85-µm polyacrylate. Note that heating is more useful with the higher boiling compounds (Boiling point of benzaldehyde is 179° C; vanillin is 285°C).

Sample Handling

Poor precision and accuracy often result from improper sample handling.

1. It is a common practice to prepare a stock solution of analytes in methanol and then add a small aliquot to water. This method is acceptable with SPME and results are the same as those obtained by adding organics directly to water if the total level of methanol is less than 1%.
2. Saturation of the standards and samples with sodium chloride or sodium sulfate is useful in two situations:
   The analytes are polar and soluble in water
   The samples contain salts and it is desired to minimize matrix differences.
3. When determining acidic compounds such as phenols, lower the pH; for basic compounds such as amines, raising the pH will enhance sensitivity.
4. It is important to keep the concentrations of all of the components in an aqueous solution sufficiently low so that they remain dissolved in water.

For volatile compounds, additional guidelines should be followed:

5. When preparing standards of volatiles in water, the liquid should fill the entire storage container without any headspace.
6. Losses can occur when diluting the high level standard in preparation for a linearity study. To minimize errors, fill the containers that are to contain diluted standards with cold water at the correct volume for the dilution, quickly pour the concentrated standard into the containers, cap, mix, and refrigerate. For example, when diluting to 1/2 and 1/4 the concentration of the highest standard, take 40 mL vials (44 mL when filled to the top), add 22 mL and 33 mL of cold water; then pour in the concentrated standard.
7. If salt is added or the pH is adjusted, great care should be taken to minimize losses. For example, if the samples and standards are to be diluted in water, the salt can be added to the water *before* the dilution is made.
8. Chill the AutoSampler vials before adding the samples. Remove the standards and samples from the refrigerator, uncap them and quickly transfer aliquots to the AutoSampler vials, using a pipette that easily fits into the neck of the vial.
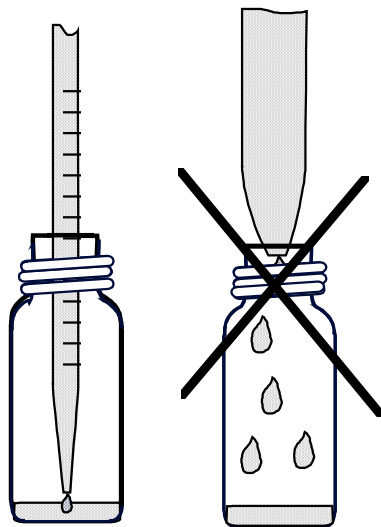
9. Cap the AutoSampler vials quickly. If solids were placed in the vial, prior to adding the liquid, mixing the vortex mixer will assure a homogeneous ample. Aqueous standards and samples remaining in the storage containers that were used to fill the AutoSampler vials, should not be used again.

Just before analysis:

One or two blanks should be run. The AutoSampler vials containing the samples, should be allowed to reach room temperature before starting the runs.

Profiling Flavors in Alcoholic and Non-Alcoholic Beverages with Automated Solid Phase Microextraction

SPME

Varian Application Note
Number 1

Zelda Penton
Varian Chromatography Systems

Key Words: solid phase microextraction, SPME, 8200 AutoSampler, beverages, food

Flavors in foods and beverages are monitored by static headspace GC and occasionally by thermal desorption or purge and trap. The result is a "fingerprint" chromatogram, that can be examined to determine if the particular sample meets the standards set by the manufacturer or if components are present that might adversely affect the taste of the product.

Solid Phase Microextraction (SPME) is a new technique for introducing analytes into a GC that can be used in this application. The technique utilizes a one-cm length of fused silica coated with an adsorbent. The coated fused silica (SPME fiber) is immersed directly into an aqueous sample or into the headspace above a liquid or solid sample. Organic compounds in the sample are subsequently adsorbed in the fiber. Finally, the fiber is inserted into a GC injector where the analytes are thermally desorbed and separated on the GC column.

Although it is possible to purchase a fiber holder for manual operation, automation is desirable to increase sample throughput and enhance repeatability. A kit is available to upgrade a Varian 8100 or 8200 CX AutoSampler for SPME sampling. The kit consists of a fiber holder and fibers, a chip to modify the AutoSampler and Windows-based software. After the modification, the AutoSampler can easily be restored to liquid sampling, if desired, in a matter of minutes.

This article illustrates that SPME is suitable for "fingerprinting" foods and flavors and offers several advantages over competing techniques.

Instrumentation and Conditions

Varian Star 3600 CX with a SPI, FID and ECD and 8200 CX AutoSampler modified for SPME. The AutoSampler was controlled by the SPME software. The GC Star Workstation ran concurrently on the same PC, controlling the GC and collecting data.

Column:	30m x 0.53 mm coated with 3 µm DB™-624, temperature program 40°C, hold 1 minute, 10°/min to 210°C, hold 7 min. Carrier gas: helium at 37 cm/s.
Injector:	SPI with insert for 530 µm columns.
Detectors:	220°C, FID at range 10^{-12} , ECD at range 10.
SPME Parameters:	Fiber coated with 100 microns polydimethylsiloxane. Adsorb in the headspace 15 minutes, desorb one minute, one sampling per vial.
Samples:	No sample preparation, 0.8 mL of food product added to a 2-mL AutoSampler vial and capped. The headspace was sampled over three alcoholic beverages and orange juice, dry coffee beans and tea leaves.

Results and Discussion

Chromatograms from the various samples are shown in the figures. Only coffee contained compounds that elicited an ECD response.
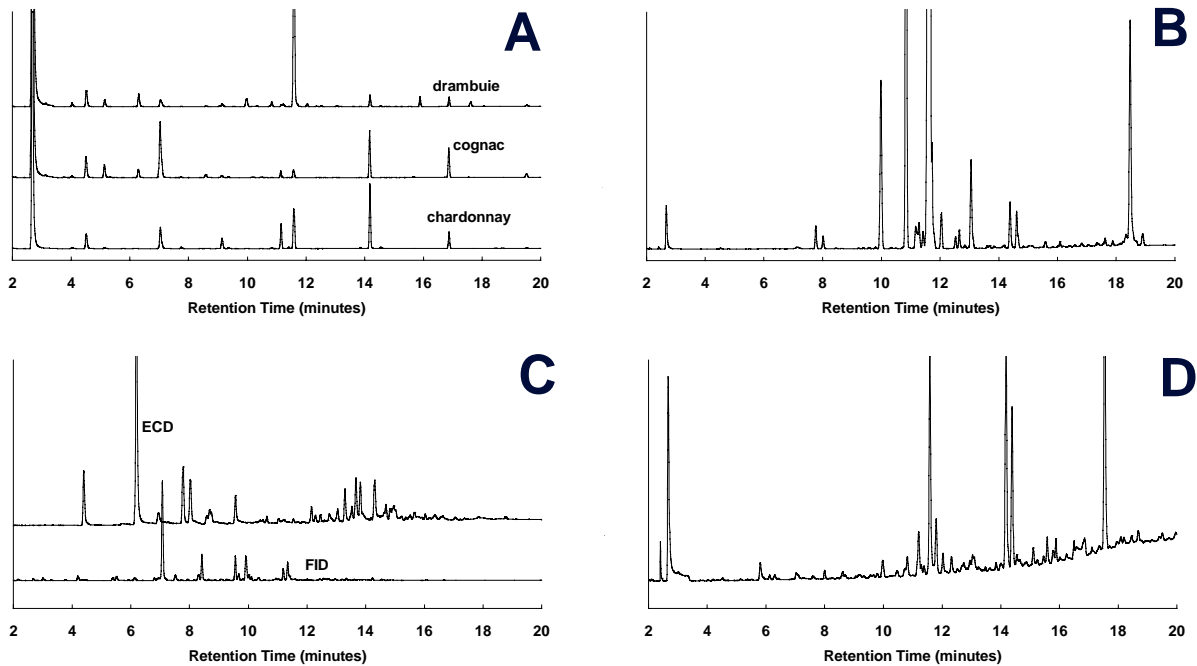
Figure 1. SPME Chromatograms of the headspace over food samples. The flame ionization detector was at range 10-12 for all of the chromatograms but the attenuation was adjusted to keep the larger peaks close to full scale. (Detector attenuation was the same for the three alcoholic beverages on the first chromatogram). The samples are: A - alcoholic beverages, B - orange juice, C -ground coffee beans and D - chamomile tea leaves.

SPME offers sensitivity at the ppb level. In addition there are several advantages over competing techniques such as static headspace, purge and trap and thermal desorption. These include no exposure of the analytes to active sites in transfer lines or collection tubes, relatively inexpensive instrumentation with full automation and no additional requirements for bench space.

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Determination of Residual Solvents in Pharmaceuticals with Automated Solid Phase Microextraction

SPME

Zelda Penton
Varian Chromatography Systems
Key Words: *Solid phase microextraction, SPME, 8200 AutoSampler, pharmaceuticals, volatiles*

Introduction

The 1995 United States Pharmacopeia (USP) National Formulary (1) lists four methods for determining organic volatile impurities in pharmaceutical compounds. All of the procedures utilize gas chromatography with flame ionization detection and either direct liquid injection or static headspace (Table 1). Table 2 lists the organic volatiles and the maximum allowable quantities in pharmaceutical compounds and in addition lists the concentration of these components in a standard solution. The area count precision required for replicate determinations of the compounds in the standard solution is 15% relative standard deviation.

Table 1. Summary of the USP methods.

Method	Column (0.53 mm fused silica)	Sample Introduction
I	5% phenyl-95% methylpolysiloxane	Direct* injection of 1 µL
IV	6% cyanopropylphenyl-94% dimethylpolysiloxane	Static headspace 1 mL
V	6% cyanopropylphenyl-94% dimethylpolysiloxane	Direct* injection of 1 µL
VI	**	Direct* injection of 1 µL

Usually water, unless another solvent is specified in the monograph for a particular drug,
**Method VI is used when a procedure is written for a particular pharmaceutical; in that case a column is specified.

The following note describes the use of solid phase microextraction (SPME) for determining solvents in pharmaceuticals.

Table 2. Organic volatile impurities and maximum allowable levels in pharmaceuticals. The concentrations in the standard solution assume a concentration of 20 mg/mL for the pharmaceutical compound.

Component	USP Limit (ppm)	Standard solution (µg/mL water)
Methylene Chloride	500*	10
Benzene	100	2
Trichloroethylene	100	2
Chloroform	50	1
1,4-Dioxane	100	2

*Effective date 1/1/95

The results, which included a recovery study on two pharmaceutical compounds, indicated that SPME is a good alternative to liquid injection or static headspace. An automated SPME system is considerably less expensive than a dedicated static headspace system and the problems of injecting aqueous samples into a GC are avoided.

Several additional solvents were considered in addition to the above to conform to compounds actually used in pharmaceutical companies. These were ethanol, acetone, isopropanol and toluene.

Instrumentation and Conditions

Instrument:	Varian Star 3600 CX with a septum-equipped temperature-programmable injector (SPI), FID and 8200 CX AutoSampler, modified for SPME. The AutoSampler was controlled by the SPME software. The GC Star Workstation ran concurrently on the same PC, controlling the GC and collecting data.
	A Varian Genesis Headspace Sampler was used for comparative studies with static headspace.
Column:	30m x 0.53 mm coated with 3 μm DBTM-624, 35°C, hold 2 minutes, 20°/min to 200°C, hold 0.75 min.
	Carrier gas: helium at 38 cm/s at 50°C.
Injector:	SPI with insert for 0.53 mm columns, 210°C, isothermal.
Detectors:	220°C, FID at range 10^{-12}
SPME Parameters:	The fiber (Supelco, Inc.) was coated with 100 μm polydimethylsiloxane.
	Adsorbed in the headspace 14 minutes, desorbed two minutes, one sampling per vial.
Standards:	A test standard was prepared in HPLC water (Table 3). The first three compounds were added directly to water; the last 6 compounds were initially dissolved in a methanol stock solution and diluted 1000-fold in water.
Samples:	Two water-soluble drugs were studied—a cholinesterase inhibitor (A) and a tricyclic antidepressant (B).
Recovery (Accuracy):	Three samples were prepared in two-mL screw-cap vials, the above test sample alone, drug A in test mix and drug B in test mix. To conform to the concentrations listed in the USP methods of 20 mg/mL, 16 mg of drug was dissolved in 0.8 mL of test sample. Blanks consisting of water and each of the two drugs in water were also prepared. To enhance the response of the polar solvents, the standards and samples were saturated with sodium sulfate (20g/100g water).
Linearity:	The above standard was prepared at 0.5, and 2 times the concentrations shown in the table and the recovery experiment above was repeated at the three concentrations. Limits of detection (LOD's) were determined, assuming a signal to noise ratio of 2.

Results and Discussion

The chromatogram in Figure 1 was obtained from sampling the headspace over Drug A, using SPME. Data for precision and recovery of the solvents in the test sample are presented in Table 3. Correlation coefficients to a straight line and LOD's are also given.

The sample was in a 2-mL vial containing 0.8 mL test standard. Drug B (16 mg) was dissolved in the test standard. Concentration of each solvent is in parenthesis next to the peak name. Compounds 5-10 were initially dissolved in a stock solution with methanol as a solvent; hence the methanol peak. FID attenuation is 10-fold more sensitive before the arrow.
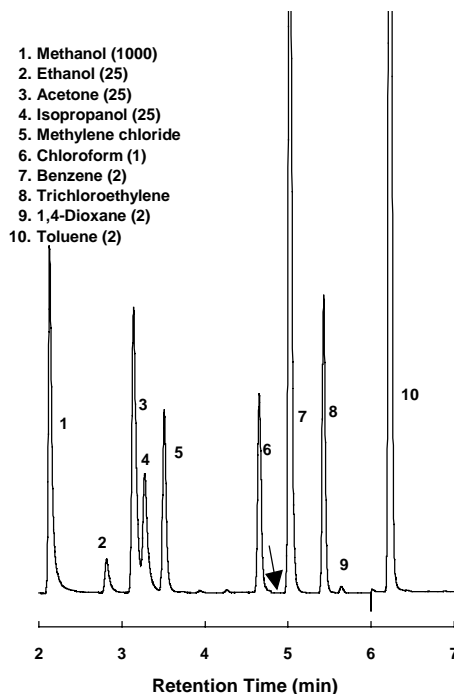
Figure 1. Automated SPME chromatogram of the headspace over a test sample containing solvents monitored in pharmaceuticals. The concentration in µg/mL is given next to each peak name.

Table 3. Precision data (%RSD area counts for 4 replicate determinations) is given for the concentrations shown in the table, linear correlation coefficients were determined by sampling at three levels—0.5 x, 1 x and 2 x the values in the table. The limits of detection (LOD's) are with FID detection (S/N=2). These values are for the standard mix; to determine the limit of detection in a drug sample, dissolved in water at a concentration of 20 mg/mL, the numbers should be multiplied by 50. Recoveries (accuracies) are calculated by comparing detector response of compounds in the standard mix to drug samples spiked with the standard mix.

| Compound | Standard Mix | | | | Drug A | | Drug B | |
	Conc. µg/mL	Precision	Corr.	LOD µg/mL	% Recovery	Precision	% Recovery	Precision
Ethanol	25	1.82	0.999	2.3	98.4	2.80	100.9	2.47
Acetone	25	1.18	0.998	0.3	103.2	0.29	101.2	0.48
Isopropanol	25	1.29	0.997	0.7	99.5	0.54	101.7	0.60
Methylene chloride	2	1.93	0.999	0.02	100.0	2.34	91.5	2.15
Chloroform	1	1.42	0.998	0.005	100.4	2.29	76.6	1.25
Benzene	2	0.49	0.999	0.0003	100.1	1.74	70.0	1.58
Trichloroethylene	2	0.50	0.999	0.001	104.0	2.43	63.4	2.00
1,4-Dioxane	2	2.18	0.995	0.04	102.8	2.88	104.2	0.44
Toluene	2	0.42	0.999	0.0001	98.5	2.65	168.6*	3.82

Blank runs of the drugs indicated that they were free of solvents with the exception of drug B which contained toluene.

Linearity and recoveries with drug A indicated no matrix effects; therefore this drug was not studied further.

Matrix effects

With Drug B, the polar solvents showed good linear response and recoveries but methylene chloride, chloroform, benzene and trichloroethylene were only partially recovered. Toluene, the solvent that was used in the purification of the drug, was still present. Moreover, the toluene was strongly retained by the drug even after the dry compound was heated in an oven at 80°C for one hour. Therefore it was felt that further study was warranted and a new toluene-free sample of this compound was purchased.

When toluene was added to the toluene-free drug, the recovery was 29%. The recovery experiment was repeated using conventional static headspace. The data in Table 4 indicates that the matrix effect is present with heated headspace and is therefore not SPME-related.

Table 4. Percentage recovery of toluene in the presence of Drug B with a heated static headspace system.

Static Headspace	
(20 min equilibration), neutral pH, saturated with sodium sulfate	
50°C	80°C
38%	46%

It was found that elimination of the sodium sulfate and lowering the pH to 2, greatly improved the recovery of toluene. The sodium sulfate was added originally per USP Method IV to improve the response of the polar compounds. Elimination of the salt and lowering of the pH increased the solubility of the drug (pKa was 9.4), thereby improving the partitioning of the toluene into the headspace and ultimately into the fiber. Under these conditions, the recovery with SPME sampling was 73%. More important, recoveries were consistent when the drug was spiked with toluene at the three levels mentioned above (linear correlation coefficient was 0.999). Quantitation could be by the method of standard additions or by comparison of a sample containing toluene with a toluene-free drug sample spiked to a known level.

Conclusion

For the determination of residual solvents in pharmaceuticals, SPME offers sensitivity and precision that greatly exceed the USP requirements. As compared to a static headspace system, SPME is compact and offers comparable sensitivity and full automation at a lower cost.

In comparison with Method I which normally involves direct injection of water, the sensitivity with SPME was greater by factors varying from 2 (dioxane) to 90 (TCE). As with all techniques, some initial method development is required to optimize results.

Acknowledgment

References and Additional Reading

1. The 1995 United States Pharmacopeia, National Formulary, USP 23, NF18, published by the United States Pharmacopeial Convention, Inc. 12601 Twinbrook Parkway, Rockville, MD 20852.

2. Z. Zhang and J. Pawliszyn, "Headspace Solid Phase Microextration", Analytical Chemistry, 1993, Vol. 65, No.14, 1843-1852.

Determination of a Wide Range of Organic Impurities in Water with Automated Solid Phase Microextraction

SPME

Varian Application Note
Number 3

Zelda Penton
Varian Chromatography Systems

Key Words: *Solid phase Microextraction, SPME, 8200 AutoSampler, Volatiles, Semivolatiles*

While gas chromatography is the instrument of choice in the determination of organic compounds in water, several methods are available for introducing the sample into the GC column. A comparison of several methods was undertaken to assess the relative merits of each technique. These were: direct aqueous injection, ambient and heated static headspace (SHS), purge and trap and finally, the automated solid phase microextraction (SPME) system described previously (1).

A test sample was prepared that contained mostly non-polar organics with a wide boiling point range (40°-170°C). The sample was analyzed utilizing each of the above sample introduction methods.

Precision and minimum detectable quantities were compared. As SPME is a relatively new technique, linearity was demonstrated; it was deemed unnecessary to verify the linearity with the other, well-established sample introduction methods.

To further verify the effectiveness of SPME for samples containing several different classes of analytes, a second test sample was prepared. This sample contained all of the compounds in the first sample plus several phenols and two polynuclear aromatic hydrocarbons (PNA's). With this sample, adsorption time versus detector response was examined and relative responses were determined for liquid and headspace SPME sampling.

Instrumentation and Conditions

Instruments:	Varian Star 3600 CX GC with a septum-equipped temperature-programmable injector (SPI), FID and 8200 CX AutoSampler. The AutoSampler was used in the liquid injection, ambient headspace and SPME modes.
	During SPME operation, the AutoSampler was controlled by the SPME software and the GC was controlled by the Star Workstation; in the liquid and headspace injection modes, the GC Star Workstation also controlled the AutoSampler. The Star Workstation and Excel Macros were used for data acquisition and summary reports.
	For comparison with static headspace and purge and trap, a Varian Genesis Headspace Sampler and a Tekmar LSC 3000 purge and trap system with AQUATek 50 Automatic Liquid Sampler were used.
Column:	30m x 0.53 mm coated with 3-µm DB-624^{TM}, 40°C, hold 1 minute, 20°/minute to 200°C, hold 0 minutes (for the second sample, the final temperature was 220°C with a 10 minute hold); carrier gas: helium, 37 cm/s at 50°C.
Injector:	SPI with SPME insert, 220°C, isothermal.
FID:	220°C, range 10^{-12}

**Sample Introduction Conditions for Sample 1
(First three methods used the 8200 CX AutoSampler)**

Direct Liquid Injection:	User-defined solvent-flush mode with 0.4 µL solvent plug (water) and lower air gap. Sample volume 1-µL.
Automated SPME:	Fibers (Supelco, Inc.) were coated with 100 µm polydimethylsiloxane. Both headspace and liquid phases were sampled. Volumes were 0.8 mL and 1.2 mL in standard 2-mL vials. Adsorption times varied for equilibration studies but were normally 10-30 minutes with 1-2 minutes desorption.
Ambient Headspace:	Same sample volume as with SPME headspace (0.8 mL). Injected 40 µL headspace.
Heated Headspace:	Samples (10 mL in a 22-mL vial) were heated to 75°C, line and valve temperatures were 85°C. Equilibration time was 4 minutes with mixing at 80% of full power for 7 minutes, stabilization time was 2 minutes. Sample loop was 500 µL.
Purge and Trap	Samples (5-mL) were purged at 30°C for 11 minutes and desorbed for 2 minutes.

**Sample Introduction Conditions
for Sample 2**

This sample was analyzed only by SPME and by static headspace (SHS). The SPME conditions were the same as for test sample 1; the SHS conditions were also the same except for the temperatures. Initially, the samples were heated to 85°C with line and valve temperatures of 95°C; then the valve was raise to 160°C and the transfer line was raised to 200°C.

Samples

The two test samples were prepared in HPLC water at the concentrations shown in Table 1. For SPME headspace and SHS determinations, the samples were saturated with Na_2SO_4. Test sample 2 was analyzed both at neutral pH and at pH 2; the low pH was required for consistent response of the phenols. Figure 1 is a SPME chromatogram of Sample 2.

Table 1. Components in the test samples.

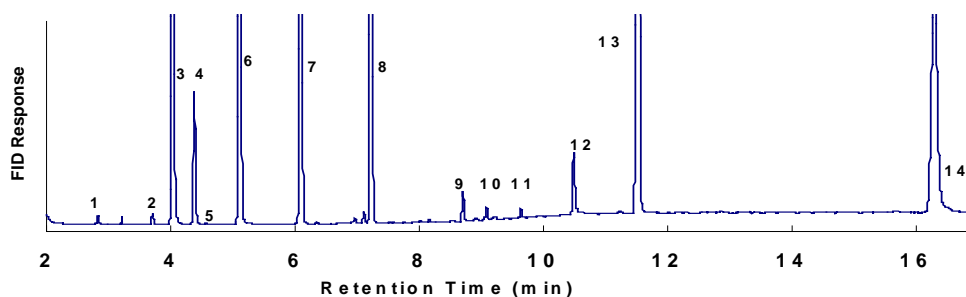|	Compound	BP (°C)	Conc (ppm) 1	2
1.	Dichloromethane (MeCl2)	40	2	0.4
2.	Chloroform	61-62	1	0.2
3.	Benzene	80	2	0.4
4.	Trichloroethylene	87	2	0.4
5.	Dioxane	101	2	0.4
6.	Toluene	111	2	0.4
7.	m-Xylene	139	2	0.4
8.	1,2,4-Trimethylbenzene (TMB)	169-171	2	0.4
9.	2,6-Dimethylphenol	201	-	0.2
10.	o-Nitrophenol	215-216	-	0.2
11.	p-Chlorophenol	220	-	0.3
12.	2,4,6-Trichlorophenol	246	-	0.2
13.	Acenapthene	279	-	0.2
14.	Phenanthrene	340	-	0.2

Figure 1. SPME sampling (liquid phase) of test sample 2 with a 100-µm polydimethylsiloxane fiber. Adsorption time was 30 minutes, desorption time was 2 minutes. The peaks are identified in Table 1. For this sample, area count precision varied from 1.7-2.7% rsd for the first 12 compounds; precision for acenaphthene was 3.5% and phenanthrene was 5.9% (5 replicates).

Results and Discussion

Adsorption Times and Relative Responses With SPME Sampling of the Liquid or Headspace Phases

When the liquid phase was sampled for various times, a leveling off of response was observed for the more volatile compounds after about 10 minutes (Figure 2). The trichlorophenol and the PNA's showed a much greater response after 30 minutes of sampling, indicating that equilibrium was not attained for these compounds; nevertheless after sampling for 30 minutes, the precision was good (Figure 1, legend).
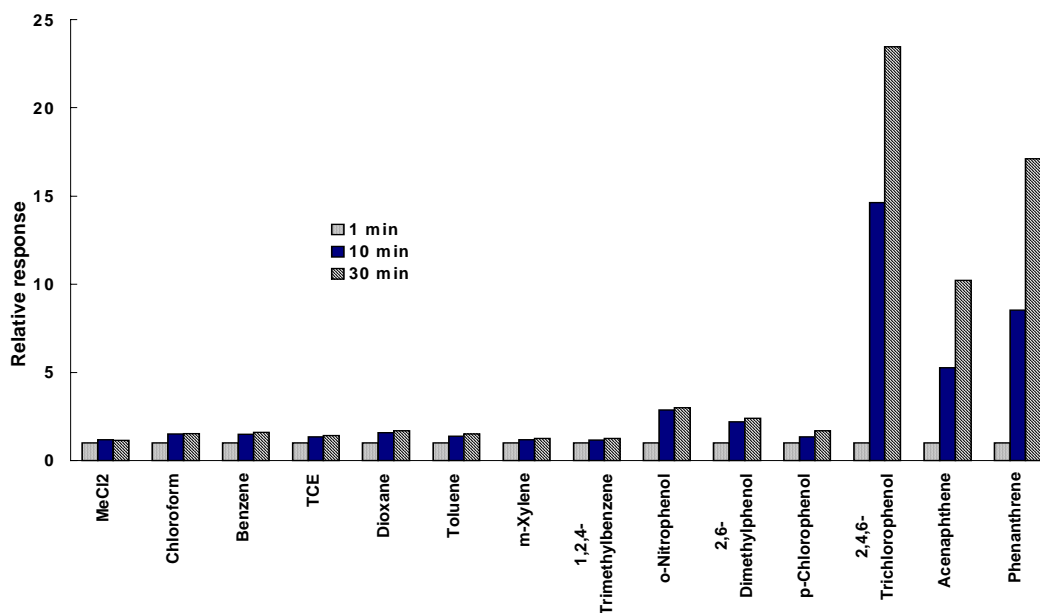

Figure 2. SPME responses for the compounds in test sample 2, after sampling the liquid phase for various times (two minutes desorption). The values at ten and thirty minutes are normalized to the values after one minute of sampling. Concentrations are in Table 1.

The relative responses with SPME after headspace and liquid sampling are shown in Figure 3. These results are totally unlike observations made with conventional static headspace sampling.

When the sample was analyzed with heated headspace at 85°C, there was no response to the phenols or PNA's, but with SPME headspace sampling at ambient temperature, there was a strong response to these compounds. With SPME, equilibrium is established between three phases and when one considers the strong affinity of the fiber for aromatic compounds, it is not surprising that there would be a good response to these compounds in spite of their low volatility.
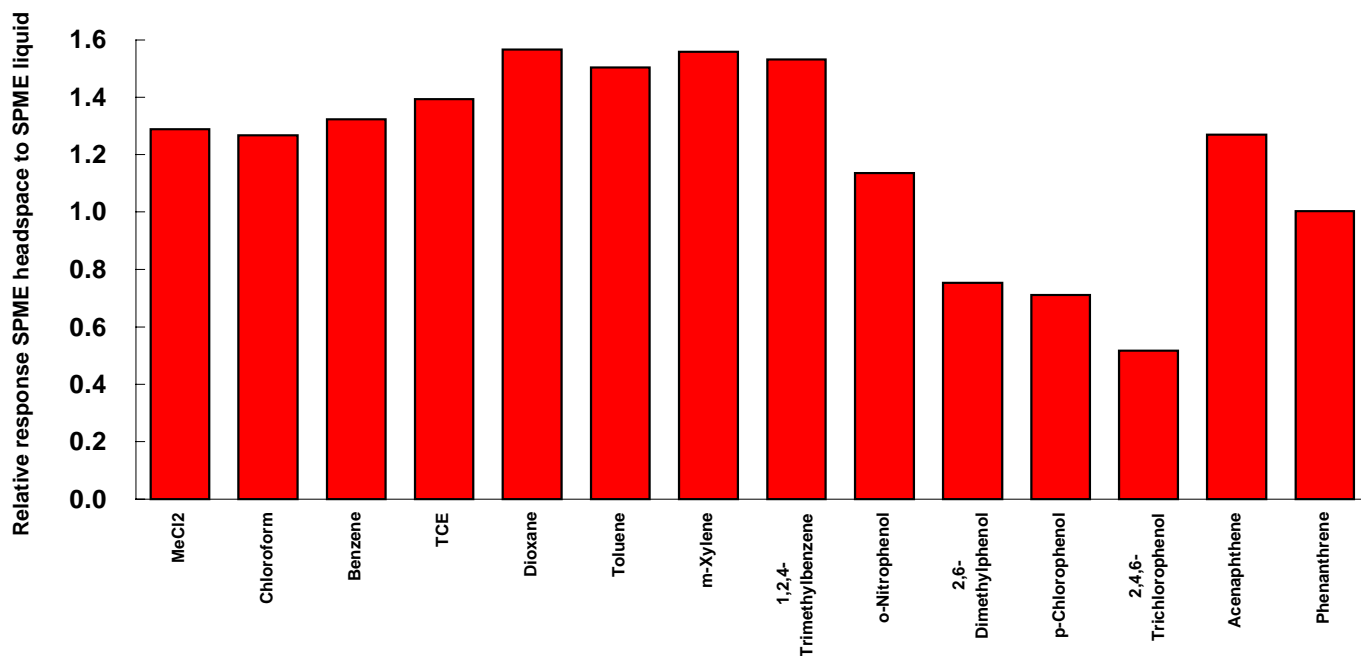

Figure 3. Responses for each of the components in test sample 2 were determined after headspace sampling over 0.8 mL and liquid sampling of 1.2 mL. Adsorption times were 10 minutes with two minutes desorption. The bars represent the FID response after headspace sampling, divided by the response from liquid sampling. These values were then multiplied by 1.5 to correct for the difference in sample volume.

Linearity and Detection Limits With Various Sample Introduction Methods
Linearity of response with headspace and liquid SPME sampling was verified with the components of test sample 1 and detection limits for these compounds with SPME were compared with other sample introduction techniques (Table 2). Precision data for these sampling methods are shown in Table 3.

Table 2. Summary of data obtained with sample 1 including correlation coefficients (r) to a straight line for SPME liquid sampling and minimum detection limits (S/N=4) with different sample introduction methods. SPME liquid and headspace values were similar.

Compound	r* SPME (liquid)	Minimum Detectable Quantities (ppb)				
		SPME (liquid)	SHS (ambient)	SHS (heated)	Purge and Trap	Direct Injection
Dichloromethane	0.9997	12	10	0.7	0.05	80
Chloroform	0.9996	8.6	20	1.5	0.04	240
Benzene	0.9989	0.3	1.4	0.1	0.003	17
Trichloroethylene	0.9989	1.2	8.5	0.8	0.01	108
Dioxane	0.9961	45	900	5.9	0.6	94
Toluene	0.9994	0.18	2.2	0.2	0.003	20
m-Xylene	0.9997	0.13	3.3	0.2	0.003	26
1,2,4-Trimethylbenzene	0.9987	0.12	3.6	0.2	0.005	29

*Eight levels, 3 samplings at each level, for concentration ranges of 20 ppb to 4 ppm (10 ppb to 2 ppm for chloroform).

With the exception of dioxane and 1,2,4-trimethylbenzene, all of the compounds in sample 1 are regulated in drinking water by the USEPA and many European countries. Of these compounds, only dichloromethane was not detected with SPME below the maximum contaminant levels. With electroconductivity detection, it would have been possible to meet the required levels (5-10 ppb) for dichloromethane.

Table 3. Area count precision for each sampling method at the 2 ppm level (1 ppm for chloroform). SPME liquid and headspace values were similar.

Compound	% RSD*				
	SPME (liquid)	SHS (ambient)	SHS (heated)	Purge and Trap	Direct Injection
Dichloromethane	0.9	1.1	1.3	7.5	2.0
Chloroform	1.1	2.0	1.4	0.6	3.3
Benzene	1.7	1.3	1.4	0.7	5.0
Trichloroethylene	2.2	1.5	1.4	1.2	8.8
Dioxane	2.0	14	2.6	10.5	7.0
Toluene	2.6	2.4	1.5	1.5	13
m-Xylene	2.8	1.7	1.7	3.0	2.8
1,2,4-Trimethylbenzene	2.7	2.2	1.8	4.4	8.2

*n=8 for SPME, SHS and purge and trap, n=3 for direct injection

Conclusions

It was shown that the automated SPME system can deliver linear and precise data with sensitivities comparable to heated headspace for volatiles and semivolatiles in water. In fact, the phenols and PNA's in the sample could be detected in the headspace with SPME but not with heated headspace. Although specialized fibers might be used to give optimum results with compounds such as phenols or PNA's (2,3), the 100-μm polydimethylsiloxane fiber was useful for a sample containing a wide range of compounds.

Finally, it appears that SPME generally meets the guidelines for contaminants in drinking water and further study for this application is warranted.

References and Additional Reading

1. "Automation and Optimization of Solid-Phase Microextraction", Arthur, C.L., Killam, L.M., Buchholz, K.D., Pawliszyn, J. and Berg, J.R., Analytical Chemistry, 64, 1992, pp 1969-66.

2. "Method Development Tips for the Automated SPME System", Penton, Z., Varian GC Advantage Note 11.

3. "Optimization of Solid Phase Microextraction Conditions for Determination of Phenols", Buchholz, K.D. and Pawliszyn, J., Analytical Chemistry, 66, 1994, pp 160-167.

Flavor Analysis of a Fruit Beverage With Automated Solid Phase Microextraction

SPME

Varian Application Note
Number 4

Zelda Penton
Varian Chromatography Systems

Key Words: Solid phase microextraction, SPME, 8200 AutoSampler, foods and flavors

Trace quantities of compounds in foods are often critical in imparting the proper taste and aroma to a product. In other cases, a very small quantity of a particular compound may be responsible for causing a food product to have an "off" taste or odor. These trace compounds are usually present in very complex mixtures and quantifying them presents an analytical challenge. GC or GC/MS, combined with static headspace, dynamic headspace or thermal desorption, is normally used in these applications.

A new sample introduction technique, solid phase microextraction (SPME), offers the possibility of becoming a strong competitor of established methods. With SPME, analytes in the liquid sample or in the headspace above the sample are adsorbed onto fused silica fibers coated with a polymer such as polydimethylsiloxane or polyacrylate. The fiber is then inserted into a GC injector for desorption. The system has been automated with the Varian 81/8200 AutoSampler (1).

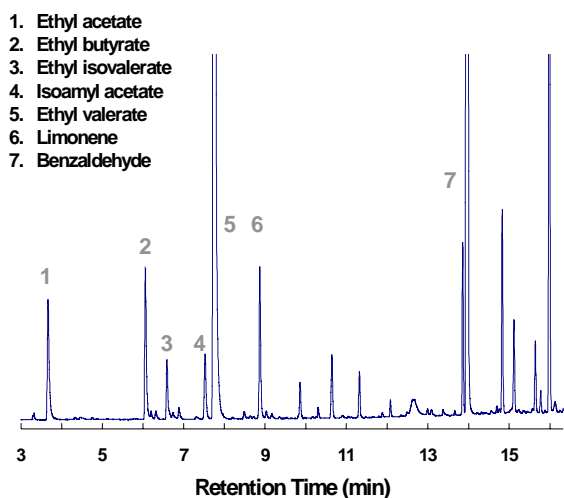In previous work (2) with volatile non-polar compounds, the automated SPME system provided excellent sensitivity, precision and good linearity for organics up to 1,2,4-trimethylbenzene, thus providing an inexpensive and compact replacement for a static headspace system. The current study extends this work to relatively polar volatiles.

A preliminary investigation (3) of the feasibility of SPME for extracting flavor components from various beverages looked promising. Therefore, a commercially available fruit beverage was studied systematically. Several key components were identified by the manufacturer as being of interest in quality control. The presence of these compounds was confirmed with GC/MS and a test sample containing known quantities of these components was prepared.

In this note, data is presented comparing the responses of these compounds on two SPME fibers with results from static headspace (SHS). Precision data is also given for the compounds in the fruit beverage with SPME and SHS.

Instrumentation and Conditions

Instruments:	Varian Saturn 3 GC/MS with a septum-equipped temperature-programmable injector (SPI), FID and 8200 CX AutoSampler, modified for SPME. The AutoSampler was controlled by SPME software. After confirmation of the identity of the critical compounds by MS, the end of the column was installed in the FID. At this point, data was collected and processed with the GC Star Workstation and Excel macros.
	A Varian Genesis Headspace Sampler was used for comparative studies with static headspace.
Column:	30m x 0.25 mm coated with 0.5-µm Supelcowax ™, 40°C, hold 2 minutes, 10°/minute to 180°C, 30°/minute to 220°C hold 3.67 minutes (total run time, 21 minutes).
	Carrier gas: helium at 41 cm/s at 50°C.
Injector:	SPI with SPME insert, 220°C, isothermal.
Mass Spec:	Electron impact ionization mode, mass range 40-250 m/z.
FID:	230°C, range 10^{-12}.
Automated SPME Conditions:	The fibers (Supelco, Inc.) were coated with 100 µm polydimethylsiloxane (PDMS) and 85 µm polyacrylate.
	Sampled the headspace over an 0.8-mL liquid sample in a 2-mL vial. Normally 20 minutes adsorption, two minutes desorption, one sampling per vial (5-60 minutes adsorption in the equilibration study).
Heated Headspace:	Samples (10 mL in a 22 mL vial) were heated to 75°C, line and valve temperatures were 85°C. Equilibration time 5 minutes, mixed at 80% of full power 7 minutes, stabilization time, 2 minutes. Sample loop was 500 µL.
Samples:	Commercially available fruit beverage. Figure 1 is a SPME chromatogram of the fruit beverage using a PDMS fiber.
	Test sample consisting of components identified in above beverage, dissolved in HPLC water (Table 1).

1. Ethyl acetate
2. Ethyl butyrate
3. Ethyl isovalerate
4. Isoamyl acetate
5. Ethyl valerate
6. Limonene
7. Benzaldehyde

Table 1. Components of the test sample. These compounds were identified in the fruit beverage by GC/MS.

Compound	Conc (ppb)
Ethyl acetate	938
Ethyl butyrate	153
Ethyl isovalerate	151
Isoamyl acetate	153
Ethyl valerate	3050
Limonene	146
Benzaldehyde	903

Figure 1. SPME sampling of the headspace over a fruit beverage with a 100-µm PDMS fiber. The compounds listed in the table were identified by GC/MS.

Results and Discussion
Comparative Response and Equilibration Times with Two Fibers
The test sample was analyzed using 100-µm PDMS and 85-µm polyacrylate SPME fibers and the comparative responses were evaluated after 20 minutes adsorption (Figure 2). The graph in Figure 3 compares detector response versus adsorption time for the two fibers.
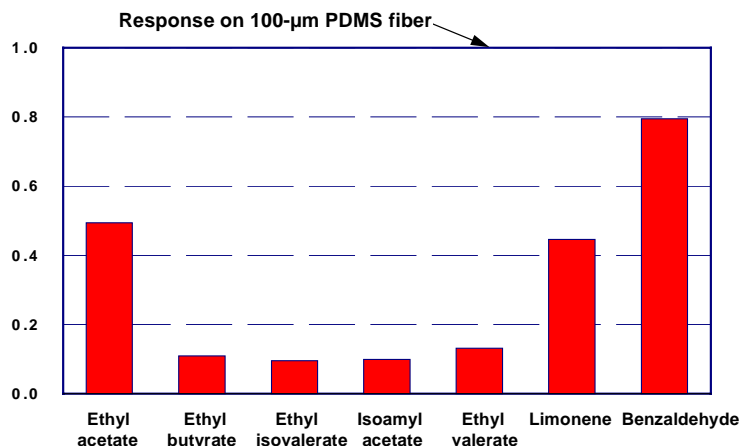

Figure 2. Response on a polyacrylate fiber (represented by the bars) normalized to the response on a PDMS fiber (20 min. adsorption).
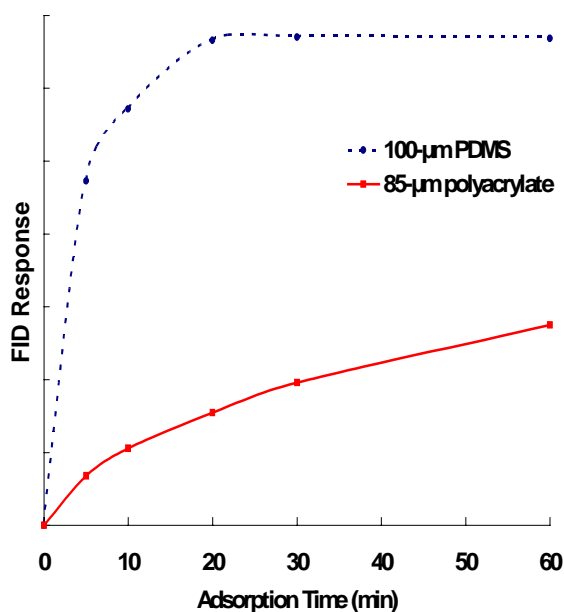

Figure 3. Equilibration study for isoamyl acetate with two SPME fibers. The headspace over the test mix was sampled. All of the compounds in the test mix, exhibited similar behavior with the two fibers.

After 20-30 minutes adsorption with the PDMS fiber, the slope of the curves leveled off, indicating that equilibrium was reached; this was not the case with the polyacrylate fiber (Figure 3). For this application, the PDMS fiber provided better sensitivity and a shorter equilibrium time than the polyacrylate fiber. However, the polyacrylate fiber has been shown to be useful for determination of phenols (4)

Comparison with Headspace
The test sample and the beverage were analyzed using static headspace and SPME with a PDMS fiber. The comparative responses (of the compounds in the test sample) are shown in Figure 4. Table 2 lists precision data for 10 replicates when the critical compounds in the fruit beverage were monitored by headspace and SPME.

Table 2. Precision of SPME and static headspace sampling of components in a fruit beverage (FID area counts, % RSD, n=10). A 100-µm PDMS fiber was used.

Compound	SPME	SHS
Ethyl acetate	1.39	3.69
Ethyl butyrate	1.42	4.46
Ethyl isovalerate	2.95	4.83
Isoamyl acetate	3.42	4.58
Ethyl valerate	1.54	4.53
Limonene	2.96	7.03
Benzaldehyde	1.28	8.34

Table 3. Minimum detectable quantities with FID and MS detection in ppb. The data is from SPME headspace sampling of the test mix, using a 100-µm PDMS fiber.

Compound	FID (s/n=4)	MS (s/n=10)
Ethyl acetate	26	2.8
Ethyl butyrate	1.3	0.4
Ethyl isovalerate	0.6	0.3
Isoamyl acetate	0.6	0.1
Ethyl valerate	1.0	0.04
Limonene	0.2	0.07
Benzaldehyde	0.2	0.02

The minimum detectable quantities of the components in the test mix are in Table 3.
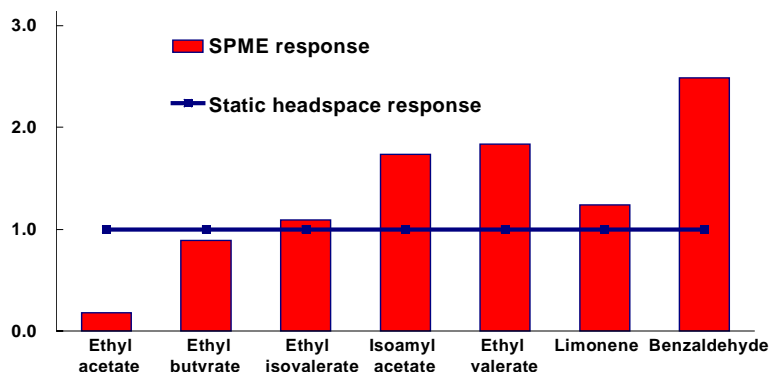
Figure 4. SPME headspace (100-µm PDMS fiber) versus conventional static headspace response. These results were derived from sampling the fruit beverage. All of the conditions are in the text.

Conclusions

SPME can deliver precise data with sensitivities comparable to heated headspace in the determination of flavor components in beverages. Instrumentation is relatively inexpensive, compact and versatile The SPME technique, shown here to be a practical and inexpensive replacement for headspace, should be widely used in analytical laboratories in the future.

References and Additional Reading

1. "Automation and Optimization of Solid-Phase Microextraction", Arthur, C.L., Killam, L.M., Buchholz, K.D., Pawliszyn, J. and Berg, J.R., Analytical Chemistry, 64, 1992, pp 1969-66.

2. "Determination of a Wide Range of Organic Impurities in Water with Automated Solid Phase Microextraction", Penton, Z, Varian GC Application Note 50.

3. "Profiling Flavors in Alcoholic and Non-Alcoholic Beverages with Automated Solid Phase Microextraction", Penton, Z., Varian GC Application Note 47.

4. "Optimization of Solid Phase Microextraction Conditions for Determination of Phenols", Buchholz, K.D. and Pawliszyn, J., Analytical Chemistry, 66, 1994, pp 160-167

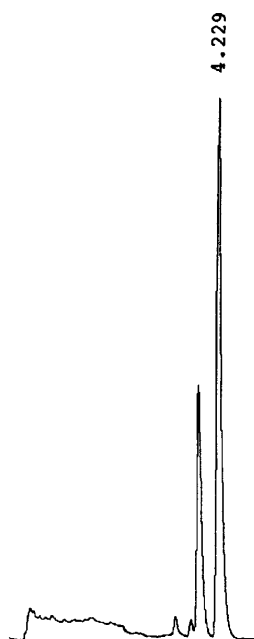# Analysis of Therminol in Process Water Using Solid Phase Microextraction

SPME

Varian Application Note
Number 5

Joyce Jennison and Colin P.R. Jennison
Varian Canada

Key Words: *Solid Phase Microextraction, SPME, Therminol, Biphenyl, Diphenyl oxide, Water*

Introduction

Figure 1. 100 ppb(ng/mL) Therminol in Water

Therminol VP-1 is a heat transfer fluid that consists of 73.5% Diphenyl oxide and 26.5% Biphenyl. This material is widely used in the chemical process industry, but must be kept out of the waste stream. Typically, residual levels of less than 4 ppb must be achieved.

The analysis of Therminol in water has previously been accomplished using liquid/liquid extraction and direct injection and analysis of the extract with gas chromatography (GC). Solid phase microextraction (SPME) is well suited for the analysis of trace organics in water.

It is a fast, simple, solvent-free extraction. Organics are adsorbed from an aqueous sample onto a fused silica fiber coated (or bonded) with a layer of liquid phase - in this example polydimethylsiloxane. After adsorption, the fiber is withdrawn into a metal sheath (needle) which protects it during withdrawal from the septum vial. The needle is then inserted through the septum into a hot injector, the fiber extended and the analytes thermally desorbed to the GC column. For the purpose of this method, development of the entire extraction and desorption process was automated with the use of standard 2-mL autosampler vials, a Varian 8200 CX AutoSampler and the appropriate software.

Instrumentation and Conditions
Instrument: Varian Star 3400 CX with an 8200 CX AutoSampler, modified for SPME. Varian Star Workstation Version 4 and SPME software.

Injector: 1077 Split/splitless injector, splitless mode, 80 mL/min vent flow, 2 minute vent timing. Temperature 220 C (7- m fiber) and 250 C (100 m fiber).

Column: 15m x 0.32 mm, 1- m DB-5, 130 C, hold 5 minutes. Helium carrier gas at 5 mL/min at 130 C.

Detector: FID, 250 C, Range 10-12.

SPME Parameters: Liquid adsorption for 10 minutes, desorbed for 2 minutes.

Results and Discussion

Initial development work was carried out with the use of a 100-μm fiber. SPME and GC conditions are described above and Figure 1 shows a 100 ppb (μg/L) standard. Although the upper temperature limit of this 100-μm fiber is 220ºC, it was found that a desorption temperature of 250ºC gave better peak shapes. The higher temperature was necessary to provide rapid desorption from this relatively thick film fiber. The high desorption temperature resulted in some fiber bleed. Quantitation was therefore based on the larger diphenyl oxide peak which was free of interference. In this manner, quantitation to < 1 ppb was readily obtained on a 1.5 mL sample volume. Figure 2 shows the calibration curve obtained for the 1 to 100 ppb concentration range.

Concerns regarding the stability of the 100 μm coated fiber, when run under high temperature conditions, prompted an evaluation of a 7 μm bonded polydimethylsiloxane fiber. The use of the thinner film bonded fiber allowed efficient desorption of the Therminol at a lower temperature (220°C) and completely eliminated fiber bleed.
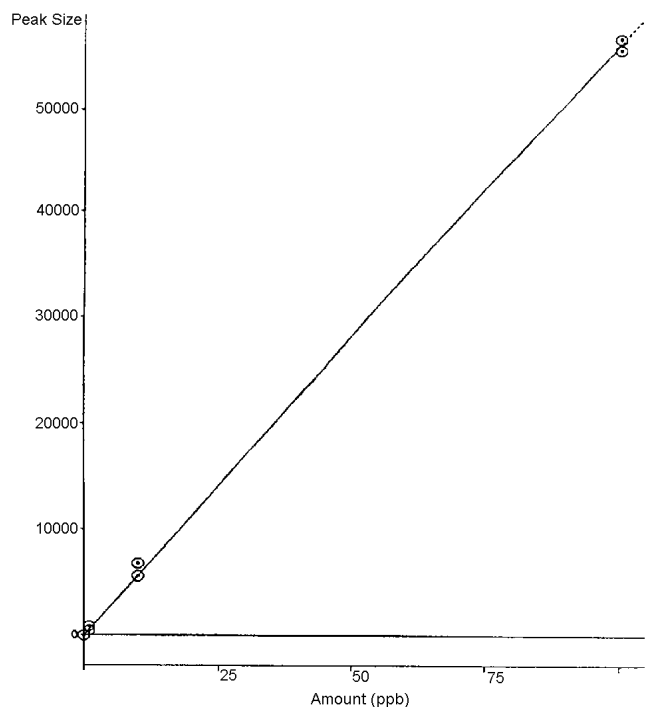
Figure 3 shows a comparison of the 100 μm versus the 7 μm fibers for a 10 ppb standard. Peak areas with the 7 μm fiber were about 3 times lower than the 100 μm fiber. However, due to a sharper peak shape the peak heights, and therefore minimum detection limits, were approximately half.
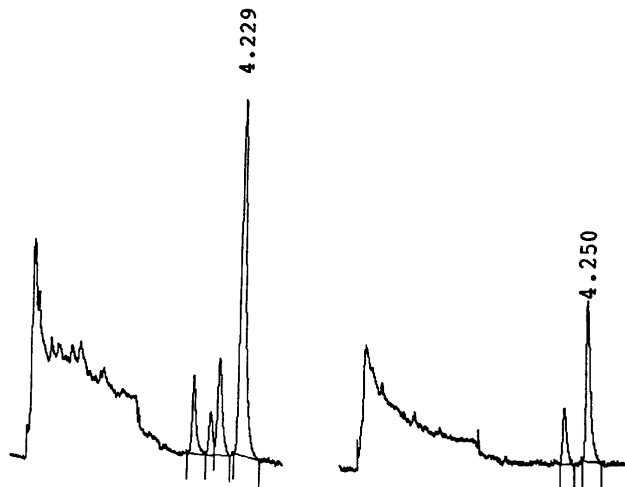

Figure 3. 10 ppb Therminol in water 100 mm fiber (left) and 7- m fiber (right)

Conclusions

SPME is a simple, sensitive, highly effective approach to the automated analysis of Therminol in water. Although a slightly lower detection limit is provided by the 100-μm fiber, the lower desorption temperature and bleed with potentially greater stability and lifetime provided by the 7μm bonded phase fiber would make it the best choice for this analysis.

Acknowledgment

The assistance of Maureen Good of Dupont Canada in the development of an automated SPME method for Therminol is greatly appreciated.

NOTE: The 100-μm fiber has been improved by Supelco, and now has a temperature limit of 250°C.

Figure 2. Calibration curve for 1 to 100 ppb therminol in water.

Characterization of Flavor Components in Wines with Solid Phase Microextraction (SPME), GC and GC/MS

SPME

Varian Application Note
### Number 6

Zelda Penton
Varian Chromatography Systems

Key Words: *Solid phase microextraction, SPME, 8200 AutoSampler, wine, foods and flavors, GC/MS*

Characterization of volatiles in wines provides important information on the origin and method of preparation. One class of volatiles, terpene alcohols (Figure 1), is critical in assuring the proper taste and aroma of wines, particularly Muscat and Cabernet Sauvignon. Previously these compounds were identified at the ppb level, using capillary GC/MS, following a tedious sample preparation method. The procedure included a 48-hour extraction with freon and fractionation using an Amberlite resin (1) .
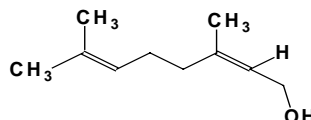

Linalool
MW 154.24
BP 198°C
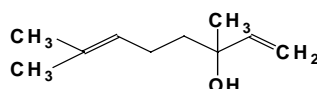
Citronellol
MW 156.24
BP 224.5°C
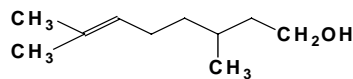
Nerol
MW 154.24
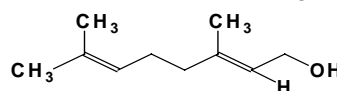**BP 225°C**

Geraniol
MW 154.24
BP 229°C

Figure 1. Structures and physical properties of terpene alcohols found in wines.

A simplification of the above sample preparation method was sought; solid phase microextraction (SPME) and static headspace (SHS) were considered. SPME offered a particularly attractive alternative; the automated system costs less and consumes far less laboratory bench space than SHS. A recent study with SPME involved determination of flavor volatiles in a fruit beverage (2). The excellent sensitivity and precision data suggested that SPME would be useful in other flavor applications.

In determining ppb levels of terpene alcohols in wines, the main question was the ability of a SPME fiber to extract these compounds from various wine matrices which contained 8-20 % ethanol. The following study showed that indeed SPME is a practical technique for this application, offering several advantages over SHS.

Instrumentation and Conditions

Instruments:	Varian Saturn 3 GC/MS with a septum-equipped temperature-programmable injector (SPI), FID and 8200 CX AutoSampler, modified for SPME (3). A 486 DX PC was used to control the GC/MS, collect MS data and control the AutoSampler in the SPME mode. The GC Star Workstation was used to collect FID data.
	A Varian Genesis Headspace Sampler was used for comparative studies with static headspace.

| Column:	30m x 0.25 mm coated with 0.25-µm Nukol™, 40°C, hold 6 minutes, 5°/minute to 180°C, hold 3 minutes, 20°/minute to 200°C hold 5 minutes (total run time, 43 minutes). Carrier gas: helium, 37 cm/s at 60°C.
Injector:	SPI with SPME insert, 200°C, isothermal, transfer line to mass spec, 220°C.
Mass Spec:	Electron impact ionization mode, mass range 45-170 u, ion trap temperature, 170°C.
	Chemical ionization mode using acetonitrile as the reagent gas for molecular weight confirmation of the terpene alcohols in the wine samples.
FID:	230°C, range 10^{-12}.
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 100-µm polydimethylsiloxane (PDMS) or 85-µm polyacrylate.
	SPME headspace: 0.8-mL liquid sample in a 2-mL vial, SPME liquid: 1.2-mL liquid sample in a 2-mL vial.
	In the linearity, precision and minimum detection level studies, 30 minutes absorption, 5 minutes desorption, one sampling per vial. These sampling times were varied in the preliminary work.
Heated Headspace:	Samples (10 mL in a 22-mL vial) were heated to 70°C, valve and transfer line temperatures were initially 85°C; but were raised to 170° and 190°C when there was no response at the lower temperatures. Equilibration time 10 minutes, mixed at 80% of full power 7 minutes, stabilization time, 2 minutes. Sample loop was 500 µL.
Samples:	Test sample consisting of purchased terpene alcohol standards (Figure 1) at various concentrations ranging from 44 ppb to 2 ppm, dissolved in HPLC water and in water containing 12% ethanol.
	Wine samples: Amber Australian Muscat with 18% alcohol (#1), light California Muscat with 9% alcohol (#2),California Cabernet Sauvignon table wine with 10-14% alcohol (#3).
SPME test plan:	Sampling: Determine if the terpene alcohols are absorbed onto the SPME fiber, effect of sampling from water versus water-ethanol. Effect of saturating samples with Na_2SO_4, headspace versus liquid sampling.
	Comparison of fibers: Response versus sampling times with PDMS and polyacrylate fibers. These preliminary studies were done with the FID.
	Identification of compounds in wine samples with ion trap detection and linearity, minimum detection limits and precision of results in spiked wine with the ion trap. Minimum detection limits with FID.

Results and Discussion

Sampling Conditions (test samples at 2 ppm): As expected, ethanol in the sampling matrix reduced the amount of terpene alcohol extracted and salting-out improved the recovery of the alcohols (Figure 2). Table 1 compares headspace versus liquid recovery for the alcohols from the water-ethanol matrix. Figure 3 shows comparative responses with the 2 fibers at different sampling times. It was decided to make a small sacrifice in sensitivity in favor of a simplified sample preparation procedure and analyze the wine samples with headspace sampling and without the addition of salt.
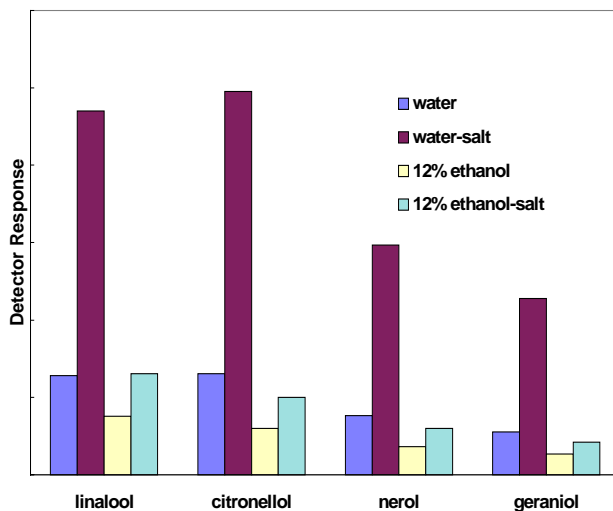
Figure 2. Relative FID responses with SPME sampling of terpene alcohols in aqueous solutions at 2 ppm each (headspace sampling, 100-µm PDMS fiber). The salt is Na_2SO_4 (saturated).

	Linalool	Citronellol	Nerol	Geraniol
Headspace Sampling (0.8 mL)	151180	119467	72386	53430
Liquid Sampling (1.2 mL)	201114	337006	185833	165728

Table 1. FID responses (area counts) after sampling terpene alcohols (2 ppm) in a 12% ethanol-water mix with a 100-μm PDMS fiber. Sampling time: 20 minutes.
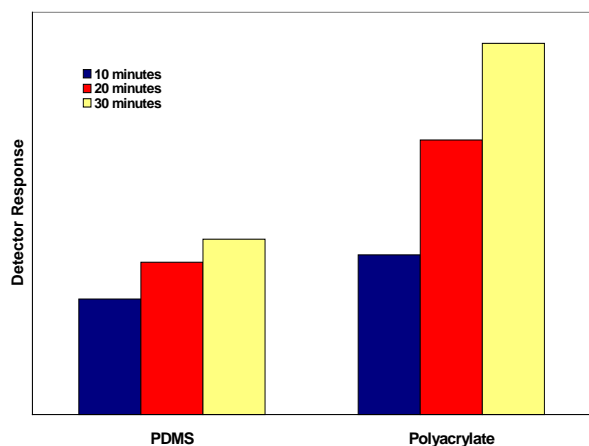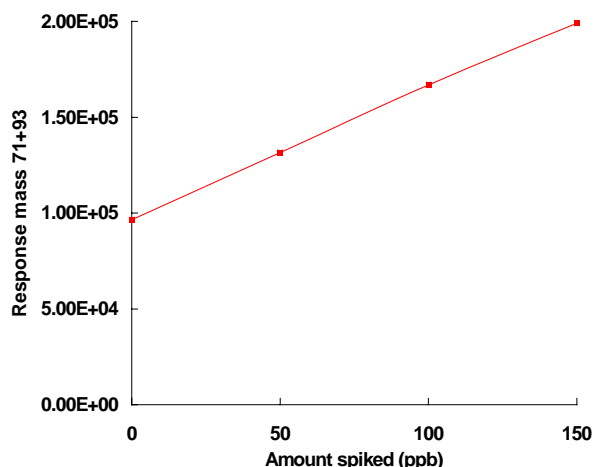

Figure 3. Comparison of FID response to linalool (2 ppm in water) after sampling the headspace with two SPME fibers for different times.

with both the polyacrylate and PDMS fibers by sampling the headspace over these samples. Figure 4 shows the linearity curve for linalool in the Australian Muscat wine.

Figure 4. Ion trap response after SPME sampling of the headspace over a Muscat wine spiked with linalool. The wine contained 150 ppb linalool before spiking.

Identification of Alcohols and Quantitation

The terpene alcohols in the wine samples were identified by comparison with pure standards and with the NIST92 library in the Saturn software. The ions used for quantitation were: 71+93 (linalool), 67+95 (citronellol), 67+69 (nerol and geraniol). For further identification, chemical ionization was also used. With acetonitrile, the mass of the main ion indicated that all of the alcohols lost water with the exception of citronellol.

The three wines and a blank 12.5% ethanol- water mix were spiked with terpene alcohol standards over the range of 0-150 ppb and linearity was confirmed

The data showed linear responses, indicating that neither fiber was saturated. As expected, the slopes of the linearity curves varied from sample-to-sample due to the different matrices. Linearity and precision data are in Table 2 and Table 3 lists the quantities of the alcohols found in the wine samples and minimum detectable quantities.

		PDMS fiber			Polyacrylate fiber		
		r	slope	% rsd*	r	slope	% rsd*
linalool	test mix	0.998	949	1.26	0.995	1840	4.22
	wine 1	0.997	686		0.993	1255	
citronellol	test mix	0.998	1269	4.73	0.995	2333	7.98
	wine 1	1.000	675		0.997	982	
nerol	test mix	0.999	586	4.72	0.997	1228	6.71
	wine 1	0.999	315		0.996	503	
geraniol	test mix	0.997	528	5.36	0.996	1015	7.81
	wine 1	0.999	266		0.996	370	

Table 2. Showing correlation coefficients to a straight line (r) after spiking 12% ethanol-water and wine #1 with the terpene alcohols (0-150 ppb). The slopes of the resulting curves are given because they are an indication of the matrix effect. For example, wine 1 contained 18% ethanol and recovery of the terpene alcohols was reduced as compared to the test sample.
*n=6

	amount present (ppb)			mdq (ppb) S/N=4	
	Wine 1	Wine 2	Wine 3	ion trap	FID
linalool	150	-	-	0.2	1.6
citronellol	12	-	3	0.3	1.9
nerol	13	-	-	0.8	2.9

geraniol	32	-	2	1.0	4.0

Table 3. Quantities of each terpene alcohol identified in the wine samples and minimum detection levels (mdq's).

The mdq's were determined with a polyacrylate fiber with the 12% ethanol-water mix. These values vary slightly, according to the sample matrix.

Static Headspace The California Muscat wine was spiked with each terpene alcohol to a concentration of 130 ppb each compound. Under the initial conditions (70 °C sample temperature, valve and line 85 °C), there was no response to the terpene alcohols. Upon heating the valve and line to 170° and 190°C, the chromatogram shown in Figure 5 resulted. This may be compared to a SPME chromatogram of the same sample. At the 2 ppm level, SHS was found to be satisfactory for the terpene alcohols; at the ppb levels required for this application, there was insufficient sensitivity due to result with SHS was adsorption of the polar terpene alcohols along the sample path.
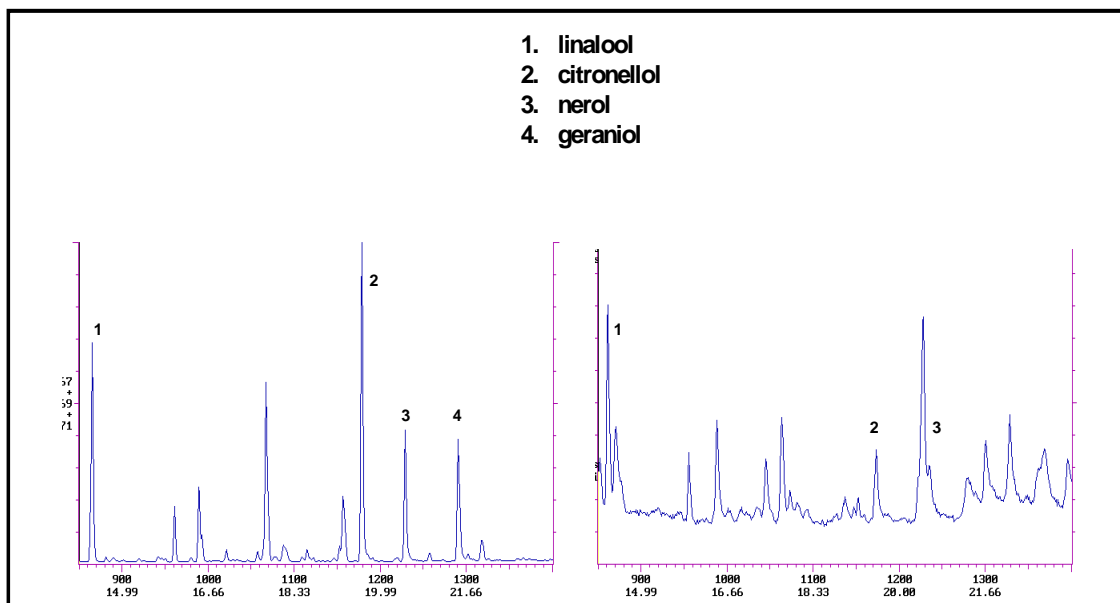
Figure 5. Ion trap chromatograms (sum of ions 67, 69 and 71) of a Muscat wine spiked with 130 ppb terpene alcohols. Left— headspace sampled with a SPME fiber, right — static headspace sampling. The scale of the chromatogram with static headspace was magnified approximately 10-fold so that the peak heights would be comparable.

Conclusions

Without any sample preparation, other than pipetting the wine into the AutoSampler vials, SPME was found to be very effective for determining trace alcohols in wine at ppb levels. Both PDMS and polyacrylate SPME fibers were useful. The linear response upon spiking with analyte indicated that in wines with up to 20% ethanol, the fibers were not saturated. The PDMS fiber resulted in slightly less sensitivity than the polyacrylate fiber but precision was slightly better. This might have been due to shorter equilibration time with this fiber.

References and Additional Reading

1. "Research on the Terpenic Composition of Galician Musts and Wines by GC-MS", Garcia-Jares, C.M., Carro-Marino, N., Muniz-Alonzo, G. and Cela-Torrijos, R., Proceedings of the Sixteenth International Symposium on Capillary Chromatography, ed by P. Sandra and G. Devos, 1994, pp 602-609.

2. "Flavor Analysis of a Fruit Beverage With Automated Solid Phase Microextraction", Penton, Z., Varian GC Application Note 51.

3. "Automation and Optimization of Solid-Phase Microextraction", Arthur, C.L., Killam, L.M., Buchholz, K.D., Pawliszyn, J. and Berg, J.R., Analytical Chemistry, 64, 1992, pp 1969-66

.

Determination of Residual Solvents and Monomers in Polymers with Solid Phase Microextraction (SPME) and GC/MS

SPME

Varian Application Note
Number 7

Zelda Penton
Varian Chromatography Systems

Key Words: *Solid Phase Microextraction, SPME, 8200 AutoSampler, Polymers, GC/MS*

Polymers are found in numerous products including food wrappings, utensils for eating and cooking, insulation, fabrics, etc. To assure the safety of the end user, as well as for quality assurance, it is critical that these compounds be monitored to verify that volatile compounds used during the manufacturing process are below a particular level in the final product. Residual solvents and monomers are normally monitored using gas chromatography with sample introduction by static headspace (SHS).

This note describes the analysis of a polystyrene polymer that was heated for different times and drawn into different shapes during the manufacturing process. The manufacturer required that volatiles in the polymer be identified and that differences in the composition of the volatiles, resulting from the variations in the process, be monitored. Laboratory personnel were planning to conduct the analysis using GC/MS and SHS; however, solid phase microextraction (SPME) was considered as a possible alternative. All of the samples were analyzed with SPME and SHS; the same compounds were recovered with both techniques. However with heated SHS, recovery was biased toward the more volatile compounds; with SPME at ambient temperatures, the recovery tended to be more uniform.

It was concluded that all of the manufacturer's requirements could be met by sampling the polymer with automated SPME, with considerable savings in equipment cost and laboratory space.

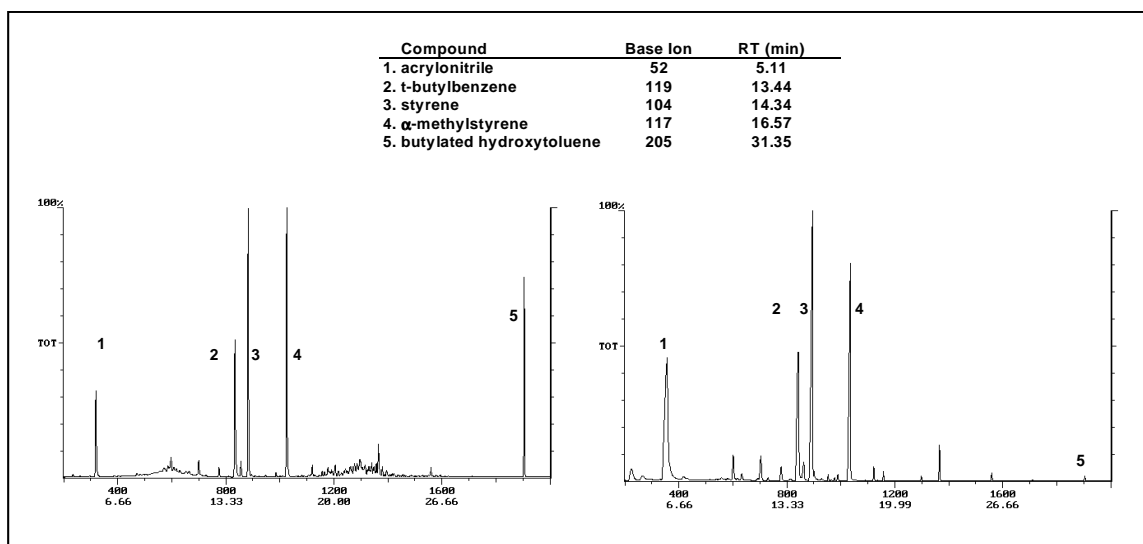| Compound	Base Ion	RT (min)
1. acrylonitrile	52	5.11
2. t-butylbenzene	119	13.44
3. styrene	104	14.34
4. α-methylstyrene	117	16.57
5. butylated hydroxytoluene	205	31.35

Figure 1: Total ion chromatogram of headspace over polymer sample #1. The chromatogram on the left resulted from sampling with a SPME fiber and the chromatogram on the right was derived from conventional heated headspace sampling. The small peaks between peaks 1 and 2 and between 4 and 5 in the SPME chromatogram appear to be derived from the polymer sample, as they were absent in blank runs.

Instrumentation and Conditions

Instruments:	Varian Saturn 3 GC/MS with a septum-equipped temperature-programmable injector (SPI), FID and 8200 CX AutoSampler, modified for SPME (1). A 486 DX PC was used to control the GC/MS and collect MS data. The same PC simultaneously controlled the AutoSampler in the SPME mode, using 8200 CX PC-control software.
	A Varian Genesis Headspace Sampler was used for comparative studies with static headspace.
Column:	30 m x 0.25 mm coated with 0.25-μm Nukol^{TM}, 40°C, hold 6 minutes, 5°/minute to 180°C, hold 3 minutes, 20°/minute to 200°C, hold 5 minutes (total run time, 43 minutes). Carrier gas: helium, 37 cm/s at 60°C.
Injector:	SPI with SPME insert, 200°C, isothermal, transfer line to mass spec, 220°C.
Mass Spec:	Ion trap temp: 170°C, electron impact ionization mode.
	Segment 1: 30 min., mass range 45-170 u, delay acquisition 1.5 min.
	Segment 2: 13 min., mass range 50-220 u.
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 100-μm polydimethylsiloxane (PDMS).
	Polymer samples (0.1-2.0 grams) were placed in the 10-mL vials; 45 minutes absorption, 5 minutes desorption, one sampling per vial.
Heated Headspace:	Polymer samples (0.1-2.0 grams in 22-mL vials) were heated to 120°C, valve and transfer line temperatures were 130°. Equilibration time was 45 minutes. Sample loop was 500 μL.
Test plan:	Identify compounds released by the polymer samples with GC/MS using SHS and SPME. Inject pure standards of the solvents found for conclusive verification of identity.
	Compare relative quantities of each compound after sample introduction with SPME and SHS.
Samples:	The polymer was made with acrylonitrile, polybutadiene, styrene, -methyl styrene and styrene butadiene rubber. Samples were as follows:

1. beads
2. beads extruded once at 220°C
3. beads extruded four times at 220°C
4. Sample #2-additional treatment (proprietary)

Results and Discussion

Identification of Solvents

Figure 1 depicts total ion chromatograms of sample #1 using SPME and SHS respectively. The compounds were identified (Figure 2) using the NIST92 library; then pure solvents were injected for additional confirmation. A significant difference between the two chromatograms is the relative recovery of butylated hydroxytoluene with SPME. This agrees with earlier studies, showing that SPME tends to yield a higher recovery with relatively nonvolatile compounds, than SHS. For example, the conditions given above for SHS, caused overload in the ion trap for the first four compounds, but very little sensitivity for the least volatile compound. One consequence of the relatively uniform recovery with SPME is ease of optimization of instrument conditions.
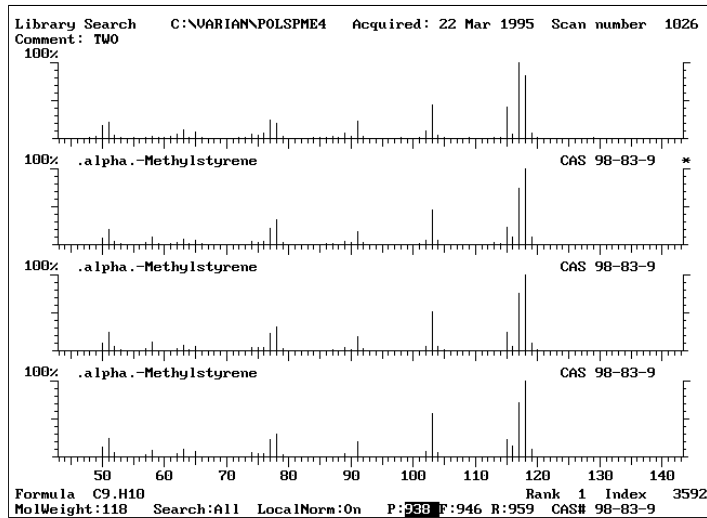
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Library Search      C:\VARIAN\POLSPME4   Acquired: 22 Mar 1995  Scan number  1026
Comment: TWO
 100%

 100%   .alpha.-Methylstyrene                                    CAS 98-83-9    *

 100%   .alpha.-Methylstyrene                                    CAS 98-83-9

 100%   .alpha.-Methylstyrene                                    CAS 98-83-9

         50    60    70    80    90   100   110   120   130   140
Formula  C9.H10                                      Rank  1  Index   3592
MolWeight:118   Search:All  LocalNorm:On   P:938 F:946 R:959  CAS# 98-83-9
```

Figure 2: Showing the results of the NIST92 Library search identifying peak #4 in the SPME chromatogram as α-methylstyrene.

Quantitation

Relative recovery after the various procedures described in the table under "samples" is shown in the graph (Figure 3). The base (most abundant) ion for each compound was selected for peak integration. Absolute quantitation is not possible in determining solvents given off by polymers. The quantity of solvent in the headspace above the polymer varies with surface area, temperature and sampling time. Therefore precision would not be expected to be as good as with other SPME or SHS applications (2). Precision of response relative to -methyl styrene varied from 3-10% relative standard deviation (sample 1, 4 replicates). To obtain some idea of the actual mass of solvents in the vial, the analyst could spike glass beads with known quantities of these solvents and compare the response to the responses of the solvents in the samples.
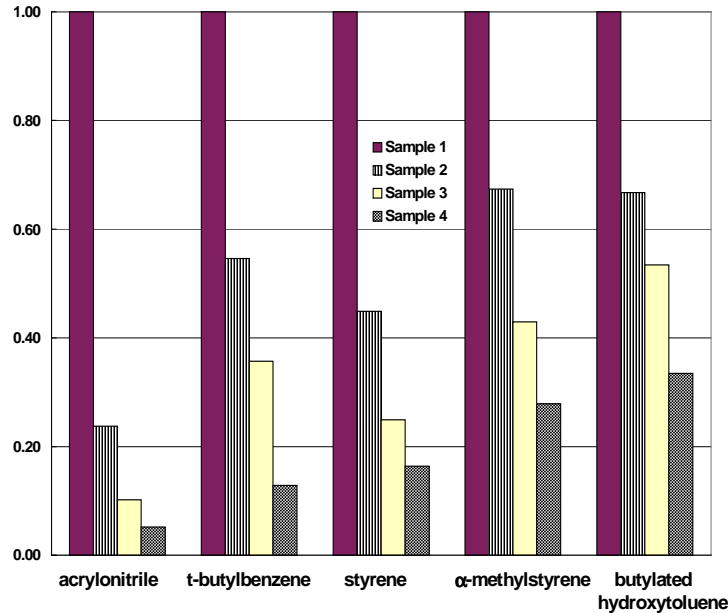

Figure 3: Showing the variation in recovery of various solvents from polymer samples after SPME sampling of the headspace. Results are normalized to sample #1, the untreated polymer. The other samples were subjected to various heat treatments described above.

Conclusions

SPME offered an attractive alternative to SHS for determining volatiles in polystyrene polymers. The automated system costs less and consumes far less laboratory bench space than SHS and the end results suggested that instrument conditions are easier to optimize with SPME.

References and Additional Reading

1. "Automation and Optimization of Solid-Phase Microextraction", Arthur, C.L., Killam, L.M., Buchholz, K.D., Pawliszyn, J. and Berg, J.R., Analytical Chemistry, <u>64</u>, 1992, pp 1969-66.

2. "Determination of a Wide Range of Organic Impurities in Water with Automated Solid Phase Microextraction", Penton, Z., Varian GC Application Note 50.

Determination of Trace Methanol in a Caustic Industrial Product with Automated Solid Phase Microextraction (SPME)

SPME

Varian Application Note
Number 8

Zelda Penton
Varian Chromatography Systems

Key Words: *Solid Phase Microextraction, SPME, 8200 AutoSampler, Methanol, Industrial Applications*

A company was required to monitor trace levels of methanol in a proprietary liquid product. The product contained 40% NaOH and other salts; therefore, it was extremely corrosive and viscous. Some of the components in the product rendered it potentially reactive. Automated solid phase microextraction (SPME) offered a possible solution for routine analysis of this sample; the sample could not be safely analyzed with an automated static headspace system.

A sample containing approximately 400 ppm methanol, was spiked with various levels of methanol and analyzed with SPME. It was demonstrated with excellent linearity and precision data, that SPME offered a practical solution to this difficult analytical problem.
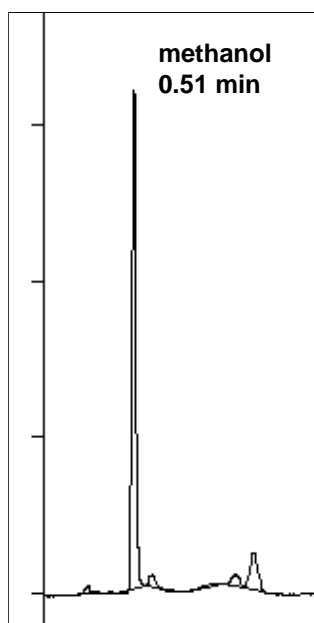

**methanol
0.51 min**

Figure 1: Chromatogram of the headspace over the unspiked caustic sample.

Instrumentation and Conditions

Instruments:	Varian 3600 CX GC with a 1078 split/splitless temperature-programmable injector, FID and PC-controlled 8200 AutoSampler. The Star workstation was used to control the instruments and collect data.
Column:	15 m x 0.53 mm coated with 1-µm DB-Wax™
GC Conditions:	Column oven: 40°C, hold 3 minutes., Carrier gas: helium, 28 mL/min, splitter flow, 67 mL/min.
	Injector: 1078 with 0.8 mm insert, 210°C, isothermal. Relay program: time 0 relay open, close at .01 minutes, open at 3 minutes.
	Detector: FID at 220°C, range 10^{-12}.
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 65-µm Carbowax/divinyl benzene; absorbed 3 minutes (headspace), desorbed 1 minute, one sampling per vial, total run time 4 min.
Sample Handling:	The sample was decanted into 24-mL plastic vials and spiked with 10 µL of methanol standards at concentrations from zero to pure methanol. Final concentrations were 0, 67.2, 168 and 336 ppm (w/v) plus the amount in the original sample.
	The samples (600 µL) were placed in 2-mL vials using a displacement pipette. It was very important to use this type of pipette for this extremely viscous sample and also to wipe off the outside of the pipette to assure that the corrosive mixture was deposited only at the bottom of the vial where it would not contact the fiber. To minimize extraneous peaks, the vial septa were baked at 150°C overnight.

Results and Discussion

Figure 1 is a chromatogram of the unspiked sample. The calibration curve is shown below (Figure 2).

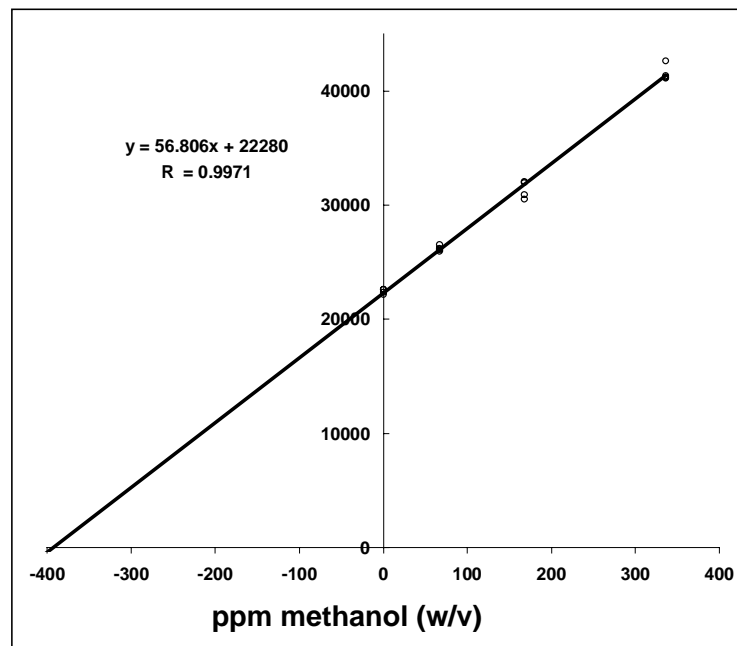y = 56.806x + 22280
R = 0.9971

ppm methanol (w/v)

Figure 2. Showing the standard additions calibration curve for spiked caustic samples. There are four points at each of four levels from 0 to 336 ppm. The "y" axis is FID response. The curve was extrapolated back to 392 ppm, representing the concentration of methanol in the sample before spiking.

The "x" intercept of the above curve was - 392, corresponding to a value of 392 ppm (w/v) methanol in the sample. Precision of the four points in the above curve varied from 0.9-2.5 % rsd. The minimum detectable quantity was calculated to be 1.2 ppm (s/n=3).

After the initial validation of the method by spiking the samples with several levels of methanol as shown here, it would be necessary, in future demonstrations, to spike only one sample with one methanol standard. Then 3-4 replicates of the spiked and unspiked sample could be run for calibration. The other samples would not require spiking since the matrix does not vary from sample to sample.

Volume in vial	Methanol Area Counts	
	200 µL	600 µL
run 1	22597	21645
run 2	22622	22095
run 3	21877	22306

Table 1. Methanol area counts after SPME sampling with 200 versus 600 µL in the 2-mL vials.

When sampling polar compounds in aqueous matrices, with static headspace or SPME headspace, the relative volume of liquid to headspace phases in the vial has little effect on sensitivity6; this is shown in Table 1. Detector response to methanol was essentially unchanged when the volume of sample in the 2-mL vial was reduced from 600 to 200 µL. Therefore, a very small volume could be sampled, minimizing risk of injury to the analyst and the SPME fiber.

The fiber was used for approximately 50 runs and then was used in another project with no discernible deterioration in performance.

Conclusions
PME offered a simple solution to a difficult analytical problem. A very corrosive sample could be analyzed in only 4 minutes with a minimum of sample handling.

[1] For a discussion of the theory, see Zhang and Pawliszyn, "Headspace Solid-Phase Microextraction", Analytical Chemistry, 65, 1993, p1843-1852.

# Blood Alcohol Determination with Automated Solid Phase Microextraction (SPME): A Comparison with Static Headspace

SPME

Zelda Penton
Varian Chromatography Systems

Key Words: SPME, 8200CX, Blood, Alcohol

Ethanol in the blood or urine of suspected intoxicated drivers is commonly measured using static headspace GC. The technique is simple and analysis time is very short. Unlike earlier methods, which involved direct injection of diluted blood, the injector insert and column remain clean and should last almost indefinitely.

It will be shown here that automated headspace solid phase microextraction (SPME) yields excellent results when determining blood ethanol, and offers several advantages over conventional static headspace (SHS) autosamplers. These include lower cost of capital equipment, no detectable sample carryover and versatility. The hardware, a modified Varian 8200 AutoSampler, can be used for either direct liquid injection or SPME; furthermore. it is installed on top of the GC, thus conserving laboratory bench space.
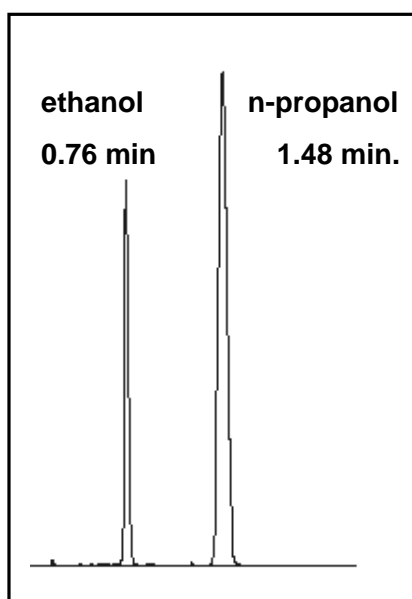
**Figure 1. SPME chromatogram of ethanol
(186 mg/dL) in blood from a California driver**

Instrumentation and Conditions

Instruments:	Varian Star 3400 GC with a septum-equipped temperature-programmable injector (SPI), FID and 8200 CX AutoSampler, modified for SPME. The Star Workstation controlled the GC and AutoSampler and acquired data. The Advanced Applications for Excel were used to generate summary reports.
	A Varian Genesis Headspace Sampler with e-form option was used for comparative studies with static headspace.
Column:	15 m x 0.53 mm coated with 1-μm DB-Wax^{TM}, 40°C, 4 minutes.
	Carrier gas: helium, .
Injector:	SPI with SPME insert at 210°C, isothermal. The carrier gas inlet of the SPI was connected to the Headspace Sampler.
Detector:	FID at range 10^{-12}, 220°C
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 65-μm Carbowax/divinylbenzene
	Headspace sampling, 3 minutes absorption, 1 minute desorption, one sampling per vial. Figure 1 is a SPME chromatogram.
Heated Headspace:	Samples were heated to 40°C, valve and transfer line temperatures were 80°C. Equilibration time was 30 minutes. Sample loop was 1 mL.

Experimental Procedure and Results

Linearity and precision SHS is a well-established technique for this application and SPME is new; therefore, linearity and precision were demonstrated only for SPME. Linearity was demonstrated over the range 0-500 mg/dL using aqueous samples (Figure 2).
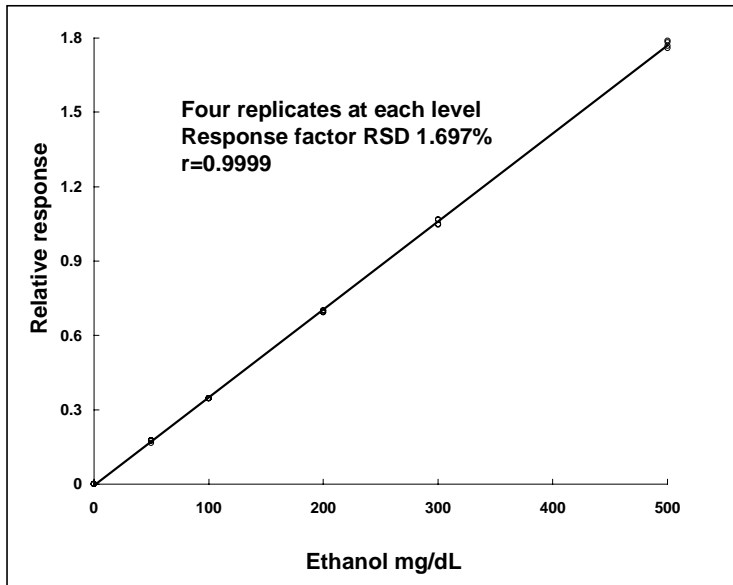

Figure 2. Linearity curve for ethanol in water, with SPME sampling

Precision data was derived from spiking both HPLC water and alcohol-free blood with ethanol and then mixing with n-propanol internal standard (Table 1).

| | % RSD Area counts | | % RSD ratio |
	ethanol	n-propanol	
Blood	2.16	1.12	1.61
Water	2.99	3.03	0.68

Table 1 Precision data for 10 SPME samplings of spiked water and spiked blood at 160 mg/dL

Comparison with Static Headspace: For comparison of SHS and SPME, the following procedure was used:

Standards: 198 µL ethanol was dispensed with a calibrated pipette into a 100-mL volumetric flask; the flask was then filled to the mark with HPLC water.

Conc: 156.2 mg/dL.

Samples: Blood and urine samples from California drivers.

Internal standard: 20 µL of n-propanol was added to 100 mL of HPLC water that was saturated with NaCl. Conc: 15.8 mg/dL.

Prior to analysis, aliquots of sample or standard were diluted ten-fold with internal standard. Two 1-mL aliquots of this mixture were dispensed into 22-mL vials for duplicate SHS analysis and two 400-µL aliquots were added to 2-mL vials for duplicate SPME determination. The purpose of the relatively high dilution was to minimize matrix differences between the various samples and the standards.

Figure 3 compares SHS and SPME results on 15 samples. The samples included an aqueous ethanol control from the College of American Pathologists. The target value was 101 mg/dL; the value determined with SHS was 97.7 and 102.7 with SPME. When a sample of unspiked water was analyzed with SPME after a standard, there was no evidence of ethanol carryover; with SHS, carryover was 0.8%.
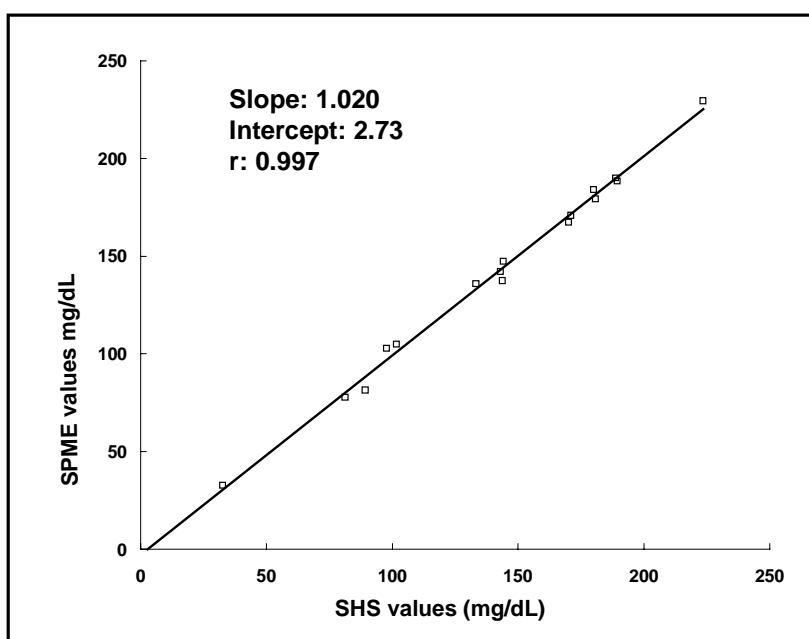
Figure 3: Comparing ethanol in blood and urine samples determined with SPME and SHS. The 15 samples included 12 blood and 2 urine specimens and an aqueous control. The samples were diluted with internal standard.

Conclusions

SPME is a practical technique for determination of ethanol in blood or urine with several practical advantages over SHS. In the study described here, the SPME system was not thermostatted; however, the use of a low molecular-weight alcohol as internal standard compensates for variations in temperature in this application (1,2). To reduce run time, sampling was interrupted before equilibrium was achieved, nevertheless the precise timing of the automated system assured good precision.

At the present time, it appears that there are additional practical applications for SPME in the toxicology laboratory. These include determination of several other volatiles in blood as well as relatively non-volatile compounds such as ethylene glycol (3).

References

1. "The Advantages of Automated Blood Alcohol Determination by Headspace Analysis", Machata, G., Z. Rechtsmed., 75, 1975, pp 229-234.

2. "Headspace Measurement of Ethanol in Blood by Gas Chromatography with a Modified Autosampler", Penton, Z., Clinical Chemistry, 31, 1985, pp 439-441.
3. Michael Butler ,Office of the Chief Medical Examiner, North Carolina Department of Environment, Health and Natural Resources, studies in progress.

Rapid Analysis of BTEX and TPH in Water using Solid Phase Microextraction (SPME) and *Fast*GC

SPME

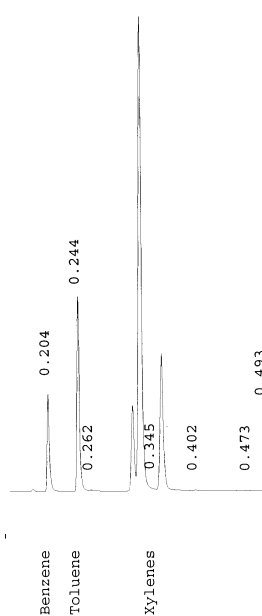## Varian Application Note
Number 10

Joy Jennison
Dr. Colin P. R. Jennison
Varian Canada Applications Lab

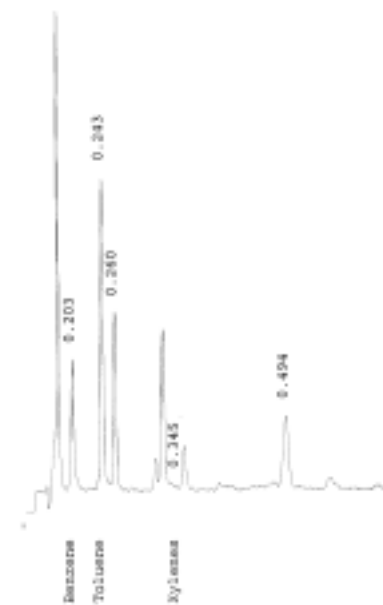***Key Words:*** *Fast*GC*, SPME, 8200CX , BTEX*

Solid Phase Microextraction (SPME) and *Fast*GC have been coupled together to enable very rapid, simple analysis of benzene, toluene, ethyl benzene and xylenes (BTEX) and volatile total petroleum hydrocarbons (TPH) in water (1). The Varian Star+ system provides a high efficiency cryofocusing inlet system with a 100,000°C/sec desorb ramp rate, virtually eliminating band broadening and allowing the use of short, conventional 0.25mm columns. In conjunction, an automated 8200 CX SPME II system, including software control via the Star Workstation, makes sample preparation fast and easy.

With SPME, analytes in the liquid sample or in the headspace above it are absorbed onto fused silica fibers coated with a polymer such as polydimethylsiloxane. The fiber is then inserted into a GC injector for desorption. The system has been automated with the Varian 8200 AutoSampler. The very rapid equilibration of non polar volatile analytes in the headspace (approximately 70% equilibrated during the first minute) allows for very short absorption

Figure 1. SPME headspace sampling of 1 ppm BTEX in water (PID)

Figure 2. SPME headspace sampling of 1 ppb BTEX in water (PID)

times. This makes headspace SPME very compatible with *Fast*GC. Using these two techniques, combined, resulted in a total of a 4 minute sample cycle time, including data processing. This allows the analysis of 48 samples in a little over 3 hours, the only sample preparation being the filling of the vials.

The exact instrument and SPME conditions used are listed below. In essence, the chromatography was carried out with a split injection to two columns and flame ionization (FID) and photo ionization (PID) detectors. This approach provides excellent selectivity and sensitivity for aromatics with the PID and an ability to simultaneously analyze TPH with FID. Figure 1 shows a PID chromatogram of a 1 ppm BTEX standard with the xylenes eluting in about 0.3 minutes. Figure 2 shows a 1 ppb BTEX standard illustrating the sensitivity of this technique. For the analysis of gasoline, quantitated as a group, total elution time is approximately 1.6 minutes as shown in Figure 3.

Instrumentation and Conditions

Instruments:	Varian Star+ FastGC with a split/splitless injector, FID and PID and 8200 CX AutoSampler, modified for SPME. The AutoSampler was controlled by SPME PC-control software. Data was collected and processed with the Star Workstation.
Columns:	10m x 0.25 mm coated with 0.25-µm DB1, 80°C, hold 2.9 minutes. Carrier gas: hydrogen at 4 mL/min measured at 80°C.
Injector:	Split/Splitless, split mode, 20 mL/min vent flow, 250°C.
FID & PID:	230°C, FID range 10-12, PID range 10-11.
Automated SPME Conditions:	The fibers (Supelco, Inc.) were coated with 100 µm polydimethylsiloxane (PDMS). Headspace sampling over an 0.8-mL liquid sample in a 2-mL vial, 2 minutes absorption, 0.7 minutes desorption.
Standards	1 ppb to 1 ppm BTEX in water, 10 ppb to 10 ppm gasoline in water (for TPH).

Results and Discussion

Linear 7 point calibration curves were generated for both BTEX (PID) and gasoline (FID). In the case of BTEX the calibration range was 1 ppb (ng/mL) to 1 ppm (µg/mL) for benzene and toluene and 3 ppb to 3 ppm for xylenes in water. Xylenes were integrated as a group. Gasoline was quantitated as a group and calibrated from 10 ppb to 10 ppm in water. A calibration curve for toluene is shown in Figure 4, benzene, xylene and gasoline calibration curves are similar. Retention time precision was excellent. Area precision was determined using six replicate injections for both BTEX and gasoline. Minimum detectable limits (MDLs) on each were also calculated, from 10 replicate runs. EPA-type MDLs were calculated using the formula MDL = s x t where s is the standard deviation of the replicate analyses and t is the students t value appropriate for a 99% confidence level. The results are listed in Table 1 below.

Table 1. Area Precision and MDLs for BTEX and TPH in Water

	Benzene	Toluene (PID)	Xylenes	Gasoline (FID)
Area %RSD (6 replicates)	3.05	3.77	3.04	2.57
MDL ppb (10 replicates)	0.19	0.11	0.41	16.65

Conclusions

SPME coupled with FastGC provides a very rapid turnaround method for the analysis of BTEX and TPH in water. Ambient headspace SPME allows rapid, selective sampling of volatiles only and, unlike purge and trap, is not subject to contamination by samples at very high concentrations (2). Linearity, precision and sensitivity are excellent and the method was found to be reliable over several hundreds of runs. There is obviously a huge time and cost saving advantage to this technique which provides a simple, rugged alternative to purge & trap and conventional GC.

References and Additional Reading

1. "Determination of a Wide Range of Organic Impurities in Water with Solid-Phase Microextraction", Penton, Z., Varian GC Application Note 50.

2. "Analysis of BTEX in Soil with Automated Headspace and PID", Jennison, Colin & Joy, Varian GC Application Note 45.
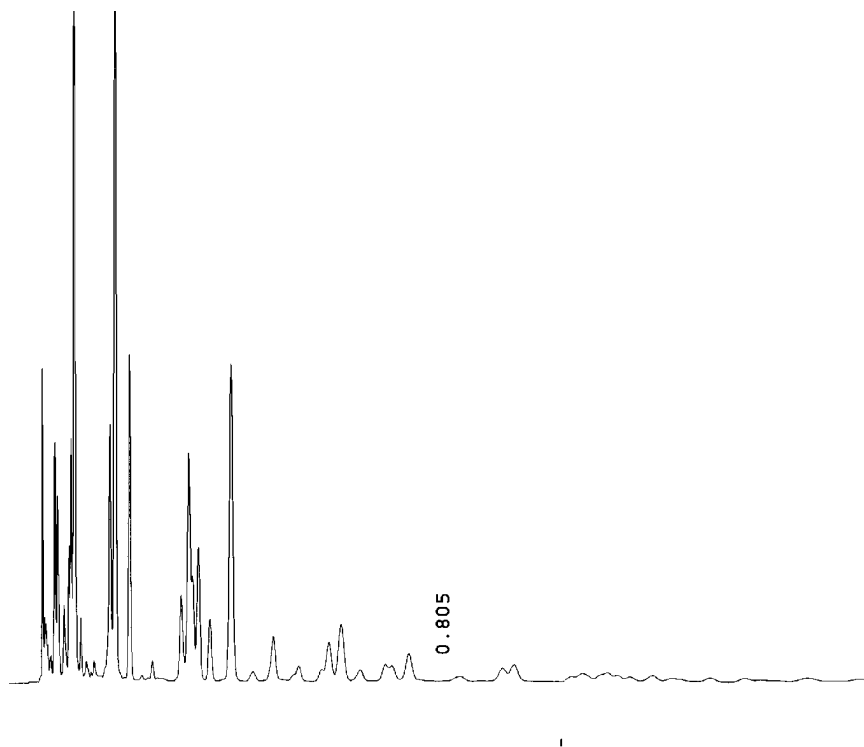
0.805

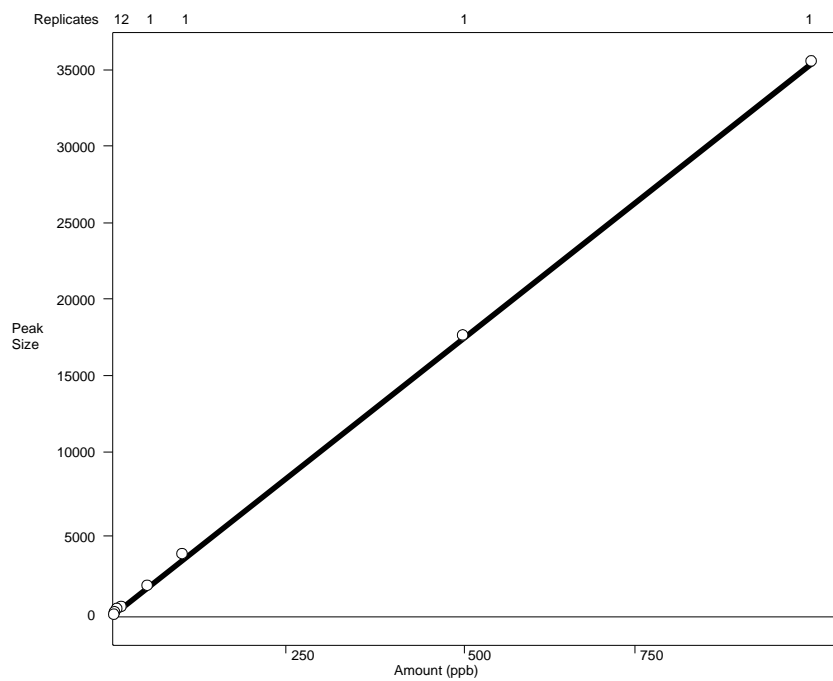**Figure 3. SPME headspace sampling of 10 ppm Gasoline in water (FID) - elution time 1.6 minutes**

Figure 4. Calibration curve for Toluene (PID) - 1 ppb to 1 ppm in water. Corr. coef. (R^2) is 0.999785

Combi PAL **69**

Determination of Phenols in Water with Automated SPME and Agitation

SPME

Varian Application Note
Number 11

Zelda Penton
Varian Chromatography Systems

Key Words: *SPME, 8200CX, Blood, Phenols*

The extraction of phenols from water was easily accomplished with automated solid phase microextraction (SPME). The 8200 CX AutoSampler, upgraded for automated SPME with agitation, was used in the analysis. With the new agitation capability, the SPME fiber is vibrated during the absorption step. This has the effect of disrupting the depleted layer of water that tends to accumulate around the fiber during static sampling1 (Figure 1) and increasing the amount of phenol absorbed in a given time.

The only sample preparation required was to adjust the pH of the sample to 2.0, thus converting the phenols to the non-ionized acid state, and to saturate the sample with Na_2SO_4. The addition of salt had the effect of reducing the solubility of the phenols in water.

Phenols were detected at the low ppb level with good precision and linearity.
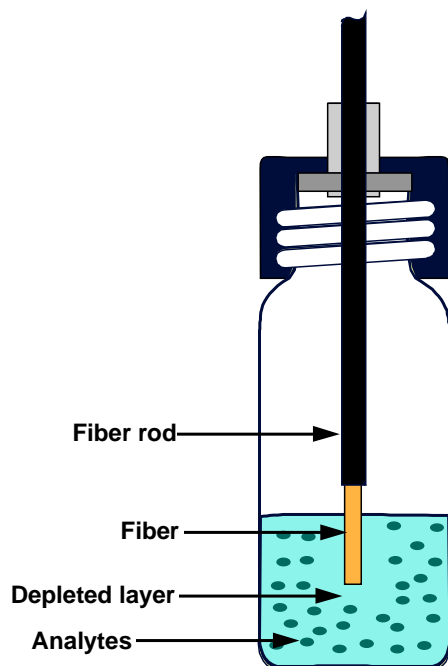

Figure 1
**Schematic of SPME sampling, showing the depletion of slow-diffusing analytes around the SPME fiber.**

Labels in figure: Fiber rod, Fiber, Depleted layer, Analytes

Instrumentation and Conditions

|	
Instruments:	Varian Star 3400 GC with a 1078 temperature-programmable split/splitless injector, FID and 8200 CX AutoSampler, modified for SPME. The Star Workstation controlled the GC and AutoSampler and acquired data. The Advanced Applications for Excel were used to generate summary reports.
	The new SPME agitation option was installed on the AutoSampler.
Column:	30 m x 0.25 mm coated with 0.25-μm DB-5^{TM}, 40°C, 4 minutes, 12°C/minute to 260°C, hold 1.67 minutes for a run-time of 24 minutes.
	Carrier gas: helium, 37 cm/s at 60°C.
Injector:	1078 with SPME insert at 280°C, isothermal. Splitless mode: close split relay at 0.01 minutes, open at 3 minutes. Flow through splitter: 94 mL/minute.
Detector:	FID at range 10^{-12}, 300°C
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 85-μm polyacrylate
	Liquid sampling for various times ranging from 5-60 minutes, 3 minutes desorption, one sampling per vial. Sampling was with and without agitation for comparison.
	A one mL volume of liquid sample in a 2.0 mL vial was found to give the best precision when agitating the fiber.
Sample:	A test sample (Supelco) containing 18 phenols in isopropanol at a concentration of 2 mg/mL each compound, was diluted in HPLC grade water to concentrations of 10, 50, 100 and 200ppb. The water was adjusted to pH 2 with HCl and saturated with Na_2SO_4.

Experimental Procedure and Results

The chromatogram in Figure 2 shows chromatograms of the compounds in the test sample with and without agitation. Note the significant enhancement in response with agitation, particularly at the end of the chromatogram where the less volatile phenols were eluted.

Compound	R.T.
1. phenol	9.069
2. 2-chlorophenol	9.194
3. 2-methylphenol	10.300
4. 3-methylphenol	10.626
5. 4-methylphenol	10.626
6. 2-nitrophenol	11.530
7. 2,4-dimethylphenol	11.729
8. 2,4-dichlorophenol	12.074
9. 2,6-dichlorophenol	12.564
10. 4-chloro-3-methylphenol	13.664
11. 2,4,5-trichlorophenol	14.553
12. 2,4,6-trichlorophenol	14.639
13. 2,4-dinitrophenol	16.348
14. 4-nitrophenol	16.480
15. 2,3,4,6-tetrachlorophenol	16.946
16. 2-methyl-4,6-dinitrophenol	17.523
17. pentachlorophenol	19.087
18. dinoseb	19.531
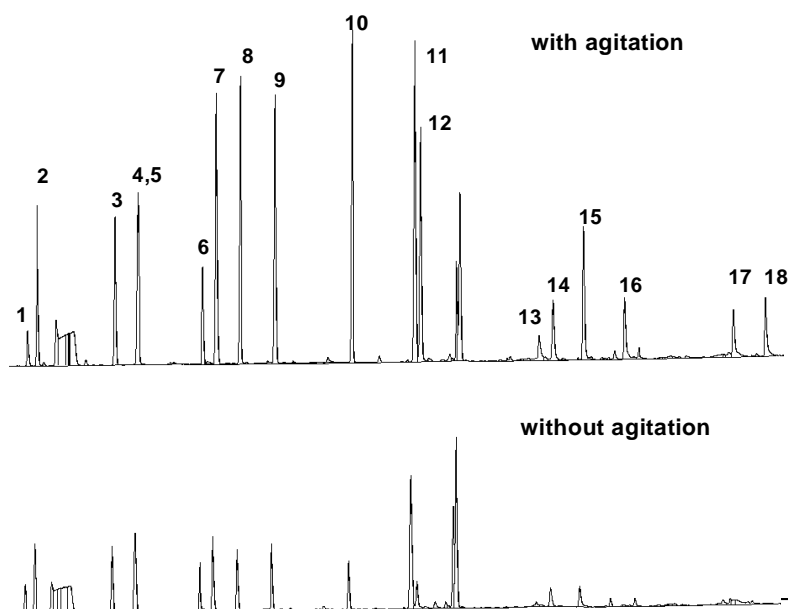
Figure 2
SPME chromatograms of the phenol test mixture at 50 ppb with and without agitation.
The absorption time for the polyacrylate fiber was 20 minutes and desorption time was 3 minutes.

The relative effectiveness of agitation for compounds of different volatilities is shown in more detail in Figure 3, where the responses of two phenols with different boiling points, are compared.
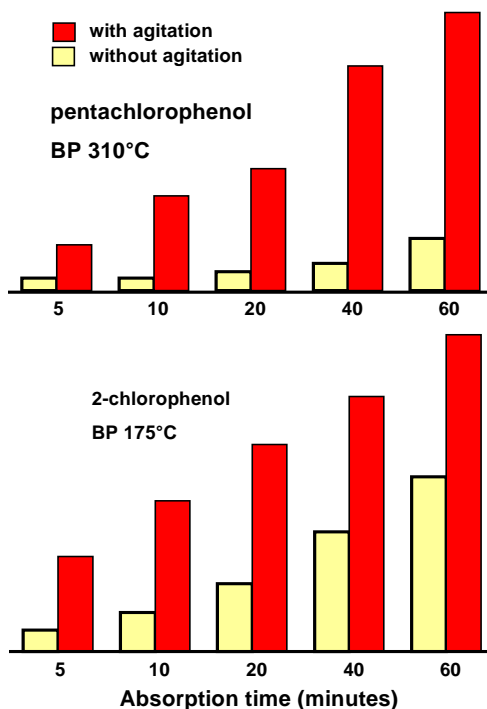
Figure 3
Details of the effect of agitating the fiber during SPME sampling for various times on the response of two phenols of different volatilities. The bars represent detector response after SPME sampling the of the phenol mixture at 100 ppb. Agitating the fiber always increased the response but the effect was greater for pentachlorophenol, the less volatile compound.

The precision of replicate analyses is shown in Table 1. To examine linearity, the mix was sampled at concentrations of 0, 10, 50, 100 and 200 ppb. Correlation to a straight line varied from 0.991 to greater than 0.999 for the phenols in the mix.

Compound	mdq (ppb)	%rsd n=6
phenol	1.40	1.52
2-chlorophenol	0.32	2.69
2-methylphenol,	0.34	2.16
3-methylphenol,	0.62	2.32
4-methylphenol		
2-nitrophenol	0.56	5.26
2,4-dimethylphenol	0.20	3.61
2,4-dichlorophenol	0.17	6.11
2,6-dichlorophenol	0.19	5.38
4-chloro-3-methylphenol	0.15	6.17
2,4,5-trichlorophenol	0.21	5.55
2,4,6-trichlorophenol	0.20	6.80
2,4-dinitrophenol	1.28	4.88
4-nitrophenol	1.15	4.42
2,3,4,6-tetrachlorophenol	0.35	7.66
2-methyl-4,6-dinitrophenol	0.49	2.67
pentachlorophenol	0.95	10.02
dinoseb	0.65	8.01

Table 1. Minimum detectable quantities (S/N=4) and area count precision (at 100 ppb) of phenols in water with SPME sampling and agitation of the fiber.

Conclusions

With agitation, the scope of automated SPME has been extended to semi-volatiles. Phenols with boiling points up to well over 300°C could be detected in water at levels below 1 ppb with FID detection. Sample preparation was minimal.

The ruggedness of the SPME technique was demonstrated here. During this study, only one fiber was used. There was no sign of deterioration of performance after repeated immersions (approximately 80 runs) in water that was at pH 2 and was saturated with sodium sulfate. The 1078 injector did not require maintenance — the same insert and septum were used throughout the project.

Reference

1. C. L. Arthur, L. M. Killam, S. Motlagh, M. Lim, D. W. Potter and J. Pawliszyn, ANALYSIS OF SUBSTITUTED BENZENE COMPOUNDS IN GROUNDWATER USING SOLID-PHASE MICROEXTRACTION, Environ. Sci. Technol., 26 (5),1992, pp 979-983.

Performance of Automated SPME: A Comparison of Results with an Interlaboratory GCMS Pesticide Study
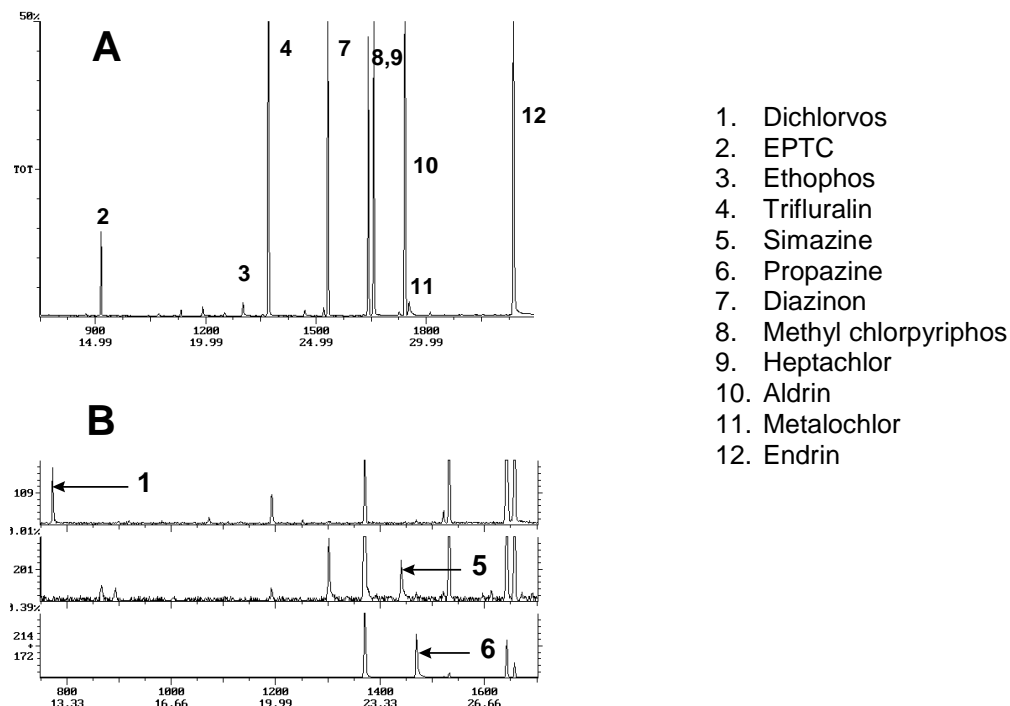
Zelda Penton
Varian Chromatography Systems

Key Words: *SPME, 8200CX, Pesticides, Saturn*

Solid phase microextraction (SPME) is a rapidly growing sample preparation method, used most frequently for extracting trace organics in aqueous matrices, prior to injection into a GC. One measure of the validity of a new analytical method is to determine if several different laboratories will agree closely with each other and with the "true" value when analyzing an unknown sample. Therefore, an interlaboratory study (1) was conducted by Górecki, Mindrup and Pawliszyn to determine if manual SPME, combined with GCMS, is a useful technique for the determination of trace pesticides in water. A detailed experimental protocol was provided to 11 participating laboratories and all of the participating laboratories received a fused-silica column, SPME fibers and samples from Supelco, Inc. The protocol specified SPME extraction of 25-mL pesticide samples in 40-mL vials with magnetic stirring.

This laboratory was not one of the participants in the test. However, a kit with the same column, SPME fibers and test sample was used to collect data for comparison of results with the 11 laboratories. The specified procedure was followed but the automated SPME III system with agitation was used with 1-mL samples in 2-mL vials.

It will be shown below that the automated system with small vials, produced results very close to the mean of the other laboratories and to the "true" values.

1. Dichlorvos
2. EPTC
3. Ethophos
4. Trifluralin
5. Simazine
6. Propazine
7. Diazinon
8. Methyl chlorpyriphos
9. Heptachlor
10. Aldrin
11. Metalochlor
12. Endrin

Figure 1:   Total ion chromatogram of the test sample at 30 ppb (A).
The pesticides with relatively low responses can be seen in the selected ion chromatograms (B).

Instrumentation and Conditions

Instrument:	Varian Saturn 2000 GCMS equipped with an automated SPME III system.
Column:	30 m x 0.25 mm coated with 0.25-µm SPB-5™, 40°C, 5 minutes, 30°C/min to 100°, 5°/min to 250°, 50°/min to 300°C, hold one minute.
	Carrier gas: helium at 41 cm/s at 60°C.
Injector:	1078 with SPME insert at 250°C, isothermal.
Ion trap:	Electron impact ionization mode, mass range 50-400 m/z, ion trap temperature, 200°C.
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 100-µm Polydimethylsiloxane
	Liquid sampling with agitation, 45 minutes absorption, 5 minutes desorption, one sampling per vial. One-mL sample in 2-mL vials
Samples:	Two pesticide samples were provided—the first was a standard with 12 pesticides (Table 1) at known concentrations; the second contained the same pesticides at unknown concentrations.
	Following the procedure, the standard (10 ppm in methanol) was diluted to 1,10 and 30 ppb in water. The unknown sample was prepared by diluting 1:1000. A freshly diluted sample was to be prepared prior to each determination; this was not considered practical for an automated system and all of the samples were placed in the autosampler carrousel, prior to the analysis. The protocol mentioned that addition of salt might increase the sensitivity of the method but specified that salt was <u>not</u> to be added to the samples in this test.
	Each of the calibration standards and the unknown sample was run in triplicate.

The GC conditions and SPME sampling and desorption times given above were specified in the study protocol.

Experimental Procedure and Results

The instructions for the manual extraction were followed, using the automated system as described above. A blank injection preceded the calibration and another blank followed the calibration. Each of the pesticides was identified by comparison with the spectra provided and in addition, by comparison with the spectra in the NIST92 library. Figure 1 A is a total ion chromatogram of the 30 ppb standard; some of the pesticides gave a very small response. This may have been due partly to deterioration of the sample and to weak affinity of the SPME fiber for these particular compounds. Nevertheless, these pesticides were easily seen in the selective ion chromatograms (Figure 1 B). Blank runs confirmed the absence of sample carryover.

<u>Linearity</u> According to the instructions, the correlation coefficients to a straight line for each pesticide should have been 0.980 or better; the actual values in this lab varied from 0.986 to 1.000.

<u>Accuracy</u> The values of the pesticides in the unknown sample are listed in Table 1, along with the "true values" and the average of the values submitted by the other labs.

Pesticide	Retention Time (minutes)	Quantitation Ion(s)	"True" Values (ppb)	Values with Automated System	Average Values of 11 Labs with Manual SPME
Dichlorvos	12.53	109	25	29.5	27.3
EPTC	15.15	128	10	10.3	9.9
Ethophos	21.41	158	17	18.4	15.5
Trifluralin	22.51	264+306	2	1.4	1.6
Simazine	24.01	201	25	29.5	23.6
Propazine	24.30	172+214	10	11.3	9.5
Diazinon	25.34	137+179	10	10.8	8.2
Methyl chlorpyriphos	27.23	286	2	2.4	1.6
Heptachlor	27.39	272	10	9.9	8.9
Aldrin	29.03	66+263	2	1.8	2.0
Metalochlor	29.13	162+238	17	19.8	15.7
Endrin	33.58	281+317	10	10.2	8.8

Table 1. Pesticides in the test sample. The retention times, quantitation ions and values (mean of 3 determinations) with the automated system are from the Varian applications laboratory. The values with manual SPME are the means of the results submitted by the 11 participating laboratories.

Note that the values listed in Table 1 for the 11 participating laboratories are averages—not individual values. When the correlations between the "true values" for each pesticide and the individual values submitted by each laboratory were calculated, the correlation coefficients "r" varied from 0.8634 to 0.9907, with six of the labs showing a value for r, greater than 0.98. With the automated system, r was 0.9974.

Precision and Relative Response of Automated SPME Compared to Manual SPME

In order to compare manual sampling with magnetic stirring and automated sampling and agitation on the same GCMS and with the same samples, 1-mL samples at the 30 ppb level were placed in 2-mL vials and sampled both ways. With the manual sampling, the rotation speed of the stirring bars was increased to the maximum that allowed a smooth rotation in the sampling vials (~90% of full capacity). Precision and average responses are shown in Table 2.

Pesticide	Mean Area Counts (%rsd, n=6)		Limit of Detection (s/n=3)
	Manual SPME	Automated SPME	with automated SPME(ppt)
Dichlorvos	595 (6.8)	2068 (6.2)	1300
EPTC	36678 (5.3)	118974 (3.3)	9
Ethophos	6281 (16.0)	29173 (5.0)	53
Trifluralin	902449 (8.6)	1252269 (5.0)	0.1
Simazine	194 (27.5)	636 (7.2)	1400
Propazine	4022 (14.9)	15912 (5.2)	92
Diazinon	112938 (13.2)	311953 (5.6)	4.5
Methyl chlorpyriphos	186655 (12.0)	449245 (5.3)	1
Heptachlor	155441 (11.5)	186629 (7.2)	8.5
Aldrin	148538 (11.3)	207265 (6.7)	3.7
Metalochlor	25776 (18.5)	105790 (5.3)	650
Endrin	88822 (17.4)	150120 (7.4)	11

Table 2. Precision and relative responses with automated and manual SPME sampling of 1-mL pesticide samples in 2-mL vials. If required, the limit of detection could be lowered by using the larger AutoSampler vials (10-mL sample) and/or by utilizing MSMS.

Conclusions

As concluded by Górecki et al, manual SPME is a valid method for determining trace amounts of semi-volatile pesticides in water. The performance with the automated system was comparable that achieved by the eleven labs in the study, with no carryover and good linearity and precision. In addition, the automated system yielded values that were in excellent agreement with the "true values".

Reference

1. T. Górecki, R. Mindrup, J. Pawliszyn, "Pesticides by SPME, Results of the Round Robin Test", submitted for publication to THE Analyst.

# Screening Packaging Materials with Automated SPME and GC/MS

SPME

Varian Application Note
Number 13

Zelda Penton
Varian Chromatography Systems

Key Words: *SPME, 8200CX, Polymers, Saturn*

Solid phase microextraction (SPME) was used to compare various packaging materials to assess their suitability for storing and shipping analytical materials. In a previous publication (SPME Application Note #7), polymeric beads that had been subjected to various heat treatments were compared; in this note, finished sheets were examined. The various materials showed specific repeatable contamination patterns. The technique was very simple—approximately 1-cm^2 of the various samples were placed into 2-mL screw cap vials and the air in the vials was sampled at ambient temperature.
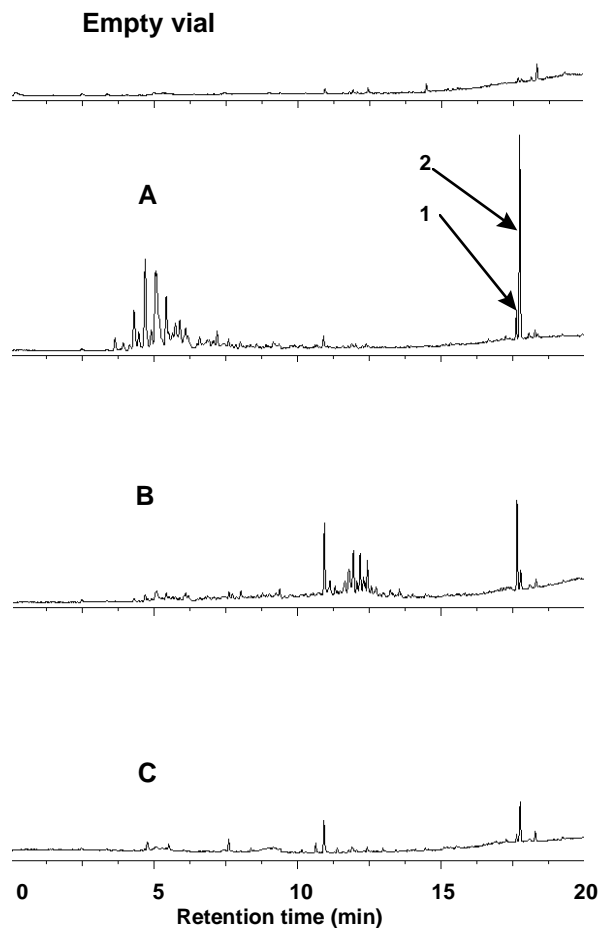
Figure 1: Total ion chromatograms of air sampled with a SPME fiber from a blank vial and vials containing three different packaging materials. Peaks 1 and 2 were tentatively identified as butylated hydroxytoluene and 2,6-bis (1,1-dimethylethyl)-4-ethylphenol.

Instrumentation and Conditions

Instrument:	Varian Saturn 2000 GCMS equipped with an automated SPME III system.
Column:	30 m x 0.25 mm coated with 0.50-µm Supelcowax 10^{TM}, 50°C, 1 minute, 10°C/min to 210°, hold 8 min. Carrier gas: helium, 41 cm/s at 60°C.
Injector:	SPI with SPME insert at 210°C, isothermal.
Ion trap:	Electron impact ionization mode, mass range 50-250 m/z, ion trap temperature, 200°C.
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 100-µm Polydimethylsiloxane. Headspace sampling without agitation in 2-mL vials, 30 minutes absorption, 2 minutes desorption, one sampling per vial.
Samples:	Three different packaging materials.

Results and Discussion

The samples were cut into one-cm squares and placed in the vials (one piece per vial). Samples were run in duplicate, with an empty vial at the beginning and end of the series. The total ion chromatograms were inspected at comparable attenuation. Figure 1 clearly shows the differences in the packaging materials. Note that duplicates of the same sample were virtually identical (Figure 2). The method is not quantitative, as one would expect the quantities of the various compounds released from the packaging material to be proportional to the surface area, but if similar-sized pieces of the materials are placed in the sampling vials, the relative cleanliness of the different samples becomes obvious.
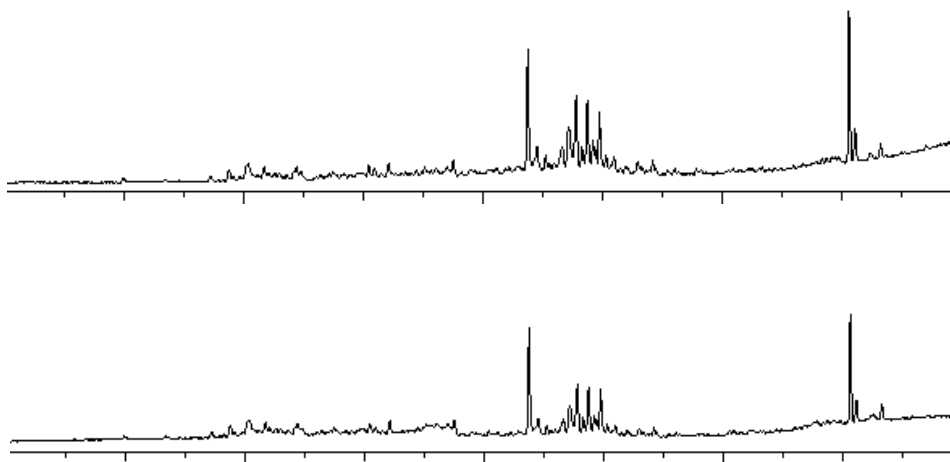

Figure 2: Total ion chromatograms of two samples of packaging material "B".

Conclusions

SPME is a very simple and effective technique for rapidly evaluating the cleanliness of packaging materials.
A simple GC-FID system may be used for fingerprinting, or if identification of the contaminants is required, GC/MS should be utilized.

03-91483500:1

Determination of Acetone and C$_1$ - C$_4$ Alcohols using Automated SPME

SPME

Varian Application Note
Number 14

Sonia Magdic and Janusz Pawliszyn
University of Waterloo, Chemistry Department,
Waterloo, Ontario, Canada
Varian CSB Contact: Zelda Penton

Key Words: *SPME, 8200CX, Alcohol, Beverages*

Introduction

Solid phase microextraction (SPME) is a solvent-free analytical technique that is significantly more rapid and simple than the conventional methods currently employed to determine alcohol (1). The SPME device is commercially available and is comprised of two major components: the syringe assembly and fiber assembly. The syringe serves as a holder for the fiber assembly which is comprised of a needle that protects a small diameter fused silica fiber that has been coated with a liquid polymeric stationary phase. During sampling the coated fiber is exposed directly to the sample (2) or to the headspace above the sample (3,4), allowing absorption of the analytes according to their affinity toward the fiber coating. The analytes are thermally desorbed from the fiber in the hot injector of a GC and are subsequently analyzed. The fiber can be used immediately for a succeeding analysis.

Direct and headspace SPME have been successfully applied to the determination of alcohols (5-7). These results were obtained by performing both manual and automated sampling from various matrices. Concentrating on the results obtained by headspace analysis, the % RSD values that were determined, ranged between 1.2 - 10.1% for manual SPME and 0.7 - 3.0% with automation.

The target analytes selected for this investigation are listed in Table 1, and a chromatogram is shown on the right.

1. methanol
2. ethanol
3. acetone
4. 2-propanol
5. n-butanol
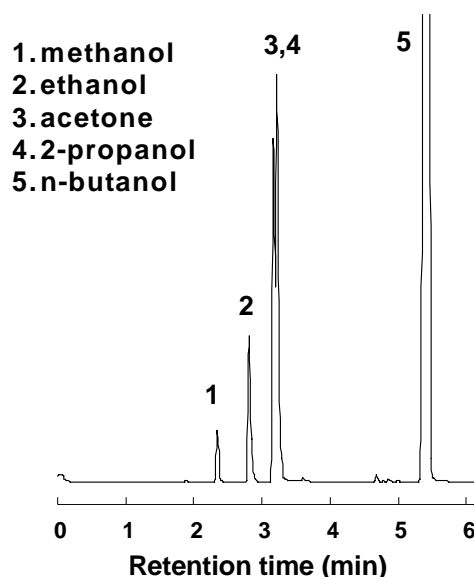
Figure 1: Chromatogram of a SPME extraction of a 1000-ppm standard aqueous solution using an 85-μm polyacrylate fiber.

Analyte	Formula	BP (°C)	MW	Solubility/Volatility
Acetone	C$_3$H$_6$O	56.5	58.08	miscible/very
Methanol	CH$_4$O	64.7	32.04	miscible/very
Ethanol	C$_2$H$_6$O	78.5	46.07	miscible/very
Iso-propanol (2-propanol)	C$_3$H$_8$O	82.5	60.09	miscible/very
***n*-Butanol**	C$_4$H$_{10}$O	117-118	74.12	9.1% v/v /very

Table 1: Chemical and physical parameters of the target analytes.

Instrumentation and Conditions

Instrument:	Varian Star 3400 GC with an 8200CX AutoSampler modified for SPME (SPME II). A Varian Star Workstation was used to run the AutoSampler and analyze the data.
Injector:	Isothermally held at 220°C for the length of the run.
Column:	SPB-5™, 30 m x 0.25 mm with a 1-µm film thickness, 30°C hold for 3 min, ramp to 100°C at 15°C/min (total run time 7.7 min), GC oven cool-down: 2 min, total time 9.7 min.
Detector:	FID, 10^{-12}, 300°C
Automated SPME Conditions:	85-µm polyacrylate coating, 10 min absorption (no stirring), 2 min desorption. Headspace sampling over 0.5 mL liquid sample in 2.0-mL vials, and over 5.0 mL liquid sample in 16-mL vials.
Sample Preparation	
small vials:	2.5 g of NaCl was added to 10 mL of a 1000-ppm standard.
large vials:	13.5g of NaCl was added to 50 mL of a 1000-ppm standard.
Standard Preparation	A 2000 ppm standard containing the 5 target analytes from Table 1 was prepared in the following manner: 2 mL of each component was pipetted into a 1000-mL volumetric flask. Milli-Q water was added to the flask to fill it to the mark. A series of dilutions was performed to generate 1000-ppm, 500-ppm, 250-ppm, 100-ppm, and 10-ppm standards.

Experimental Criteria

All experiments were performed using the Varian 8200CX AutoSampler with SPME II. The linearity of the method was tested in duplicate by extracting standards with increasing concentrations over a range typically between 10-2000 ppm. Detection limits and the limits of quantitation were determined from the linear range and based on 3 x S/N ratio and 5 x S/N ratio respectively. The precision of the method was determined by performing a minimum of 7 extractions at one concentration (1000 ppm) on one day. This experiment was also done in duplicate to verify the results obtained. A comparison was done between the precision obtained when extracting from 16-mL vials and 2-mL vials.

Results and Discussion

Solid phase microextraction is based on an equilibrium process rather than an exhaustive extraction. Direct sample extraction and headspace extraction under non-stirred conditions indicated that equilibrium conditions were attained after 10 minutes. Therefore, this was chosen as the extraction time. This extraction time was also optimal since the total time for the GC analysis is also 10 minutes. A chromatogram of the 10 minute extraction of a 1000-ppm aqueous standard is illustrated in Figure 1. The results obtained for precision and linearity are listed in Table 2. The linearity was also determined for both vial sizes.

Target Analyte	Small Vials				Large Vials			
	r^2 value	precision %RSD	LOD (ppm)	LOQ (ppm)	r^2 value	precision %RSD	LOD (ppm)	LOQ (ppm)
Methanol	0.999	1.6	40	65	0.993	1.5	20	30
Ethanol	0.995	5.6	13	20	0.997	1.5	7	11
Acetone	0.995	1.8	7	12	0.996	3.8	4	6
Iso-propanol	0.989	1.7	5	8	0.997	4.2	2	4
n-**Butanol**	0.989	0.8	0.7	1.2	0.966	2.0	0.3	0.6

Table 2: Linearity, precision, and limits of detection and quantitation.

The method was found to be linear over the range tested from 10 ppm to 2000 ppm with the GC/FID.

Conclusions

The determination of alcohols using the SPME AutoSampler with headspace sampling was proven to be successful. The extraction of alcohols from aqueous media was performed using an 85-μm polyacrylate coating. Automation reduces the time required by the analyst for sample preparation and analysis, as compared to manual extraction.

References

1. Zhang, Z., Yang, M., Pawliszyn, J., *Anal. Chem.*, **1994**, *66*, 844A.
2. Pawliszyn, J., *TRAC* **1995**, *14 (3)*, 113.
3. Zhang, Z., Pawliszyn, J., *J. High Resol. Chromatogr.*, **1993**, *16*, 689.
4. Zhang, Z., Pawliszyn, J., *Anal. Chem.,* **1993**, *65*, 1843.
5. Shirey, R., Mani, V., Butler, M., *The Reporter*, vol 14, no 5, **1995**.
6. Penton, Z., Varian SPME Application Note, No 8. **1995**.
7. Penton, Z, Varian SPME Application Note, No 9. **1995**

Determining Volatiles in Beer with Automated SPME and GC/MS/ECD

SPME

Zelda Penton
Varian Chromatography Systems

Key Words: *SPME, 8200CX, Food, Saturn*

Volatile compounds are monitored in beer to detect components causing "off" flavors as well as to assure uniformity of product. Compounds of particular interest are 2,3 butanedione (diacetyl), 2,3-pentanedione, trans-2-nonenal, trans, trans-2,4-decadienal , and ethyl esters (Figure 1). Solid phase microextraction (SPME) was evaluated for determining these volatiles.

Several different SPME fibers were compared for relative efficiency in extracting the analytes of interest; then linearity and precision were studied. A comparison was made with conventional static headspace (SHS) for this application.
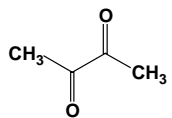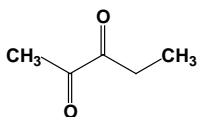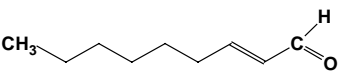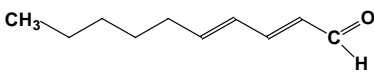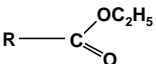
<u>ECD Detection</u>	<u>MW</u>	
2,3-butanedione (diacetal)	**86.09**	
2,3-pentanedione	**100.12**	
<u>MS Detection</u>		
trans-2-nonenal	**140.2**	
trans, trans-2,4-decadienal	**152.2**	
ethyl esters		

Figure 1:   Structures of compounds monitored in beer.

Instrumentation and Conditions

While the aldehydes are easily detected at very low levels with the ion trap detector, the diones fragment into small ions (butanedione: mass 43 and pentanedione: masses 43 and 57). Since the background contains nu*merous interfering ions with the same masses, sensitivity is poor. For the same reason, sensitivity is not improved in the chemical ionization mode (CH_4).

However, the diones give a strong signal with ECD detection; this signal is temperature dependent with significantly more sensitivity at a detector temperature of 150°C than at 220°C (Figure 2) .

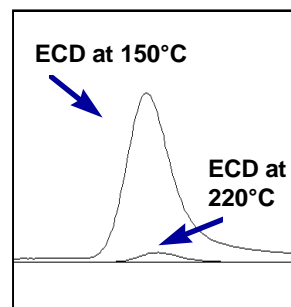Therefore, the system was configured so that both ECD and ion trap detection could be used.

ECD at 150°C

ECD at 220°C

Figure 2. Response for butanedione vs ECD temperature.

A 4-port switching valve (Figure 3) allowed the effluent from the analytical column to pass through the ECD to detect the early-eluting diones — after eight minutes, the effluent was directed into the ion trap where the later-eluting aldehydes as well as various ethyl esters were detected.
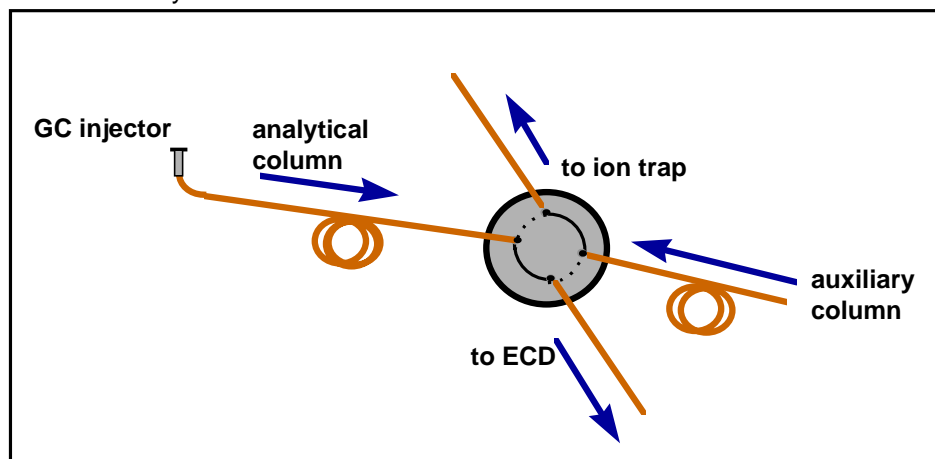
Figure 3. Schematic of system for beer analysis The sample is introduced into the GC injector and flows into the ECD where the diones are detected.

After 8 minutes, the valve is activated and directs the sample into the ion trap to detect the aldehydes and esters.

Another possible approach for combining ECD and ion trap detection would have been to split the effluent between the ECD and the ion trap. This was rejected for two reasons:

1. It was necessary to keep the ECD at a low temperature to maximize sensitivity. If the effluent were split, high boiling compounds would have entered the cold ECD, causing contamination.

2. Splitting the effluent would have decreased sensitivity for all of the compounds.

Instruments:	Varian Saturn 2000 GCMS equipped with an ECD and two injectors, a SPI and a 1078. A 4-port 1/32 inch high temperature mass spec leak-tested Valco valve was mounted in the column oven. Automated SPME III system. Varian Genesis static headspace sampler with electroform nickel sample path
Column:	30 m x 0.25 mm coated with 0.50-µm Supelcowax 10^{TM}, temperature program 50°C, 1 minute, 5°C/min to 200°, hold 9 min., carrier gas: helium, 41 cm/s at 60°C. Two pieces of 35-cm 0.25 mm deactivated fused silica tubing were used to connect the valve to the ECD and to the ion trap. An auxiliary column (0.25 mm) was also required (see Figure 2).
Injector:	SPI (isothermal mode) with SPME insert, 210°C— 230°C with the Carboxen-PDMS fiber
ECD:	Range 10, temperature 150°C, sampled first 8 minutes of the run.
Ion trap:	Electron impact ionization mode, mass range 45-300 m/z, ion trap temperature, 150°C, transfer line temperature 180°C, acquisition delay time 8 minutes.
Automated SPME Conditions:	Fibers (Supelco, Inc.) were coated with 100µm polydimethylsiloxane (PDMS), 85µm polyacrylate and 65µm Carboxen-PDMS. Headspace sampling over 0.8 mL in 2-mL vials, 38 minutes absorption, 3 minutes desorption, one sampling per vial.
Conventional Static Headspace:	10-mL samples in 22-mL vials were heated to 70°C, line and valve temperatures were 90°C, equilibration time one hour.
Beer Samples:	Michelob Amber Bock, Budweiser Light
Standards:	Pure standards of the four compounds of interest :2,3 butanedione, 2,3-pentanedione, trans-2-nonenal, trans, trans-2,4-decadienal were dissolved in purge and trap grade methanol to concentrations of 1 mg/mL each compound and then diluted into the beer samples at the level desired for the particular experiment.

Results and Discussion

Establishment of SPME Sampling Conditions

To optimize SPME sampling, the following parameters were studied: response with various fibers, effect of saturating the beer with salt (Na_2SO_4), liquid versus headspace sampling and agitation. Table 1 shows the results of these investigations. Quantitative data for the aldehydes was obtained using mass 81 for trans-2-nonenal and the sum of masses 81 and 83 for trans, trans-2,4-decadienal.

Parameter	Area Count Ratio	1	2	3	4
Salt saturation (Na_2SO_4)	Salt/no salt	1.6	2.0	1.0	1.5
Fiber coating	Polyacrylate /100µm PDMS	2.0	1.5	0.53	0.64
	Carboxen-PDMS/100µm PDMS	147	24.6	0.35	0.07
Phase sampled	Liquid/headspace	1.2	1.1	1.8	2.2
	Liquid plus agitation/headspace	1.4	1.3	1.6	2.8

Table 1. Effect of varying SPME parameters on area count ratios of the four compounds of interest in spiked beer: 2,3-butanedione (1), 2,3-pentanedione (2), trans-2-nonenal (3) and trans, trans-2,4-decadienal (4).

The data in the table shows a very significant enhancement of response for the diones with the carboxen fiber. Saturating with salt also enhanced the response for these compounds; however it was felt that saturation with salt was too inconvenient for routine monitoring.

Figure 4 is a chromatogram of beer spiked with the four compounds of interest.
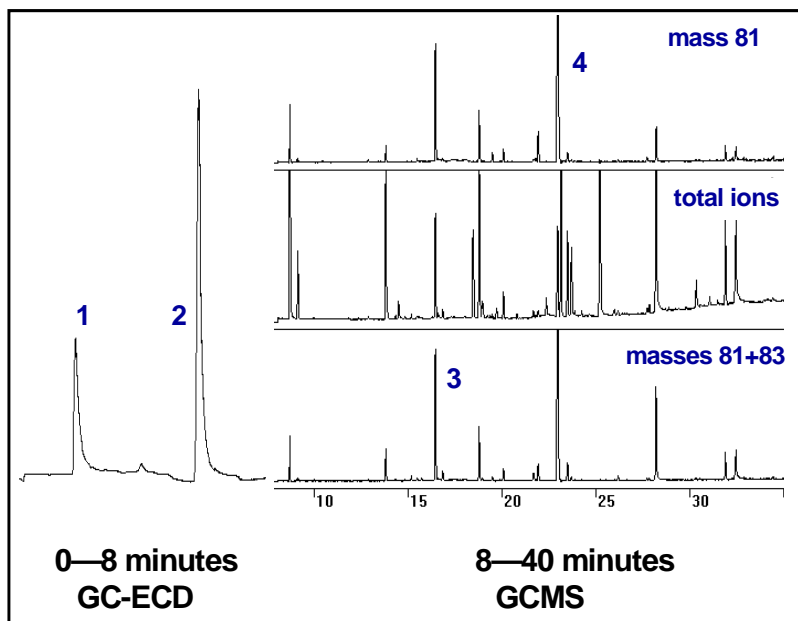
Figure 4. SPME chromatogram of headspace over beer sampled with a polyacrylate fiber. The beer was spiked with 100 ppb 2,3-butanedione (1), 2,3-pentanedione (2), trans-2-nonenal (3) and trans, trans-2,4-decadienal (4).

Linearity, Precision and Minimum Detectable Quantities

The Michelob Amber Bock beer samples were spiked with the compounds of interest at levels of 25 ppb to 1 ppm for linearity determinations with the polyacrylate fiber and SHS. For sampling with the Carboxen-PDMS fiber, the spiking level was 10 ppb to 250 ppb. (The ECD was saturated above this level.)

In all cases, the ECD response curves for the diones showed a better fit to a quadratic curve than to a linear curve. For example, when 2,3-butanedione was sampled with the polyacrylate fiber, the correlation coefficient (r^2) to a straight line was 0.9990; r^2 was 0.9997 for a quadratic curve fit. The r^2 values were 0.9988 (linear) and 0.9998 (quadratic) with SHS and 0.9959 (linear) and 0.9987 (quadratic) for SPME sampling with the Carboxen-PDMS fiber.

The unspiked beer samples contained 25-50 ppb 2,3-butanedione and 5-18 ppb 2,3-pentanedione. To establish a blank value, it was necessary to sample bottled drinking water that had been vigorously boiled to remove interfering compounds.

The ion trap responses to the two aldehydes were linear with SPME sampling. The correlation coefficients to straight lines were 1.000 for both compounds when sampled with the polyacrylate fiber. These compounds were not reliably detected with SHS at the levels studied. The unspiked beer samples did not show any trace of these compounds with SPME sampling although the detection limits were less than 1 ppb.

Table 2 gives the precision and minimum detectable values for the compounds.

| %rsd	1	2	3	4
Polyacrylate fiber*	3.08	2.14	2.93	3.31
Carboxen-PDMS fiber**	3.56	2.82	5.70	8.59
SHS*	5.20	5.73	n.d.	n.d.
mdq (ppb, s/n=3)				
Polyacrylate fiber	10	3	0.6	0.2
Carboxen-PDMS fiber	0.2	0.1	1.4	2.1
SHS	4	3	n.d.	n.d.

 *6 samplings at 100 ppb
**8 samplings at 50 ppb

Table 2. Precision and minimum detectable quantities for the four compounds of interest in spiked beer: 2,3-butanedione (1), 2,3-pentanedione (2), trans-2-nonenal (3) and trans, trans-2,4-decadienal (4).

Compounds 1 and 2 were detected with an ECD; 3 and 4 were detected with the Saturn.

In addition to the four compounds that were spiked into the beer samples for the study, additional compounds were identified in the beer samples. These are shown in the chromatogram (Figure 5) .
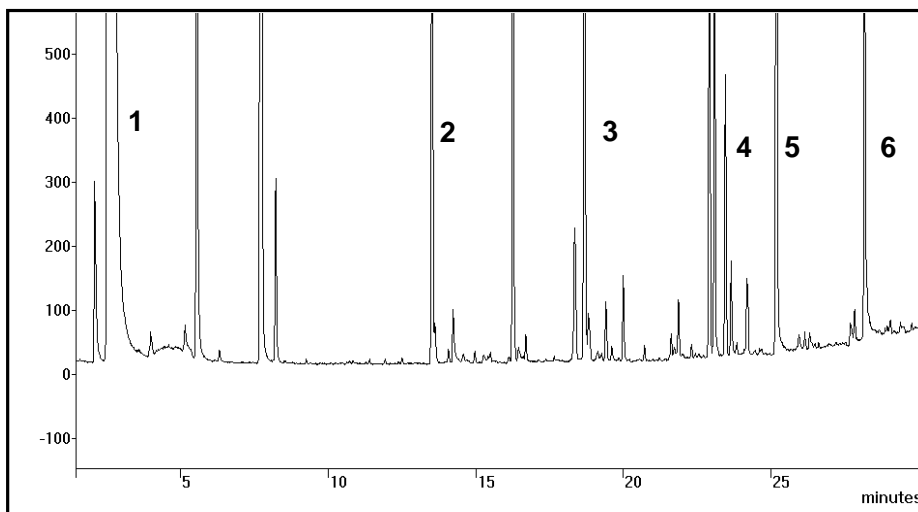
Figure 5. Total ion chromatogram of beer sampled with the polyacrylate fiber. The compounds were identified as 1. ethanol, 2. ethyl octanoate, 3. ethyl decanoate, 4. ethyl dodecanoate, 5. phenylethanol and 6. octanoic acid.

Conclusions

The data indicated that SPME is a practical technique for detecting diones and aldehydes that are monitored in beer. The polyacrylate fiber was useful for general screening of all of the compounds in beer including the less volatile ethyl esters. The carboxen fiber would be the natural choice to determine the diones at very low levels although this fiber was less efficient at extracting the less volatile compounds.

Both SPME fibers were able to sample a wider range of compounds than the conventional static headspace autosampler.

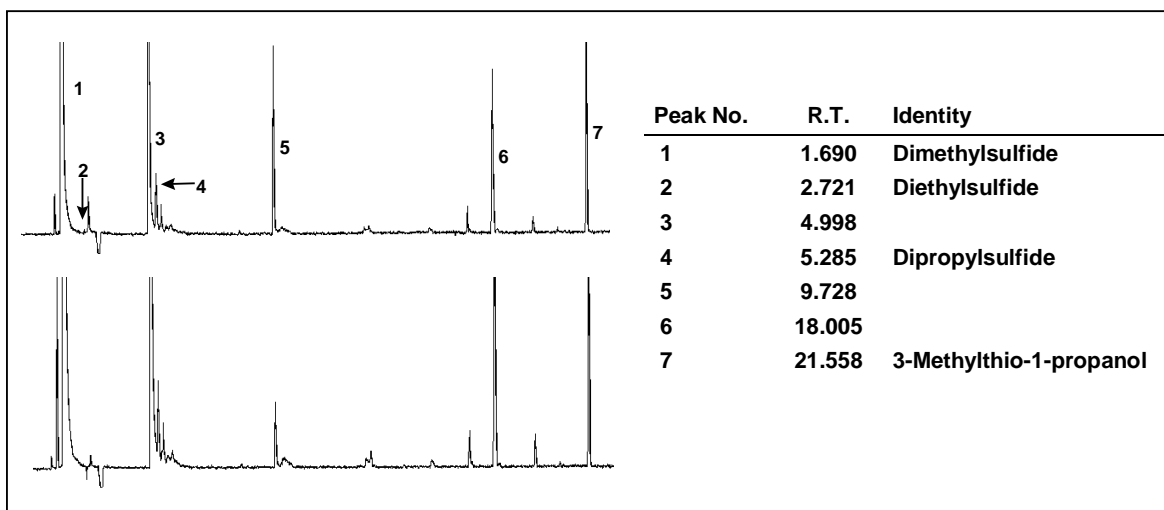# Determining Sulfur Volatiles in Beer with Automated SPME and PFPD Detection

SPME

Varian Application Note
Number 16

Zelda Penton
Varian Chromatography Systems

Key Words: *SPME, 8200CX, Food, PFPD, Sulfur*

Volatile sulfur compounds are routinely monitored in beer and other beverages because their presence even at trace levels can affect the flavor. In this note, beer was sampled using a Carboxen-PDMS SPME fiber that has a strong affinity for highly volatile compounds. A highly selective pulsed flame ionization detector (PFPD) was used to detect sulfur volatiles. Some of the compounds in the beer were tentatively identified by matching retention times to sulfur standards. Ion trap chromatograms of the beer samples were studied and on this basis, an additional compound was identified.

Peak No.	R.T.	Identity
1	1.690	Dimethylsulfide
2	2.721	Diethylsulfide
3	4.998	
4	5.285	Dipropylsulfide
5	9.728	
6	18.005	
7	21.558	3-Methylthio-1-propanol

Figure 1: Profile of sulfur compounds in two beer samples (top: Budweiser Light; bottom: Michelob Amber Bock) screened by headspace SPME using a Carboxen-PDMS fiber and PFPD detection.

Instrumentation and Conditions

The beer samples and standards were extracted with SPME headspace as described below.

Instruments:	Varian 3600 GC equipped with a PFPD and a 1078 injector.
	Automated SPME III system.
	Varian Star Workstation to control the GC and SPME autosampler and to collect data.
Column:	30 m x 0.25 mm coated with 0.50-µm Supelcowax 10^{TM}, temperature program 50°C, 1 minute, 5°C/min to 200°, hold 8 min., carrier gas: helium, 41 cm/s at 60°C.
Injector:	1078 (isothermal splitless mode) with SPME insert, 220°C
	Relay program: close splitter at 0.01 minutes, open at 2 minutes.
PFPD:	Sulfur mode, range 10, temperature 200°C.
Automated SPME Conditions:	75µm Carboxen-PDMS fiber Headspace sampling over 0.8 mL in 2-mL vials,15 minutes absorption, 3 minutes desorption, one sampling per vial.
Beer Samples:	Michelob Amber Bock, Budweiser Light

Results and Discussion

Standard compounds were dissolved in water and retention times were matched to the compounds in the beer. The standards are listed in Table 1.

Compound	Retention Time	Identified in Beer
H2S, COS, Ethyl mercaptan	1.633	-
Dimethyl sulfide	1.695	+
Isopropyl mercaptan	1.787	-
n-Propyl mercaptan	2.171	-
Sec-butyl mercaptan	2.414	-
Isobutylmercaptan	2.667	-
Diethyl sulfide	2.725	+
n-Butyl mercaptan	3.158	-
Di-n-propylsulfide	5.278	+
n-Hexyl mercaptan	6.858	-
Diallylsulfide	7.090	-
n-Heptyl mercaptan	9.488	-

Table 1. Sulfur standards and retention times

SPME Application Note 15 describes[1] the analysis of the beer samples with the ion trap detector after SPME sampling using the same column and the same chromatographic conditions described here. The ion trap chromatograms were studied and one additional peak was identified—peak 7 (Figure 2).
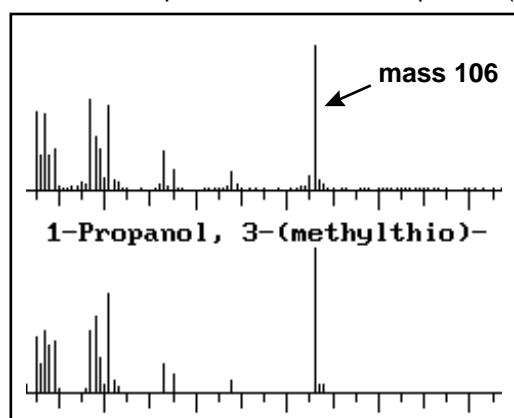

mass 106

1-Propanol, 3-(methylthio)-

Figure 2. Mass spectra of peak 7 (top) identified in a search (NIST 92 library). Conditions are in SPME Application Note 15.

Conclusions

SPME combined with the PFPD is useful for generating profiles of the sulfur compounds in beer. Some of the later-eluting sulfur compounds could be identified if the effluent from the column were split between the ion trap detector and the PFPD[2].

References

1. SPME Application Note 15 "Determining Volatiles in Beer with Automated SPME and GC/MS/ECD"

2. GC/MS Advantage Note 11 "Maximize Information by Splitting Between the Ion Trap Mass Spectrometer and a GC Detector"
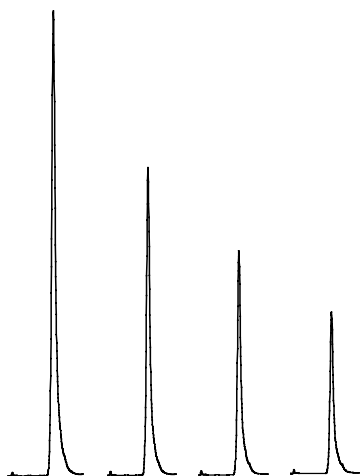
Quantitative Determination of Vinyl Chloride in a Polymer with Automated SPME

SPME

Zelda Penton
Varian Chromatography Systems

Key Words: *SPME, 8200 Standalone, Polymers*

Figure 1: Showing 4 successive extractions of vinyl chloride from a polymer with a Carboxen-PDMS fiber and FID detection. The retention time was 1.03 minutes.

Volatiles in solid samples can easily be extracted with SPME, but accurate quantitation can be quite difficult. Multiple headspace extraction, a technique that was originally developed for the quantitative determination of monomers in a polymer, with static headspace (1) can also be applied to SPME. With multiple extraction, a polymer is sealed in a vial and sampled repeatedly at equal time intervals. It is assumed that the concentration of volatiles, under these conditions, will decay exponentially (Figure 1). If an infinite number of extractions are carried out, the volatiles will be completely removed from the vial. The total area count of the analyte is equal to the sum of the areas from each individual extraction. Multiple extraction can also be applied to aqueous samples if the partition coefficient between water and the analyte is small—the analyte must favor the headspace and a substantial quantity must be removed at each extraction.

In this note, vinyl chloride monomer was determined in a finely ground sample of polyvinyl chloride material. A carboxen/ PDMS fiber was used for sampling.

Instrumentation and Conditions

Instruments:	Varian 3800 GC equipped with FID and a 1079 injector.
	Automated SPME III system.
	Varian Star Workstation to control the GC and SPME AutoSampler and to collect data.
Column:	30 m x 0.53 mm coated with 3-µm DB-624^{TM}, temperature program 60°C, 5 minutes, 20°C/min to 200°, hold 10 min.
Injector:	1079 (isothermal splitless mode) with SPME insert, 250°C.
	Relay program: inject with the split vent closed, open at 5 minutes.
EFC:	Carrier gas: helium, constant pressure mode, 7.0 PSI (flow 9.8 mL/min.)
FID:	Range 10^{-12}, temperature 200°C.
Automated SPME Conditions:	75µm Carboxen-PDMS fiber
	Headspace sampling over 1 gram in 16-mL vials, 30 minutes absorption, 2 minutes desorption, four samplings per vial. *Special tested headspace septa for large vials were used (p/n 03-926100-03).* These septa sealed after multiple samplings and allowed penetration of the SPME fiber without breakage.
Samples:	1 gram polyvinyl chloride powder weighed into 16-mL vials.
Standards	500 µL vinyl chloride injected into a Super Syringe at 500 mL, 50 µL of this mix was injected into a 16- mL vial that contained silanized glass beads, so that the headspace volume was the same in the standards and the samples.

Calculations

In practice, it is not necessary to extract more than three to six times and, using the following equation, the total area count can be calculated for each volatile in the sample:

$$A_i = \frac{A_1}{1 - e^k}$$ (1)

A_i is the total area count

A_1 is the area count of the first extraction

k is the slope of the plot obtained by plotting the natural log of area counts versus the number of extraction steps. (**k** will be a negative number)

The procedure is as follows:

1. Sample the polymer several times and determine the peak area (**A**) for each sampling.
2. Determine the natural log (ln) of **A**.
3. Plot ln **A** versus **n-1** where **n** is the number of samplings corresponding to **A**.
4. Determine the slope of the plot by linear regression.
5. Calculate the total area.
6. For calibration, prepare a vial that does not contain the matrix. The headspace volume in the vial should be equal to the headspace volume in the sample vials. (The calibration vials can be filled with glass beads with a volume that is the same as the volume of the samples.)
7. Inject a known quantity of the analyte of interest into the calibration vial.
8. Following steps 1-5, calculate the area corresponding to the known standard.
9. Calculate the amount of volatile in the unknown, by comparison of the area of the calibration standard to the area of the unknown (external standard calculation).

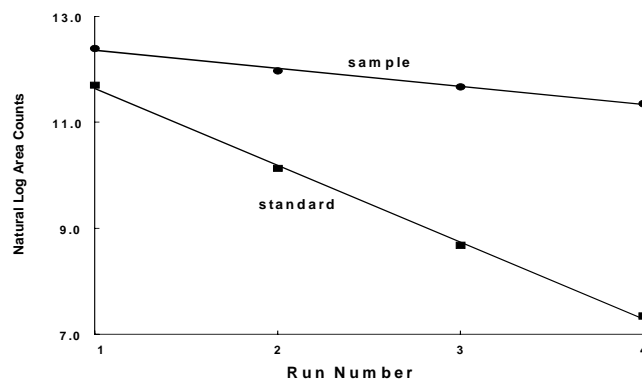Figure 2 shows a graph for a calibration standard and a sample.

Figure 2. Plot of multiple samplings of vinyl chloride from a sample of polyvinyl chloride polymer and a standard.

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After the method has been validated by demonstrating a linear response such as that shown in the graph, a simplified form of equation 1 can be used, which requires only two samplings:

$$A_i \text{ is the total area count}$$

$$(2) \qquad A_i = \frac{A_1^2}{(A_1 - A_2)} \qquad A_1 \text{ is the area count of the first extraction}$$

$$A_2 \text{ is the area count of the second extraction}$$

While results would be expected to be more accurate with more than two samplings, equation 2 is practical for routine analysis. The area counts in the calibration sample would also be determined using equation 2.

Results and Discussion

Equation 1 was used to calculate the total area counts for two replicate runs of the standard and three replicate runs of the sample. The mean of the total area counts for the standards was 157851 and the quantity of standard in each vial was 128 ng. Equation 3 was used to calculate the weight of vinyl chloride in the sample where A_{sample} is the total area count of vinyl chloride in the sample and $A_{standard}$ is the total area count of vinyl chloride in the standard.

$$(3) \qquad Wt_{Smp} = \frac{(A_{Sample})(Wt_{Std})}{(A_{Standard})}$$

Run	FID Area Counts		
	Replicate 1	Replicate 2	Replicate 3
1	241571	261362	24460
2	158710	176288	158840
3	117134	126382	113761
4	85402	94415	84961
Total area	833363	909609	827113
Weight vinyl chloride (ng) (from equation 3)	676	738	671
Weight polymer (g)	0.9963	0.9988	0.9954
ng vinyl chloride/g polymer	678	738	674
Mean (rsd)	697 (5.2)		

Conclusion

SPME is a convenient technique for determining monomers in polymers and the SPME multiple extraction method allows quantitation by minimizing the matrix effect.

Generally, when a new quantitative method is being developed using SPME or another technique, the sample should be analyzed by the new method and by a different technique and the results compared.

Reference

1. B Kolb. Multiple headspace extraction—a procedure for eliminating the influence of the sample matrix in quantitative headspace gas chromatography. Chromatographia 15:587-594, 1982.

[1]The sample time was 30 minutes to increase the probability that equilibrium was attained. It is likely that a shorter sampling time could have been used, but time and sample limitations prevented a detailed study to determine the minimum extraction time.

Determination of Phenol Content in Fibers of Industrial Interest

SPME

Varian Application Note
Number 18

Sergio PUCCI, Alessandro SABA, Andrea RAFFAELLI and Piero SALVADORI
Dipartimento di Chimica e Chimica Industriale - Università di Pisa, Centro di Studio del CNR per le Macromolecole Stereordinate ed Otticamente Attive, Via Risorgimento 35, 56126 Pisa (Italy),   Varian CSB Contact: Zelda Penton

Key Words: SPME, Saturn, Phenols

Phenol (CAS [108-95-7]) is a highly toxic organic compound. Its presence inside a working location with a concentration higher than 20 mg/m^3 can be detrimental to health (NIOSH TWA recommendations based on up to a 10-h exposure, 1978[1]). A simple and rapid analytical method capable of determining phenol in fibers of industrial interest with high sensitivity can be very important.

We developed a GC-MS method, using SPME for analyte extraction and enrichment. This method was tested on paper samples of different nature and origin, including samples of recycled paper and it proved to be very reliable and sensitive. A major advantage of SPME for this application is that sample pre-treatment is minimal, thus avoiding any alteration of the composition of the samples.

Instrumentation and Conditions

Instruments:	Varian Saturn 2000 MS/MS interfaced to a Varian 3800 GC and automated SPME III system
Column:	Varian VA-5MS, 30 m x 0.25 mm, 0.25-µm film, 50°C, hold 3 minutes, 15°/minute to 80°C, hold 1 minute, 5°/minute to 120°C, hold 1 minute, 20°/minute to 280°C, hold 1 minute (total run time, 24 minutes). Carrier gas: helium, constant flow: 1 mL/minute.
Injector:	1079 split/splitless injector with 0.8-mm SPME insert, 250°C, isothermal, splitless mode for the first 3 minutes and split mode with split ratio 20 to the end of the thermal run.
Mass Spec:	Electron impact ionization mode, ion trap temperature 150°C, transfer line 220°C.
	Full-scan mode: mass range 50-600 amu,
	MS/MS mode: parent ion mass m/z 94, mass isolation window m/z 3, wave form type resonant, excitation time 20 msec, excitation amplitude 0.35 V, mass range 65-69 amu.
Automated SPME Conditions:	Fiber (Supelco, Inc.) coated with 65-µm Carbowax/Divinylbenzene (CW/DVB).
	SPME headspace, 2-mL vial, 10 minutes absorption, 3 minutes desorption, one sampling per vial. No stirring was used.
Samples:	Test sample consisting of standard phenol solutions (water was used as solvent) at various concentrations ranging from 1 ppt to 100 ppb.
	Recycled paper samples: about 60 mg in little fragments.
SPME Test Plan:	Determination of limit of detection (LOD) in full scan mode and with MS/MS.
	Semi-quantitative determination of phenol in the real samples.

Results and Discussion

LOD in Full-Scan Mode. The LOD in full-scan mode was determined by exposing the SPME fiber to 1 μl of a water solution containing 100 pg of phenol. Under those conditions, the entire sample evaporated and there was only one phase in the vial. When ion 94 was plotted, the signal to noise ratio of phenol was 28. (Figure 1)

LOD in MS/MS Mode. Using the MS/MS feature of the Varian Saturn, the LOD was reduced to 10 pg (Fig. 2). The molecular ion at m/z 94 was isolated and the ions from 65-69 were plotted. The total ion chromatogram showed a S/N of 6 (Figure 2); the plot of the daughter ion (66 amu) showed a S/N 12.

Recycled Paper Samples. In the analysis of real samples, we found phenol concentrations in the range 150-500 ppt. Quantitation was performed by spiking the samples with 100 pg of phenol, and by comparison of the peak areas before and after spiking[*].

Conclusions

The technique presented here is extremely rapid, very reliable and very sensitive. The methods now in use are quite tedious. For instance, the I.R.S.A. (Italian Research Institute on Water), a spectrophotometric method, is not suitable for solid matrices. It requires that the phenol be extracted from the paper and dissolved in water. Furthermore, the LOD is 5 ppb as opposed to 150 ppt with SPME and GC/MS/MS.
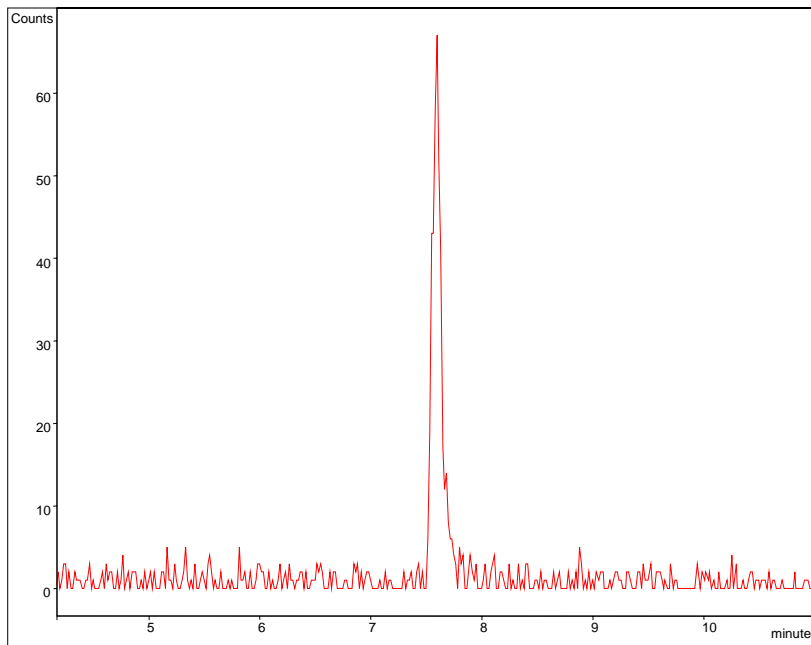

Figure 1: Plot of the ion at m/z 94, following a SPME sampling of a vial, containing 100 pg of phenol. The retention time of phenol was 7.59 minutes.
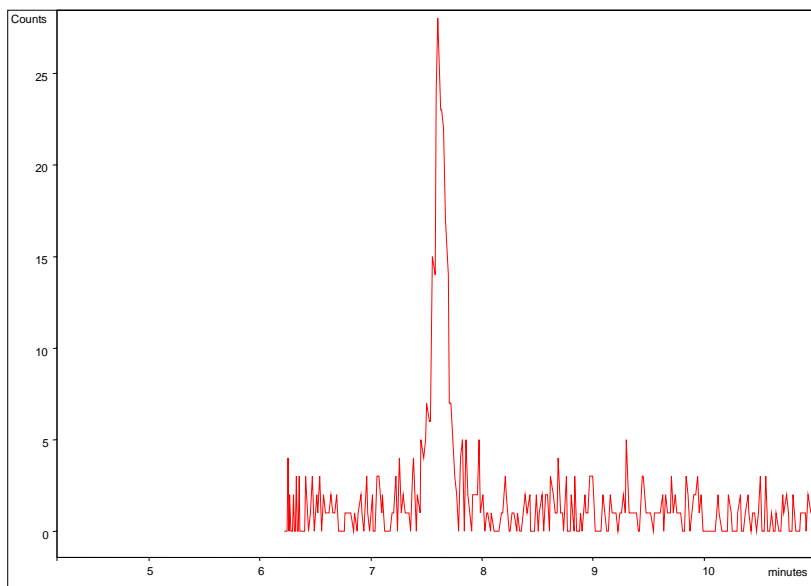

Figure 2: Narrow scan MS/MS TIC chromatogram (daughter ions of m/z 94) related to a SPME headspace GC/MS/MS analysis of a vial containing 10 pg of phenol.
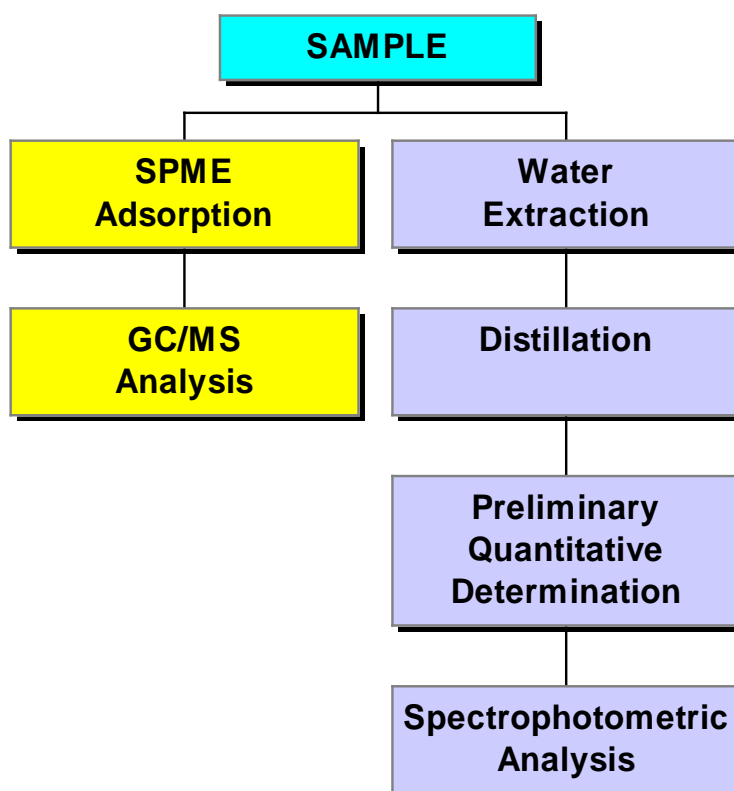
**Figure 3: Comparison between the SPME-GC/MS method
and the spectrophometric method.**

References

1. Kirk-Othmer, "Encyclopedia of Chemical Technology", 3rd Edition, Wiley Interscience - John Wiley & Sons, New York, NY, 1981, Vol. 13, pp. 253-277.

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[*]The linearity of the method was not evaluated because the producer of the recycled paper who gave us this job was interested in knowing the approximate value of phenol in the fibers and these data were sufficient for his needs.