SPME for GC Analysis

Getting Started with
Solid Phase Microextraction
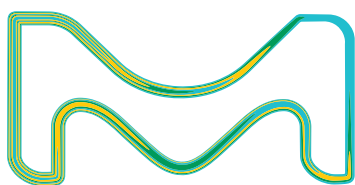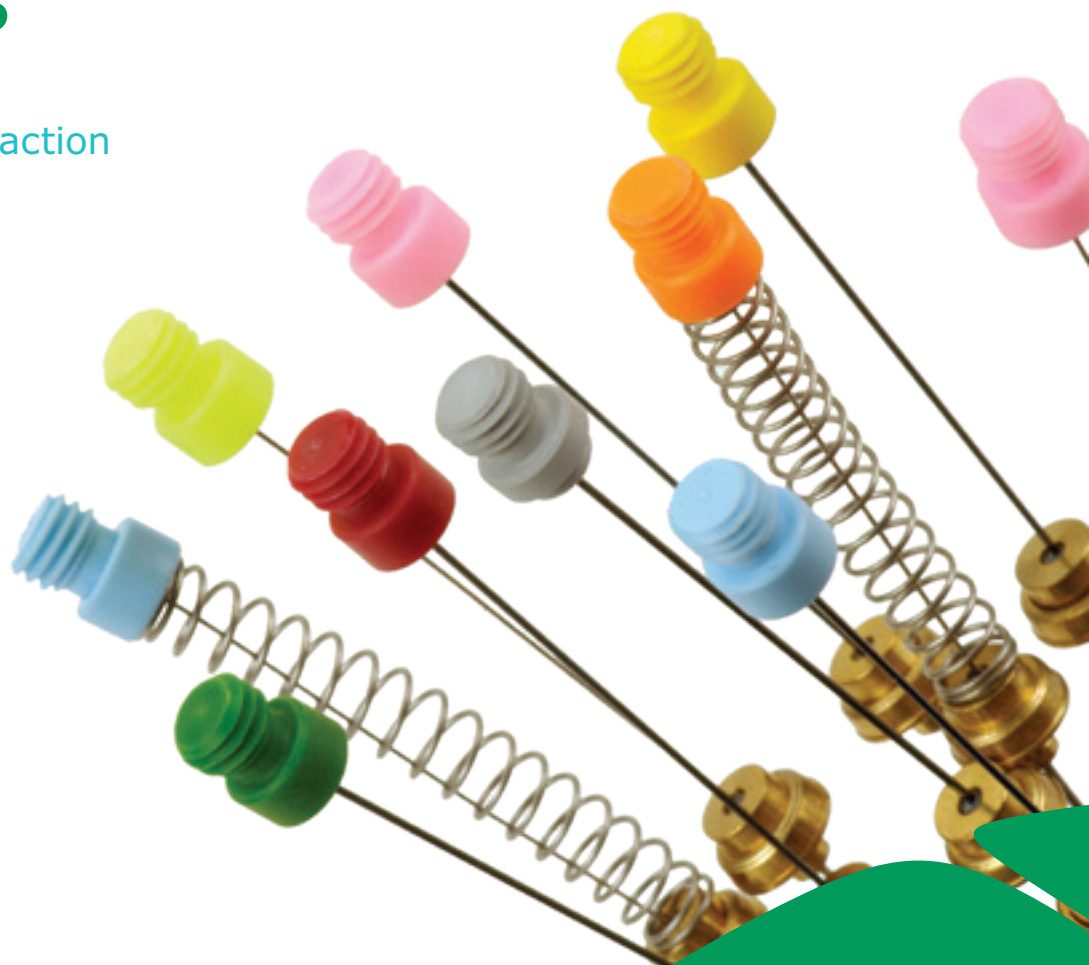
Supelco ®

Analytical Products

SAMPLE PREP MADE EASY

Solid Phase Microextraction (SPME)

Solid Phase Microextraction (SPME) is an innovative, solvent-free sample prep technology that is fast, economical, and versatile. SPME uses a fiber coated with an extraction phase comprising a pure polymer (liquid coating) or adsorptive particles embedded in a polymer (adsorbent/particle coatings). The fiber coating extracts the compounds from the sample. The SPME fiber is then inserted directly into the GC injector for thermal desorption and analysis. SPME has gained widespread acceptance as a preferred technique for many applications in the areas of food & beverage, flavors and fragrances, forensics and toxicology, and environmental volatiles (VOC) testing, to name a few.

During the SPME process, equilibria are established for the analytes between the sample, or the headspace above the sample, and the fiber. The analytes on the fiber are then directly thermally desorbed in the GC injector and transferred to the GC column. SPME concentrates analytes on the fiber, and the desorption step delivers them rapidly to the column. Thus, detection limits are minimized. SPME is compatible with analyte separation/detection by gas chromatography or HPLC, and provides quantitative results for a wide range and concentrations of analytes.

- Solvent-free extraction technique for nearly any sample or matrix
- Easy to automate
- Compatible with almost every GC instrument
- Reusable
- Inexpensive
- Fast
- Alternative to static head-space gas chromatography (GC) and solid phase extraction (SPE)
- Directly interfaced with GC analysis with desorption directly into the GC injection port.
- Non-destructive to sample

SAMPLING AND SAMPLE PREPARATION IN ONE STEP ON A SMALL FIBER

How To Get Started with SPME

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SPME in Official Methods

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Acknowledgments

Optimization of SPME Extraction Conditions

Fiber Coating Selection

Types of SPME Fiber Coatings

There are two types of SPME coatings: polymeric films for absorption of analytes, and particles embedded in polymeric films for adsorption of analytes (Table 1). The absorbent type fibers include those coated with polydimethylsiloxane (PDMS), polyacrylate (PA), and polyethylene glycol (PEG).

The adsorbent type fibers contain porous particles such as divinylbenzene (DVB), Carboxen® (CAR), or a combination of both. Typically, PDMS is used as the binder.

Typically the adsorption mechanism on a particle is a more efficient extraction mechanism, making the particle fibers more suitable for trace analysis methods at lower concentrations. However, as they have a finite surface on the particle, the linear range is typically smaller than the one from the film fibers.

When selecting the appropriate SPME fiber, consider the physical and chemical properties of the compounds being analyzed. **Figure 1** illustrates fiber coating selection based on the molecular weight as an indicator for the volatility of the analytes. **Table 2** provides more specific guidance for fiber selection based on analyte type.

In the case of the adsorptive fiber types, the Carboxen®/PDMS fiber works well for low molecular weight, highly volatile compounds. Regarding the PDMS-DVB fibers, the more macroporous DVB makes them better suited for higher MW compounds than totally microporous Carboxen®, providing efficient extraction and desorption properties. The DVB-Carboxen® fibers cover both areas and expand the MW range that can be anylzed with the same fiber; however, they have slightly lower capacity for lighter or heavier compounds in comparison to the single sorbent fibers.

In the case of the absorptive PDMS fiber, the thinner film is better suited for higher MW analytes than the 100 μm PDMS fiber. Heavier analytes will be easier to desorb off of the 7 μm compared to the 100 μm fiber.

Films – Absorption:

Coating	Polarity
7 μm Polydi methylsiloxane (PDMS)	Nonpolar
30 μm PDMS	Nonpolar
100 μm PDMS	Nonpolar
85 μm Polyacrylate (PA)	Polar
60 μm CARBOWAX™-Polyethylene Glycol (PEG)	Polar

Particles – Absorption:

Coating	Polarity
85 μm Carboxen®-PDMS	Bipolar
65 μm PDMS-DVB	Bipolar
55 μm/30 μm DVB/Carboxen®-PDMS	Bipolar

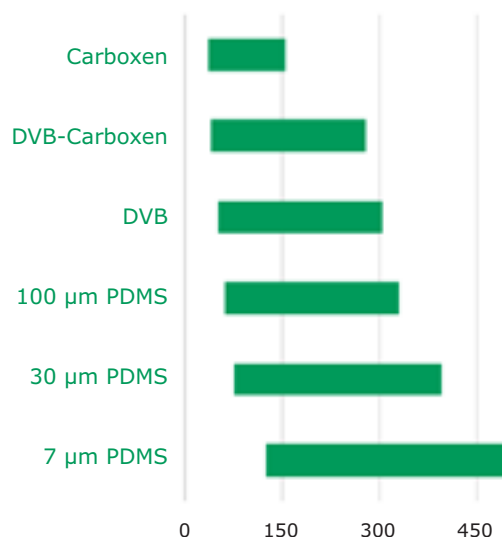*Table 1. SPME Fiber Coating Types and Polarities*

Figure 1. Analyte Molecular Weight Range (g/mol) for SPME Fibers

Optimization of SPME Extraction Conditions

Fiber Coating Selection

Analyte Typ	Molekular Weight Range (g / moll)	Recommanded Fiber
Gases and Low Molecular Weights Compound	30 - 225	75 µm/85 µm Carboxen®/polydimethylsiloxane
Volatiles	60 - 275	100 µm polydimethylsiloxane
Volatiles, Amines and Nitro-aromatic Compounds	50 - 300	65 µm polydimethylsiloxane/divinylbenzene
Polar Semi-volatiles	80 - 300	85 µm polyacrylate
Non-polar High Molecular Weight Compounds	125 - 600	7 µm polydimethylsiloxane
Non-polar Semi-Volatiles	80 - 500	30 µm polydimethylsiloxane
Alcohols and Polar Compounds	40 - 275	60 µm CARBOWAX™ (PEG)
Flavor Compounds: Volatiles and Semivolatiles, C3 - C20	40 - 275	50/30 µm divinylbenzene/Carboxen® on polydimethylsiloxane on a StableFlex® fiber
Trace Compound Analysis (ppb)	40 - 275	50/30 µm divinylbenzene/Carboxen® on polydimethylsiloxane on a 2 cm StableFlex® fiber
Amines and Polar Compounds (HPLC use only)		60 µm polydimethylsiloxane/divinylbenzene

Table 2. Fiber Coating Selection based on Analyte Properties

Fiber Core Type

There are currently three fiber core types: fused silica, StableFlex™ (SF), and metal alloy. Fused silica is the original support for SPME, and numerous methods have been developed on these types of fibers. Fused silica has the advantage of being highly inert, however, the major disadvantage of fused silica is that it is fragile. Currently, adsorbent type fibers are now available on a StableFlex™ (SF) core in addition to the standard fused silica core. A thin coat of polymer on the fused silica makes the SF core more flexible/rugged than the original fused silica because the coating tends to bond more tightly to the SF core. This creates a more stable and durable fiber phase. In addition, due to automated fiber production, SF fibers tend to give better reproducibility than the original fused silica versions.

 Fibers with metal alloy in needle, plunger, and fiber core are now available that are even more flexible than the SF fibers. The metal alloy fiber is made out of Nickel titanium and does not contain iron, which makes the core very inert, even more inert than stainless steel. The new metal alloy design includes a thicker, flexible plunger that is much less likely to kink or break, and helps to reinforce the needle especially when used in an auto-sampler with a sample agitator. A bevel has been placed on the needle to help it pierce septa materials more easily. Due to the thicker outer diameter of the needle, these highly inert, durable fibers are best used with Merlin Microseal™ or similar septum-less injection systems.

Fiber Assembly Configuration

There are fiber assemblies designed for use with the manual holder as well as fiber assemblies designed for use with automation. The manual fiber assemblies contain a spring; while those designed for automation do not contain a spring (Figure 2). Assemblies designed for automation (without a spring) may also be used in a manual holder. There are two needle gauge sizes as well: 23 Ga and 24 Ga. The 23 Ga were designed to be used for septum free injectior ports to ensure a proper seal during desorption in the GC.

Figure 2. Manual Fiber Assembly (Left) and Automated Fiber Assembly (Right)

Fiber Installation

To begin to using SPME, attach the fiber assembly to the SPME holder. The steps below describe the process for inserting a fiber to a manual holder.

1. Unscrew the black cylinder-like depth gauge from the holder **(A)**.

2. Unscrew the threaded end-cap **(B)** on the end of the holder.

3. Push the black plunger **(C)** forward through theZ-slot on the base of the holder to expose the end of the plunger. Note internal threads inside of the plunger **(D)** will accept the threaded fiber assembly **(E)**.

4. Screw the fiber assembly into the end of the plunger.

5. Retract the plunger by pulling it back through the Z-slot and slide the threaded end-cap over the needle. Screw the threaded end-cap tightly onto the end of holder.

6. Screw the black depth gauge onto the end of the holder over the threaded end-cap.

7. Test the holder/fiber by pushing the plunger forward until the fiber is exposed from the protective needle. Stop at the Z-slot **(F)** to hold the fiber in the exposed position during sampling and injection in the GC.

8. To retract the fiber, move the plunger out of the Z-slot and draw it back.

The fiber assembly is attached the same way to the autosampler holder. Remove hexagonal nut and push black plunger down to expose threaded port for fiber assembly attachment. No spring is used with the autosampler fiber assemblies because the autosampler controls the movement of the plunger and fiber. Autosampler fiber assemblies can be used with manual holders, but the manual fiber assemblies cannot be used with the autosampler holders because of the spring. To see a video of fiber installation, visit is at SigmaAldrich.com/spme-videos and select "Fiber Installation."
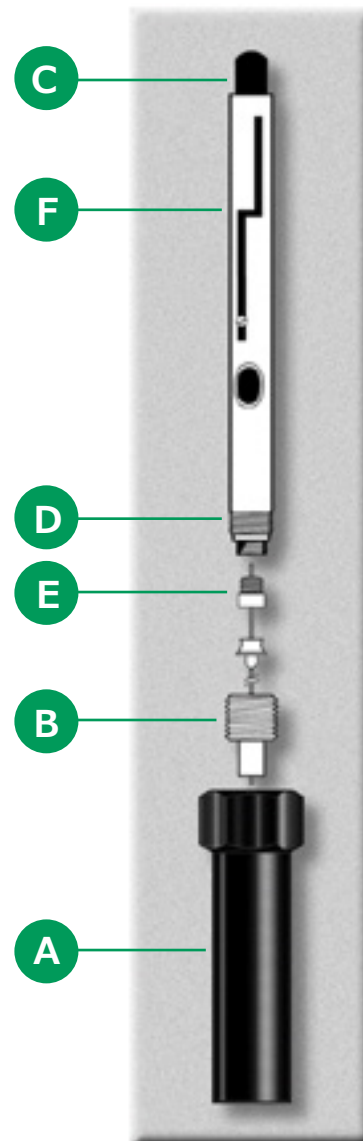

Figure 3. Diagram Illustrating Attachment of the SPME Fiber Assembly to the Holder

Optimization of SPME Extraction Conditions

Precondition the Fiber

It is essential to precondition the SPME fiber before use. In Gas Chromatography (GC), this is performed thermally by exposing the fiber in the GC injection port (alternatively in a conditioning station of the autosampler). Follow the conditioning guidelines in Table 3 to thermally clean the SPME fibers. When conditioning, be sure to open the splitter to reduce the amount of impurities entering the column. Always ramp the oven temperature after fiber conditioning to remove any contaminants that may have entered the column.

To reduce septa inlet coring we strongly recommend using molded LB-2 septa with injection hole. Also, make sure that the injection port contains an appropriate liner. It is strongly recommend that a liner designed specifically for SPME with a narrow I.D. (0.75 mm - 1.0 mm), and compatible with the particular GC instrument be used. This will reduce band broadening and sharpen analyte peaks. Refer to the SPME Product Offering section for recommended inlet liners for various instruments. Do not insert an SPME fiber into a liner containing glass wool. If the fiber contacts the wool, the fiber's coating could be damaged or the fiber can break.

Follow the conditioning guidelines in Table 3 to thermally clean the SPME fibers before use. Insert the fiber into the injection port at the appropriate needle depth by adjusting the black needle guide so that the top is between 3.5 to 4 on the vernier gauge on the holder (suitable for most instruments). If a fiber becomes contaminated after use, these steps can be repeated. If the contamination is severe, the fibers can be thermally cleaned for an extended period of time at a temperature 20 °C below the listed conditioning temperature in Table 3. If this does not clean the fibers, solvent cleaning can be attempted. Please follow the guidelines for solvent cleaning of specific fiber coatings in the troubleshooting section. A conditioned fiber should be stored clean (e.g. in a empty closed vial with clean nitrogen) to prevent un necessary contamination.

Fiber Coating	Film Thickness	Conditioning Temperature (°C)	Conditioning Time (Hrs)
PDMS	100 μm	250	0.5
PDMS	30 μm	250	0.5
PDMS	7 μm	320	1
PDMS/DVB+OC	65 μm	250	0.5
Polyacrylate	85 μm	280	0.5
Carboxen/PDMS	all	300	0.5
PEG	60 μm	240	0.5
DVB/CAR/PDMS	50/30 μm	270	0.5

Table 3. Temperature and Conditioning Guidelines for SPME Fiber Coatings

Extraction Mode

When choosing the extraction mode, the physical and chemical properties of the and the composition of the matrix must be considered. Headspace (HS) and direct immersion (DI) are the two extraction modes for SPME. If the analytes of interest are reasonably volatile, HS is the best choice. HS is the cleaner of the extraction modes because the fiber does not come in contact with the matrix or non-volatile components. It is exposed to the air surrounding the matrix. HS extraction mode is useful when sampling from solid or very dirty matrices. Often matrix modifications must be performed to help drive analytes into the headspace (see Matrix Modification sections).

For compounds having low to medium volatility and high to medium polarity, DI is the preferred extraction mode. When performing DI, the fiber is submerged directly into the matrix. In most cases, the extraction efficiency is much higher in DI as compared to HS. Please refer to Table 4 to determine the right mode for the application of interest.

When performing DI in very dirty or complex matrices, a fiber that is more physically robust and less prone to fouling is preferred. The overcoated SPME fiber (SPME-OC), also known as the matrix-compatible PDMS/DVB/PDMS fiber, has an extra outer layer of PDMS to make it compatible with complex matrices. For more information on this fiber, please visit SigmaAldrich.com/spme-ocf.

Extraction Mode Selection	Direct Immersion (DI)	Headspace (HS)
Analyte Properties	Low-to-medium volatility high-to-medium polarity	High-to-medium volatility low-to-medium volatility
Sample Matrix	Simple or complex liquid	Simple and complex liquid; solid
Advantages	Higher extraction efficiency	Very helpful with dirty and complex matrices, long fiber lifetime
Disadvantages	Fouling, shorter fiber lifetime, sample pretreatment	Sample modification needed to improve mass transfer in HS

Table 4. Choosing an Extraction Mode Based on Application

Agitation Method

Agitation of the sample facilitates the mass transport between the sample and the fiber coating (improving the kinetics), and, therefore, may provide shorter extraction times as well as greater sensitivity in pre-equilibrium extractions (see Extraction Time section). There are a variety of agitation methods, each having advantages and disadvantages. For reproducible results, it is essential to maintain the same agitation method and agitation intensity. Please refer to **Figure 4** to choose the one best suited for the application.
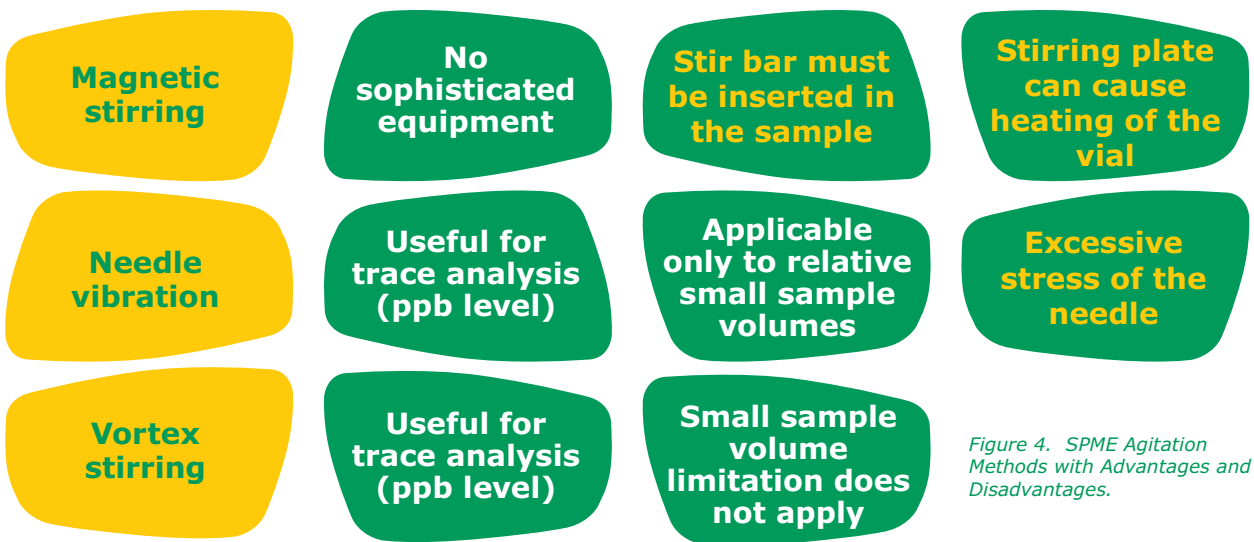
Magnetic stirring	No sophisticated equipment	Stir bar must be inserted in the sample	Stirring plate can cause heating of the vial
Needle vibration	Useful for trace analysis (ppb level)	Applicable only to relative small sample volumes	Excessive stress of the needle
Vortex stirring	Useful for trace analysis (ppb level)	Small sample volume limitation does not apply	

Figure 4. SPME Agitation Methods with Advantages and Disadvantages.

AgitaExtraction Time

The extraction time is a critical parameter in the SPME sampling process. **Figure 5** shows the typical relationship of extraction time to analyte absorbed on the fiber.

The optimum extraction time depends on the objective(s) of the analysis. If the main goal is high-throughput, choose the shortest extraction time possible, and work under pre-equilibrium conditions. In this case, it is imperative to keep the extraction time and agitation exactly the same for each sampling. If the time that the fiber is exposed during sampling varies, the concentration of the analyte on the fiber will also vary, resulting in poor reproducibility. Therefore, when working under pre-equilibrium conditions, it is highly recommended to utilize SPME automation in order to attain good reproducibility.

If sensitivity is the most important factor, make sure to operate at equilibrium conditions. Use an extraction time after which the uptake of analyte onto the fiber remains constant. Furthermore, the equilibrium can be shifted to provide higher extraction efficiencies by variation of temperature and sample modification (ionic strength and pH).

If reproducibility is the main objective, operate at or very close to equilibrium conditions (if performing the extraction manually), or in pre-equilibrium conditions using an autosampler.

Pre-Equilibrium

Analyte Absorbed

Time Control Not As Critical. Small change in time results in small or no change in analyte absorbed.

Time Control Is Critical. Small change in time results in large change in analyte absorbed.

Equilibrium Reached
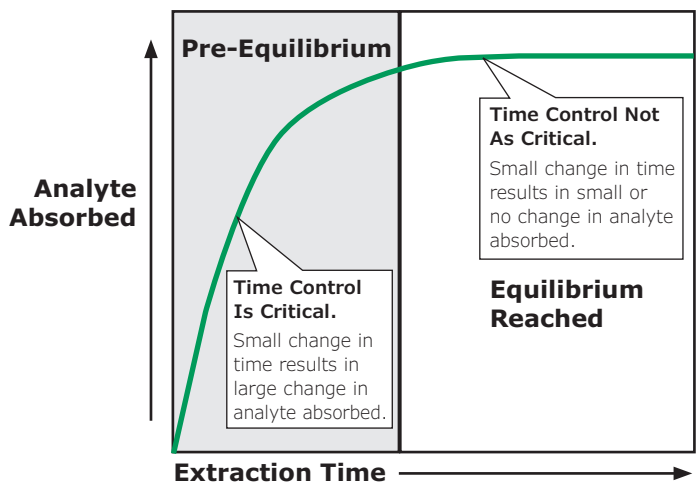
Extraction Time

Figure 5. Effect of Extraction Time on Amount of Analyte Absorbed.

Optimization of SPME Extraction Conditions

Sample Volume

Figure 6 shows the equation governing the equilibrium between analytes extracted by SPME and the initial concentration of analytes in the sample. This distribution is dependent on different parameters: the partition coefficient between the coating and the sample, the volume of the sample, and the volume of the coating. In the case of large sample volumes (greater than 10 mL), the amount of analyte extracted is not influenced by sample volume changes anymore. Therefore, the amount of analyte extracted increases with the sample size up to a point, after which the sensitivity does not increase with further increases in sample volume. An important aspect of SPME is that it allows for field analysis and in vivo analysis without having to know the precise sample volume.

When selecting an appropriate sample volume, take into account the following:

- Sample availability

- System compatibility, in the case of automation (e.g. vial size, extraction conditions, etc.)

- Sufficient volume to cover the coating reproducibility for direct immersion extraction

- Sufficient headspace to avoid fiber spilling (in the case of headspace extraction)

$$n_e = \frac{K_{es}\, V_e\, V_s\, C_o}{K_{es}\, V_e + V_s} \quad \text{If} \quad V_s \gg K_{es}\, V_e$$

$$\text{then}\ V_s / (K_{es}\, V_e + V_s) = 1$$

1. **field & in vivo;**
2. **analytes with small K_{es}**

$$V_s \gg K_{es}\, V_e \quad \longrightarrow \quad n_e = K_{es}\, V_e\, C_o$$

n_e = number of moles of analyte extracted at equilibrium

V_e = fiber coating volume

V_s = sample volume

C_o = initial concentration of a target analyte

K_{es} = distribution constant

Figure 6. Correlation Between the Amount of Analytes Extracted by SPME and Initial Concentration of Analytes in the Sample

Sample Matrix Modification

pH

Sensitivity of SPME is maximum when extracting neutral or undissociated analytes. Thus, pH modification can improve the method's sensitivity for basic or acidic compounds. When dealing with acidic compounds, select a pH that is less than the pKa of the compound (minus two units or less). When dealing with basic compounds, select a pH that is greater than the pka of the compound (plus two units or more).

When performing headspace SPME (HS-SPME) the matrix can be adjusted to any pH value without damaging the fiber. However, if performing direct immersion SPME (DI-SPME) care must be taken when adjusting the pH. Very low and very high pH levels may damage the fiber coating. **Table 5** illustrates the recommended pH range of operation for the respective SPME fibers when performing DI-SPME.

Fiber Coating	Film Thickness	pH
PDMS	100 µm	2 to 10
PDMS	30 µm	2 to 11
PDMS	7 µm	2 to 11
PDMS/DVB+OC	65 µm	2 to 11
Polyacrylate	85 µm	2 to 11
Carboxen/PDMS	all	2 to 11
PEG	60 µm	2 to 9
DVB/CAR/PDMS	50/30 µm	2 to 11

Table 5. Recommended pH Range of Operation for the Respective SPME Fibers when Performing Direct Immersion SPME (DI-SPME).

Ionic Strength

In certain applications, the addition of salt to an aqueous solution minimizes the variability in ionic strength in the sample, helping to normalize the results obtained. Increasing the ionic strength of a sample induces an effect referred to as the salting out effect. This is mostly beneficial for compounds with log P < 3. It is recommended to saturate with salt to ensure same ionic strength from sample to sample, ensuring reproducibility.

Adding salt to the sample matrix may:

- Improve sensitivity in most applications by driving analytes toward the fiber

- Promote the mass transfer of analytes to the headspace, in the case of headspace analysis

- Improve reproducibility for samples containing salt.

Although sodium chloride (NaCl) is the most commonly used salt to adjust ionic strength, it is wise to explore the use of other salts. Other salts may have different abilities to salt out analytes, especially when dealing with complex matrices such as food.

Cautions to take when adding salt to the sample:

- Increasing the ionic strength may decrease solubility of some analytes.

- For some analytes, the addition of salt may also decrease the amount of analyte extracted; something to be especially aware of with analytes that are very polar.

- While salt may improve the SPME extraction of the desired analytes, it could also cause co-extraction of more matrix interferences or undesired compounds.

Sample Dilution

For complex matrices, sample dilution may prove to be beneficial. Sample dilution can help to release the analytes from the matrix, increasing analyte extraction. Dilution will also help to minimize fouling of the coating and provide a longer fiber lifetime when performing extractions by direct immersion. In addition, dilution enhances the amount of analyte available for extraction by the fiber by helping to free it from the matrix. It also helps to avoid saturation of adsorptive coatings (especially for complex matrices). However, one must be careful to avoid excessive dilution as it will essentially increase the detection limit by diluting the amount of analyte in the sample, leading to less sensitivity.

Sample Temperature

There are a number of factors to keep in mind when deciding upon the best temperature to perform an SPME extraction. Increasing the extraction temperature has a positive effect on the overall extraction kinetics by increasing the analyte diffusion coefficient, the headspace capacity, and the extraction rate and speed (decreasing the time at which equilibrium is reached). Conversely, increasing the temperature decreases the partition coefficients as well as the amount of analyte extracted at equilibrium when working with absorptive fibers. Summarizing, one must consider the priorities of the experiment when selecting an appropriate temperature.

Sample Matrix Modification

Organic Solvent Content

When adding spiking solutions in organic solvent to samples prior to SPME, keep the amount of organic solvent added to samples to a minimum; less than 5% v/v is recommended. The ideal value is 1% v/v. Also, try to keep the amount of organic solvent added to samples and standards constant (within 0.5% RSD), as wide variations can negatively affect reproducibility. If alcoholic beverages are investigated, a dilution to a lower alcohol content is advisable to achieve better extraction results.

Analyte Derivatization

Because derivatization reagents can bring additional sources of interference and errors into the system, derivatization should only be carried out when strictly necessary.

Analytes may be derivatized to:

- Enhance extraction efficiency
- Enhance detection sensitivity
- Make compounds more amenable to a particular mode of analysis or detection

Different options include derivatization of analytes prior to the extraction, during the extraction, or post-extraction. Derivatization prior to or during the extraction can affect extraction amounts, chromatographic behavior, and detection. Post-extraction derivatization can affect only chromatographic behavior and detection.

Desorption Condition Optimization

Desorption In GC Analysis

When desorbing analytes into the GC instrument, factors to take into consideration include carrier gas flow rate, desorption temperature, and desorption time. A high carrier gas flow rate, as well as the use of narrow bore liners in splitless mode during desorption is recommended for the best results. Please see the SPME Product Offering section for recommended inlet liners.

Regarding the desorption temperature, **Table 6** lists the recommended desorption coating for each fiber coating type. Typically particle fibers should be desorbed at high temperatures to ensure immediate release of the volatile analytes. Determine the optimal desorption time by performing a desorption time profile, making sure to quantitate desorption. Also, make sure to test carry over by performing a consecutive desorption of the coating and monitor for any targeted peak detected.

Typically with SPME the initial oven temperature of the GC is low (< 50°C) to allow a refocussing of the semi and less volatile analytes at the column entrance (hold for min. 1.5 min). For more volatile compounds thicker GC columns films (> 1 μm) are recommended to ensure good peak shapes.

Fiber Coating	Film Thickness	Recommanded Desorption Temperature (°C)
PDMS	100 μm	200 to 280
PDMS	30 μm	200 to 280
PDMS	7 μm	220 to 320
PDMS/DVB+OC	65 μm	200 to 270
Polyacrylate	85 μm	220 to 280
Carboxen/PDMS	all	250 to 310
PEG	60 μm	200 to 250
DVB/CAR/PDMS	50/30 μm	230 to 270

Table 6. Recommended Desorption Temperature SPME Fiber Coatings.

Advances in SPME

Overcoated SPME (Matrix Compatible PDMS/DVB/PDMS SPME)

Complex matrices, such as many foods, pose a challenge to direct immersion SPME (DI-SPME) due to the presence of fats, sugars, pigments, and other matrix-specific compounds. These compounds can stick to the SPME fiber, in particular to particle fibers, and reduce its usable life or be transferred to the GC where they may interfere with chromatographic analysis.

A new development in SPME fiber technology allows DI-SPME in complex matrix samples. Overcoated SPME, also known as matrix compatible PDMS/DVB/PDMS SPME, incorporates an additional protective PDMS overcoating on the fiber.

The PDMS overcoating seals the tip of the fiber, preventing wicking of matrix components and analytes into the fiber core. It also seals the connection of the fiber to the assembly, preventing fiber detachment due to corrosion by the sample. As a result, the overcoated fiber is physically more robust, and typically lasts two to three times longer than the conventional SPME equivalent.

The overcoating repels unwanted matrix components and allows for an efficient and quick wash step before analyte desorption into the GC. Washing or wiping the matrix components off of the fiber gives fewer interferences and decreases system fouling.

Overcoating also reduces competition between the analytes and matrix components for adsorption sites on the fiber's particle surface, resulting in increased analyte extraction and improved sensitivity.

Figure 7. Overcoated SPME Fiber Assembly (PDMS/DVB/PDMS)

SPME Portable Field Sampler

The SPME portable field sampler providesan efficient and economical way of extracting and transporting volatile and semivolatile compounds from the field. Extracted compounds are safely sealed behind a replaceable septum. The sampler can be reused up to 50-100 times (depending on the sample matrix), and is disposed of when the fiber is no longer usable. The portable field sampler also efficiently collects organic compounds from air. Four fiber phases are available: a polydimethylsiloxane (PDMS)/Carboxen fiber for trace levels of volatiles, a general purpose PDMS fiber and, a PDMS/DVB fiber for semi-volatiles and larger volatiles.
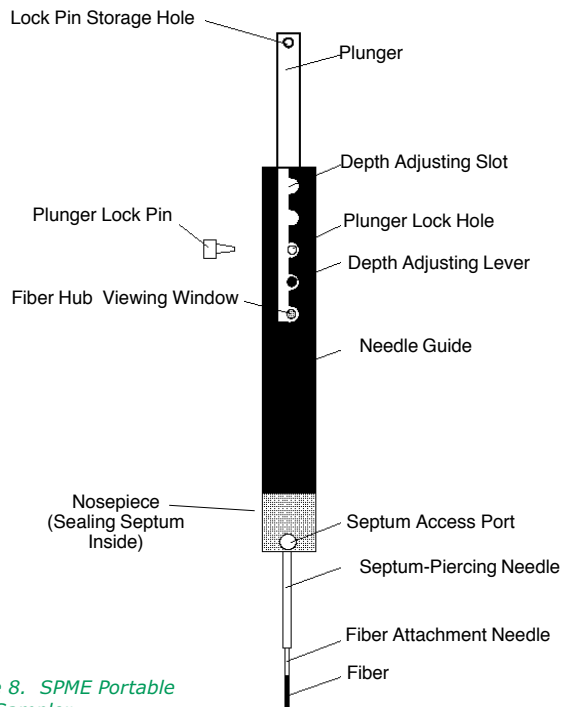

Figure 8. SPME Portable Field Sampler

Advances in SPME

BioSPME

Although BioSPME is used prior to LC or direct MS analysis, it is an advance in SPME of which all SPME users should be aware. SPME-GC users aware of this option too. BioSPME offers an innovative approach to bioanalytical microsampling and sample preparation in one step. It enables the direct measurement of free analyte fraction. This technology allows for the selective extraction of a broad range of analytes from biological samples, and the quick and easy procedure results in clean samples ready for LC/MS or direct MS (DART/DESI) analysis. The C18 and PDMS/DVB-coated fibers and tips are available for a wide range of applications for both in vitro and in vivo extractions. These products are listed in the SPME Product Offering section under the heading BioSPME Fibers and Probes for HPLC and Direct MS Analysis.

Figure 9. BioSPME LC 96-well Tips

Figure 10. BioSPME LC Needle Probes for in vivo

SPME Troubleshooting

The following section gives some helpful troubleshooting suggestions to combat factors that may prove to be problematic in SPME extractions. Review these suggestions before performing method development work.

General Precautions for Using SPME Fibers

1. Keep in mind that there are a number of factors that may affect reproducibility, including:
   - Variable content of organic solvent in spiked samples
   - Variable vial shape and headspace volumes
   - Inconsistent agitation speed during extraction
   - Heating of the stirring plate when using magnetic stirrers
   - Incorrect or irreproducible sampling depth inside a vial

2. Help to maintain reproducibility by:
   - Using an internal standard for extraction (add the internal standard to sample before extraction)
   - Keeping the organic solvent content the same for all samples when optimizing the method and constructing a calibration curve
   - Using the same type of vial for all samples in a batch and always piercing the vial in the same position (use recommended SPME vials)
   - Keeping sample agitation constant during extraction of one sample or multiple samples in a batch
   - Isolating the vial from the stirring plate by placing a septum between a vial and stirrer surface
   - Keeping the fiber coating sampling depth in the vial constant for all samples
   - Avoiding splashing of the coating in HS-SPME during agitation
   - Immersing the coating entirely in the sample for DI-SPME
   - Using an internal standard for extraction (add the internal standard to sample before extraction)
   - Avoid sample heat from other ambient sources

3. Do not soak any SPME fiber in chlorinated solvents.

4. All SPME fiber coatings are bonded; however, bonded fibers will still swell in certain solvents.

Polar fibers such as polyacrylate will swell more in the presence of polar solvents and, likewise, nonpolar fibers such as PDMS in the presence of nonpolar solvents. In some cases, the swelling can be enough that the coating will strip off when the fiber is retracted back into the needle. The swelling may occur in both headspace and immersion modes. In some samples the organic compounds can be concentrated in the headspace and swell the fiber even more than if the fiber was immersed. It is important to determine compatibility of samples with the fiber coatings.

If a fiber becomes contaminated after use, repeat the cleaning procedure listed in the "Preconditioning "section. If the contamination is severe, the fibers can be thermally cleaned for an extended period of time at a temperature 20 °C below the listed conditioning temperature in Table 3. If this does not clean the fibers, solvent cleaning can be attempted. Please follow the guidelines for solvent cleaning of specific fiber coatings in the troubleshooting section entitled "Fiber Specific Precautions , Solvent Cleaning, and Compatibility."

5. To prevent fiber breakage when autosamplers are used:
   - Check often for proper alignment of the fiber needle with the injector of the GC-MS, vial trays, and vials. Improper alignment is one of the main causes of fiber breakage.
   - Avoid stress of the needle by keeping agitation speed into the agitator/heater module of the autosampler below 600 rpm. Higher speeds can be used if the extraction time is short (< 10 min).
   - Be sure that the tension chords contained in some autosamplers are at the proper tension and functioning properly.
   - It is highly recommended to use screw capped vials with 1.3 or 1.5 mm thick silicone septa.
   - Make sure that the insertion depths into the injection port and vials are properly set.
   - It is recommended to use assemblies with 23 gauge needles for increased strength and less bending

Blank Analysis

After conditioning the SPME fiber, it is advisable to run a fiber blank to gauge the amount of background. Prior to running a fiber blank analysis, be sure that the GC column has been thermally conditioned to the desired upper temperature of the method.

1. Create an appropriate GC method for SPME analysis of the samples. This same program will be used to run a blank analysis. The starting temperature of the oven program should be low enough to help focus the analytes onto the column (generally a temperature of < 50 °C works in most cases). After 1.5 - 5 minutes at the starting temperature the column oven temperature can be ramped at a rate or multiple rates necessary to achieve the desired separation and elution of analytes. For splitless injections (which are commonly used in SPME), set the vent to open after 1 - 3 minutes.

2. Insert the SPME fiber into the injection port at the appropriate depth and start the GC.

3. Run the GC program until completed.

4. There are typicallysome extraneous peaks in the initial runs.

5. Repeat the step again to see if there is a reduction in the size and number of peaks.

6. Depending upon the sensitivity of the instrument, the peaks may be extremely low in intensity. It is important to run a sample to determine if the peaks are too large.

7. Please note the fibers may introduce oxygen and water into the GC which may produce extraneous peaks.

8. Contact technical service to obtain assistance if the peaks are too large.

9. Also consider running a vial blank. This would involve performing SPME on a capped, empty. Use the same SPME parameters as for the samples. This will help to gauge the level of background coming from the septa used on the sample vials.

10. Store conditioned fibers clean (not in the box) by flushing a vial with clean nitrogen and close with a septum cap. "Inject" the fiber assmebly into the vial without exposing the fiber. The opening of the fiber will only be exposed to clean nitrogen. If this is not possible, a small cap, of any inert material e.g. Teflon™ can be inserted at the tip of the fiber assembly to protect the inner SPME coating. Before using a stored fiber, perform a blank run to recondition and check for cleanliness. If the fiber assembly has not been used for several hours, it is best to desorb the fiber with the split vent open for several minutes prior to extraction

SPME Troubleshooting

Fiber Specific Precautions , Solvent Cleaning, and Compatibility

PDMS (Polydimethylsiloxane) Absorbent Fiber Coatings

- For solvent cleaning, PDMS fibers can be immersed in water-soluble organic solvents such as methanol, acetonitrile, acetone or ethanol, especially if it is a mixture of water with the organic solvent. The addition of water helps to reduce swelling. Usually 15 - 30 min is sufficient to clean the fibers.

- Keep in mind that PDMS fibers may swell when exposed to silylating reagents.

- Do not place PDMS fibers in non-polar solvents or samples containing high levels of non-polar solvents such as hexane, methylene chloride and diethyl ether.

- Heated headspace extraction of samples with high concentration (>100 ppm) of non-polar solvents and terpenes can swell PDMS coatings. The 30 µm PDMS is less likely to be stripped than the 100 µm PDMS when the fiber is retracted. Consider this fiber as an option when evaluating such samples.

PEG (Polyethylene glycol, CARBOWAX™®) Fiber Coatings

- For solvent-cleaning PEG fibers, place the fibers in a 1% methanol:water solution containing a minimum of 15% NaCl for 15 to 30 minutes. It is important to have the salt present when soaking to reduce swelling of the PEG coating.

- PEG fibers can be immersed in hydrocarbon solvents and will not swell.

- It is highly recommended that PEG fibers not be immersed in samples with water-soluble organic concentrations above 1% (total water soluble organic) unless the water sample contains at least 15% NaCl or other salts. The degree of swelling will vary depending upon the solvent(s) in the water. In many cases there will not be sufficient swelling to damage the fiber, but in some cases the fiber coating can be stripped or damaged when the fiber is retracted.

- It is recommend that the PEG fiber should not be exposed to the headspace of samples with a water-soluble organic concentration higher than 2% v/v. The organic analytes will be concentrated in the heated headspace and can swell the phase that can result in stripping when the fiber is retracted into the needle.

- Methanol may be produced when the fiber is exposed to acidic samples. This is due to the presence of an inhibitor in the starting material. Most of the inhibitor has been removed, but several additional extractions in an acidic solution will remove any remaining inhibitor. Solvent cleaning (step 1) is usually sufficient for removal of the inhibitor from the fiber coating.

Polyacrylate Fiber Coatings

- For cleaning, soak the fiber in a water miscible organic solvent for 30 min, followed by immersion in water to reduce any swelling. It is best to place the fiber in water prior to retracting the fiber.

- The polyacrylate fiber can be immersed in aliphatic hydrocarbon solvents (e.g. hexane, heptane) without swelling.

- Polyacrylate coating may darken with use. This is not unusual and does not affect fiber performance unless the coating becomes black. This indicates that oxygen is present in the injection port. If the fiber is desorbed at 280°C or lower, the discoloration will be reduced.

Adsorbent/Particle Type Fibers

- Carboxen® containing SPME fibers can retain solvents in the micropores, so it is generally not advisable to soak this fiber in solvents. It could take multiple desorption cycles to remove the solvent. Fibers will not swell in water-soluble organic solvents to an appreciable degree. To quicker clean the fiber from strong contamination, one may expose it to the headspace above a solvent (e.g. benzene or toluene), wait for 10-15 min, and then perform repeated thermal desorption.

- For PDMS-DVB fibers, follow the guidelines for PDMS fibers. Methanol is the best option.

Overcoated (OC) SPME Fiber Assemblies

- OC fiber assemblies have a thin layer of PDMS applied over the adsorbent coating. The overcoat provides a barrier to non-volatile matrix components and increases the durability of the adsorbent coating on the fiber.

- To help further lengthen the fiber coating life, the fiber coating should be rinsed after each extraction by dipping the fiber into clean water for 10 - 30 seconds prior to desorption. For very complex matrices, it is recommended to wipe the fiber with clean water. The rinse/wipe step will help remove sugars and other water-soluble, non-volatile components. The length of the rinse time is dependent upon the volatility of the analytes that are being sampled.

- The overcoat changes the extraction properties of the adsorbent coating by slightly reducing fiber affinity/selectivity for polar analytes and slightly increasing equilibrium time. For many analytes the changes in extraction properties between the OC and standard adsorbent coatings are very subtle.

SPME in Official Methods

ISO 27108 Water quality (derived from DIN 38407-F34)
Determination of selected plant treatment agents and biocide products - Method using solid-phase microextraction (SPME) followed by gas chromatography-mass spectrometry (GC-MS)

ISO 17943 (derived from DIN 38407-41)
Determination of selected easily volatile organic compounds in water - Method using gas chromatography (GC-MS) after solid-phase micro extraction (SPME)

OENORM A 1117, 2004-05-01
Determination of volatile compounds in cellulose-based materials by Solid Phase Micro Extraction (SPME)

UNICIM 2237 / 2009 – Italian Method on SPME for air sampling
Determinazione Delle Aldeidi Aerodisperse – Methodo per microestrazione su fase solida (SPME) ed analisi mediante gascromatografia accoppiata a spettrometria di massa (GC-MS)

Determination of air borne aldehydes by SPME/GC-MS using derivatisation on fiber with PFBHA

ASTM D 6438, 2005
Standard Test Method for Acetone, Methyl Acetate, and Parachlorobenzotrifluoride Content of Paints, and Coatings by Solid Phase Microextraction-Gas Chromatography

ASTM D 6520, 2000
Standard Practice for the Solid Phase Micro Extraction (SPME) of Water and its Headspace for the Analysis of Volatile and Semi-Volatile Organic Compounds

ASTM D 6889, 2003
Standard Practice for Fast Screening for Volatile Organic Compounds in Water Using Solid Phase Microextraction (SPME)

ASTM D 7363 - 11
Standard Test Method for Determination of Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water Using SPME and GC/MS in Selected Ion Monitoring Mode

EPA Method 8272 (Dec 2007)
Parent and Alkyl Polycyclic Aromatics in Sediment Pore Water by SPME GC/MS

ASTM E 2154, 2001
Standard Practice for Separation and Concentration of Ignitable Liquid Residues from Fire Debris Samples by Passive Headspace Concentration with SPME

SPME Product Offering and Related Products

SPME Fibers Holders for GC Analysis

Description	Cat. No.
USPME Fiber Holders	
For use with manual sampling	57330-U
For use with Varian Autosampler or HPCL	57331
For use with CTC CombiPAL, Gerstel MPS2 and Thermo TriPlus Autosamplers	57347-U

SPME Fibers Holders for GC Analysis

Fiber Coating and Thickness	Fiber Core/ Assembly Type	Hub Description	Sampling and Analysis Mode			
			Manual Holder (w/spring)		Autosampler	
			23 Ga*	24 Ga*	23 Ga*	24 Ga*
Carboxen®/Polydimethylsiloxane (PDMS)						
75 µm Carboxen/PDMS	Fused Silica/SS	Black/plain	57344-U	57318	57343-U	57319
85 µm Carboxen/PDMS	Metal alloy/Metal alloy**	Lt. Blue/plain	—	—	57906-U	—
85 µm Carboxen/PDMS	Stableflex/SS	Lt. Blue/plain	—	57334-U	57295-U	57335-U
Polydimethylsiloxane (PDMS)						
7 µm PDMS	Fused Silica/SS	Green/plain	—	57302	57291-U	57303
30 µm PDMS	Fused Silica/SS	Yellow/plain	—	57308	57289-U	57309
100 µm PDMS	Metal alloy/Metal alloy** Fused	Red/plain	—	—	57928-U	—
100 µm PDMS	Silica/SS	Red/plain	57342-U	57300-U	57341-U	57301
Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)						
65 µm PDMS/DVB	Metal alloy/Metal alloy**	Pink/plain	—	—	57902-U	—
65 µm PDMS/DVB	Fused Silica/SS	Blue/plain	57346-U	57310-U	57345-U	57311
65 µm PDMS/DVB	StableFlex/SS	Pink/plain	—	57326-U	57293-U	57327-U
65/10 µm PDMS/DVB-OC**	StableFlex/SS	Pink/notched	—	—	57439-U	—
Polyacrylate						
85 µm Polyacrylate	Fused Silica/SS	White/plain	—	57304	57294-U	57305
Divinylbenzene/Carboxen/Polydimethylsiloxane						
50/30 µm DVB/CAR/PDMS	Metal alloy/Metal alloy (1 cm)**	Gray/plain	—	—	57912-U	—
50/30 µm DVB/CAR/PDMS	Metal alloy/Metal alloy (2 cm)**	Gray/notched	—	—	57914-U	—
50/30 µm DVB/CAR/PDMS	StableFlex/SS (1 cm)	Gray/plain	—	57328-U	57298-U	57329-U
50/30 µm DVB/CAR/PDMS	StableFlex/SS (2 cm)	Gray/notched	—	57348-U***	57299-U	—
Polyethylene Glycol (PEG)						
60 µm PEG	Metal alloy/SS	Purple/plain	57355-U	—	57354-U	—
Bare Fused Silica						
Bare Fused Silica	Fused Silica/SS	Orange/plain	—	7316-U	—	—

*Ga – Needle gauge.
**Metal alloy fiber assemblies are provided 1/pk.
***No spring included.
****PDMS overcoated – see back side for description.

SPME Product Offering and Related Products

SPME Fiber Assortment Kits

The SPME fiber assortment kits consist of 1 fiber each of the types listed below:

Descrption		Cat. No.
SPME Fiber Assortment Kit 1: For Volatiles and Semi-volatiles		
85 µm polyacrylate coated fiber	For use with manual holder, needle size 24 ga	57306
100 µm PDMS coated fiber	For use with autosampler, needle size 24 ga	57307
7 µm PDMS coated fiber	For use with autosampler, needle size 23 ga	57285-U
SPME Fiber Assortment Kit 2: For Volatile or Polar Organics in Water		
75 µm Carboxen®/PDMS coated fiber	For use with manual holder, needle size 24 ga	57320-U
65 µm PDMS/DVB coated fiber	For use with autosampler, needle size 24 ga	57321-U
85 µm Polyacrylate coated fiber	For use with autosampler, needle size 23 ga	57286-U
SPME Fiber Assortment Kit 3: For SPME/HPLC Analysis		
60 µm PDMS/DVB coated fiber		
85 µm Polyacrylate coated fiber		
100 µm PDMS coated fiber	For use with autosampler, needle size 24 ga	57323-U
SPME Fiber Assortment Kit 4: For Flavors and Odors		
100 µm PDMS coated fiber	For use with manual holder, needle size 24 ga	57324-U
65 µm PDMS/DVB coated fiber	For use with autosampler, needle size 24 ga	57325-U
75 µm Carboxen®/PDMS coated fiber	For use with autosampler, needle size 23 ga	57287-U
SPME Fiber Assortment Kit 5: For Flavors and Odors		
100 µm PDMS coated fiber		
65 µm PDMS/DVB coated fiber		
85 µm Carboxen®/PDMS coated fiber		
50/30 µm DVB/PDMS coated fiber	For use with autosampler, needle size 23 ga	57362-U
SPME StableFlex™ Fiber Assortment Kit		
65 µm PDMS/DVB coated fiber	For use with manual holder, needle size 24 ga	57550-U
50/30 µm DVB/Carboxen®/PDMS coated fiber	For use with autosampler, needle size 24 ga	57551-U
85 µm Carboxen®/PDMS coated fiber	For use with autosampler, needle size 23 ga	57284-U
85 µm polyacrylate coated fiber		

SPME Portable Field Samplers

Description	Cat. No.
Portable Field Samplers	
SPME Portable Field Sampler coating Polydimethylsiloxane (PDMS)	504823
SPME Portable Field Sampler coating Carboxen/Polydimethylsiloxane (CAR/PDMS)	504831
SPME Portable Field Sampler coating Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)	57359-U
SPME Septum Removing Tool For Portable Field Sampler	504858
Thermogreen® LB-2 Septa, solid discs diam. 5 mm (3/16 in.), pkg of 50 ea	20638

BioSPME Fibers and Probes for HPLC and Direct MS Analysis

Description	Cat. No.
SPME-LC Needle Probes	
functional group C18, pkg of 5 ea	57281-U
SPME-LC Pipette Tips	
functional group C18, 96-tip array	57234-U
mixed mode PDMS/DVB, 96-tip array	57248-U
IonSense® SPE-it Tips	
C18 SPE-it Tips pkg of 96 ea	57264-U
PDMS/DVB SPE-it Tips pkg of 96 ea	57249-U

SPME Compatible GC Inlet Liners

Description	Cat. No.
Agilent®/HP (5880, 5890 Series, 6890) Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2637501
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2637505
Direct (SPME) Type, Straight Design (unpacked) pkg of 25 ea	2637525
PerkinElmer® Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) technique used for SPME, pkg of 5 ea	2631205
Shimadzu™ GC Models 14/15A/16 (SPL-14 Injector) Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2633501
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2633501
Shimadzu™ GC Models 17A (SPL-17 Injector) Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2633901
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2633905
Direct (SPME) Type, Straight Design (unpacked) pkg of 25 ea	2633925
Shimadzu™ GC Models 9A/15A/16 Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2632901
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2632901
Varian® 1075/1077 Injectors Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2635801
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2635805
Direct (SPME) Type, Straight Design (unpacked) pkg of 25 ea	2635825
Varian® 1078/1079 Splitless Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2637801
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2637805
Varian® 1093-94 SPI Injector Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2636401
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2636405
Direct (SPME) Type, Straight Design (unpacked) pkg of 25 ea	2636425

SPME Product Offering and Related Products

SPME Accessories

| Description	Cat. No.
Varian® 1075/1077 Injectors Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2635801
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2635805
Direct (SPME) Type, Straight Design (unpacked) pkg of 25 ea	2635825
Varian® 1078/1079 Splitless Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2637801
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2637805
Varian® 1093-94 SPI Injector Inlet Liner	
Direct (SPME) Type, Straight Design (unpacked) pkg of 1 ea	2636401
Direct (SPME) Type, Straight Design (unpacked) pkg of 5 ea	2636405
Direct (SPME) Type, Straight Design (unpacked) pkg of 25 ea	2636425

SPME Portable Field Samplers

Description	Cat. No.
Portable Field Samplers	
SPME Portable Field Sampler coating Polydimethylsiloxane (PDMS)	504823
SPME Portable Field Sampler coating Carboxen/Polydimethylsiloxane (CAR/PDMS)	504831
SPME Portable Field Sampler coating Polydimethylsiloxane/Divinylbenzene (PDMS/DVB)	57359-U
SPME Septum Removing Tool For Portable Field Sampler	504858
Thermogreen® LB-2 Septa, solid discs diam. 5 mm (3/16 in.), pkg of 50 ea	20638

SPME Accessories

Description	Cat. No.
SPME Sampling Stand	
SPME Sampling Stand for use with 4 mL vials	57333-U
SPME Sampling Stand for use with 15 mL vials	57357-U
SPME sampling stand holder & rod assembly for use with SPME Sampling Stand	57364-U

Acknowledgments
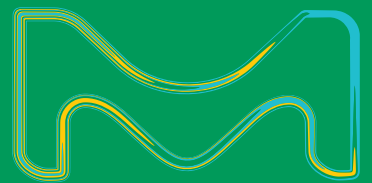
For further information, please contact your local
VWR organization or have a look at the VWR webpages:
vwr.com