Designation: D6520 – 06 (Reapproved 2012)

Standard Practice for the Solid Phase Micro Extraction (SPME) of Water and its Headspace for the Analysis of Volatile and Semi-Volatile Organic Compounds[1]

This standard is issued under the fixed designation D6520; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This practice covers procedures for the extraction of volatile and semi-volatile organic compounds from water and its headspace using solid-phase microextraction (SPME).

1.2 The compounds of interest must have a greater affinity for the SPME-absorbent polymer or adsorbent or combinations of these than the water or headspace phase in which they reside.

1.3 Not all of the analytes that can be determined by SPME are addressed in this practice. The applicability of the absorbent polymer, adsorbent, or combination thereof, to extract the compound(s) of interest must be demonstrated before use.

1.4 This practice provides sample extracts suitable for quantitative or qualitative analysis by gas chromatography (GC) or gas chromatography-mass spectrometry (GC-MS).

1.5 Where used, it is the responsibility of the user to validate the application of SPME to the analysis of interest.

1.6 The values stated in SI units are to be regarded as the standard.

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For specific hazard statements, see Section 10.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D3370 Practices for Sampling Water from Closed Conduits

D3694 Practices for Preparation of Sample Containers and for Preservation of Organic Constituents

D3856 Guide for Management Systems in Laboratories Engaged in Analysis of Water

D4210 Practice for Intralaboratory Quality Control Procedures and a Discussion on Reporting Low-Level Data (Withdrawn 2002)[3]

D4448 Guide for Sampling Ground-Water Monitoring Wells

3. Terminology

3.1 *Definitions*—For definitions of terms used in this practice, refer to Terminology D1129.

4. Summary of Practice

4.1 This practice employs adsorbent/liquid or adsorbent/gas extraction to isolate compounds of interest. An aqueous sample is added to a septum-sealed vial. The aqueous phase or its headspace is then exposed to an adsorbent coated on a fused silica fiber. The fiber is desorbed in the heated injection port of a GC or GC-MS or the injector of an HPLC.

4.2 The desorbed organic analytes may be analyzed using instrumental methods for specific volatile or semi-volatile organic compounds. This practice does not include sample extract clean-up procedures.

5. Significance and Use

5.1 This practice provides a general procedure for the solid-phase microextraction of volatile and semi-volatile organic compounds from an aqueous matrix or its headspace. Solid sorbent extraction is used as the initial step in the extraction of organic constituents for the purpose of quantifying or screening for extractable organic compounds.

5.2 Typical detection limits that can be achieved using SPME techniques with gas chromatography with flame ionization detector (FID), electron capture detector (ECD), or with a

[1] This practice is under the jurisdiction of ASTM Committee D19 on Water and is the direct responsibility of Subcommittee D19.06 on Methods for Analysis for Organic Substances in Water.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] The last approved version of this historical standard is referenced on www.astm.org.

mass spectrometer (MS) range from mg/L to µg/L. The detection limit, linear concentration range, and sensitivity of the test method for a specific organic compound will depend upon the aqueous matrix, the fiber phase, the sample temperature, sample volume, sample mixing, and the determinative technique employed.

5.3 SPME has the advantages of speed, no desorption solvent, simple extraction device, and the use of small amounts of sample.

5.3.1 Extraction devices vary from a manual SPME fiber holder to automated commercial device specifically designed for SPME.

5.3.2 Listed below are examples of organic compounds that can be determined by this practice. This list includes both high and low boiling compounds. The numbers in parentheses refer to references at the end of this standard.

Volatile Organic Compounds (1,2,3)
Pesticides, General (4,5)
Organochlorine Pesticides (6)
Organophosphorous Pesticides (7,8)
Polyaromatic Hydrocarbons (9,10)
Polychlorinated biphenyls (10)
Phenols (11)
Nitrophenols (12)
Amines (13)

5.3.3 SPME may be used to screen water samples prior to purge and trap extraction to determine if dilution is necessary, thereby eliminating the possibility of trap overload.

6. Principles of SPME

6.1 SPME is an equilibrium technique where analytes are not completely extracted from the matrix. With liquid samples, the recovery is dependent on the partitioning or equilibrium of analytes among the three phases present in the sampling vial: the aqueous sample and headspace (Phase 1), the fiber coating and aqueous sample (Phase 2), and the fiber coating and the headspace (Phase 3):

$$(\text{Phase 1})\ K_1 = C_L/C_g \quad (1)$$

$$(\text{Phase 2})\ K_2 = C_F/C_L \quad (2)$$

$$(\text{Phase 3})\ K_3 = C_F/C_G \quad (3)$$

where C_L, C_G and C_F are the concentrations of the analyte in these phases.

6.1.1 Distribution of the analyte among the three phases can be calculated using the following:

$$C_0V_L = C_GV_G + C_LV_L + C_FV_F \quad (4)$$

6.1.2 Concentration of analyte in fiber can be calculated using the following:

$$C_F = C_0V_LK_1K_2/V_G + K_1V_L + K_1K_2V_F \quad (5)$$

7. Interferences

7.1 Reagents, glassware, septa, fiber coatings and other sample processing hardware may yield discrete artifacts or elevated baselines that can cause poor precision and accuracy.

7.1.1 Glassware should be washed with detergent, rinsed with water, and finally rinsed with distilled-in-glass acetone. Air dry or in 103°C oven. Additional cleaning steps may be required when the analysis requires levels of µg/L or below. Once the glassware has been cleaned, it should be used immediately or stored wrapped in aluminum foil (shiny side out) or under a stretched sheet of PTFE-fluorocarbon.

7.1.2 Plastics other than PTFE-fluorocarbon should be avoided. They are a significant source of interference and can adsorb some organics.

7.1.3 A field blank prepared from water and carried through sampling, subsequent storage, and handling can serve as a check on sources of interferences from the containers.

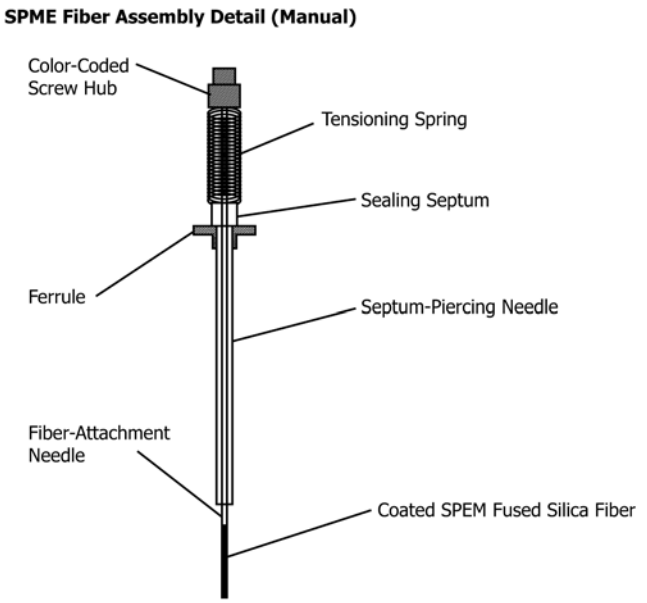
7.2 When performing analyses for specific organic compounds, matrix interferences may be caused by materials and constituents that are coextracted from the sample. The extent of such matrix interferences will vary considerably depending on the sample and the specific instrumental analysis method used. Matrix interferences may be reduced by choosing an appropriate SPME adsorbing fiber.

8. The Technique of SPME

8.1 The technique of SPME uses a short, thin solid rod of fused silica (typically 1-cm long and 0.11-µm outer diameter), coated with a film (30 to 100 µM) of a polymer, copolymer, carbonaceous adsorbent, or a combination of these. The coated, fused silica (SMPE fiber) is attached to a metal rod and the entire assembly is a modified syringe (see Fig. 1).

8.2 In the standby position, withdraw the fiber into a protective sheath. Place an aqueous sample containing organic analytes or a solid containing organic volatiles into a vial, and seal the vial with a septum cap.

8.3 Push the sheath with fiber retracted through the vial septum and lower into the body of the vial. Inject the fiber into the headspace or the aqueous portion of the sample (see Fig. 2). Generally, when 2-mL vials are used, headspace sampling requires approximately 0.8 mL of sample and direct sampling requires 1.2 mL.



NOTE 1—This figure is Fig. 5, p. 218, Vol 37, Advances in Chromatography, 1997. Used with permission.

FIG. 1 SPME Fiber Holder Assembly

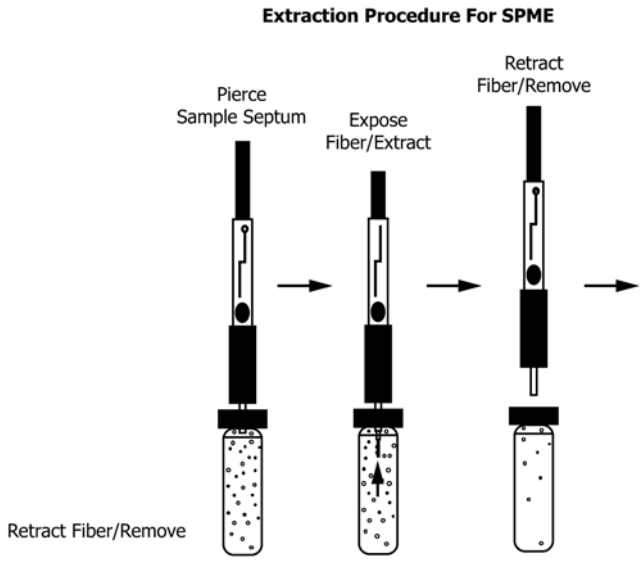


FIG. 2 Process for Adsorption of Analytes from Sample Vial with SPME Fiber

8.4 Organic compounds are absorbed onto the fiber phase for a predetermined time. This time can vary from less than 1 min for volatile compounds with high diffusion rates such as volatile organic solvents, to 30 min for compounds of low volatility such as PAHs.

8.5 Withdraw the fiber into the protective sheath and pull the sheath out of the sampling vial.

8.6 Immediately insert the sheath through the septum of the hot GC injector (see Fig. 3), push down the plunger, and insert the fiber into the injector liner where the analytes are thermally desorbed and subsequently separated on the GC column.

8.6.1 The blunt 23-gage septum-piercing needle of the SPME is best used with a septumless injector seal. These are manufactured by several sources for specific GC injectors.

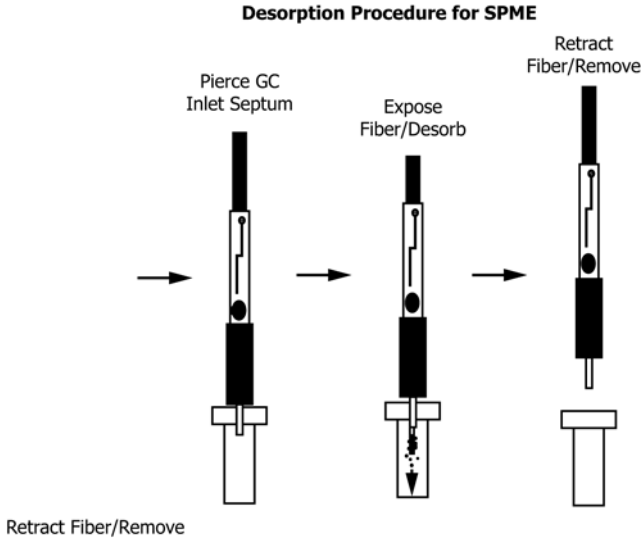


FIG. 3 Injection Followed by Desorption of SPME Fiber in Injection Port of Chromatograph

8.6.2 A conventional GC septum may be used with SPME. A septum lasts for 100 runs or more. To minimize septum failure, install a new septum, puncture with a SPME sheath three or four times, and remove and inspect the new septum. Pull off and discard any loose particles of septum material, and reinstall the septum.

8.6.3 The user should monitor the head pressure on the chromatographic column as the fiber sheath enters and leaves the injector to verify the integrity of the seal. A subtle leak will be indicated by unusual shifts in retention time or the presence of air in a mass spectrometer.

8.7 Ensure that the injector liner used with SPME is not packed or contains any physical obstructions that can interfere with the fiber. The inner diameter of the insert should optimally should be about 0.75 to 0.80 mm. Larger inserts (2 to 4 mm) may result in broadening of early eluting peaks. SPME inserts are available commercially and may be used for split or splitless injection. With splitless injection, the vent is timed to open at the end of the desorption period (usually 2 to 10 min).

8.8 Injector temperature should be isothermal and normally 10 to 20°C below the temperature limit of the fiber or the GC column (usually 200 to 280°C), or both. This provides rapid desorption with little or no analyte carryover.

9. Selection of Fiber Phase

9.1 The selection of the fiber phase depends on several factors, including:

9.1.1 The media being extracted by the fiber, aqueous or headspace,

9.1.2 The volatility of the analyte such as gas phase hydrocarbons to semivolatile pesticides, and

9.1.3 The polarity of the analyte.

9.2 A selection of fiber phases and common applications is shown in Table 1.

10. Apparatus

10.1 *SPME Holder,* manual sampling or automated sampling.

10.2 *SPME Fiber Assembly.*

10.3 *SPME Injector Liner,* that is, inserts for gas chromatographs.

10.4 *Septum Replacement Device,* Merlin or Jade.

10.5 *Vials,* with septa and caps, for manual or automation. For automation, use either 2- or 10–mL vials.

11. Reagents

11.1 *Purity of Water*— Unless otherwise indicated, reference to water shall be understood to mean reagent water that meets the purity specifications of Type I or Type II water, presented in Specification D1193.

11.2 Chemicals, standard materials and surrogates should be reagent or ACS grade or better. When they are not available as reagent grade, they should have an assay of 90 % or better.

11.3 *Sodium Chloride (NaCl),* reagent grade, granular.

TABLE 1 Commercially Available SPME Fibers for GC and GC/MS

Phase	Polarity	Features and Applications
Polydimethylsiloxane, 100 µM (PDMS)	Non-polar	High sample capacity, wide variety of applications; volatile organics to semivolatiles
PDMS, 30 µM	Non-polar	Semivolatiles, pesticides. Faster desorption, carryover minimized
PDMS, 7 µM	Non-Polar	Semivolatiles, higher desorption temperatures (320°C), reduces sample capacity
Polyacrylate, 85 µM[A]	Polar	Phenols, polars, semivolatiles
Carbowax/divinyl benzene, 65 µM (CW-DVB)	Polar	Alcohols
CW-templated resin, 50 µM	Polar	Surfactants
PDMS-DVB, 65 µM	Bi-Polar	Alcohols, amines
PDMS-DVB, 60 µM	Bi-Polar	For HPLC, special more durable phase
Carboxen™ 1006-PDMS	Bi-Polar	Bi-polar light hydrocarbons, polar solvents, VOCs; sulfur gases, useful for air monitoring
DVB-Carboxen™—PDMS	Bi-Polar	volatiles

[A]Phase more of a solid, so slower diffusion rates.

12. Hazards

12.1 The toxicity and carcinogenicity of chemicals used in this practice have not been precisely defined. Each chemical should be treated as a potential health hazard. Exposure to these chemicals should be minimized. Each laboratory is responsible for maintaining awareness of OSHA regulations regarding safe handling of chemicals used in this practice.

12.2 If using either solvent, the hazard of peroxide formation should be considered. Test for the presence of peroxide prior to use.

13. Sample Handling

13.1 There are many procedures for acquiring representative samples of water. The choice of procedure is site and analysis specific. There are several ASTM guides and practices for sampling.[4] Two good sources are Practices D3370 and Guide D4448.

13.2 The recommended sample size is 40 to 100 mL. More or less sample can be used depending upon the sample availability, detection limits required, and the expected concentration level of the analyte. VOA vials of 40-mL capacity are commonly used as sampling containers. Any headspace should be eliminated if volatiles analysis is required.

13.3 *Sample Storage:*

13.3.1 All samples must be iced or refrigerated to 4°C from the time of collection until ready for extraction.

13.3.2 Samples should be stored in a clean, dry place away from samples containing high concentrations of organics.

13.4 *Sample Preservation:*

13.4.1 Some compounds are susceptible to rapid biological degradation under certain environmental conditions. If biological activity is expected, adjust the pH of the sample to about 2 by adding HCI. The constituent of concern must be stable under acid conditions. For additional information, See Practice D3694.

13.4.2 If residual chlorine is present, add sodium thiosulfate as a preservative (30 mg per 4 oz bottle).

14. Optimizing SPME Sampling Parameters

14.1 Liquid sampling and headspace sampling give approximately the same recovery for volatiles but not for semi-volatiles. Semi-volatiles are best extracted with SPME liquid sampling. Headspace sampling is desirable if samples contain nonvolatile compounds such as salts, humic acids, or proteins.

14.2 Sample mixing is effective in increasing the response of semi-volatile analytes. It reduces the equilibrium time for the adsorption of the semi-volatile components. Mixing reduces any analyte depleted area around the fiber phase and increases the diffusion of larger molecules from the aqueous matrix. Mixing is much less effective for volatiles and is generally not required.

14.3 Matrix modification through the addition of salt to the aqueous phase may be used to drive polar compounds into the headspace. It has very little effect on nonpolar compounds. Adding salts to the sample also minimizes matrix differences when there are sample to sample variations in ionic strength

14.4 Heating the sample is often used to increase the sensitivity in static headspace; it is much less effective with SPME. The equilibrium tends to be shifted to the headspace rather than to the fiber.

14.5 *Ratio of Liquid to Headspace* —With nonpolar analytes, the sensitivity is enhanced when the proportion of liquid phase is increased. The magnitude of the enhancement depends upon the partition coefficient.

14.6 *Vial Size*—Larger sampling vials are not effective in increasing the sensitivity if the relative volumes of headspace and liquid are the same. The precision of measurements is not affected by vial size with direct aqueous sampling. The relative standard deviation of sampling the headspace is lower with the larger vials (>10 mL) than smaller ones (2 mL). Larger vials are easier to fill with solid and semisolid samples.

14.7 *Acidity of Sample*—When determining acidic compounds, such as phenols, or basic compounds, such as amines, the pH of the sample should be adjusted so that the analytes are in the nonionic state.

15. Quality Control

15.1 Minimum quality control requirements are: an initial demonstration of laboratory capability; analysis of method blanks; a laboratory fortified blank; a laboratory fortified sample matrix; and, if available, quality control samples. For a general discussion of good laboratory practices, see Guide D3856 and Practice D4210.

[4] Refer to the *Annual Book of ASTM Standards*, Vol 00.01, or the ASTM Homepage on the internet at www.astm.org to find titles of specific standards.

15.2 Select a representative spike concentration (about three times the estimated detection limit or expected concentration) for each analyte. Extract according to Section 13 and analyze.

15.3 Method blanks must be prepared using reagent grade water and must contain all the reagents used in sample preservation and preparation. The blanks must be carried through the entire analytical procedure with the samples. Each time a group of samples are run that contain different reagents or reagent concentrations, a new method blank must be run.

15.4 All calibration and quality control standards must be extracted using the same reagents, procedures, and conditions as the samples.

15.5 Precision and bias must be established for each matrix and laboratory analytical test method.

15.5.1 Precision should be determined by splitting spiked samples or analytes in the batch into two equal portions. The replicate samples should then be extracted and analyzed.

15.5.2 Bias should be determined in the laboratory by spiking the samples with the analytes of interest at a concentration three times the concentration found in the samples or less.

16. Procedure

16.1 Remove samples from storage and allow them to equilibrate to room temperature.

16.2 Remove the container cap from the sample container. Make a volumetric transfer of a portion of this sample to either a 2- or 10–mL volume septum-capped vial. The volume transferred depends upon whether SPME extraction is from the headspace or direct from the sample. For headspace sampling, the nominal volume of sample is 40 % of the vial volume. For direct sampling of the liquid, the nominal volume of sample is 60 % of vial volume.

16.3 If acid neutral or base compounds are of interest, adjust the pH to <2 for acid neutral and >11 for base compounds. If salt is required to aid in analyte extraction from headspace, add approximately 0.1 g NaCl per 1 mL of sample.

16.4 If sample is to be extracted at an elevated temperature, heat sample to this temperature and hold as required.

16.5 Insert SPME shaft through septum into either headspace above sample or directly into sample.

16.6 Depress plunger either manually or automatically and expose fiber coating to headspace or aqueous sample. The extraction time can vary from 2 to 30 min depending upon application.

16.7 If mixing is required, initiate after plunger is depressed.

16.8 Following extraction, retract fiber into protective sheath and remove from vial.

16.9 Inject sheath through GC septum and depress plunger into heated injector insert or liner, desorbing analytes to column. This time is generally less than 2 min.

16.10 Analyze desorbed analytes by GC or GC-MS.

17. Calibration, Standardization and Analysis

17.1 While the recovery of analytes with a SPME fiber is relatively low, the degree of extraction is consistent so that SPME is quantitative with linearity, precision and accuracy.

17.2 Determine the appropriate SPME extraction fiber and optimize the SPME extraction parameters as described in Section 14. Next, select the applicable calibration procedure depending upon the complexity of the sample matrix. For simple or clean sample matrices such as drinking water, external or internal standard calibration procedures may be used. For more complex matrices such as certain waste waters, the matrix can effect the equilibrium so that quantitation may require matrix modifiers or the method of standard additions.

17.3 For clean sample matrices, prepare calibration standards by spiking the blank or reagent water with portions of the stock standard solution. Prepare a blank and at least three calibration standards in graduated amounts in the appropriate range. Space the calibration standards evenly in concentration from 0 to 20 % greater than the highest expected value.

17.4 Beginning with the blank or reagent water and working toward the highest standard, analyze the solutions and record the readings. Repeat the operation a sufficient number of times to obtain a reliable average reading for each solution.

17.5 Construct an analytical curve by plotting the concentrations of the standards versus their responses as provided by the instrument workstation. Analyze the unknown using the same procedure and determine the analyte concentration.

17.6 For more complex matrices, matrix modification and standard additions may be employed where analyte recovery and equilibration with the SPME fiber is matrix dependent. Modifiers should be chosen that enhance the release of analytes from the matrix while reducing the differences between samples and standards. Modifiers for SPME include salts such as NaCl and non-volatile acids.

17.7 Standard additions may be used where matrix modification is either not effective or not feasible. Four sample aliquots are generally required. Dilute the first aliquot to a known volume with water. Then add increasing amounts of the unknown analyte to the second, third and fourth aliquot before they are diluted to the same volume. Determine the detector response of the analyte in each solution and plot versus quantity added. Extrapolate the resulting curve back to the zero response. This intercept with the abscissa on the left of the ordinate will be the concentration of the unknown.

18. Precision and Bias

18.1 Precision and bias cannot be determined directly for this practice. Precision and bias should be generated in the laboratory on the parameters of concern. Examples of this type of data may be found in the literature for volatile organic compounds and pesticides, see Refs (**1**) and (**2**) respectively.

19. Keywords

19.1 extraction; sample preparation; semivolatile; solid phase microextraction (SPME); water; volatile

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