Application Note 99

SPME/HPLC: A Rapid and Sensitive Analysis of Polynuclear Aromatic Hydrocarbons in Water

An SPME/HPLC interface enables HPLC analysts to take advantage of the time and solvent savings offered by SPME. Peaks for 16 polynuclear aromatic hydrocarbons were sharp and symmetric, confirming efficient transfer from the SPME fiber to the HPLC column. Relative to peaks for directly injected analytes, peaks for most of the extracted analytes were significantly larger, indicating a concentrating effect that can increase the sensitivity of the analysis for trace-level analytes.

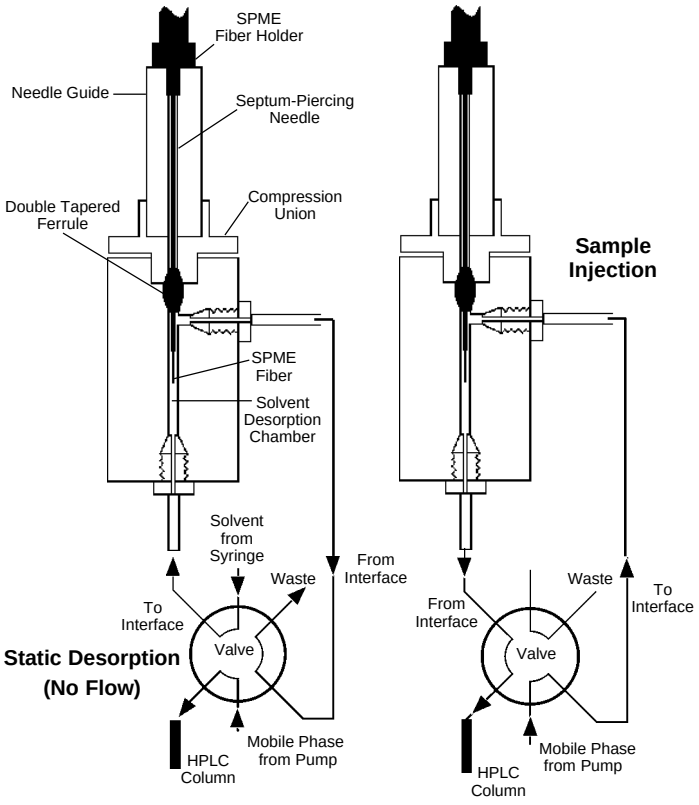
Key Words:
- polynuclear aromatic hydrocarbons
- sample extraction
- solid phase microextraction
- SPME

Solid phase microextraction – SPME* – has rapidly been established among the practical alternatives for sample preparation for gas chromatography. Polynuclear aromatic hydrocarbons and other weakly volatile or thermally labile compounds are more effectively monitored by HPLC but, initially, there was no simple way to introduce analytes extracted by SPME onto an HPLC column.

An SPME/HPLC interface developed at Supelco enables HPLC analysts to take advantage of the time and solvent savings offered by SPME. Easily installed and removed, the interface consists of a six-port injection valve and a desorption chamber (Figure A) that replaces the injection valve in the HPLC system. The SPME fiber is introduced into the desorption chamber under ambient pressure when the injection valve is in the "load" position. After the fiber is inserted through the ferrule, the unit is made leak-tight by closing a sealing clamp and compressing the ferrule against the SPME needle. All surfaces which contact the SPME fiber or the mobile phase are stainless steel or VESPEL®. Within the interface, mobile phase contacts the SPME fiber, removes the adsorbed analytes, and delivers them to the column for separation. Analytes can be removed in a moving stream of mobile phase (dynamic desorption) or, when analytes are more strongly adsorbed to the fiber, the fiber can be soaked in mobile phase before the injection is made (static desorption).

Analysts at the University of Waterloo (Ontario, Canada) analyzed polynuclear aromatic hydrocarbons, using SPME/HPLC (1). Chemists in Supelco laboratories also have evaluated the practicality of the technique for monitoring PAHs in water, using SPME fibers with 7µm, 30µm, or 100µm polydimethylsiloxane coating to extract the 16 PAHs listed in US Environmental Protection Agency methods 610 and 8310. All but three of the analytes were present at a concentration of 100ppb or 200ppb. Equilibration was fastest

Figure A. SPME/HPLC Interface: Desorption Chamber



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with the 7µm coating, but the 100µm coating provided the highest extraction levels (i.e., greatest sensitivity). After a 30-minute immersion in the sample, the fiber was introduced into the SPME/HPLC interface, and with the injection valve in the "load" position, the desorption reservoir was filled with 200µL of 40:60 acetonitrile:water. The fiber was soaked in the solvent for 2 minutes, then the analysts switched the valve to the "inject" position and began integration. During the first 2 minutes, the analytes were delivered to the column at a low flow rate (0.2mL/min), to minimize band broadening on the column. After 2 minutes, the valve was returned to the "load" position and the desorption chamber was flushed with 500µL of acetonitrile to eliminate the possibility of analyte carry-over to the next extraction. The desorption chamber was flushed and refilled with 500µL of 40:60 acetonitrile:water, the fiber was removed, and the flow

Figure B. PAHs Effectively Extracted from Water

Sample: 5µL PAH mix (Cat. No. 48743) in 5mL water (SPME) or acetonitrile:water, 40:60 (conventional injection)
•100ng/mL ■200ng/mL ••1000ng/mL ■■2000ng/mL
SPME Fiber: 100µm polydimethylsiloxane
Cat. No.: **57301**
Extraction: immersion, 30 min (rapid stirring)
Desorption: static, 200µL acetonitrile:water, 40:60, 2 min
Column: **SUPELCOSIL LC-PAH, 15cm x 4.6mm ID, 5µm particles**
Cat. No.: **58318**
Mobile Phase: acetonitrile:water gradient (see program)
Flow Rate: 0-2 min: 0.2mL/min
2-45 min: 1.0mL/min
Det.: UV, 254nm

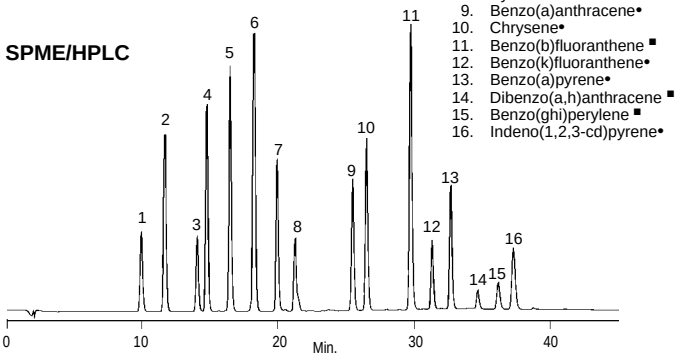
Gradient Program

Time (min)	% ACN
0	50
5	50
30	100
45	100

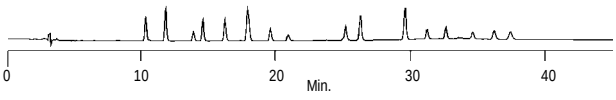
Flow increased at 2.0 min

Analyte
1. Naphthalene••
2. Acenaphthylene■■
3. Acenaphthene••
4. Fluorene ■
5. Phenanthrene•
6. Anthracene•
7. Fluoranthene ■
8. Pyrene•
9. Benzo(a)anthracene•
10. Chrysene•
11. Benzo(b)fluoranthene ■
12. Benzo(k)fluoranthene•
13. Benzo(a)pyrene•
14. Dibenzo(a,h)anthracene ■
15. Benzo(ghi)perylene ■
16. Indeno(1,2,3-cd)pyrene•

SPME/HPLC

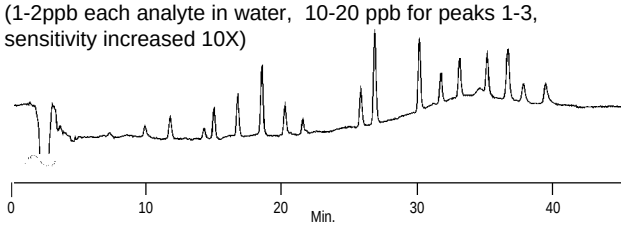


Conventional Injection (200µL of sample injected onto column)



SPME/HPLC

(1-2ppb each analyte in water, 10-20 ppb for peaks 1-3, sensitivity increased 10X)



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rate was increased to 1mL/min. A 1-minute air drying period was sufficient to prepare the fiber for the next extraction.

The analytes were best separated on a 15cm SUPELCOSIL™ LC-PAH column. Sharp, symmetric peaks indicate efficient transfer of the analytes from the SPME fiber to the HPLC column (Figure B). 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. Subsequent extraction of the same analytes at 1-2ppb each clearly demonstrates the effectiveness and sensitivity of the extraction technique (Figure B).

If your HPLC analyses call for time-consuming and expensive solvent-based extractions, SPME may very well be a better approach. In addition to analyses of PAHs, investigators have shown that SPME can be effective for monitoring drugs and drug metabolites in biological fluids (for an example – amphetamines in urine – request Application Note 83). The SPME/HPLC interface will accelerate the development of many new environmental, pharmacological, and food and beverage applications.

Ordering Information:

Description	Cat. No.
SPME/HPLC Interface with Valco® Valve	**57350-U**
SPME/HPLC Interface with Rheodyne® Valve	**57353**
SPME Holder Initially you must order both holder and fiber assembly. Holder is reusable indefinitely. For HPLC (also for Varian 8100/8200 AutoSampler)	**57331**
SPME Fiber Assembly 100µm polydimethylsiloxane, pk. of 3 For HPLC (also for Varian 8100/8200 AutoSampler)	**57301**
SUPELCOSIL LC-PAH Column 15cm x 4.6mm ID, 5µm particles	**58318**
EPA 610 Polynuclear Aromatic Hydrocarbons Mix 16 analytes at concentrations indicated on Figure B in methanol:methylene chloride, 50:50. 1mL	**48743**

To see our complete selection of SPME fibers, refer to the Supelco catalog, or call our Technical Service chemists.

Reference

1. Chen, J. and J. Pawliszyn, *Anal. Chem.* **67:** 2530-2533 (1995).

Reference not available from Supelco. For information, 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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