ISO 9001 REGISTERED

SUPELCO

Supelco Park
Bellefonte, PA 16823-0048 USA
Telephone 800-247-6628 • 814-359-3441
Fax 800-447-3044 • 814-359-3044
email: supelco@sial.com
http://www.sigma-aldrich.com/supelco

Bulletin 929

P000800

A Practical Guide to Quantitation with Solid Phase Microextraction

Solid Phase Microextraction* (SPME) is an innovative, solvent free technology that is fast, economical, and versatile. SPME has gained wide spread acceptance as the technique of preference for many applications. This guide presents a practical introduction to quantitation using the technique based on your type of sample. We present the factors that will influence your accuracy and precision and the different quantitation approaches that you can use. To help you further, we provide specific examples for each of the different approaches discussed and suggested references for additional reading.

SPME

SUPELCO

Introduction

SPME is a fiber coated with a liquid (polymer), a solid (sorbent), or a combination of both. The fiber coating removes the compounds from your sample by absorption in the case of liquid coatings or adsorbing in the case of solid coatings. Traditional sample preparation methods try to completely remove the analytes of interest from the sample. SPME does not work this way. With SPME, the amount of analyte removed by the fiber is proportional to the concentration of the compound in the sample. This is true when the fiber and the sample reach equilibrium or before equilibrium, as long as you carefully control the sampling parameters. The ability to use SPME quantitatively before you reach equilibrium permits much shorter sampling times producing a fast, economical, and versatile technique.

The decision of which quantitation approach to choose when using SPME will depend on the sample matrix, its complexity, and the extraction method being used (1). Qualitatively optimize the SPME parameters to determine the best fiber and sampling conditions to use before choosing a quantitation approach and calibrating the instrument. Once you have the conditions optimized, choose an appropriate calibration approach if you need quantitative results. We discuss three common approaches, external calibration, internal standard comparison, and the method of standard addition.

Quantitation Guide Table

Matrix	Type	Method	Recommended Approach		
			External	Internal	Standard Addition
Gas	Simple	Headspace	✓		
	Complex	Headspace	✓	✓	
Liquid	Simple	Headspace	✓		
	Immersion		✓	✓	
	Complex	Headspace		✓	✓
	Immersion				✓
Solid	Simple	Headspace		✓	✓
	Complex	Headspace			✓

How to Use the Quantitation Guide Table

Matrix

First, select the sample medium you will be using. The sample will fall into one of the three general categories of gas, liquid, or solid.

Gas (indoor air, breath, atmosphere, insect spray)

Liquid (drinking water, fruit juice, blood, groundwater, milk, soda, coffee, wastewater, wine, beer, vegetable oil, urine, saliva, salt water)

Solid (soil, cheese, sludge, tobacco, vegetables, flowers, fruits, pharmaceuticals, polymers, paint, insects, hair, fire debris, fish tissue)

Type

Next, select the complexity of the sample you are working with as simple or complex. You should determine this based on your knowledge of the sample and any historical data available.

Simple sample types are those that are fairly consistent with low total organic content and particulate matter (e.g.- drinking water, indoor air).

Complex sample types are those that are more variable with regards to total organic content, pH, and particulate matter (e.g.- wastewater, soil); or they contain lipids, proteins, or other potentially interfering components (e.g.- biological fluids, milk).

Method

Next, select the sampling method you will use, headspace or direct immersion. You will base this on the compounds of interest and results from your optimization tests with the sample.

Approach

Lastly, select the recommended quantitation approach based on the box checked in the table. We list the approaches in order of their complexity to perform from external to standard addition. Start with the easiest quantitation approach suggested. Go to the section in the guide that presents the suggested approach for an explanation of the technique, examples of it's use, and references for additional reading and examples.

Call us at 800-359-3041 if you need assistance with selecting an appropriate quantitation approach for your sample.

Approaches to Quantitation

Method of External Standardization

Description of Method

External standard calibration compares detector responses from the sample to the responses from the target compounds in the calibration standards. Create standard mixtures over the range of concentration expected in the sample. Extract and analyze each standard mixture by SPME. Create the calibration curve from the detector response for each analyte concentration in the calibration standard. Comparison of the sample extract's detector response to the calibration curve determines the amount of analyte in the unknown sample

Best Used For

Simple sample matrices, such as, gaseous or liquid samples, that do not have interferences or high levels of organic solvents. Also, samples that are homogeneous and do not vary in the type and total number of compounds that are present. Prepare calibration standards in a clean matrix sample for external calibration. Choose spiked water standards to represent aqueous samples.

Not Recommended For

We do not recommend samples with complex matrices like protein, fat, or humic material. These samples adsorb target analytes or vary greatly in the type and total number of compounds that are present.

Example of Use

We applied external standardization to a volatile organics in water application (2). Different aliquots of a volatile standard mixture (100mg/mL) were added to 4mL of phosphate buffer containing 25% NaCl. The final concentration of the mixtures covered a concentration range from 5 to 100,000ppb. We extracted each standard mixture using the immersion method with a Carboxen fiber for 15 min. Figure A shows the external calibration curves. The plot shows the detector response for each analyte versus its concentration. We used a log-log plot because of the large concentration range analyzed. You can use a linear plot with a narrower concentration range.

References for Additional Reading

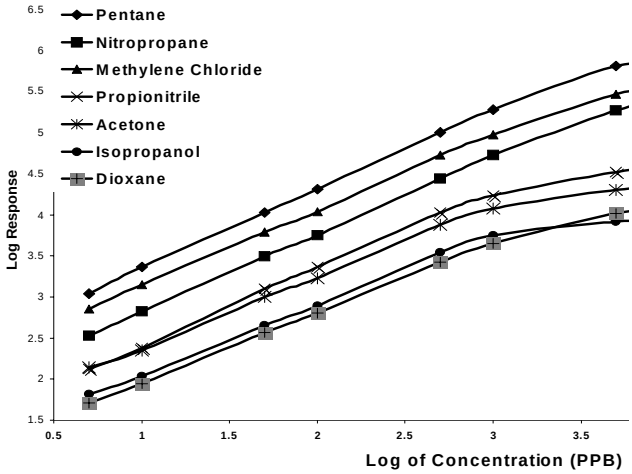
Gaseous Samples – determination of volatile organic compounds compared to NIOSH sampling approach. "Air Sampling and Analysis of Volatile Organic Compounds with SPME", Koziel, J., *Anal. Chem. 2000, 72, 5178-5186.*

"Calibration of Solid Phase Microextraction for Air Analysis based on Physical Chemical Properties of the Coating", Martos, P, *Anal. Chem 1997, 89, 206-215.*

Liquid Samples – BTEX determination at pg/mL in water "Detection of substituted benzenes in water at the pg/ml level using SPME and GC-ion trap mass spectrometry", D. Potter and J. Pawliszyn, J. Chromatography 625 (1992) 247-255.

Figure A. External Standard Approach

Sample:	Water containing 25% NaCl and 0.05M phosphate buffer, spiked with analytes to a final concentration of 2ppm
Fiber:	85µm StableFlex™ Carboxen/polydimethylsiloxane
Cat. No.:	**57335-U (automated sampling)**
Extraction:	headspace, ambient, 15 minutes with agitation
Desorption:	2 minutes
Column:	30m x 0.32mm x 4.0µm SPB™-1 SULFUR (24158)
Oven:	40°C (2 min) to 140°C at 8°C/min (1 min)
Det.:	FID
Inj.:	splitless, closed 0.5 min, 0.75mm ID liner



Method of Internal Standardization

Description of Method

Internal standard calibration requires the addition of a known amount of a known compound into the calibration standards and samples. You should select internal standards that are similar in analytical behavior to the target analytes but not found in the sample. The ideal internal standard is an isotopically labeled analogue of the analyte of interest, e.g. toluene-d_8 for toluene and other similar volatile aromatics. This approach will help compensate for sample to sample variations in extraction and desorption efficiency caused by the sample matrix.

Best Used For

A sample matrix that are complex gaseous or liquid mixtures or less complex solid samples that you can disperse in a liquid and perform headspace sampling.

Not Recommended For

Samples where you can use the easier external standardization approach or more complex liquid or solid matrices like protein, fat, or humic material that adsorb target analytes.

Example of Use

Staff members of the Fukuoka University School of Medicine used the internal standard approach in urine to measure amphetamine and methamphetamine levels (3). They used the deuterated amphetamine analogs as internal standards in the study. Figure B shows the linear plot used for determination of the amphetamines over a range of 0.2-10mg/L. Correlation coefficients for both amphetamines were 0.9999.

References for Additional Reading

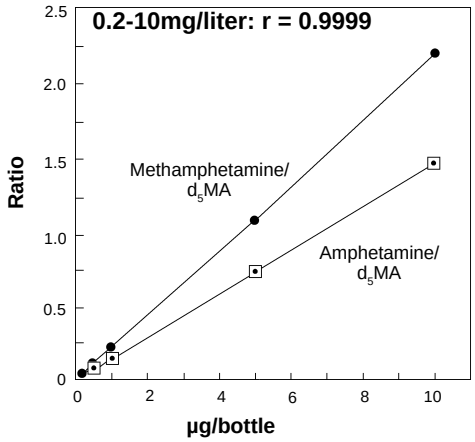
Gaseous Samples – C1-C6 sulfur compounds determined in beer, "Determination of sulphur compounds in beer using headspace SPME and GC analysis with pulsed Flame photometric detection", P. Hill, R. Smith, J.Chromatogr. A 872(2000) 203-213.

Liquid Samples – determination of hydrocarbon fuels in wastewater, "Quantitative analysis of fuel-related hydrocarbons in surface water and wastewater samples by SPME", J. Langenfeld, S. Hawthorne, D. Miller, Anal. Chem. (1996), 68, 144-155.

Solid Samples – determination of semivolatile organics from soil samples, "Coupled subcritical water extraction with SPME for determining semivolatile organics in environmental solids". K. Hageman, L. Mazeas, C. Grabanski, D. Miller, S. Hawthorne, Anal. Chem (1999), 68, 3892-3898.

Figure B. Internal Standard Approach

Sample:	1mL urine (100µg each analyte, 5µg d_5-methamphetamine, 0.7g K_2CO_3)in 12mL vial
SPME Fiber:	**100µm polydimethylsiloxane**
Cat. No.:	**57300-U** (manual sampling)
Extraction:	headspace, 80°C, 5 min (sample incubated 20 min)
Desorption:	3 min, 250°C
Column:	polydimethylsiloxane, 15m x 0.53mm ID, 2.0µm film
Oven:	110°C
Carrier:	nitrogen, 25mL/min
Det.:	FID, 250°C
Inj.:	splitless, 250°C



795-0597

Method of Standard Additions

Description of Method

The technique of standard addition uses the addition of known concentrations of the actual analyte of interest to multiple aliquots of the sample (4). The sample alone is then analyzed. You then plot the detector response versus the amount spiked for each analysis. A straight line is drawn and the x intercept is determined. This value is the amount in the unknown.

Best Used For

A sample matrix where a blank matrix in not available or ones that vary greatly in the type and total number of compounds that are present.

Not Recommended For

A sample matrix where you can use the easier external or internal standardization approaches.

Example of Use

Varian developed an application note that describes the "Determination of Methanol in a Caustic Industrial Product with Automated SPME" (5). The sample contained 40% NaOH and high level of salt. Determination of the amount of methanol in the sample was done by the standard addition approach. The chemist spiked aliquots of the sample with varying levels of methanol and extracted them by SPME. Figure C shows the plot of the detector response versus the methanol concentration. The detector response of the methanol in the unspiked sample was set at zero on the x-axis. The concentration of methanol in the sample solution was determined by extending the calibration curve to zero response (400ppm methanol). Always check linearity of detector response when using standard solutions. The standard solutions should be at a concentration representing the combined concentration of the native analyte and the spiked standard.

References for Additional Reading

Liquid Samples – Oxidative by-products from milk, "Comparison of SPME and dynamic headspace methods for the GC-MS analysis of light-induced lipid oxidation products in milk", Marsili, R., J. Chromatogr. Sci. (1999), 37,17-23.

Flavor volatiles in 40% ethanol solution, "Characterization of Commercial Vodkas by SPME and GC-MS Analysis", Ng, L., J Sci Food Agric 1996, 70, 380-388.

Solid Samples – determination of chlorophenols in wetlands soil, "Determination of chlorophenols in soils using accelerated solvent extraction combined with SPME", L. Wennrich, P. Popp, M. Moder, Anal. Chem (2000), 72, 546-551.

Flavor additives used with tobacco for cigarettes. "Qualitative and quantitative analysis of flavor additives on tobacco products using SPME-GC-Mass Spectroscopy", Clark, TJ., Bunch, J., J. Agric. Food Chem, (1997) 45, 844-849.

Figure C. Standard Addition Approach

Sample:	0.6mL of the product spiked with 10µL of the methanol standards in 2-mL sampling vials. To minimize extraneous peaks, the vial septa were baked at 150°C overnight
Fiber:	65µm Carbowax/divinylbenzene
Cat. No.:	**57313 (automated sampling)**
Extraction:	headspace, ambient, 3 minutes
Desorption:	1 minute, 210°C
Column:	15m x 0.53mm, 1µm poly(ethylene glycol)
Oven:	40°C, hold 3 minute
Det.:	FID, 220°C, range 10 – 12
Inj.:	1078 with 0.8mm insert, 210°C, isothermal. Relay program: time 0 relay open, close at .01 minutes, open at 3 minutes

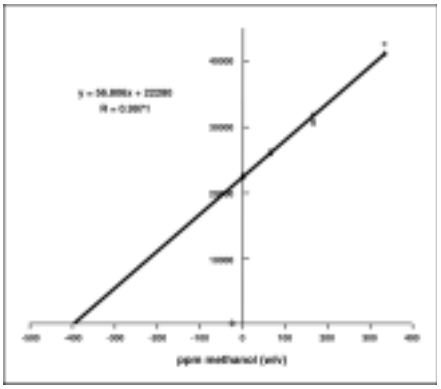


Figure C courtesy of Zelda Penton, SPME Application Note 8, Varian, Inc.

G001487

Surrogates and Matrix Spike Compounds

Chemists use surrogate standards and matrix spike compounds to assess matrix interferences that would bias quantitation. Surrogates are compounds that are chemically similar to the target analytes but not expected to occur in the sample. You should add the surrogates to each sample and calibration standard just before extraction. The recovery of the surrogate standard is an indicator of any unusual matrix effects.

Chemist will also use matrix spike compounds to assess matrix interferences that would bias quantitation. Matrix spike compounds are selected target compounds that you spike into a second aliquot of the sample just before extraction. Typically, you should select one out every ten or twenty samples for use as a matrix spike sample. The recovery of the matrix spike compounds is an indicator of any unusual matrix effects. The use of surrogates and matrix spike compounds is applicable for all three approaches to quantitation and are good tools to help determine matrix-related sources of bias.

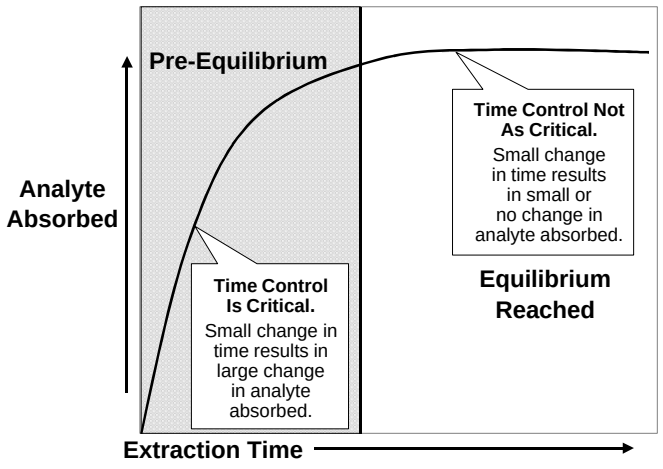
Tips to Improve Quantitation

You can improve your reproducibility and quantitation with SPME through careful control and monitoring of time, temperature, and technique during sample extraction.

Time

The extraction time is a critical parameter in the SPME sampling process. Figure D shows the typical relationship of extraction time to analyte absorbed on the fiber. If you vary the time that you expose the fiber during sampling you will vary the analyte concentration on the fiber, until you reach equilibrium. Once the analyte is at equilibrium between the fiber and the sample, its concentration will become constant. Consequently, controlling your extraction time is critical when working in the pre-equilibrium period. Use a stopwatch to time each extraction precisely.

Figure D. Effect of Time on Amount of Analyte Absorbed



Temperature

Temperature also effects the equilibrium during extraction. If you increase the temperature, you change the equilibrium distribution of analytes in the sample and the headspace. During the sampling process, the fiber also establishes equilibrium with the sample and headspace. Variations in temperature will change the equilibrium and the resulting concentration of analyte on the fiber. You need to stabilize the sample at the determined optimal temperature before exposing the fiber. Be aware that variations in room temperature can cause non-reproducible results if you use an ambient sampling temperature. Use a calibrated thermometer along side the sample to insure a constant extraction temperature.

Technique

The technique used in sampling will also influence your reproducibility. A reliable, reproducible technique is important whether using a headspace or direct immersion sampling approach. Be consistent with your fiber position and applying agitation, salting, or pH adjustments. You can dramatically improve your reproducibility if you just pay close attention to time, temperature, and technique throughout the sampling procedure. Be careful to add the same amount of salt to each sample and to stir the sample at the same speed during each extraction. Use a sampling stand to position the fiber above or in the sample consistently.

Conclusion

SPME is a fast, economical, and versatile technique that can be very reproducible and quantitative when you establish and follow good procedures. The properties of the target analytes and sample matrix will influence precision and accuracy as with any sample preparation technique. Using the optimal approach to quantitation for your particular sample matrix is important. Homogeneous type matrices require only external calibration standards to achieve reproducible quantitation. Internal standards or standard addition work best with more heterogeneous samples where the sample's composition is not as predictable. Controlling and monitoring the sampling parameters of time, temperature, and technique are critical to achieving reproducible SPME results.

References

1. Pawliszyn, J. Application of Solid Phase Microextraction (Book)- Chapters 1 & 2 on Quantitation, Royal Society of Chemistry, ISBN 0-85404-525-2 (1999)
2. Shirey, R. *Extech 2000 presentation titled "Analyte response vs. Conc (Carboxen-PDMS) T400156*
3. Yashiki, M., Detection of Amphetamines in Urine Using Head Space SPME and Chemical Ionization Selected Ion Monitoring, *Forensic Sci Intl., 76(2), 169-177 (1995).*
4. Bader, M., - Paper on conducting Standard Addition Calibration, *J. Chem. Ed. 57 (10)(1980) pg. 703*
5. Varian, Inc., Z. Penton, Application Note 8, Determination of Trace Methanol in a Caustic Industrial Product with Automated Solid Phase Microextraction (SPME).

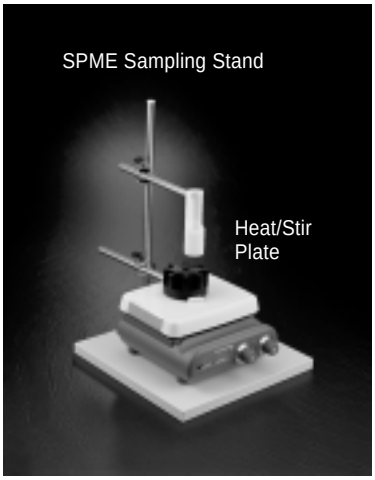
9970045

Helpful Products

StableFlex Fibers

StableFlex fibers are more durable than the standard fused silica SPME fibers. They are coated with the same polymeric coatings but on a flexible fused silica core. The increased flexibility provides better performance and reproducibility in your sample extractions. StableFlex fibers are available in the manual and automated version. The new assortment kit provides you the opportunity to try the four StableFlex coatings to optimize your application. Fibers are 1cm long unless otherwise noted.

	Manual sampling		Automated sampling	
Needle size:	**24 gauge**	**23 gauge**	**24 gauge**	**23 gauge**
StableFlex Fiber Assemblies				
65µm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)	**57326-U**		**57327-U**	
85µm Carboxen/Polydimethylsiloxane (CAR/PDMS)	**57334-U**		**57335-U**	
70µm Carbowax/Divinylbenzene (CW/DVB)	**57336-U**	**57338-U**	**57337-U**	**57339-U**
50/30µm DVB/Carboxen/PDMS (DVB/CAR/PDMS)	**57328-U**		**57329-U**	
50/30µm DVB/Carboxen/PDMS on a 2cm length fiber	**57348-U**			
SPME StableFlex Fiber Assortment Kit 1 (kit contains one each of the four StableFlex fiber coatings)	**57550-U**		**57551-U**	



P000123

NEW! 40mL Vial Holder

Use this aluminum block for heating/stirring during headspace SPME sampling of odors or other volatiles.

40mL Vial Holder	**33313-U**

SPME Sampling Stand

Holds eight vials while supporting the SPME syringe for consistent fiber immersion depth. Cat. No. **57333-U** accommodates 4mL vials only; Cat. No. **57357-U** accommodates 15mL vials. Order the 15mL vial puck (Cat. No. **57358-U**) as a replacement for the 15mL unit, or to use 15mL vials with the 4mL unit.

for 4mL vials	**57333-U**
for 15mL vials	**57357-U**
Vial puck for 15mL vials	**57358-U**

Heat/Stir Plate

Fits compactly on the base of the SPME sampling stand. Heating range is 40-550°C, stirring range is 60-1200rpm.

Corning heat/stir plate, 120VAC	**Z262129**

Magnetic Stirring Bars

Fits 4mL vials, 10 x 3mm, pk. of 3, PTFE covered	**Z11,8877-3EA**

Visit our website (www.sigma-aldrich.com/supelco) for a complete listing of PTFE and glass covered magnetic stirring bars.

Thermometer

For monitoring sample temperature when using the SPME sampling stand and a heat/stir plate.

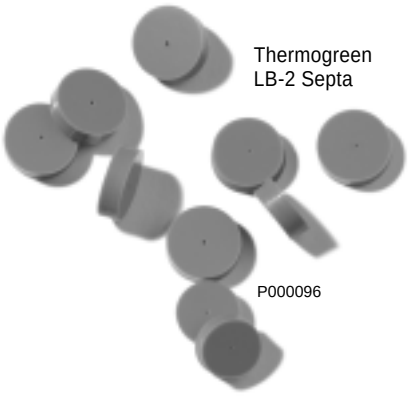
5" thermometer	**57332**

910-0045

Stopwatch

Performs many functions: time of day, 1/100 second timing, split (lap) times, day, date and month, time in/time out, two finishing times, night light, 0.003% accuracy. Includes battery.

LCD Digital Stopwatch	**23011**



Pre-Drilled Thermogreen LB-2 Septa for SPME

Easier needle penetration and high puncture tolerance – ideal for autosamplers. Reduce septum coring that can cause extraneous peaks. Already conditioned, ready-to-use. Extremely low bleed over a wide range of inlet temperatures – from 100°C to 350°C. Rubber formulation exclusive to Supelco.

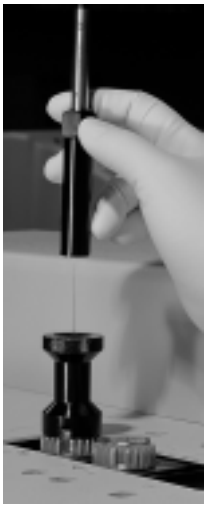
9.5mm (pk. of 25)	**23161**
9.5mm (pk. of 50)	**23162-U**
11mm (pk. of 25)	**23167**
11mm (pk. of 50)	**23168**

SPME Inlet Guide

Secures the SPME fiber holder in the injection port during the thermal desorption process. Interchangeable among Merlin Microseal sealing system and most Varian and Hewlett-Packard chromatographs.

SPME inlet guide	**57356-U**

SPME Inlet Guide



P000047

Merlin Microseal High Pressure Septa

Eliminate siloxane background, prolong septum lifetime.

To eliminate septum coring during SPME injections, use the Merlin Microseal system, a patented long-life replacement for the standard septum and septum nut on a capillary or purged packed inlet system. Two sequential seals provide a much longer life than conventional septa. The new high pressure units allow operation at 2-100psi. Use only with 23 gauge SPME fiber assembly.

For Hewlett-Packard GC Models 5800, 5900 series, 6890	
1 nut and 2 septa	**24814-U**
1 nut and 1 septum	**24815-U**
1 replacement septum	**24816-U**
For Varian GC Models 3400, 3800	
1 Varian nut, 1 septum, and 1 inlet adapter	**24817-U**
1 replacement septum	**24818-U**

Contact Us:

Please contact us to order SPME products or for more information about the SPME product line.

Ordering / Customer Service ..... **800-247-6628 / 814-359-3441**
Technical Service ..... **800-359-3041 / 814-359-3041**
web ..... **www.sigma-aldrich.com/supelco**

SPME

SUPELCO

Books on SPME

Solid Phase Microextraction: A Practical Guide - Sue Ann Sheppers Wercinski, ed. 1999, 242pp. This reference book contains extensive descriptions of proven sampling methods for chemical analysis, focusing on SPME application.
26610-U

Solid Phase Microextraction: Theory and Practice - Janus Pawliszyn, 1997, 241pp. This book describes the operating principles and construction of SPME devices, theory, method development, and applications.
26591-U

Applications of Solid Phase Microextraction - Janus Pawliszyn, 1999, 653 pp. A compilation of 46 invited chapters describing applications of SPME for foods, forensics, environmental samples, and other areas.
26611-U

Techniques for Analyzing Food Aroma - Ray Marsilli, ed. 1997, 371 pp. This book discusses the analytical methods for food flavors and aromas, showing how to select appropriate techniques for resolving the problems of major food trends.
26589-U

SPME Literature on CD

This CD includes the SPME Application Guide, 3rd Ed. with over 750 literature references using SPME technology (151 new), and our full library of SPME Application Notes and Bulletins. Request T199925 (CJQ)

SPME Troubleshooting Guide

SPME Troubleshooting Guide, Bulletin 928 (T101928), is a new guide that provides tips and troubleshooting guidance for new or experienced SPME users. This guide is not contained on the 3rd edition CD, please request it separately, by asking for T101928 (EDV).

Patents

*Solid Phase Microextraction (SPME) Technology licensed exclusively to Supelco. U.S. patent #5,691,206; European patent #523092.

**Merlin Instrument Co., US Patent #4,954,149.

Trademarks

Carbowax - Union Carbide
Carboxen, SPB, StableFlex & Thermogreen - Sigma-Aldrich Co.
Microseal - Merlin Instrument Company
Teflon - E.I. duPont de Nemours & Co., Inc.

www.sigma-aldrich.com/supelco

Order/Customer Service 800-247-6628, 800-325-3010 ● Fax 800-325-5052 ● E-mail supelco@sial.com
Technical Service 800-359-3041, 814-359-3041 ● Fax 800-359-3044, 814-359-5468 ● E-mail techservice@sial.com
SUPELCO ● Supelco Park, Bellefonte, PA 16823-0048 ● 814-359-3441

ISO 9001 REGISTERED

We are committed to the success of our Customers, Employees and Shareholders through leadership in ***Life Science****,* ***High Technology*** *and* ***Service****.*

The SIGMA-ALDRICH Family SIGMA ALDRICH Fluka Riedel-de Haën SUPELCO

T101929
EDW