

# TheReporter

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# Solid Phase Microextraction: Rapid and Versatile Extraction for GC or HPLC Applications

C. Woolley, R. Mindrup

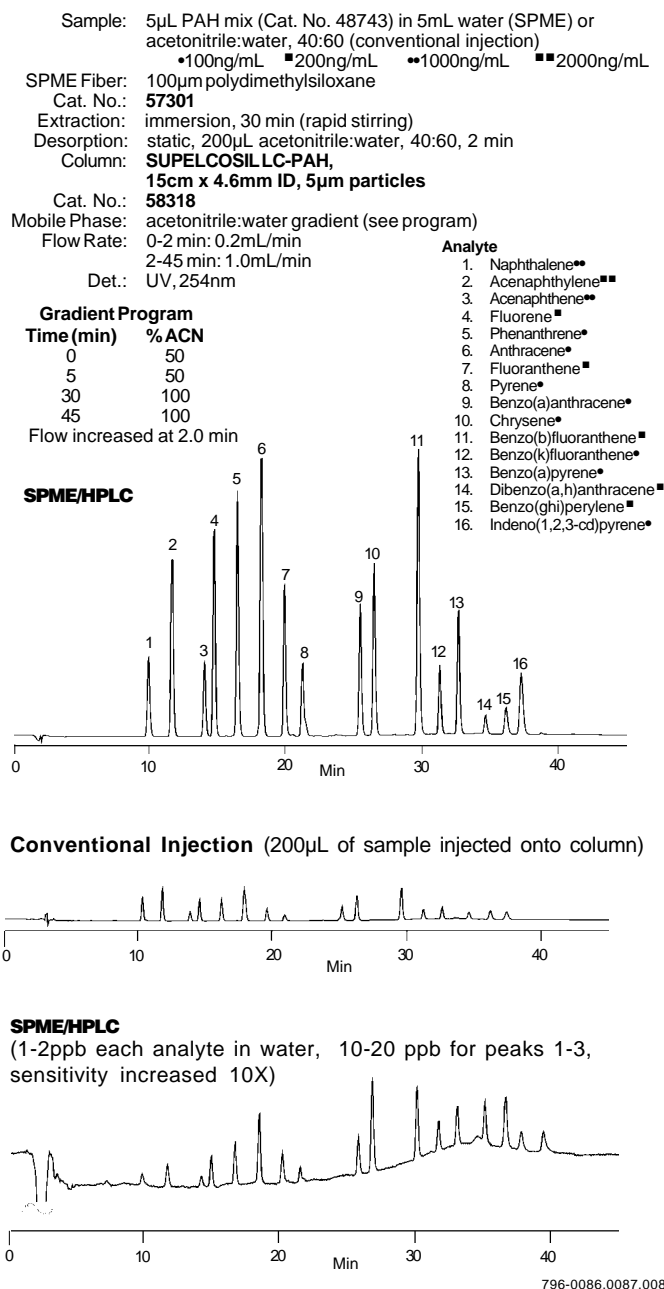
*Solid phase microextraction is a fast, solventless alternative to conventional sample extraction techniques. Analytes are concentrated on the SPME fiber, then are rapidly delivered to a capillary GC column or an HPLC column. In monitoring analytes in biological fluids, SPME is not only simpler and faster, it produces cleaner extracts than liquid-liquid or solid phase extraction. For sampling airborne compounds, such as organic pollutants or insect pheromones, the technique is very sensitive. This article summarizes several new applications for SPME/chromatography.*

Because analytes can be rapidly extracted from an aqueous matrix with virtually no solvent consumption, solid phase microextraction (SPME\*) saves preparation time and solvent purchase and disposal costs, and can improve the limits of detection in an analysis. The technique has rapidly been established among the practical alternatives for sample preparation for gas chromatography. Our new SPME/HPLC interfaces now allow SPME to be incorporated into the analyses of many weakly volatile or thermally labile compounds, including pharmaceutical compounds, polynuclear aromatic hydrocarbons, and numerous other analytes (1). The technique now can be used with any HPLC system, as well as with GCs and GC-mass spectrometer systems. The four examples which follow show new applications for this versatile extraction technique.

## Monitoring PAHs in Water, Using SPME/HPLC

Analysts at the University of Waterloo (2) and in Supelco laboratories (3) have extracted and analyzed polynuclear aromatic hydrocarbons in water, using SPME/HPLC. The Supelco chemists found a 100 $\mu$ m polydimethylsiloxane coating provided the highest extraction levels (i.e., greatest sensitivity) for the 16 PAHs listed in US Environmental Protection Agency methods 610 and 8310. The analytes were best separated on a 15cm x 4.6mm ID SUPELCOSIL™ LC-PAH column (5 $\mu$ m particles). Sharp, symmetric peaks indicate efficient transfer of the analytes from the fiber to the column (Figure A). Relative to peaks for directly injected analytes, peaks for the extracted analytes were significantly larger, indicating a concentrating effect that can increase the sensitivity of the analysis for trace-level analytes. Extraction of the same analytes at 1-2ppb each clearly demonstrates the sensitivity of the extraction technique (Figure A).

**Figure A. PAHs in Water, Using SPME/HPLC and Conventional Injection**



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### SPME as a Concentration and Injection Technique for GC Analysis of Whole Air Samples

Organic compounds in air normally are adsorbed and concentrated on an adsorbent-filled sampling tube, then are extracted in solvent or thermally desorbed for analysis. Solvent extraction requires expensive and sometimes toxic solvents, and the sample dilution entailed can reduce the sensitivity of the analysis. Thermal desorption requires specialized equipment, and some analytes must be refocused cryogenically. A whole air sample can be introduced directly onto the column from a sampling bag or canister, but sensitivity of this approach is lower than that of sample-concentrating methods.

Investigators at BOVAR-CONCORD Environmental (Toronto, Ontario, Canada) collected air samples and prepared BTEX (benzene, toluene, ethylbenzene, xylene) gas standards in 1-liter, 3-liter, and 10-liter sampling bags, then exposed a 100 $\mu$ m polydimethylsiloxane-coated SPME fiber to the samples in the bags (4). Adsorbed analytes were desorbed from the fiber onto a Carbowax<sup>®</sup>-type capillary GC column. Relative to direct injection of 0.5mL of whole air samples – the maximum volume the authors felt the column would accept without loss of sample resolution – the SPME approach increased sensitivity for test compounds by factors of 1.2 (benzene) to 14.6 (o-xylene), as shown in Table 1. Relative standard deviations for the compounds also were smaller (1.5–4.7% for SPME, versus 3.6–10.5% for direct injection). Similar results for using SPME to monitor airborne organic compounds are presented in (5).

#### Detecting Cocaine in Urine

In analyses of drugs in urine, blood, etc., interfering components of the complex matrix can be extracted along with the analytes of interest, complicating the analysis. SPME typically eliminates this problem, while providing linear results over wide concentrations of analytes (typically to parts per million/parts per billion levels). When matrix components interfere with the analysis, the analytes usually can be extracted from the headspace above the sample, rather than from the liquid sample itself.

Investigators from the Departments of Legal Medicine at Showa University School of Medicine and Hamamatsu University School of Medicine developed a method for detecting cocaine in urine, by combining SPME with capillary GC/nitrogen-phosphorus detection (6). In this application headspace extraction gives poor recovery values for cocaine, and the analysts immerse the 100 $\mu$ m polydimethylsiloxane SPME fiber into the samples.

The analysts add 0.5mL urine (plus internal standard, cocapropylene, prepared according to reference 7) to a 1mL vial containing 20 $\mu$ L of 2.5% sodium fluoride solution and a small magnetic stirring bar. They immerse the SPME fiber in the sample for 30 minutes, then expose the fiber in the heated injection port for 3 minutes to ensure complete desorption of the extracted analyte. Figure B shows chromatograms for cocaine and the internal standard as extracted from spiked urine (250ng each), and an extract from a urine blank. The extracts are very clean – urine background does not interfere with the analysis. Recovery values for cocaine and the internal standard, determined by comparing peak areas for the extracts to those for standards in a methanol

**Table 1. Sampling for BTEX Compounds in Air**

	Direct Injection		SPME		Response Ratio SPME:Direct
	Mean Area	RSD (%)	Mean Area	RSD (%)	
Benzene	450	7.4	557	1.6	1.24
Toluene	906	10.5	3128	1.8	3.45
Ethylbenzene	164	5.0	1178	4.7	7.19
m-Xylene	278	4.2	2906	4.5	10.5
o-Xylene	192	3.6	2802	1.5	14.6
Mean		6.1		2.8	

n = 3; area counts x 10<sup>3</sup>

Sample: air containing 175mg/m<sup>3</sup> benzene, 350mg/m<sup>3</sup> toluene, 87mg/m<sup>3</sup> ethylbenzene, 131mg/m<sup>3</sup> m-xylene, 87mg/m<sup>3</sup> o-xylene  
0.5mL by direct injection or:

SPME Fiber: **100 $\mu$ m polydimethylsiloxane**

Cat. No.: **57300-U** (manual sampling)

Extraction: 10 min, 23°C

Desorption: 2 min, 200°C

Column: **Carbowax-type, 15m x 0.53mm ID, 1.0 $\mu$ m film**

Oven: 40°C (2 min) to 150°C at 10°C/min

Carrier: helium, 10mL/min

Det.: FID, 200°C

Inj.: 35mL/min split flow, 20mL/min make-up gas, 200°C

Data obtained by M. Chai and Y.-Z. Tang, BOVAR-CONCORD Environmental, 2 Tippet Road, Toronto, Ontario, Canada M3H 2V2.

solution, were 20% and 30%, respectively. Extractions were linear from 30ng–250ng/0.5mL urine, and the detection limit for cocaine was approximately 6ng/0.5mL urine. A 100 $\mu$ m polydimethylsiloxane SPME fiber has been used to monitor other drugs of abuse in urine, including methadone, amphetamines, cannabinal, and methaqualone, after adjusting the samples to pH 12 (8). Additional drug applications for SPME are described in Bulletin 901 (free on request).

#### SPME in Chemical Ecology: Recovery of Pheromones

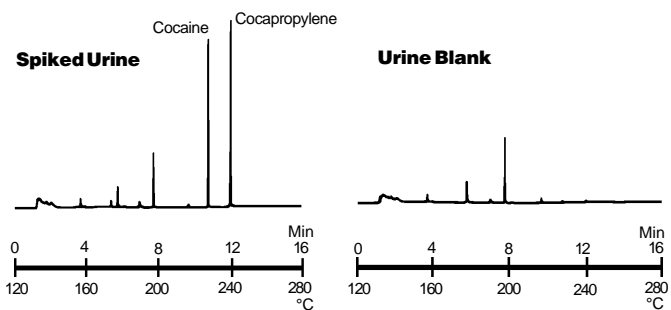
Ecologists have long appreciated the importance of chemical signals among and between insects and plants. Signal chemicals vary among populations and within individuals under different stimuli and at different stages in their life cycles. SPME gives these investigators the ability to collect volatiles emitted by individual insects and plants, without disturbing the organism. Investigators at the Royal Institute of Technology in Stockholm have investigated the suitability of SPME for monitoring chemical signals in several situations (9, 10). Some of their results are summarized here.

Analyses of pheromones of leaf miner moths (*Phyllonorycter* species) have been based on solvent extracts of the abdominal glands of signaling females. Multiple moths have been needed to provide enough material for a single injection onto a GC column. SPME has enabled the analysts at the Royal Institute of Technology to study the pheromone output by individual female *P. sylvella* moths during 1–3 hour sampling periods. To collect a sample, the moth is placed in a glass bottle with the SPME fiber a few millimeters from its abdominal glands. Other teams of investigators also have used SPME to monitor insect pheromones (11).

*Ophrys* flowers release volatiles that mimic the pheromones of female hymenopteran insects, and are pollinated by corresponding males – a relationship which exists between various other plant and

## Figure B. Cocaine in Urine

Sample: 0.5mL urine (250ng each analyte, 20 $\mu$ L 2.5% NaF) in 1mL vial  
 SPME Fiber: **100 $\mu$ m polydimethylsiloxane**  
 Cat. No.: **57300-U** (manual sampling)  
 Extraction: immersion, 30 min  
 Desorption: 3 min, 240°C  
 Column: polydimethylsiloxane, 30m x 0.32mm ID, 0.25 $\mu$ m film  
 Oven: 120°C to 280°C at 10°C/min  
 Carrier: helium, 3mL/min  
 Det.: NPD, 280°C  
 Inj.: splitless (splitter opened after 1 min), 240°C



796-0156, 0157

Figure provided by T. Kumazawa and K. Sato, Dept. Legal Medicine, Showa University School of Medicine, Tokyo, Japan and K. Watanabe, H. Seno, A. Ishii, and O. Suzuki, Dept. Legal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan.

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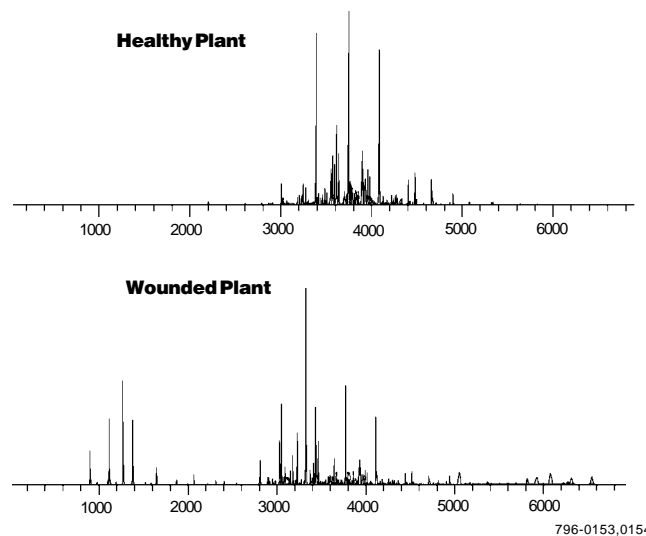
insect combinations. In earlier studies, the investigators had to combine volatiles released from several *Ophrys* plants to ensure sufficient material for analysis. SPME now enables them to monitor emissions from a single plant, or from individual flowers on one plant.

Changes in chemical signals caused by wounding a plant are known to have attracting effects on insects. The Stockholm investigators have used SPME to monitor the volatiles emitted by healthy and wounded spruce seedlings. Differences in the emission profiles are easily detected (Figure C). Wounded plants emit substantial amounts of mono- and sesquiterpenes, whereas healthy plants emit only aliphatic hydrocarbons and small amounts of sesquiterpenes.

As the examples briefly described here indicate, solid phase microextraction is being used in an increasingly wide variety of applications. If your analyses call for time-consuming and expensive solvent-based extractions, SPME may very well be a better approach. Or, as the Swedish applications show, SPME may be the approach to an analysis that, until now, has been difficult or impossible to perform. We feel the SPME/HPLC interface will further accelerate the development of new environmental, pharmacological, and food and beverage applications for SPME.

## Figure C. Chemical Signals from Spruce Seedlings

Sample: headspace surrounding *Picea abies* seedlings  
 SPME Fiber: **100 $\mu$ m polydimethylsiloxane**  
 Cat. No.: **57300-U** (manual sampling)  
 Extraction: headspace, 60 min  
 Desorption: 30 sec, 225°C  
 Column: poly(5% diphenyl/95% dimethylsiloxane), 30m x 0.25mm ID, 0.25 $\mu$ m film  
 Oven: 40°C (4 min) to 200°C at 4°C/min, hold 16 min  
 Carrier: helium, 10psi  
 Det.: EID, 70ev  
 Inj.: splitless (splitter opened after 30 sec), 225°C



796-0153, 0154

Figure provided by A.-K. Borg-Karlson, The Royal Institute of Technology, Department of Chemistry, Stockholm, Sweden.

### References

- For additional information on the SPME/HPLC interfaces, request Product Specification 496049.
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Only references 1 and 3 are available from Supelco.

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Description	Cat. No.
<b>SPME Holder**</b> For manual sampling For HPLC or Varian 8100/8200 AutoSampler	<b>57330-U</b> <b>57331</b>
<b>SPME/HPLC Interface</b> with Valco® Valve with Rheodyne® Valve	<b>57350-U</b> <b>57353</b>
<b>SPME Fiber Assembly Kit</b> One fiber assembly each: 100µm polydimethylsiloxane (for volatile analytes), 7µm polydimethylsiloxane (for nonpolar-intermediate polarity semivolatiles), 85µm polyacrylate (for polar semivolatiles) For manual sampling For HPLC or Varian 8100/8200 AutoSampler	<b>57306</b> <b>57307</b>
<b>SPME Sampling Stand</b> For consistent fiber immersion. Holds eight 4mL vials.	<b>57333-U</b>
<b>Heat/Stir Plate</b> 110 VAC 220 VAC (CE marked)	<b>Z110957-1EA</b> <b>Z262137-1EA</b>
<b>Magnetic Stirring Bars</b> 10 x 3mm, pk. of 3	<b>Z118877-3EA</b>
<b>SUPEL COSIL LC-PAH Column</b> 15cm x 4.6mm ID, 5µm particles	<b>58318</b>
<b>SUPEL COWAX™ 10 Capillary Column</b> 15m x 0.53mm ID, 1.0µm film	<b>25300-U</b>
<b>SPB™-1 Capillary Column</b> 30m x 0.32mm ID, 0.25µm film	<b>24044</b>
<b>SPB™-5 Capillary Column (5% diphenyl phase)</b>	<b>24034</b>
<b>EPA 610 Polynuclear Aromatic Hydrocarbons Mix</b> 16 analytes at concentrations indicated on Figure A, in 1mL methanol:methylene chloride, 50:50.	<b>48743</b>

For our complete selection of nonpolar, intermediate polarity, and polar SPME fibers, see the Supelco catalog, or call our Technical Service chemists.



## Acknowledgments

The procedure for detecting cocaine in urine was developed by T. Kumazawa and K. Sato, Department of Legal Medicine, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142, Japan and K. Watanabe, H. Seno, A. Ishii, and O. Suzuki, Department of Legal Medicine, Hamamatsu University School of Medicine, 3600 Handa-cho, Hamamatsu 431-31, Japan.

The procedure for monitoring BTEX compounds in air was developed by M. Chai and Y.-Z. Tang, BOVAR-CONCORD Environmental, 2 Tippet Road, Toronto, Ontario, Canada M3H 2V2.

Figures C and D were provided by A.-K. Borg-Karlson, The Royal Institute of Technology, Department of Chemistry, Organic Chemistry, S-100 44 Stockholm, Sweden.

For information on references 2 and 5, contact the authors at The Guelph-Waterloo Centre for Graduate Work in Chemistry, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

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\*\* Initially you must order both holder and fiber assembly. Holder is reusable indefinitely. Use with AutoSampler requires Varian SPME upgrade kit (available from Varian).