

SPME Resolves Difficult Matrix Problems

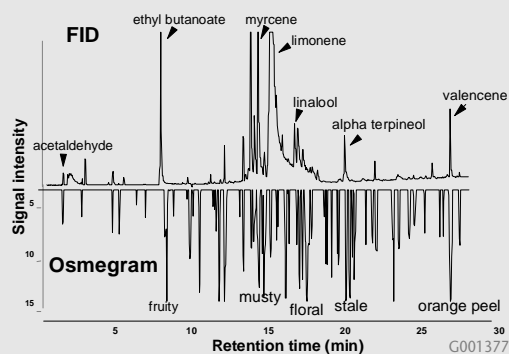
In the flavor and fragrance industry, analytical chemists are often required to isolate key ingredients, off-flavor components, or trace organic compounds from their sample matrices without altering their structure or composition. Traditional approaches to isolating the analytes of interest include solvent extraction, purge and trap analysis and static headspace analysis. **Solid Phase MicroExtraction (SPME)** offers an approach that provides the benefits of these methods while at the same time eliminates the use of solvents and reduces analysis costs and the time required for sample preparation. Essentially all sample matrices, whether liquid, solid or gaseous, can be sampled by SPME.

SPME is a solvent-less extraction procedure that involves the exposure of a coated fused silica fiber to a gaseous or liquid sample or the headspace above a liquid or solid sample. The fiber coating is typically an immobilized polymer, a solid adsorbent or a combination of the two. Published articles, many of which are referenced in our SPME application guide, have cited the use of SPME for sampling complex matrices or matrices containing interfering organic compounds. Past issues of the Supelco SPME Newsletter have demonstrated the success of SPME in resolving difficult matrix problems such as the isolation of pyrazines from peanut butter, fatty acids from milk products, and amphetamines from biological fluids. By sampling the headspace above a complex sample matrix, SPME helps to minimize effects due to interfering organic compounds. Also, headspace-SPME can provide a representation of the organic composition of the sample for screening applications or can isolate and concentrate specific trace analytes of interest for quantitative applications. Through the evaluation and adjustment of sample conditions such as temperature, pH and fiber exposure time, SPME extraction efficiency and sensitivity can be optimized. Likewise, sample agitation and analyte solubility reduction through salting effects are common practices that improve the extraction efficiency.

In a recent publication by Dr. Rouseff from the University of Florida, SPME was used for the identification of volatile flavor components in an orange juice matrix (1). Dr. Rouseff compared the results of SPME extraction to gas chromatography-olfactometry (GCO), a technique that utilizes trained odor panelists for the identification of volatile flavor components. SPME was demonstrated to be able to detect concentrations below FID response levels, yet verifiable by GCO at a sensor port. Since SPME does not utilize solvents, early eluting volatile components are not masked by a solvent peak, a common problem encountered when using other extrac-

Fig. 1 – Complex Orange Juice Sample Extraction with SPME

Sample: 25ml orange juice
 Fiber: 75µm Carboxen-PDMS
 Extraction: headspace, 30 min. @ 40°C stirred
 Desorption: 3 min., 320°C
 Column: 30m x 0.25mm, 5% methyl-phenyl siloxane
 Oven: 3 min. @ 32°C, 6°C/min to 200°C
 Carrier: Helium @ 29cm/sec.
 Injection: 320°C, 0.75mm ID inlet liner, splitless
 Detector: FID



tion techniques. The chromatogram in Figure 1 shows a typical orange juice extract obtained using SPME and the Carboxen/PDMS fiber. Headspace sampling was conducted over the orange juice sample for 15 minutes. This allowed the extraction of the volatile compounds such as acetaldehyde as well as the lower volatility terpenes and sesquiterpenes. By increasing the exposure time of the fiber, extraction of the higher boiling compounds is enhanced at the sacrifice of the more volatile aldehydes. The dual coated DVB/Carboxen/PDMS fiber is under investigation to determine if it offers the advantage of extracting a broader range of analytes from the citrus sample matrix.

Another complex matrix problem handled by SPME was the extraction of volatile sulfur compounds from saliva and breath (oral malodors). This was demonstrated by Richard Payne of the Colgate Palmolive Company at the ACS National meeting in August, 1999 (2). Payne faced significant challenges in dealing with the complex nature of the oral cavity. Not only does the sample matrix change with time, but there were also difficulties to overcome in physically sampling and concentrating the sulfur malodors. Extraction techniques such as off-

(continued on page 4)

SPME
 issue

SPRING EDITION 2000

SOLID PHASE
 MICROEXTRACTION

SPME
 Sample Prep / Made Easy

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ExTech Conference
University of Waterloo,
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SPME ON THE ROAD

PITTCON 2000

This year at PITTCON 2000, Bob Shirey, SPME R&D chemist, delivered a presentation on "SPME Adsorption Versus Absorption: Which Fiber Type is Best for Your Application"

Bob discussed the capacity and selectivity of different fiber coatings relative to molecular structure and size. The results of his study indicated the following:

- Carboxen-PDMS is the best choice for extracting small analytes, e.g. < 5 carbon atoms
- Carboxen-PDMS is linear over the 1-10,000 ppb concentration range
- Carboxen-PDMS becomes saturated at high analyte concentrations without displacement
- The linear range of Carboxen-PDMS fibers can be extended if you reduce the extraction time, e.g. 2 min. vs. 15min. allows a 10 fold increase in the upper range but with some sacrifice on the lower detection limit.

Relative to extraction of larger molecules (semivolatiles), Bob found that:

- Fiber polarity has little effect on the extraction of small analytes, but does have an effect for larger molecules.
- Liquid phase coated fibers (PDMS) yield higher minimum detection limits (MDLs).
- Liquid phase coated fibers have greater capacity and wider linear range (> 100ppm)

In general, adsorbent coatings are best for trace concentration levels, while polysiloxane (PDMS) coatings extend the useful range of SPME to sub-percent levels.

For more information request T400011.

ExTech Conference

The ExTech Conference is dedicated to the discussion of sample preparation techniques. SPME will be a prominent subject with presentations from noted scientists. Bob Shirey, Supelco R&D Chemist, will make a presentation on "A Systematic Approach to Selecting the Appropriate SPME Fiber".

Bob will expound on the extraction of volatile and semi-volatile compounds utilizing various fiber coatings. He found that most volatile compounds are extracted easily using the Carboxen/PDMS fiber, semi-volatile compounds using the Carboxen/DVB/PDMS fiber and amines using the DVB/PDMS fiber.

The results of this paper will be discussed in a future Reporter-SPME edition.

For more information on ExTech, contact Heather Lord, University of Waterloo, at 519-885-1211.

NEW LITERATURE

The Revised SPME Application Guide is now available on request. 110 new references have been added to the guide bringing the total to 600. The references have been categorized according to application, analyte/matrix, and extraction condition.



To request a copy of the Updated SPME Guide, request T199925.

NEW!

SPME UPDATED INTERNET PAGE

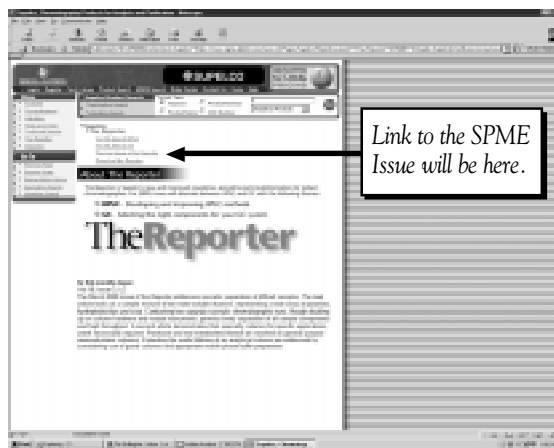
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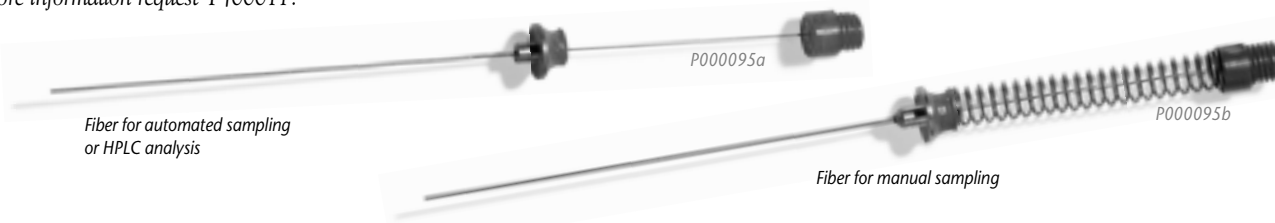
New Stableflex SPME Fibers

Bob Shirey's Pittcon presentation demonstrated that the headspace extraction of most analytes from complex matrices could be accomplished by using just two fibers: the 65 μ m Carboxen/PDMS for concentration of volatile compounds and the dual coated 50 μ m/30 μ m DVB/Carboxen/PDMS fiber for semi-volatile compounds.

Both fibers are available in the new, rugged Stableflex version of SPME fibers.

Automated versions of the fibers are also available for use with the Varian 8200 and Combi PAL auto-injection systems.

☞ For more information request T400011.



	Type	Description	Cat. No.
Stableflex	manual	65 μ m DVB/PDMS	5-7326-U
	automated		5-7327-U
Stableflex	manual	85 μ m Carboxen/PDMS	5-7334-U
	automated		5-7335-U
Stableflex	manual	50 μ m/30 μ m DVB/Carboxen/PDMS	5-7328-U
	automated		5-7329-U
Stableflex	manual	70 μ m Carbowax/DVB	5-7336-U
	automated		5-7337-U

SPME PERFORMANCE TIP

Selecting the Appropriate SPME Fiber

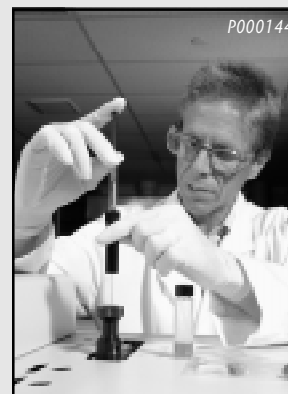
In his Pittcon paper, Bob Shirey discusses the various SPME fibers and their capacity for collection of volatile and semi-volatile compounds. When attempting to collect volatile analytes, the Carboxen/PDMS fiber is shown to be ideal for adsorption of volatile compounds. Adsorption type fibers are also better for extracting analytes present at low concentration levels and often provide lower MDLs for many analytes. To improve sampling of volatiles in complex matrices, place the sample in a closed vial with 2/3 of the vial filled with the sample and 1/3 remaining as headspace. Note that excessive headspace volume reduces the analyte concentration in the headspace and reduces the extraction efficiency. For the majority of volatile analytes evaluated in this study, the linear concentration range was 1ppb to 10ppm; however, this range will vary depending upon the analyte polarity.

If you are attempting to extract semi-volatile compounds in the headspace, use an absorptive fiber coating such as PDMS or polyacrylate. Heating the sample slightly will increase the volatility of the analytes. To insure reproducibility, the sample must be stabilized at the extraction temperature before inserting the fiber and initiating extraction of the sample. Even analytes with high boiling points can be extracted from the headspace if they have sufficient vapor pressure. For example, PCBs can be extracted from soils into the headspace by heating the sample at only

50°C. Absorptive fibers have greater capacity and linear concentration ranges than adsorptive fibers, since they utilize partitioning for the extraction.

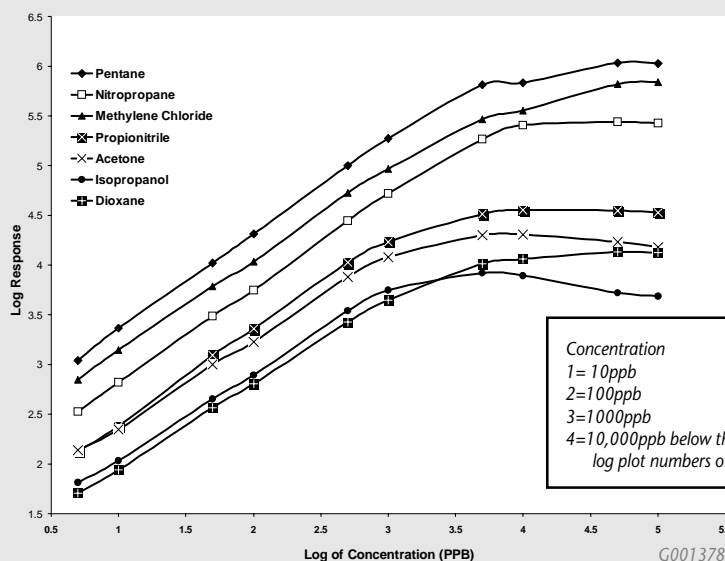
For some polar analytes having low vapor pressure, polar fibers such as Carbowax DVB and polyacrylate may be the better choice. Immersion into solution may be necessary. In general, Absorption fibers or adsorptive fibers with large pores (DVB for example) are better for extracting and releasing large analytes (>150 amu).

☞ For more information on fiber selection and capacity, request Bob Shirey's Pittcon paper, T400011.



Bob Shirey - SPME R&D Chemist

Fig. 3 - Volatiles extracted with Carboxen/PDMS fiber



G001378

References for:

SPME Resolves Difficult Matrix Problems

1. Bazemore, R., Goodner, K., Rouseff, R. Volatiles from Unpasteurized and Excessively Heated Orange Juice Analyzed with Solid Phase Microextraction and GC-Olfactometry, *J. Food Sci.* 64: 800-803 (2000)
2. Payne, R., Labows, J., Liu, X. Released Oral Malodor Measured by Solid Phase Microextraction/Gas Chromatography Mass Spectrometry (HS-SPME-GC-MS) *Proceeding of ACS - Flavor Release No. 0841236925*

Acknowledgement

Information and illustrations in this article were submitted by:

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Mr. Richard Payne, Colgate Palmolive Co., Piscataway, NJ

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Patents:

SPME - Technology licensed exclusively to Supelco. US patent #5,691,206; European patent #523092.

SPME Resolves Difficult Matrix Problems (continued from page 1)

closed-loop trapping, purge and trap, and direct injection of mouth air samples have been tried in the past. The approach taken by Payne was to collect saliva samples from human subjects, incubate the saliva overnight at 37°C, and conduct dynamic headspace sampling over the sample. The Carboxen/PDMS fiber was positioned in a specially designed sampling tube that allowed the fiber to collect the malodors from a stream of air passing over the saliva sample. Sample collection was performed for one minute prior to GC-MS analysis. Figure 2 shows the results of the extraction. The ability of the Carboxen/PDMS fiber to collect a variety of sulfur-containing compounds was demonstrated. Hydrogen sulfide, considered to be one of the prime components of oral malodors, did not show good extraction efficiency with

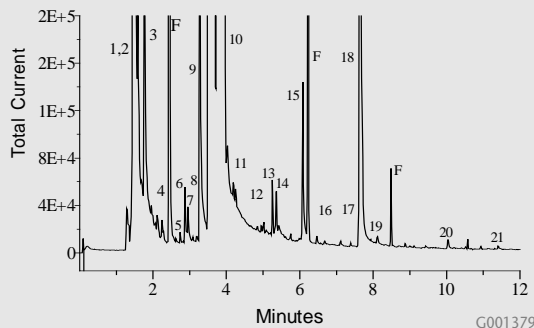
the Carboxen/PDMS fiber. However, many new compounds were identified from the SPME headspace sampling approach and are indicated in Figure 2 with a (*) symbol.

In both examples above, SPME was used to overcome the complexity of the sample matrix by sampling the headspace above the sample. Eliminating the interfering matrix components improves the ability of the analyst to identify key compounds that may otherwise be masked. SPME fibers coated with Carboxen/PDMS have been demonstrated as the best fiber choice for the collection of volatile organics.

If your needs are to identify volatile organic compounds in complex sample matrices, consider the advantages of SPME, **Sample Preparation Made Easy.**

Fig. 2. Compounds in Salivary Headspace by Dynamic HS-SPME

Sample: 3ml. saliva solution w/thioglycollate medium
Fiber: 75µm Carboxen-PDMS
Extraction: headspace, 15 min. @ 22°C, stirred
Desorption: 1 min., 250°C
Column: 30m x 0.25mm x 0.25µm, Supelcowax 10
Oven: 50-200°C, 10°C/min hold 5min. @ 200°C
Carrier: Helium @ 30cm/sec.
Injection: 250°C, 0.75mm ID inlet liner, splitless
Detector: GC-MS ion trap



Peak No.	Compounds	Ret. Time (min)	ID ^a
2	Methyl Mercaptan	1.55	rt,ms
3	Dimethyl Sulfide	1.63	rt,ms
4	Acetone	1.82	rt,ms
5	Butanone ^b	2.15	rt,ms
6	Z-1-(methylthio)-1-propane*	2.77	T,ms
7	E-1-(methylthio)-1-propane*	2.97	T,ms
8	S-methyl ethanethioate	3.28	T,ms
9	Dimethyl disulfide	3.55	rt,ms
10	Limonene ^b	4.97	rt,ms
11	Amyl alcohol ^b	5.02	rt,ms
12	S-methyl pentanethioate*	5.25	T,ms
13	S-Methyl 3-methyl butanethioate*	5.35	T,ms
14	Thiocyanic acid methyl ester*	6.08	T,ms
15	2,5-dimethyl pyrazine ^b	6.68	rt,ms
16	5-methyl-6-hepten-2-one ^b	6.88	T,ms
17	4-hydroxy-4-methyl-2-pentanone ^b	7.25	T,ms
18	Dimethyl trisulfide	7.65	rt,ms
19	Alkyl Benzene ^b	8.12	T,ms
20	Acetic acid	8.58	rt,ms
21	Dimethyl sulfoxide*	10.02	rt,ms
22	Methyl(methylthio)methyl disulfide	11.14	T,ms
23	Benzyl alcohol ^b	13.85	rt,ms
24	Phenol	15.32	rt,ms
25	Indole	23.32	rt,ms

^a Identification was by GC retention times (rt) and mass spectrometry (ms) of authentic compounds. Tentative (T) identification by mass spectrometry only when authentic compound was not available.

^b Probable exogenous sources.

* Not previously identified in saliva.

CASE STUDY 1

Determining Off-Flavor Chemicals in Milk

Dr. Ray Marsili at Dean Foods Technical Center was confronted with the problem of determining the cause of off-flavors in milk products exposed to fluorescent lights in supermarket dairy cases. The isolation and identification of the malodorous chemicals in milk requires high sensitivity. Dynamic headspace-gas chromatography/mass spectrometry (DH-GC/MS) has been the method of choice, but it is time consuming and lacks good precision. Also, dynamic headspace apparatus are expensive and require careful maintenance.

Dr. Marsili investigated the feasibility of using SPME as a substitute sample preparation device prior to GC-MS analy-

sis. The Carboxen/PDMS fiber was evaluated for the extraction of low ppb levels of pentanal, hexanal, and dimethyldisulfide. The results of the comparison of SPME to dynamic headspace analysis indicate that SPME-GC/MS is more accurate and precise while providing sensitivities equivalent to DH-GC/MS.

Review the details of Dr. Marsili's evaluation of SPME by visiting www.sigma-aldrich.com/TheReporter and follow the link to the Case Study.


For more information request T498259 and refer to Marsili's paper in *J. Chromatogr. Sci.* 37: (17-23) 1999.



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