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New Pharmaceutical and Food & Beverage Applications for SPME/HPLC

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Solid phase microextraction (SPME) has been established as a practical alternative for sample preparation for gas chromatography. A new SPME/HPLC interface now allows SPME to be used in analyses of many weakly volatile or thermally labile compounds, such as pharmaceutical compounds. New SPME/HPLC applications include monitoring tricyclic antidepressant drugs in serum and antioxidants and preservatives in foods and beverages.

Because analytes are rapidly extracted from a sample matrix, with virtually no solvent consumption, solid phase microextraction (SPME)* has become established as a practical approach to sample preparation for gas chromatography. SPME saves preparation time and solvent purchase and disposal costs, and can improve the limits of detection. Now, our SPME/HPLC interface enables analysts to use SPME in analyses of numerous weakly volatile or thermally labile analytes (1). New applications for SPME/HPLC are summarized here.

Tricyclic Antidepressant Drugs in Serum

In analyses of drugs in blood, urine, or other biological fluids sample preparation usually consists of removing the analytes through liquid-liquid extraction, solid phase extraction, or other techniques. These methods can involve excessive preparation time, and require expensive organic solvents. SPME eliminates these drawbacks.

Investigators from the departments of legal medicine at Showa University School of Medicine (Tokyo) and Hamamatsu University School of Medicine (Hamamatsu) developed a headspace SPME/capillary GC method for extracting the most volatile tricyclic antidepressants (amitriptyline, chlorimipramine, imipramine, trimipramine) from urine (2). In developing their method, these analysts extracted four additional tricyclic antidepressants (carpipramine, clocapramine, desipramine, lofepramine) from urine samples, but these compounds decomposed in the GC and eluted as multiple peaks. Such heat-sensitive analytes should be analyzed by combining SPME extraction with HPLC analysis. Our SPME/HPLC interface makes this combination possible.

We have developed a procedure for SPME/HPLC analysis of three nonvolatile tricyclic antidepressants, desipramine, nordoxepin, and nortriptyline, in serum. We add methanol to the serum sample to denature the serum proteins and release protein-bound drugs, then extract the drugs by immersion SPME, using a specially prepared 40µm polydimethylsiloxane/divinylbenzene SPME fiber. Extraction and analytical conditions are summarized in Figure A. The data summarized in Table 1 show that the extraction is consistent and sensitive, and the analytes are efficiently delivered to the HPLC column.

In addition to ensuring speed and economy in extractions of pharmaceuticals from biological fluids, SPME can be more effective than liquid-liquid extractions in separating analytes of interest from potentially interfering matrix components or coadministered

Figure A. Tricyclic Antidepressant Drugs in Serum

Sample:	0.8mL thawed serum containing 100ng/mL each analyte, 0.16mL methanol (20% of serum volume) added to denature proteins/release protein-bound drugs
SPME Fiber:	**40µm PDMS/DVB-HPLC**
Cat. No.:	**57317**
Extraction:	immersion, 30 min (rapid stirring)
Desorption:	dynamic, methanol:acetonitrile, 60:40, 10 min
Column:	**SUPELCOSIL™ LC-PCN,** 25cm x 4.6mm ID, 5µm particles (with Supelguard™ LC-PCN guard column)
Cat. No.:	**58378**
Mobile Phase:	acetonitrile:methanol:0.01M K_2HPO_4 (pH 7.0 with phosphoric acid), 60:15:25
Flow Rate:	2mL/min
Temp.:	40°C
Det.:	UV, 215nm

1. Nordoxepin
2. Nortriptyline
3. Desipramine
4. Protriptyline (int. std.)

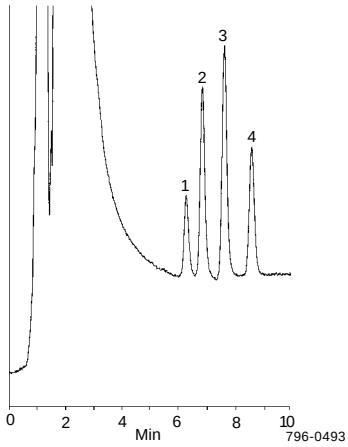

Table 1. Consistent Recovery of Tricyclic Antidepressants by SPME/HPLC

	Summary of Response Factors			
	Precision^		Calibration Curve^^	
Analyte	Mean ±Std. Dev.	%RSD	Mean ±Std. Dev.	%RSD
Nordoxepin	0.68 ±0.06	10.0	0.74 ±0.07	9.9
Nortriptyline	1.51 ±0.14	9.1	1.52 ±0.06	4.1
Desipramine	1.86 ±0.09	5.2	1.90 ±0.09	4.9

^100ng/mL each analyte in serum, n = 8 extractions (4 fibers, 2 extractions each).
^^50-400ng/mL each analyte in serum.
Internal standard: protriptyline. Conditions listed in Figure A.

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drugs (2).*Antioxidants and Preservatives in Foods and Beverages*

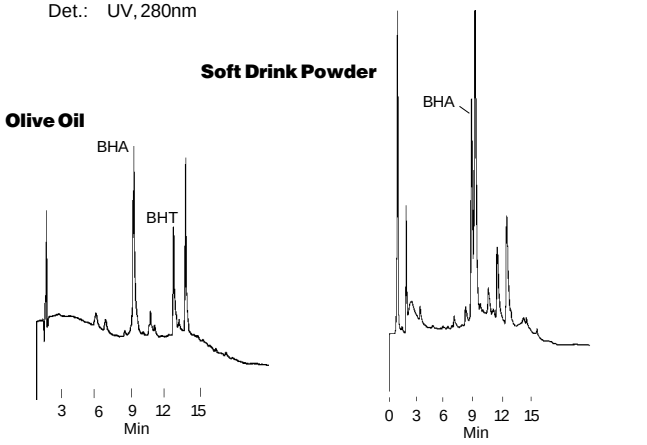
Antioxidants and preservatives maintain the quality of food products during storage. These additives must be monitored to ensure that they are within specified limits. Currently, BHA (2- and 3- tert-butyl-4-hydroxyanisole) and BHT (3,5-di-tert-butyl-4-hydroxytoluene) in oils and fats are analyzed through a process that includes tedious liquid extraction/concentration (3). SPME/HPLC offers a considerable savings of time and solvents, while providing very satisfactory results, as shown by analyses of BHA and BHT extracted from olive oil and soft drink powder (Figure B).

The procedure for monitoring benzoic acid in foods includes analyte derivatization, as well as liquid extraction and concentration (4). SPME/HPLC eliminates all of these steps. Note in Figure C that caffeine, as well as benzoic acid, is extracted by the 40µm PDMS/DVB-HPLC fiber.

These examples show SPME now offers a faster, better approach to sample preparation for HPLC. Alternatively, SPME may be the key to an analysis that, until now, has been difficult or impossible to perform. In either case, the combination of SPME with HPLC should prove valuable in many environmental, pharmacological, and food and beverage applications.

Figure B. Antioxidants in Foods

Sample: 3mL olive oil containing 1mg/g each analyte
0.6g soft drink powder in 3mL water
SPME Fiber: **40µm PDMS/DVB-HPLC**
Cat. No.: **57317**
Extraction: immersion, 15 min (olive oil) or 20 min (powder)(rapid stirring)
Desorption: static, acetonitrile:methanol, 50:50, 5 min (olive oil) or 3 min (powder)
Column: **SUPELCOSIL LC-18, 15cm x 4.6mm ID, 5µm particles**
Cat. No.: **58230**
Mobile Phase: A = 5% acetic acid in water
B = acetonitrile:methanol, 1:1
30% B for 2 min, then to 100% B over 10 min, hold 10 min
Flow Rate: 2mL/min
Det.: UV, 280nm



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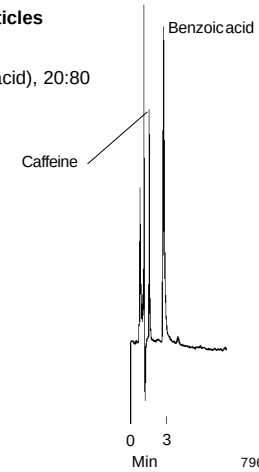
Trademarks
Rheodyne – Rheodyne, Inc.
SUPELCOSIL, Supelguard – Sigma-Aldrich Co.
Valco – Valco Instruments Co., Inc.

Figure C. Benzoic Acid in Diet Cola

Sample: cola in water, 1:10 (pH to 2.2 with acetic acid), 3mL
SPME Fiber: **40µm PDMS/DVB-HPLC**
Cat. No.: **57317**
Extraction: immersion, 25 min (rapid stirring)
Desorption: static, mobile phase, 1 min
Column: **SUPELCOSIL LC-18, 15cm x 4.6mm ID, 3µm particles**
Cat. No.: **58985**
Mobile Phase: acetonitrile:0.02M sodium acetate (pH 4.3 with acetic acid), 20:80
Flow Rate: 1.5mL/min
Det.: UV, 254nm



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Ordering Information:

Description	Cat. No.
SPME Holder**	
For manual sampling	**57330-U**
For HPLC	**57331**
SPME/HPLC Interface	
with Valco® valve	**57350-U**
with Rheodyne® valve	**57353**
SPME/HPLC Fiber Assembly	
40µm PDMS/DVB-HPLC	**57317**
HPLC Columns	
SUPELCOSIL LC-PCN 25cm x 4.6mm ID, 5µm particles	**58378**
SUPELCOSIL LC-18 15cm x 4.6mm ID, 5µm particles	**58230-U**
SUPELCOSIL LC-18 15cm x 4.6mm ID, 3µm particles	**58985**

References

1. For additional information about the HPLC/SPME interface request Product Specification 496049.
2. Supelco Bulletin 901 (available on request).
3. AOAC Official Method 983.15, *AOAC Official Methods of Analysis,* Supplement March 1995.
4. AOAC Official Method 983.16, *AOAC Official Methods of Analysis,* Supplement March 1995.

References 3 and 4 not available from Supelco (obtain from Association of Official Analytical Chemists, Arlington, VA USA).

For descriptions of our SPME fibers and accessories, request publication 413019.

For chemical standards for tricyclic antidepressants, refer to the Supelco catalog. For BHA, BHT, and other antioxidant/preservative standards, request our Food & Beverage Analysis Guide.

* Technology licensed exclusively to Supelco. US patent #5,691,206; European patent #0523092.

** Initially you must order both holder and fiber assembly. Holder is reusable indefinitely. Cat. No. 5-7331 also is used with Varian 8100/8200 AutoSampler (requires Varian SPME upgrade kit, available from Varian).

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