

Sample Prep

Perspectives

**Maria Eugênia C. Queiroz
and Fernando M. Lanças**

This month's guest authors review the application of solid-phase microextraction (SPME) to the analysis of drugs in human plasma. They discuss important factors in the optimization of extraction efficiency such as coating (stationary phase) fiber types, extraction time, matrix pH, ionic strength, effect of organic additives, temperature, degree of agitation, use of derivatization, influence of protein, and desorption conditions. The authors also review published applications in the areas of clinical pharmacology, social toxicology, and forensic science, and round out the column with a discussion of method validation issues.

Ronald E. Majors
Sample Prep Perspectives Editor

Practical Tips on Preparing Plasma Samples for Drug Analysis Using SPME

Measurement of drug concentration in biological samples (especially in plasma) from patients commonly is performed in clinical medicine to ensure a proper therapeutic level while minimizing the incidence of toxicity. Sample preparation is an important part of these analyses when using chromatographic methods. The aim of a sample preparation is to eliminate interfering compounds from the matrix using a minimum number of steps, resulting in a reproducible methodology. Solid-phase microextraction (SPME) has been evaluated as a suitable sampling technique for a wide range of applications (1) and has proven to be a very effective, highly sensitive, solvent-free approach (1–3).

In SPME, sample analytes are extracted and concentrated by the fiber coating and then introduced into the chromatograph (Figure 1). Although this technique was introduced originally for the extraction of organic compounds from environmental samples (1,4), since 1995 it also has been applied to various biological matrices (5,6). The use of SPME for assaying drugs in plasma is summarized in Table I (7–25).

Parameters Affecting the Extraction of Analytes by SPME

Many variables have been studied for the optimal extraction of analytes from liquid matrices by direct immersion SPME and in liquid and solid matrices by the application of headspace SPME. When dealing with the analysis of drugs in biological fluids, some of these parameters are more important than others. We will now discuss these parameters.

Fiber Coating

The affinity of the coating for an analyte is the most important factor in the successful use of SPME. Selection of the coating is based primarily on the polarity and volatility of the analyte. Both the coating thickness and distribution constant determine

the sensitivity of the method and the extraction time. Thick coatings offer increased sensitivity but require much longer equilibration times. Therefore, it is important to use the appropriate coating for a given application (1–3).

The main commercially available coatings offer only a few alternatives: polydimethylsiloxane of different film thicknesses (7, 30, and 100 μm), 85- μm polyacrylate, 65- and 60- μm polydimethylsiloxane–divinylbenzene, 75- μm Carboxen–polydimethylsiloxane, 65- μm Carbowax–divinylbenzene, and 50- μm Carbowax–templated resin. There is a substantial difference in performance between liquid and solid coatings. In liquid coatings, the analytes partition onto the extraction phase, while in solid sorbents, the coating has a well-defined, dense crystalline structure that substantially reduces the diffusion coefficients within the structure with extraction occurring only on the surface coating (1–3).

The most popular fibers are those coated with 7–100 μm polydimethylsiloxane as the extraction medium. SPME partitioning equilibrium on this phase has been correlated with octanol–water distribution coefficients (k_{ow}) (1, 26). These studies demonstrated that for solutes with low k_{ow} , low recoveries are obtained. This low recovery is mainly due to the phase ratio between the aqueous and polydimethylsiloxane phases. The amount of polydimethylsiloxane used in SPME is typically on the order of 0.5 μL or less, thereby limiting the enrichment on the polydimethylsiloxane fiber. Based upon this observation, a new approach using stirring bars coated with polydimethylsiloxane was developed (27). This approach uses 50–300 μL polydimethylsiloxane coatings. Consequently, the sensitivity is increased by a factor of from 100 to 1000. Complete recovery is possible for solutes with k_{ow} larger than 500. This technique, named stir-bar sorptive extraction, has been applied successfully to the

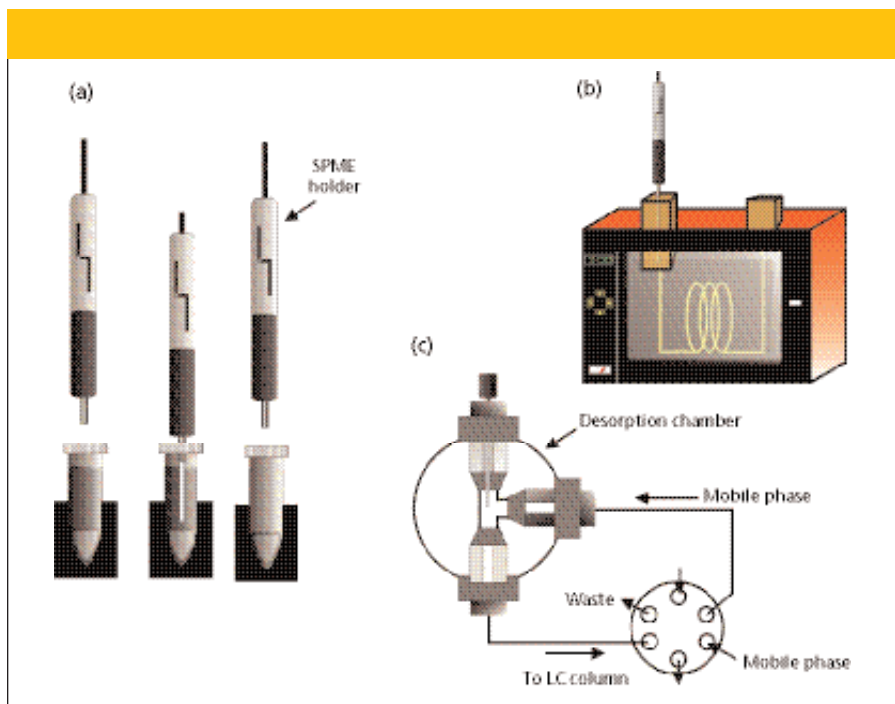


Figure 1: Schematics of (a) the fiber SPME extraction procedure, (b) thermal desorption on a GC injection port, and (c) solvent desorption using an SPME interface.

analysis of biological samples (28–31).

Ionic Strength

Altering the ionic strength of the matrix also can optimize SPME methods. Salt ions in solution have two opposing effects on drugs in solution. As salt is added to solution, water molecules are tied up in hydration spheres around the salt ions. This reduces the availability of free water molecules for dissolving the drugs. As a consequence, the activity, or effective concentration of drugs in solution, increases and more drugs will distribute into the fiber (1–3), according to the salting-out effect. Conversely, as salt concentration continues to increase, the salt itself can interact with the drugs in solution through electrostatic, covalent (as in the case of organic salts), or ion-pairing interactions. This reduces the ability of the drug to move into the fiber coating, reducing the amount extracted (12,32). Caution should be taken as a high salt concentration in the sample matrix facilitates salt deposition on the fiber, which decreases extraction efficiency over time.

For anticonvulsants analysis (SPME–liquid chromatography [LC]) in plasma samples, the first step to optimize SPME conditions was to evaluate the influence of ionic strength of the matrix on extraction (Figure 2a). For that purpose, the addition of sodium chloride (0, 10, 20, and 30%) was investigated. In a conic glass tube (5 mL),

3 mL of phosphate buffer (0.01 mol/L) was added to 1 mL of the drug-free plasma spiked with working standards, and the sample was vortexed for 10 s before extraction. The fiber, Carbowax-templated resin, then was immersed in the sample with stirring at room temperature for 10 min. For the condition in which the solution was saturated with 40% sodium chloride, the mass extracted decreased for drugs. Thus, 30% sodium chloride was selected as the best condition.

Matrix pH

Matrix pH can be adjusted to optimize the SPME of either acidic or basic drugs. This is related to the fact that, unless an ion-exchange coating is used, SPME can extract only neutral (nonionic) species from the matrix. By properly adjusting the pH, weak acids and bases can be converted to their neutral forms, which enables them to be extracted by the SPME fiber. The matrix pH effect on the extraction efficiency of the anticonvulsants using different pH values adjusted with phosphate buffer is shown in Figure 2b. This direct extraction was achieved with Carbowax-templated resin fiber (50 μm film thickness) by modifying 1.0 mL of sample plasma matrix to 30% sodium chloride, adding 3 mL phosphate buffer (pH 3.0, 5.0, 7.5, 9.0, and 11.0) at room temperature, and stirring for 10 min. The majority of anticonvulsants evaluated

were weakly acidic, so the best results were obtained at pH 5.0. Care should be taken when direct-immersion SPME is used, because extreme pH values (less than 2 and greater than 10) can damage the coating and make it difficult to implement large pH changes (13). The pH of the sample should be adjusted after adding salt or, if a small amount of organic additive is used (see the following), before organic solvent addition.

Extraction Time

Drug extraction is optimized by determining the time required for an analyte to reach equilibrium between the sample matrix, gaseous headspace, and fiber coating. Figure 2c shows the effect of the equilibrium time (5, 10, 20, 30, and 50 min) on the extraction efficiency of the anticonvulsants in a plasma sample (1 mL) that was modified with 30% sodium chloride and 3 mL phosphate buffer (pH 5.0) at room temperature and stirred periodically. The extraction equilibrium time was reached at 30 min for all investigated drugs. When equilibration times are excessively long, shorter extraction times can be used. However, in such cases, the extraction time and mass transfer conditions must be controlled strictly to assure good precision. The extraction equilibrium time usually is reached in 15–30 min for most drugs in plasma; a relatively short extraction time is one of the main advantages of SPME.

Organic Additives

The use of a small volume of an organic additive such as methanol is recommended for plasma samples. It is suggested that the binding of target analyte to the protein can be decreased and therefore, the sensitivity of the method can be increased substantially (10).

Temperature

By increasing the extraction temperature, the distribution constant of drug between the fiber coating and the extraction mixture decreases. However, in contrast, an increase in temperature also can increase the analyte diffusion rate by lowering the viscosity, which shortens the equilibrium extraction time. This temperature might be more important for plasma than for water or urine because of its higher viscosity. Consequently, SPME methods can be optimized by selecting extraction temperatures with which satisfactory sensitivity is achieved in an acceptable time period. Figure 2d shows the effect of 30–70 $^{\circ}\text{C}$ temperatures on the

Table I: Application of SPME in plasma samples

Analyte	Extraction Mode Fiber Coating (thickness, mm)	Analytical System (LOQ or LOD)	Remarks	References
Valproic acid	Direct immersion PDMS (100)	GC-FID (LOD: 1 mg/mL)	Equilibrium dialysis followed SPME	Krogh et al., 1995 (7)
Aniline, phenols, nitrobenzenes	Direct immersion PA (85)	GC-MS	Protein binding study, determination of free concentrations	Vaes et al., 1996 (8)
Antidepressants	Direct immersion PDMS (100)	GC-NPD, GC-MS (LOQ:90–200 ng/mL)	Theoretical model for influence of proteins	Ulrich and Martens, 1997 (9)
Diazepam	Direct immersion PA (85) PDMS (7, 100)	GC-FID (LOQ:0.25 nmol/mL)	1-Octanol-modified PA fiber, pretreated plasma (TCA)	Krogh et al., 1997 (10)
Benzodiazepines	Direct immersion PA (85)	GC-FID (LOQ: 0.01–0.48 mmol/mL)	1-Octanol-modified PA fiber, pretreated plasma (TCA)	Reubsaet al., 1998 (11)
Clozapine	Direct immersion PDMS (100)	GC-NPD (LOD: 30 ng/mL)	Influence of proteins and triglycerides	Ulrich et al., 1999 (12)
Lidocaine and three of its metabolites	Direct immersion CW-DVB (65) PA (85) PDMS (100)	GC-NPD (LOQ: 8–21 ng/mL)	Effect of different fiber coating	Abdel-Rehim et al., 2000 (13)
Lidocaine	Direct immersion PDMS (100)	GC-FID (LOD: 5 ng/mL)	Analysis of free, protein-bound, and total amount of lidocaine in human plasma	Koster et al., 2000 (14)
Anesthetics	Direct immersion CW-DVB (65) PA (85) PDMS (100)	GC-NPD (LOQ: 0.5 mmol/mL)	Study of protein-binding ultra filtrate plasma	Abdel-Rehim et al., 2000 (15)
Gamma- hydroxybutyric acid	Derivatization headspace	GC-PICI-MS (LOQ: 1 mg/mL)	Conversion of gamma-hydroxybutyric to gamma-butyrolactone	Frison et al., 2000 (16)
Methadone and its main metabolite	Direct immersion PDMS (100)	GC-MS (LOD: 40 ng/mL)	Application to methadone -treated patients	Bermejo et al., 2000 (17)
Levomopromazine	Direct immersion PDMS (100)	GC-NPD (LOQ: 5 ng/mL)	Application to therapeutic drug monitoring	Kruggel and Ulrich, 2000 (18)
Midazolam	PA (85)	GC-MS (SIM) (LOD: 1.0 ng/mL)	Application to therapeutic drug monitoring	Frison et al., 2001 (19)
Anticonvulsants	Direct immersion CW-TPR (50)	LC-UV (LOQ:0.05–1.0 mg/mL)	Off-line desorption	Queiroz et al., 2002 (20)
Anticonvulsants	Direct immersion CW-DVB (65)	GC-TSD (LOQ:0.05–0.2 mg/mL)	Application to therapeutic monitoring	Queiroz et al., 2002 (21)
Thymol	Headspace PDMS-DVB (65)	GC-FDI (LOQ: 8.1 ng/mL)	Enzymatic cleavage of thymol sulfate	Kohlert et al., 2002 (22)
Sulfentanil	Direct immersion PDMS-DVB (65)	GC-MS (LOQ: 6.0 ng/mL)	Influence of pH and ionic strength	Paradis et al., 2002 (23)
Amitraz	Direct immersion PDMS (100)	GC-TSD (LOQ: 20 ng/mL)	Application to toxicity studies in dogs	Queiroz et al., 2003 (24)
Busulphan	Direct immersion CW-DVB (65)	GC-MS (LOQ: 20 ng/mL)	In-vial derivatization	Abdel-Rehim et al., 2003 (25)

PDMS = polydimethylsiloxane, PA = polyacrylate, CW = Carbowax, DVB = divinylbenzene, FID = flame ionization detection, NPD = nitrogen-phosphorus detection, TSD = thermionic specific detection, LOQ = limit of quantitation, LOD = limit of detection, TCA = trichloroacetic acid, PICI-MS = positive ion chemical MS, SIM = selected ion monitoring

SPME efficiency of the anticonvulsants in the plasma sample (1 mL) modified with 30% sodium chloride and 3 mL of phosphate buffer (pH 5.0) and stirred for 30 min. Based upon the results shown in Figure 2, we concluded that the best experimental conditions among those investigated for the SPME procedure were the following: direct extraction with Carbowax-templated resin fiber (50 μ m film thickness), 1.0 mL of sample plasma

matrix modified with 30% sodium chloride and 3 mL of phosphate buffer (pH 5.0), with the extraction temperature at 30 °C and stirring for 30 min.

Agitation

The SPME of plasma samples was performed in two different ways: by direct extraction and by headspace extraction (Table I). In direct extraction mode, the coated fiber is inserted into the liquid sam-

ple, and analytes migrate between the sample matrix, gaseous headspace, and fiber coating until equilibrium is reached. Drug equilibration time depends upon its mass transfer rate in sample matrix. Stirring, fiber vibration, and sonication enhances drug transfer from the matrix to fiber coating, which then reduces the effect of the “depletion zone” produced close to the fiber as a result of slow diffusional transport of analyte through the stationary layer

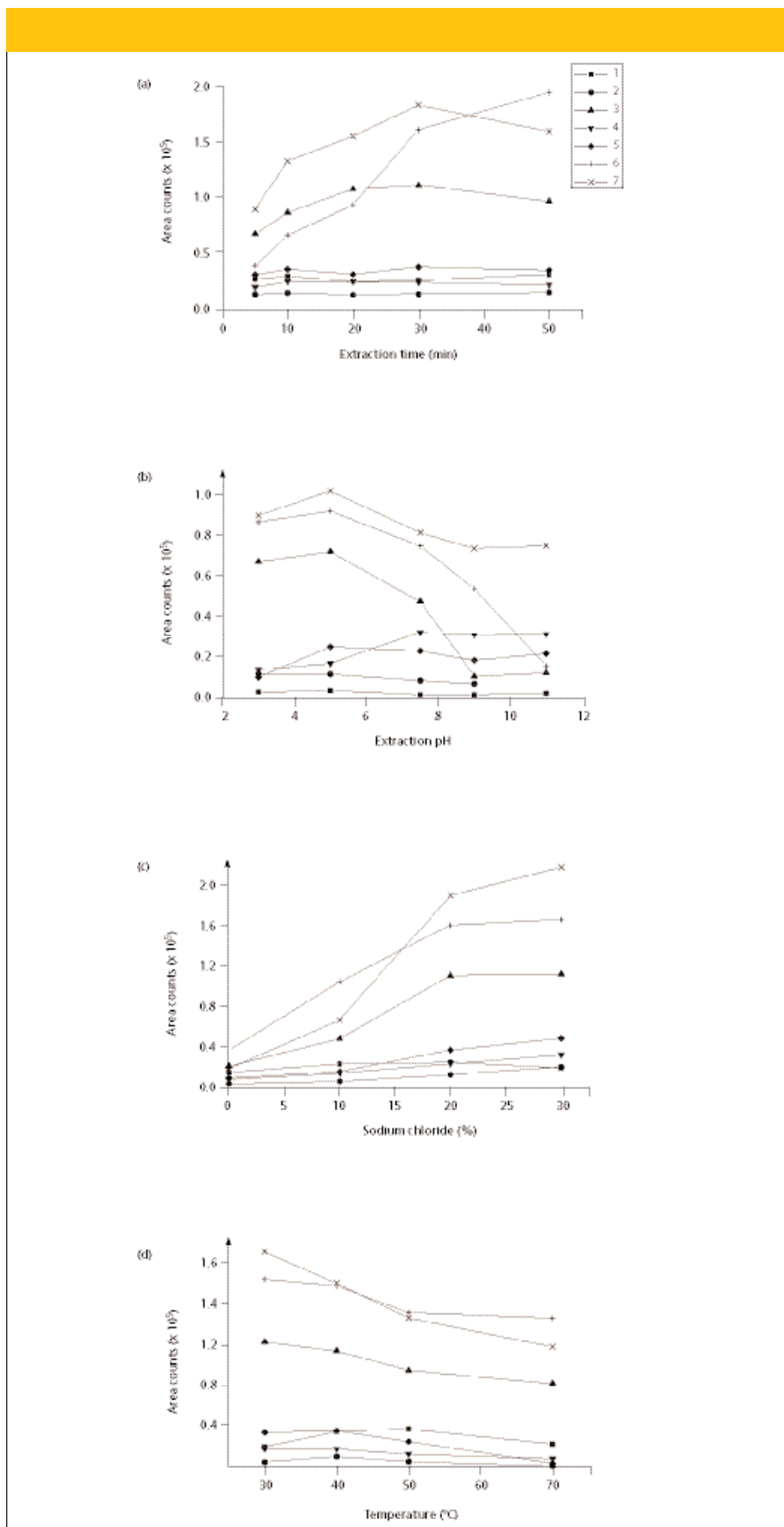


Figure 2: Effect of the major experimental variables (a) extraction time, (b) pH, (c) sodium chloride concentration, and (d) temperature on the efficiency of direct SPME of anticonvulsants in a plasma sample. Fiber: Carbowax-templated resin (50 μm film thickness). (See text for further details.) Drugs: 1 = phenylethylmalonamide, 2 = phenobarbital, 3 = primidone, 4 = lamotrigine, 5 = carbamazepine epoxide, 6 = phenytoin, 7 = carbamazepine.

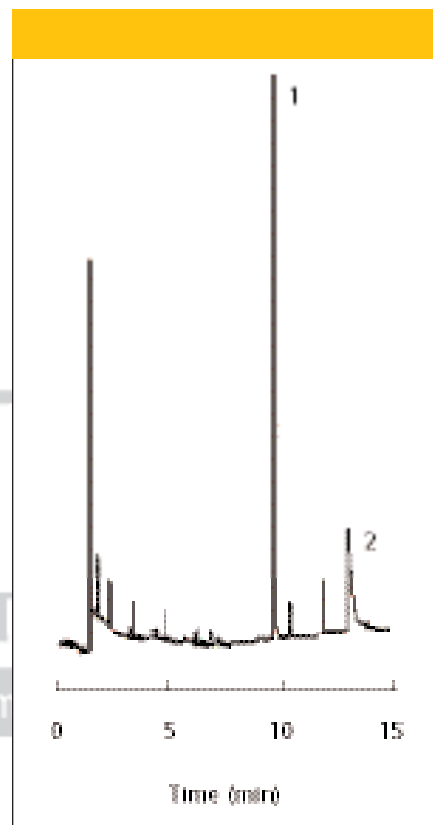


Figure 3: Capillary GC-thermionic specific detection chromatogram for the SPME extracts of dog plasma that was dipped in an amitraz bath, 292 mg/kg of body weight, resulting in plasma levels of 41.29 ng/mL. Peaks: 1 = internal standard (chlorpyrifos), 2 = amitraz.

of liquid matrix surrounding the fiber (22,1).

Derivatization

Derivatization can be performed as follows: directly in the sample by adding appropriate reagents followed by extraction (33); into the gas chromatography (GC) injector by extracting and derivatizing within the GC inlet (34); and in the SPME fiber by extracting, then exposing the fiber-containing extract to the derivatizing reagent (35).

Influence of Plasma Protein on SPME

Plasma proteins decrease the extraction recovery, which indicates a disturbance of the extraction process. High molecular weight compounds such as proteins can adsorb irreversibly to the fiber, thus changing the properties of the stationary phase and rendering it unusable. Protein precipitation has been used to release drugs from the plasma by acidification (9,11,14,19,22) and the addition of methanol (10). When the plasma samples were spiked with drugs after protein precipitation (remaining aqueous phase from plasma), the peak

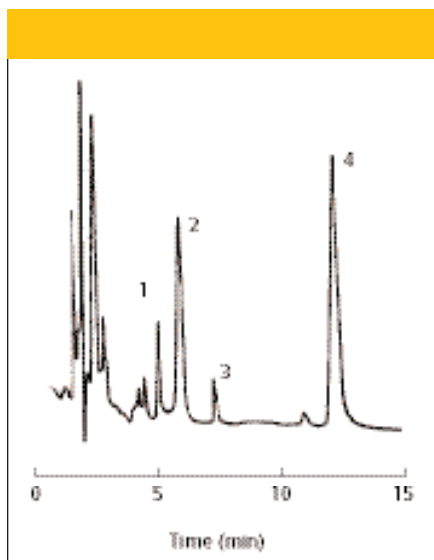


Figure 4: SPME-LC-UV (off-line) chromatogram of human plasma collected from a patient with epilepsy. The drugs were administered orally at concentrations of 4.44 mg/kg/day (lamotrigine) and 31.10 mg/kg/day (carbamazepine), resulting in plasma levels of 2.24 $\mu\text{g/mL}$ of the lamotrigine, 10.2 $\mu\text{g/mL}$ of the carbamazepine, and 4.16 $\mu\text{g/mL}$ of the carbamazepine 10,11-epoxide. Peaks: 1 = lamotrigine, 2 = internal standard, 3 = carbamazepine 10,11-epoxide, 4 = carbamazepine.

areas obtained were comparable to those values obtained by spiking pure water (9,21).

The high protein binding of antidepressants (9), clozapine (12), and anticonvulsants (21) appeared to be the main limiting factor concerning a faster extraction and higher recovery in plasma (9,12,21).

The sensitivity of SPME can be improved considerably by dilution of plasma samples with either buffer solution or water (12,16–18,20,21,24). The dilution increases the diffusion coefficient of the drugs from plasma samples to the polymeric coating. Compared with water, diffusion coefficients are smaller in the more viscous protein solution.

Analyte Desorption

Gas chromatography: When GC is used for analyte separation and quantitation, the fiber is inserted into a hot injector, where thermal desorption of the trapped analytes takes place (Figure 1b). Opening the split line during SPME injection is not practical because it results in reduced sensitivity. Efficient desorption and rapid transfer of the analytes from the injector to the column require high linear flow rates of the carrier gas around the coating. This can be accomplished by reducing the internal

diameter of the injector insert, matching it as closely as possible to the outside diameter of the coated fiber. Narrow-bore inserts for SPME are available commercially for a range of GC instruments (1–3). Temperature, time of desorption, and the position of the needle in the GC injector are the main factors affecting the thermal desorption of analytes from the SPME fiber.

SPME desorption of drugs usually uses an injection temperature close to 250 °C and a normal desorption time in the splitless mode from 2 to 4 min. An example of a successful SPME-GC application for the analysis of a drug in dog plasma can be seen in Figure 3.

Liquid chromatography: In LC, a specially designed desorption interface is used for the analysis of nonvolatile and thermally labile compounds with SPME preconcentration. A typical SPME-LC interface consists of a desorption chamber and a six-port injection valve (Figure 1c). The optimization of the desorption conditions is the most critical step in SPME-LC. One of the main difficulties that limits the wide application of SPME-LC is imposed by the on-line coupling of these methods, as well as their subsequent operation (36), and absence of a suitable commercially available stationary phase that not only has high extraction ability for polar analytes but also is stable in solutions of various matrices.

The number of SPME-LC applications is substantially lower than for SPME-GC, despite its potential for plasma analysis. However, most LC applications were developed in recent years, clearly indicating increasing interest in the technique.

Queiroz and colleagues (20) described a specific and very simple set up for off-line coupling SPME and LC that does not require any interface. Currently it is employed successfully in the determination of lamotrigine simultaneously with carbamazepine and carbamazepine 10,11-epoxide, the main metabolite of carbamazepine, an antiepileptic drug (Figure 4). The SPME-LC off-line desorption of anticonvulsants in plasma samples was achieved by exposing the fiber to 50 μL of the mobile phase for 10 min using a 0.1-mL glass vial (V-shape). This volume enables the fiber coating to be immersed completely in the solvent. This off-line desorption procedure also was used to analyze tricyclic antidepressants in plasma samples by SPME-LC-UV (Figure 5).

Moreover, an SPME-LC approach known as in-tube SPME was developed

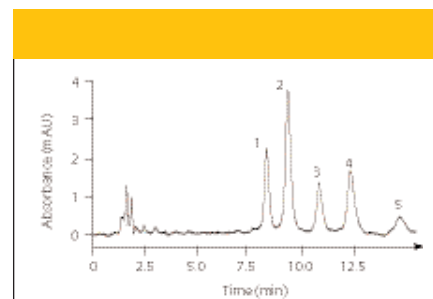


Figure 5: SPME-LC-UV (off-line) chromatogram of a plasma sample spiked with tricyclic antidepressants at 250 ng/mL. Peaks: 1 = desipramine, 2 = nortriptyline, 3 = imipramine, 4 = amitriptyline, 5 = clomipramine (internal standard).

recently using an open tubular fused-silica capillary column with an internal coating as the SPME device instead of SPME fiber. The combination between in-tube SPME and HPLC or LC-MS can be done easily by fixing this capillary column as the SPME device between the injection loop and injection needle of the HPLC autosampler. The in-tube SPME technique has been applied successfully to polar and nonpolar compounds in biological samples (37).

Analytical Validation

Most of the described methods (Table I) showed high chromatographic selectivity, linearity, precision (coefficient of variation less than 15%), and high sensitivity, in-line with the international criteria for validation such as for therapeutic drug monitoring (7,10,12–14,16,18–21,23,25), clinical toxicology (9,16,18,24), forensic toxicology (10,16), social toxicology (17), bioavailability, and pharmacokinetics (22).

SPME of drugs in plasma samples (Table I) showed low recoveries in most cases. Namara and colleagues (38) showed that SPME analysis of drugs from biological fluids gave recoveries ranging from 0.03 to 12.9%. Low recovery does not necessarily imply insufficient precision of the method. Changes in the recovery due to changes in the matrix, changes in the fiber, or due to small deviations from the standard procedure (for example, extraction time or properties of the fiber) are compensated for by the use of an internal standard quantitation procedure (12). The choice of the internal standard is critical for the development of the SPME method for human plasma because it requires a chemical structure and physicochemical properties similar to those of the analyte. The calibration curve should be con-

structed by using blank plasma samples (free from the analytes to be quantified) spiked with analytical standards of the target drugs at different concentrations.

Conclusion

For the analysis of drugs in plasma, SPME has many advantages over conventional extraction methods, such as simplicity — all of the sample preparation is integrated in one step and in one device — low cost, compatibility with most analytical systems, automation, the absence of extraction solvent, and a relatively short extraction time.

The sensitivity of SPME methods can be improved considerably by protein precipitation or dilution of plasma samples with buffer solution or water, as well as adjusting extraction parameters such as fiber type, extraction time, ionic strength, pH, temperature, and agitation.

The number of SPME–LC applications is substantially less than SPME–GC at the moment. This is due to difficulties imposed by the on-line coupling of these methods, as well as their subsequent operation, and the absence of a suitable stationary phase that not only has high extraction ability for the polar analytes but also is stable in solutions of various matrices.

The SPME methods described here support the international criteria for validation of the analysis of drugs in plasma in several areas, including clinical toxicology (therapeutic drug monitoring, bioavailability, and pharmacokinetics), forensic toxicology, and social toxicology (abuse drugs).

Acknowledgements

The authors wish to thank the Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for financial support.

References

- J. Pawliszyn, in *Applications of Solid Phase Microextraction* (Royal Society of Chemistry, Cambridge, UK, 1999).
- J. Pawliszyn, *J. Chromatogr. Sci.* **38**, 270–278 (2000).
- H. Lord and J. Pawliszyn, *J. Chromatogr., A* **885**, 153–193 (2000).
- M.E.C. Queiroz, S.M. Silva, D. Carvalho, and F.M. Lanças, *J. Environ. Sci. Health* **36**, 517–527 (2001).
- G. Theodoridis, E.H.M. Koster, and G.J. Jong, *J. Chromatogr., B* **745**, 49–82 (2000).
- N.H. Snow, *J. Chromatogr., A* **885**, 445–455, (2000).
- M. Krogh, K. Johansen, and K. E. Rasmussen, *J. Chromatogr., B* **673**, 299–305 (1995).
- W.H.J. Vaes, E.U. Ramos, H.J.M. Verhaar, W. Seinen, and J.L.M. Hermens, *Anal. Chem.* **68**, 4458–4462 (1996).
- S. Ulrich and J. Martens, *J. Chromatogr., B* **696**, 217–234 (1997).
- M. Krogh, H. Grefslie, and K.E. Rasmussen, *J. Chromatogr., B* **689**, 357–364 (1997).
- K.J. Reubsæet, H.R. Norli, P. Hemmersbach, and K.E. Rasmussen, *J. Pharm. Biomed. Anal.* **18**, 667–680 (1998).
- S. Ulrich, S. Kruggel, H. Weigmann, and C. Hiemke, *J. Chromatogr., B* **731**, 231–240 (1999).
- M. Abdel-Rehim, M. Bielenstein, and T. Arvidsson, *J. Microcolumn Sep.* **12**, 308–315 (2000).
- E.H.M. Koster, C. Wemes, J.B. Morsink, and G.J. Jong, *J. Chromatogr., B* **739**, 175–182 (2000).
- M. Abdel-Rehim, G. Carlsson, M. Bielenstein, T. Arvidsson, and L.G. Blomberg, *J. Chromatogr. Sci.* **38**, 458–464 (2000).
- G. Frison, L. Tedeschi, S. Maietti, and S.D. Ferrara, *Rapid Commun. Mass Spectrom.* **14**, 2401–2407 (2000).
- A.M. Bermejo, R. Seara, M.J. Tebernero, P. Fernandez, and R. Marsili, *J. Anal. Toxicol.* **24**, 66–69 (2000).
- S. Kruggel and S. Ulrich, *Ther. Drug Monit.* **22**, 723–728 (2000).
- G. Frison, L. Tedeschi, S. Maietti, and S.D. Ferrara, *Rapid Commun. Mass Spectrom.* **15**, 2497–2501 (2001).
- M.E.C. Queiroz, S.M. Silva, D. Carvalho, and F.M. Lanças, *J. Sep. Sci.* **25**, 91–95 (2002).
- M.E.C. Queiroz, S.M. Silva, D. Carvalho, and F.M. Lanças, *J. Chromatogr. Sci.* **40**, 219–223 (2002).
- C. Kohlert, G. Abel, E. Schmid, and M. Veit, *J. Chromatogr., B* **767**, 11–18 (2002).
- C. Paradis, C. Dufresne, M. Bolon, and R. Boulieu, *Ther. Drug Monit.* **24**, 768–774 (2002).
- M.E.C. Queiroz, C.A. Valadão, A. Farias, D. Carvalho, and F.M. Lanças, *J. Chromatogr., B* **794**, 337–342 (2003).
- M. Abdel-Rehim, Z. Hassan, L. Blomberg, and M. Hassan, *Ther. Drug Monit.* **25**, 400–406 (2003).
- J. Beltran, F.J. Lopez, O. Cepria, and F. Hernandez, *J. Chromatogr., A* **808**, 257–263 (1998).
- E. Baltussen, P. Sandra, F. David, and C. Cramers, *J. Microcolumn Sep.* **11**, 737–747 (1999).
- B. Tienpont, F. David, K. Desmet, and P. Sandra, *Anal. Bioanal. Chem.* **373**, 46–55 (2002).
- T. Benijts, J. Vercaemmen, R. Dams, H.P. Tuan, W. Lambert, and P. Sandra, *J. Chromatogr., B* **755**, 137–142 (2001).
- B. Tienpont, F. David, K. Desmet, and P. Sandra, *J. Pharm. Biomed. Anal.* **32**, 569–579 (2003).
- M. Kawaguchi, K. Inoue, N. Sakui, R. Ito, and H. Nakazawa, *J. Chromatogr., B* **799**, 119–125 (2004).
- H.L. Lord and J. Pawliszyn, *Anal. Chem.* **69**, 3899–3906 (1997).
- H.G. Uglund, M. Krogh, and K.E. Rasmussen, *J. Pharm. Biomed. Anal.* **19**, 463–475 (1999).
- A. Namera, M. Yashiki, J. Liu, K. Okajima, K. Hara, T. Imamura, and T. Kojima, *Forensic Sci. Int.* **109**, 215–223 (2000).
- G. Gmeiner, C. Krassnig, E. Schmid, and H. Tausch, *J. Chromatogr., B* **705**, 132–138 (1998).
- Y. Saito, M. Kawazoe, M. Hayashida, and K. Jinno, *Analyst* **125**, 807–809 (2000).
- K. Hiroyuki, *Anal. Bioanal. Chem.* **373**, 31–45 (2002).
- A. Namara, M. Yashiki, J. Liu, K. Hara, T. Imamura, and T. Kojima, *Forensic Sci. Int.* **109**, 215–223 (2000).

Fernando M. Lanças is a professor with the Institute of Chemistry at São Carlos, University of São Paulo, Brazil. His interests are in sample preparation, instrumental development for chromatography and allied techniques, and capillary chromatography (GC, LC, SFC, and CEC). He is the chairman of the Latin American Symposium on Chromatography (COLACRO) and has published more than 200 scientific papers and supervised more than 100 thesis and postdoctoral students. He is the author of two books and several chapters of books on chromatography.

Maria Eugenia C. Queiroz is a professor with the Department of Chemistry, University of São Paulo, Brazil. Her interests are in biological sample preparation for analysis of drugs through chromatographic methods for chemical area (therapeutic drug monitoring and pharmacokinetics).

Ronald E. Majors

*"Sample Prep Perspectives" Editor
Ronald E. Majors is business development manager, Consumables and Accessories Business Unit, Agilent Technologies, Wilmington, Delaware, and is a*



member of LCGC's editorial advisory board. Direct correspondence about this column to "Sample Prep Perspectives," LCGC, Woodbridge Corporate Plaza, 485 Route 1 South, Building F, First Floor, Iselin, NJ 08830, e-mail lcgedit@lccmag.com.