**Table 1** Applications of MSPD to the analysis of residues in various matrices

| *Analyte(s)* | *Matrix* |
|---|---|
| Aminoglycosides | Bovine kidney |
| Benzimidazoles | Beef liver |
| Benzimidazoles | Swine muscle |
| Beta-agonists | Bovine liver |
| Carbofuran | Corn |
| Chloramphenicol | Milk |
| Chlorsulfuron | Milk |
| Chlorsulon | Milk |
| Clenbuterol | Bovine liver |
| Furazolidone | Chicken muscle |
| Furazolidone | Milk |
| Furazolidone | Swine muscle |
| Ivermectin | Fish muscle |
| Ivermectin | Milk |
| Ivermectin | Bovine liver |
| Moxidectin | Bovine tissues |
| Nicarbazin | Animal tissues |
| Oxolinic acid | Catfish |
| Oxytetracycline | Catfish muscle |
| PCBs | Fish |
| Pesticides | Beef fat |
| Pesticides | Catfish muscle |
| Pesticides | Crayfish |
| Pesticides | Fish |
| Pesticides | Fruit, vegetables |
| Pesticides | Milk |
| Pesticides | Oysters |
| Sulfa drugs | Chicken tissues |
| Sulfadimethoxine | Catfish muscle |
| Sulfadimethoxine | Catfish, plasma |
| Sulfonamides | Infant formula |
| Sulfonamides | Milk |
| Sulfonamides | Salmon muscle |
| Sulfonamides | Swine muscle |
| Sulfonamides | Eggs |
| Tetracyclines | Milk |

with such methods on several levels and should be considered as an alternative when pursuing new analytical methodology. This is especially the case for solid or semi-solid biological materials.

*See also:* **II / Extraction:** Solid-Phase Extraction; Solvent Based Separation. **III / Solid-Phase Extraction with Cartridges. Sorbent Selection for Solid-Phase Extraction.**

## Further Reading

Barker SA (1992) Application of matrix solid-phase dispersion (MSPD) to the extraction and subsequent analysis of drug residues in animal tissues. In: Agarwal VK (ed.) *Analysis of Antibiotic Residues in Food Products of Animal Origin*, pp. 119–132. New York: Plenum Press.

Barker SA and Floyd ZE (1996) Matrix solid-phase dispersion (MSPD): implications for the design of new bonded-phase surface chemistries. In: Pesek JJ, Matyska MT and Abuelafiya RR (eds) *Chemically Modified Surfaces*: *Recent Developments*, pp. 66–71. Cambridge, UK: Royal Society of Chemistry.

Barker SA and Long AR (1992) Tissue drug residue extraction and monitoring by matrix solid-phase dispersion (MSPD)-HPLC analysis. *Journal of Liquid Chromatography* 15: 2071–2089.

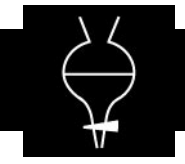
Barker SA, Long AR and Hines ME (1993) The disruption and fractionation of biological materials by matrix solid-phase dispersion. *Journal of Chromatography* 629: 23–34.

Barker SA, Long AR and Short CR (1989) Isolation of drug residues from tissues by solid-phase dispersion. *Journal of Chromatography* 475: 353–361.

Crouch MC and Barker SA (1997) Analysis of toxic wastes in tissues from aquatic species: applications of matrix solid-phase dispersion. *Journal of Chromatography* 774: 287–309.

US Patent # 5 272 094. Issued 21 December (1993) A bonded-phase matrix dispersion and extraction process. Isolation of drugs, and drug residues, from biological specimens, and tissues (Dr Steven A Barker; co-patent applicant, Dr Austin R Long, Louisiana State University).

# SOLID-PHASE MICROEXTRACTION



## Biomedical Applications

**H. Kataoka**, Okayama University, Tsushima, Okayama, Japan
**H. L. Lord and J. Pawliszyn**, University of Waterloo, Ontario, Canada



Analysis of drugs in biological samples is growing in importance owing to the need to understand the therapeutic and toxic effects of drugs and to continue the development of more selective and effective drugs. Furthermore, the screening and confirmation of abused drugs in body fluids is important for the detection of potential users of drugs and the control of drug addicts following withdrawal therapy. Simultaneous analysis of these drugs in biological samples

is required in many circumstances, such as clinical control for diagnosis and treatment of diseases, doping control, forensic analysis and toxicology. Although high efficiency instruments have been developed, most analytical instruments cannot handle the sample matrix directly. Therefore, sample preparation is very important to achieve a practical and reliable method for the analysis of complex matrices such as biological samples. In general, over 80% of analysis time is spent on sampling and sample preparation steps such as extraction, concentration and isolation of analytes. However, previous sample preparation techniques, such as liquid–liquid extraction and solid-phase extraction, have their problems. These techniques are generally time-consuming and require large volumes of samples and solvents. For example, a long sample preparation time limits the number of samples that can be analysed, and multistep procedures are prone to loss of analytes. Furthermore, the use of a large amount of solvent influences trace analysis, and also causes environmental pollution and health concerns. Ideally, sample preparation techniques should be fast, easy to use, inexpensive and compatible with a range of analytical instruments.

Solid-phase microextraction (SPME), developed by Pawliszyn and co-workers in 1990, is a new sample preparation technique using a fused-silica fibre that is coated on the outside with an appropriate stationary phase. The analyte in the sample is directly extracted onto the fibre coating. The method saves preparation time, solvent purchase and disposal costs, and can improve the detection limits. It has been used routinely in combination with gas chromatography (GC) and GC/mass spectrometry (GC/MS), and successfully applied to a wide variety of compounds, especially for the extraction of volatile and semi-volatile organic pollutants from water samples. SPME was also introduced for direct coupling with high performance liquid chromatography (HPLC) and LC/MS in order to analyse weakly volatile or thermally labile compounds not amenable to GC or GC/MS. The SPME/HPLC interface, equipped with a special desorption chamber, is utilized for solvent desorption prior to HPLC analysis, instead of thermal desorption in the injection port of the GC. Moreover, a new SPME/HPLC system known as in-tube SPME, was recently developed using an open-tubular fused-silica capillary column as the SPME device in place of the SPME fibre. In-tube SPME is suitable for automation, and automated sample handling procedures not only shorten the total analysis time, but also usually provide better accuracy and precision relative to manual techniques.

In this article, we review SPME techniques coupled with various analytical instruments and the applications of these techniques to drug analysis. The review consists of two main parts. In the first part, general aspects of SPME techniques are surveyed for fibre and in-tube SPME methods coupled with various instruments. In the second part, applications of the SPME methods in drug analysis are considered according to the drug type.

## SPME Techniques Coupled with Various Analytical Instruments

### Fibre SPME

The fibre SPME device consists of a fibre holder and fibre assembly with built-in fibre inside the needle. In fibre SPME, analytes are extracted directly from the sample onto a polymeric stationary phase coated on the fibre. When the fibre is inserted into the sample, the target analytes partition from the sample matrix into the stationary phase until equilibrium is reached. Two types of fibre SPME techniques can be used to extract analytes: headspace SPME and immersion SPME. In headspace SPME, the fibre is exposed in the headspace of gaseous, liquid or solid samples. In immersion SPME, the fibre is directly immersed in liquid samples. The fibre with concentrated analytes is then transferred to an instrument for desorption, followed by separation and quantification. Headspace and immersion SPME techniques can be used in combination with any GC, GC/MS, HPLC and LC/MS system. The process of the fibre SPME/GC method is shown in **Figure 1**.

In fibre SPME, the amount of analyte extracted onto the fibre depends on the polarity and thickness of the stationary phase coating on the fibre, extraction time, and the concentration of analyte in a sample. In general, volatile compounds require a thick polymer coat and a thin coat is effective for semi-volatile compounds. Extraction of analytes is also typically improved by agitation, addition of salt to the sample, changing the pH, and temperature. Although full equilibration is not necessary for accurate and precise analysis by SPME, consistent extraction time and other SPME parameters are essential. Furthermore, it is important to keep a consistent vial size and sample volume. In general, immersion SPME is more sensitive than headspace SPME for analytes predominantly present in the liquid. On the other hand, headspace SPME is suitable for the extraction of more volatile compounds. Extractions from biological samples by headspace SPME exhibit lower background than extractions obtained by immersion SPME. Because the headspace and immersion SPME techniques differ in kinetics, both approaches should be evaluated to optimize fibre SPME conditions for

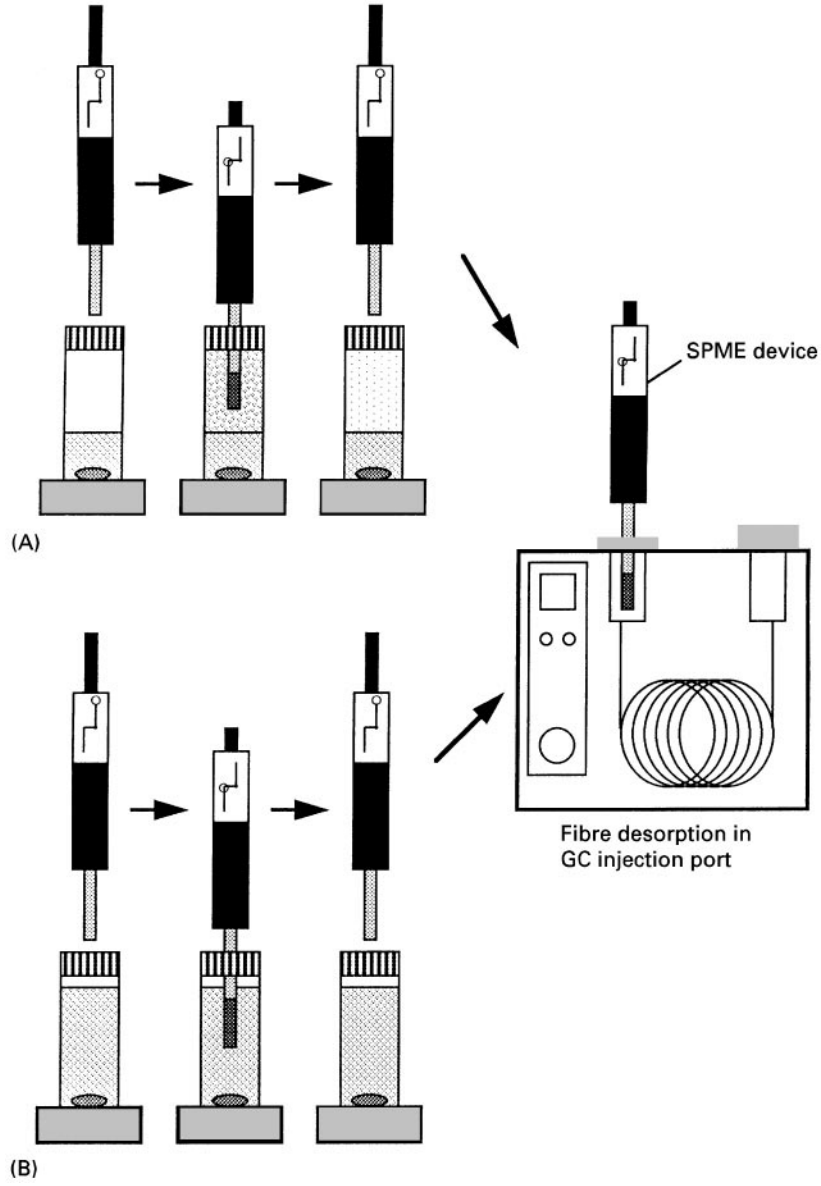



**Figure 1** Schematic illustration of headspace and immersion SPME/GC methods. (A) Headspace SPME; (B) direct immersion SPME.

analytes. Fibre SPME techiques in combination with GC or GC/MS are unsuitable for the extraction of less volatile or thermally labile compounds. Thus derivatization approaches are frequently used to extract polar compounds from biological samples. Four types of derivatization techniques in combination with SPME are implemented. Direct derivatization in the sample matrix is similar to well-established approaches used in solvent extraction. Analytes are extracted by SPME after derivatization in the vial. For in-coating derivatization with the fibre-doping method, simultaneous derivatization and extraction are directly performed in the fibre coating by a two-step process: (1) dope fibre with derivatization agent and (2) expose doped fibre to sample for extraction. This technique can be used for polar volatile compounds. Another in-coating derivatization technique is performed by the following two-step process: (1) dope fibre to sample for extraction and (2) expose doped fibre in the headspace of derivatizing agent. For derivatization in the injection port, the analyte extracted by SPME is desorbed in a GC injection port and then derivatized with additional reagent.

The desorption of analyte from the fibre coating is performed by heating the fibre in the injection port of a GC or GC/MS, or by loading solvent into the desorption chamber of the SPME/HPLC interface. Efficient thermal desorption of an analyte in a GC

injection port is dependent on the injection depth, injector temperature, and exposure time. A narrow-bore GC injector insert is required to ensure high linear flow and the fibre needs to be exposed immediately after the needle is introduced into the insert. Needle exposure depth should be adjusted to place the fibre in the centre of the hot injector zone. Desorption time is determined by the injector temperature and the linear flow rate around the fibre. The HPLC interface, on the other hand, consists of a six-port injection valve and a special desorption chamber, and requires solvent desorption of the analyte prior to HPLC or LC/MS analysis. A typical SPME/HPLC interface is shown in **Figure 2**. The desorption chamber is placed in the position of the injection loop. After sample extraction, the fibre is inserted into the desorption chamber at the 'load' position under ambient pressure. When the injector is changed to the 'inject' position, mobile phase contacts the fibre, desorbs the analytes, and delivers them to the HPLC column for separation. Two desorption techniques can be used to remove the analytes from the fibre: dynamic desorption and static desorption. In dynamic desorption, the analytes can be removed by a moving stream of mobile phase. When the analytes are more strongly adsorbed to the fibre, the fibre can be soaked in mobile phase or other strong solvent for a specified time by static desorption before injection onto the HPLC column. In each desorption technique, rapid and complete desorption of analytes using minimal solvent is important for optimizing the SPME/HPLC or SPME/LC/MS methods.

### In-tube SPME

In-tube SPME using an open-tubular capillary column as the SPME device was developed for coupling with HPLC or LC/MS. It is suitable for automation, and can continuously perform extraction, desorption and injection using a standard autosampler. With the in-tube SPME technique, organic compounds in aqueous samples are directly extracted from the sample into the internally coated stationary phase of a capillary column, and then desorbed by introducing a moving stream of mobile phase or static desorption solvent when the analytes are more strongly absorbed to the capillary coating. A schematic diagram of the automated in-tube SPME/LC/MS system is shown in **Figure 3**. The capillaries selected have coatings similar to those of commercially available SPME fibres. The capillary column is placed between the

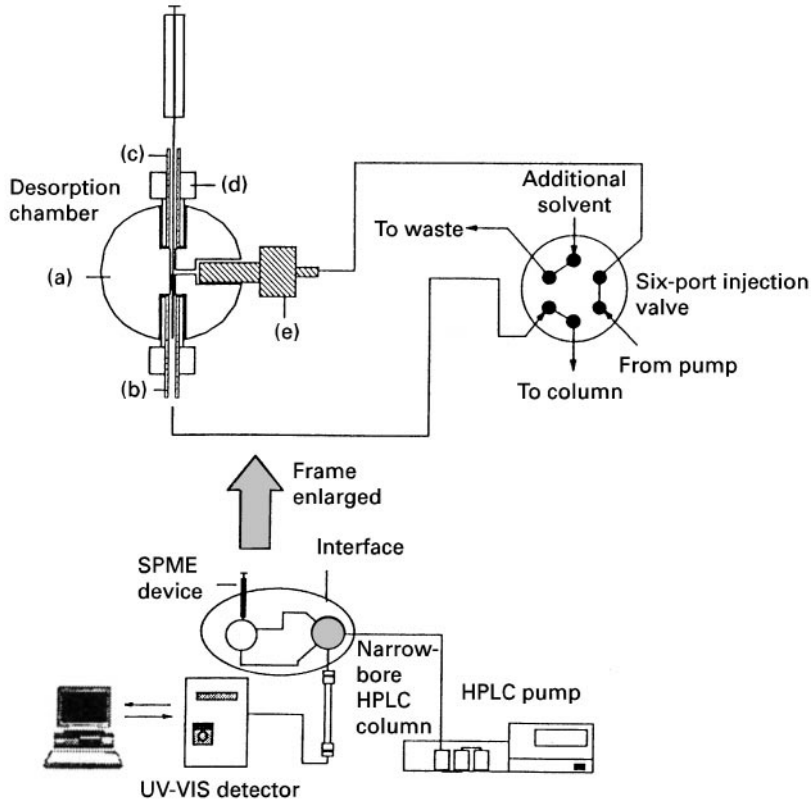



**Figure 2** Schematic of the SPME-HPLC system. (a) Stainless steel (SS) 1/16 inch tee joint; (b) 1/16 inch o.d., 0.02 inch i.d., SS tubing; (c) 1/16 inch o.d. poly(ether ether ketone) (PEEK) tubing (0.02 inch i.d.); (d) two-piece finger-tight PEEK union; (e) PEEK tubing (0.005 inch i.d.) with a one-piece PEEK union. (Reproduced with permission from Pawliszyn J (1997) *Solid Phase Microextraction: Theory and Practice*. Translated by permission of John Wiley & Sons, Inc. All rights reserved.)

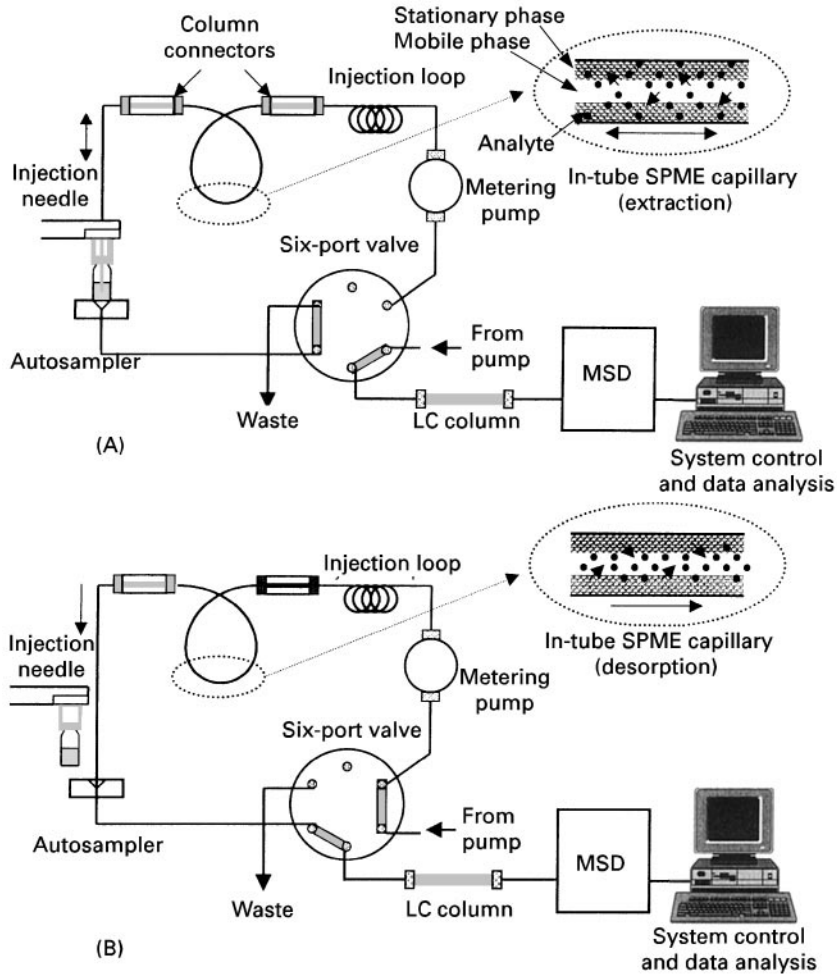



**Figure 3** Schematic of the in-tube SPME/LC/MS system. (A) Load position (extraction phase); (B) injection position (desorption phase). (Reproduced with permission from Kataoka H, Narimatsu S, Lord HL and Pawliszyn J (1999) *Analytical Chemistry* 71: 4237. Copyright American Chemical Society.)

injection loop and the injection needle of the HPLC autosampler. While the injection syringe repeatedly draws and ejects sample from the vial under computer control, the analytes partition from the sample matrix into the stationary phase until equilibrium is reached. Subsequently, the extracted analytes are directly desorbed from the capillary coating by mobile phase flow or by aspirating a desorption solvent. The desorbed analytes are transported to the HPLC column for separation, and then detected with UV or a mass selective detector (MSD).

In in-tube SPME, the amount of analyte extracted by the stationary phase of the capillary column depends on the polarity of capillary coating, number and volume of draw/eject cycles and the sample pH. A capillary column 50–60 cm long is optimal for extraction. Below this level, extraction efficiency is reduced, and above this level, peak broadening is observed. In general, complete equilibrium extraction is not obtained for any of the analytes, because the analytes are partially desorbed into the mobile phase during each eject step. The target analytes with higher *K*-values need longer equilibration times. Although an increase in number and volume of draw/eject cycles can enhance the extraction efficiency, peak broadening is often observed in this case. The optimal flow rate of draw/eject cycles is 50–100 $\mu L\ min^{-1}$. Below this level, extraction requires an inconveniently long time, and above this level, bubbles form on the inside of the capillary and extraction efficiency is reduced. The in-tube SPME technique does not need a special SPME/HPLC interface for desorption of analytes. The analytes extracted onto the capillary coating can be easily desorbed by a moving stream of mobile phase or desorption solvent when the analytes are more strongly adsorbed to the capillary coating. Carryover in the in-tube SPME method is lower or eliminated in comparison with the fibre-SPME method.

Although the theories of fibre and in-tube SPME methods are similar, the significant difference between these methods is that the extraction of analytes

is performed on the outer surface of the fibre for fibre-SPME and on the inner surface of the capillary column for in-tube SPME. Therefore, with the in-tube SPME method, it is necessary to prevent plugging of the capillary column and flow lines during extraction, and typically particles must be removed from samples by filtration before extraction. On the other hand, with the fibre-SPME method, it is not necessary to remove particles before extraction because they are removed by washing the fibre with water before insertion into the desorption chamber of the SPME/HPLC interface. Another significant difference between in-tube SPME and manual fibre-SPME/HPLC is the possible decoupling of desorption and injection with the in-tube SPME method. In the fibre-SPME method, analytes are desorbed during injection as the mobile phase passes over the fibre. On the other hand, in the in-tube SPME method, analytes are desorbed by mobile phase or by aspirating a desorption solvent from a second vial, and then transferred to the HPLC column by mobile-phase flow. The fibre-SPME/HPLC method also has the advantage of eliminating the solvent peak from the chromatogram, but peak broadening is sometimes observed because analytes can be slow to desorb from the fibre. With the in-tube SPME method, peak broadening is not observed because analytes are completely desorbed before injection.

## Biomedical Applications: Drug Analysis

SPME methods applied to the analysis of various abused and therapeutic drugs in biological samples are listed in **Table 1**, according to the drug type, sample type, extraction device, extraction mode, and analytical technique. The SPME methods using 100-μm polydimethylsiloxane (PDMS) fibres in combination with GC or GC/MS are widely used for the analysis of various drugs. The SPME methods coupled with HPLC or LC/MS are used for the analysis of less volatile or thermally labile drugs. For recent reviews of some of these methods for drug analysis see Pawliszyn, Lord and Pawliszyn, Namera *et al.*, Junting *et al.*, Kataoka *et al.* and Sporkert and Pragst in the Further Reading section.

### Amphetamines and Related Compounds

Yashiki and co-workers developed a simple and rapid method for analysing amphetamine (AM) and methamphetamine (MA) in urine and blood samples by headspace SPME and GC/MS-selected ion monitoring (SIM). In order to move the analytes into the headspace, the sample was heated at 80°C for 20 min under $K_2CO_3$ or NaOH alkaline conditions. Subsequently, a 100-μm PDMS fibre was exposed to the headspace for 5 min, and then inserted into the injection port of GC/MS for desorption. The method was twenty times more sensitive than the conventional headspace method. Lord and Pawliszyn optimized several extraction parameters for the analysis of AM and MA in urine samples by headspace SPME/GC-flame ionization detection (FID). Centini *et al.* and Battu *et al.* reported simultaneous analysis of amphetamines and their analogues, such as 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyethylamphetamine (MDEA), in urine samples by headspace SPME using a 100-μm PDMS fibre. As shown in **Figure 4**, a clean total-ion chromatogram is obtained from a urine sample spiked with 100 ng $mL^{-1}$ of each of the 21 central nervous system stimulants and extracted by the headspace SPME method. Koide *et al.* applied this technique to the analysis of amphetamines in hair samples.

Degel, Penton, Ishii *et al.*, Makino *et al.* and Myung *et al.* used the direct immersion technique in order to improve the extraction efficiency and sensitivity. The extraction recoveries of AM and MA by the immersion SPME method are several times higher than those by the headspace SPME method. Ugland *et al.* reported an SPME technique in combination with derivatization. After derivatization with alkylchloroformate, amphetamines and their methylenedioxy analogues were analysed by immersion-fibre SPME/GC-nitrogen-phosphorus detection (NPD) or GC/MS.

Kataoka *et al.* developed an in-tube SPME/LC/MS method for the analysis of amphetamines and their methylenedioxy analogues using Omegawax (Supelco, Bellefonte, PA, USA) capillary as the extraction device. As shown in **Figure 5**, these drugs spiked into urine samples were selectively analysed without interference peaks by SIM-mode detection.

### Anaesthetics

Kumazawa *et al.* developed headspace and direct-immersion-SPME methods for the analysis of ten local anaesthetics in blood samples. These drugs were extracted with 100-μm PDMS fibres after deproteinization of the sample with perchloric acid. Heating in a NaOH and $(NH_4)_2SO_4$ solution during headspace SPME gave the best recoveries of the drugs and the cleanest backgrounds. The recoveries for all drugs in the sample mixture at neutral pH in the presence of NaCl were greater than for that of a sample at the same pH without NaCl (see **Figure 6**). Although a small amount of background noise appeared in the direct immersion-SPME method, the advantage of using immersion-SPME is that recovery is much better than that of headspace-SPME.

**Table 1** SPME methods for the analysis of drugs in biological samples

| *Drugs* | *Specimen* | *Extraction device* | *Extraction mode*[a] | *Hyphenated analysis* | *Reference* |
|---|---|---|---|---|---|
| *Amphetamines and related compounds* | | | | | |
| AM, MA | Urine | 100-μm PDMS fibre | HS | GC/MS | *Forensic Sci. Int.* (1995) 76: 169. |
| AM, MA | Blood | 100-μm PDMS fibre | HS | GC/MS | *Forensic Sci. Int.* (1996) 78: 95. |
| AM, MA, MDMA, MDEA | Urine | 100-μm PDMS fibre | HS | GC/MS | *Forensic Sci. Int.* (1996) 83: 161. |
| AM, MA | Urine | 100-μm PDMS fibre | DI | GC/MS | *Clin. Biochem.* (1996) 29: 529. |
| AM, MA | Urine | 100-μm PDMS fibre | DI | GC/FID | *Can. Soc. Forensic Sci. J.* (1996) 29: 43. |
| AM, MA | Urine | 65-μm PDMS/DVB fibre | DI | GC/NPD | *Jpn. J. Forensic Toxicol.* (1996) 14: 228. |
| AM, MA | Urine | 100-μm PDMS fibre | HS | GC/FID | *Anal. Chem.* (1997) 69: 3899. |
| AM, MA | Urine | 100-μm PDMS fibre | D + DI | GC/NPD<br>GC/MS | *J. Chromatogr. B* (1997) 701: 29. |
| MA | Urine | 100-μm PDMS fibre | DI | GC/NPD<br>GC/MS | *Chromatography* (1997) 18: 185. |
| AM, MA, MDA, MDMA, MDEA etc. | Urine | 100-μm PDMS fibre | HS | GC/MS | *J. Chromatogr. Sci.* (1998) 36: 1. |
| AM, MA | Hair | 100-μm PDMS fibre | HS | GC/NPD | *J. Chromatogr. B* (1998) 707: 99. |
| AM, MA, MDMA | Urine | 100-μm PDMS fibre | DI | GC/MS | *J. Chromatogr. B* (1998) 716: 359. |
| AM, MA, MDA, MDMA, MDEA | Urine | 100-μm PDMS fibre | D + DI | GC/NPD<br>GC/MS | *J. Pharm. Biomed. Anal.* (1999) 19: 463. |
| AM, MA, MDA, MDMA, MDEA | Urine | Omegawax 250 capillary | IT | LC/MS | *J. Chromatogr. B* (submitted).<br>*J. Anal. Toxicol.* (2000) 24: in press. |
| *Anaesthetics* | | | | | |
| Lidocaine etc. | Blood | 100-μm PDMS fibre | HS | GC/FID | *Jpn. J. Forensic Toxicol.* (1995) 13: 182. |
| Lidocaine etc. | Blood | 100-μm PDMS fibre | DI | GC/FID | *Chromatographia* (1996) 43: 59. |
| Phencyclidine | Urine, blood | 100-μm PDMS fibre | HS | GC/SID | *Chromatographia* (1996) 43: 331. |
| Lidocaine etc. | Blood | 100-μm PDMS fibre | HS | GC/MS | *J. Chromatogr. B* (1998) 709: 225. |
| Lidocaine | Urine | 100-μm PDMS fibre | DI | GC/FID<br>HPLC/UV | *Chromatographia* (1998) 47: 678. |
| *Anorectics* | | | | | |
| Fenfluramine | Urine | 30-μm PDMS fibre | DI | GC/MS | *J. Microcolumn Sep.* (1997) 9: 249. |
| *Antidepressants* | | | | | |
| Amitriptyline, imipramine etc. | Urine | 100-μm PDMS fibre | HS | GC/FID | *Jpn. J. Forensic Toxicol.* (1995) 13: 25. |
| Amitriptyline | Urine | 100-μm PDMS fibre | DI | GC/MS | *Clin. Biochem.* (1996) 29: 529. |
| Amitriptyline, imipramine etc. | Plasma | 100-μm PDMS fibre | DI | GC/NPD | *J. Chromatogr. B* (1997) 696: 217. |
| Amitriptyline, imipramine etc. | Blood | 100-μm PDMS fibre | HS | GC/FID | *J. Chromatogr. Sci.* (1997) 35: 302. |
| Maprotiline, mianseline, seliptiline | Blood | 100-μm PDMS fibre | HS | GC/MS | *J. Anal. Toxicol.* (1998) 22: 396. |
| *Antiepileptics* | | | | | |
| Valproic acid | Plasma | 100-μm PDMS fibre | DI | GC/FID | *J. Chromatogr. B* (1995) 673: 299. |

**Table 1** *Continued*

| *Drugs* | *Specimen* | *Extraction device* | *Extraction mode*[a] | *Hyphenated analysis* | *Reference* |
|---|---|---|---|---|---|
| *Antihistaminics* | | | | | |
| Diphenhydramine etc. | Urine, blood | 100-μm PDMS fibre | HS | GC/FID | *J. Chromatogr. Sci.* (1997) 35: 275. |
| Ranitidine | Urine | Omegawax 250 capillary | IT | LC/MS | *J. Chromatogr. B* (1999) 731: 353. |
| *Antihypertensives* | | | | | |
| Propranolol etc. | Urine, serum | Omegawax 250 capillary | IT | LC/MS | *Anal. Chem.* (1999) 71: 4237. |
| *Antipsychotics* | | | | | |
| Promazine etc. | Urine, blood | 100-μm PDMS fibre | HS | GC/FID | *Jpn. J. Forensic Toxicol.* (1996) 14: 30. |
| *Barbiturates* | | | | | |
| Barbital etc. | Urine | 65-μm Carbowax/DVB fibre | DI | GC/MS | *J. Chromatogr. A* (1997) 777: 275. |
| *Benzodiazepines* | | | | | |
| Diazepam | Urine | 100-μm PDMS fibre | DI | GC/MS | *Clin. Biochem.* (1996) 29: 529. |
| Diazepam | Plasma | Solvent-modified 85-μm PA fibre | DI | GC/NPD | *J. Chromatogr. B* (1997) 689: 357. |
| Diazepam etc. | Urine | 65-μm PDMS/DVB fibre | DI | GC/FID | *Jpn. J. Forensic Toxicol.* (1997) 15: 16. |
| Diazepam etc. | Urine | 85-μm PA fibre | DI | HPLC/UV | *Chromatography* (1997) 18: 244. |
| Diazepam etc. | Urine, serum | 65-μm Carbowax/DVB fibre | DI | GC/FID<br>GC/MS | *J. Microcolumn Sep.* (1998) 10: 193. |
| Benzodiazepine metabolites | Urine | 100-μm PDMS, 85-μm PD fibre | DI | GC/ECD | *J. Anal. Toxicol.* (1999) 23: 54. |
| *Narcotics and other illicit drugs* | | | | | |
| Cocaine | Urine | 100-μm PDMS fibre | DI | GC/NPD | *Jpn. J. Forensic Toxicol.* (1995) 13: 207. |
| Meperidine | Urine, blood | 100-μm PDMS fibre | HS | GC/FID | *Jpn. J. Forensic Toxicol.* (1995) 13: 211. |
| Cocaine | Urine | 100-μm PDMS fibre | DI | GC/NPD<br>GC/MS | *Chromatography* (1997) 18: 185. |
| Morphine, heroin etc. | Urine | 65-μm PDMS/DVB 100-μm PDMS fibre | HS<br>DI | GC/FID | *Anal. Chem.* (1997) 69: 3899. |
| Cannabinoids | Saliva | 100-μm PDMS fibre | DI | GC/MS | *Anal. Chem.* (1998) 70: 1788. |
| Cannabinoids | Hair | 30-μm PDMS fibre | DI | GC/MS | *J. Anal. Toxicol.* (1999) 23: 7. |
| *Nicotine* | | | | | |
| Nicotine , Cotinine | Urine | 100-μm PDMS fibre | HS | GC/MS | *Jpn. J. Forensic Toxicol.* (1995) 13: 17. |
| Nicotine, Cotinine | Urine | 100-μm PDMS fibre | DI | GC/FID | *Clin. Biochem.* (1996) 29: 529. |
| *Steroids* | | | | | |
| Estrone, estradiol etc. | Serum | 85-μm PA fibre | DI + D | GC/MS | *J. High Resolut. Chromatogr.* (1997) 20: 171. |
| Corticosteroids | Urine | 65-μm Carbowax/ DVB fibre | DI | LC/MS | *Rapid Commun. Mass Spectrom.* (1997) 11: 1926. |

[a]HS: headspace; DI: direct immersion; IT: in-tube; D: derivatization.

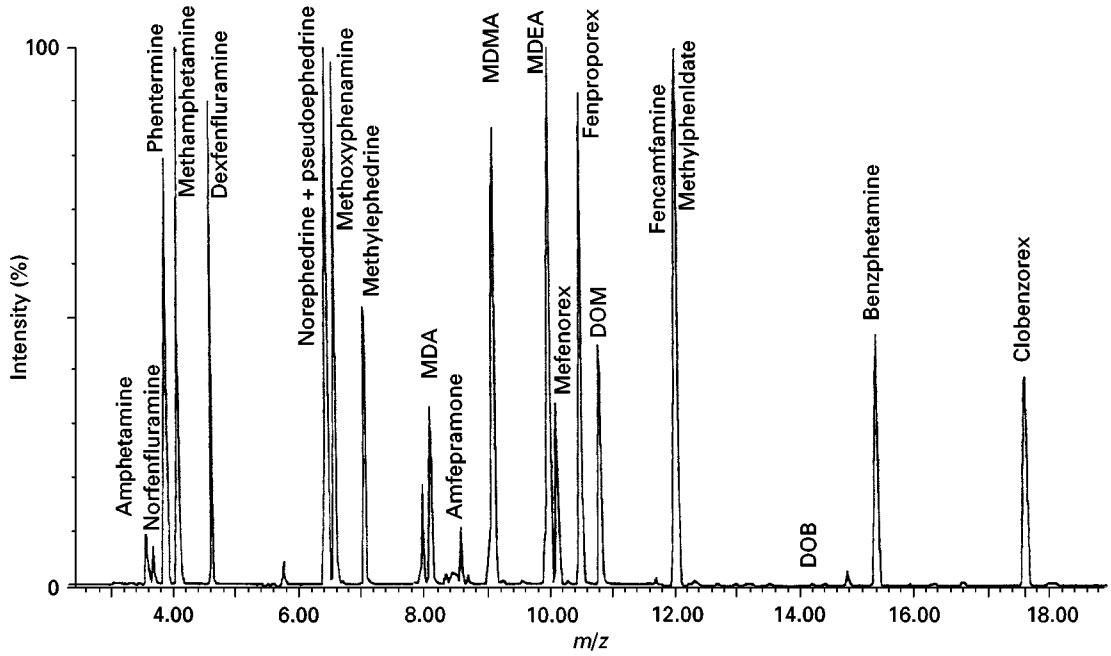



**Figure 4** Total-ion chromatogram of a urine sample extract spiked with 21 central nervous system stimulants at 1000 μg $L^{-1}$. SPME conditions: fibre, 100 μm PDMS; extraction, at 80°C headspace for 10 min with stirring; desorption, exposure for 10 min in GC injection port. GC/MS conditions: column, PTA-5 (30 m × 0.32 mm i.d., 0.5 μm film thickness); injector, splitless mode at 200°C; split opening time, 2 min; oven temperature, programme from 60 to 120°C at 30°C $min^{-1}$, then to 210°C at 5°C $min^{-1}$, and finally to 280°C at 30°C $min^{-1}$ and hold at 280°C for 5 min; transfer line and detector temperature, 280°C; helium flow-rate, 1.3 mL $min^{-1}$, ionization, 70 eV. (Reproduced with permission from Battu C, Marquet P, Fauconnet AL, Lacassie E and Lachâtre G (1998) *Journal of Chromatographic Science* 36: 1, by permission of Preston Publications, A Division of Preston Industries, Inc.)

Furthermore, Kumazawa *et al.* reported a method for analysis of phencyclidine in urine and whole blood by headspace-SPME and GC with a surface ionization detector (SID). Watanabe *et al.* developed a simple method for analysis of five local anaesthetics in blood samples by headspace SPME using a 100-μm PDMS fibre and GC/MS-SIM. Koster *et al.* reported direct immersion-SPME methods coupled with GC-FID and HPLC-UV for the determination of lidocaine in urine samples. Desorption of the PDMS fibre in HPLC is more complicated than the desorption in GC, because it is dependent on the composition of the mobile phase or the desorption solvent.

### Antidepressants

Kumazawa *et al.* developed a simple headspace-SPME method for the analysis of four tricyclic antidepressants in urine and whole-blood samples. These drugs were extracted with a 100-μm PDMS fibre after heating at 100°C in the presence of a NaOH solution. Namera *et al.* reported a headspace-SPME/GC-MS method for the analysis of three tetracyclic antidepressants in whole-blood samples, and its application to a medicolegal case of setiptiline intoxication. Ulrich and Martens developed a direct immersion-SPME method for the simultaneous analysis of ten antidepressant drugs and metabolites in plasma samples, and applied the method to toxicological analysis after the accidental or suicidal intake of higher doses. The sample was extracted with a 100-μm PDMS fibre for 10 min and the fibre was exposed in the GC injection port at 260°C for 1 min after washing in 50% methanol and subsequent drying at room temperature. As shown in **Figure 7**, these drugs in plasma samples were selectively analysed by NPD without interference peaks. However, the recoveries of antidepressants from plasma samples were very low due to the high protein binding of these drugs. The limits of quantification for these drugs in plasma samples were 90–200 ng $mL^{-1}$. The sensitivity can be considerably improved by increasing the extraction time and dilution of plasma samples with water.

### Benzodiazepines

Krogh *et al.* developed a direct immersion-SPME method in combination with GC-NPD for the analysis of diazepam in plasma samples. The polyacrylate (PA) fibre doped with 1-octanol was used to extract diazepam from the samples. The solvent-modified PA fibre was found to be more efficient in the extraction of diazepam than the untreated PA and PDMS fibres. This technique offers sufficient enrichment for bioanalysis, high selectivity, and short sample preparation time. However, the potential of the

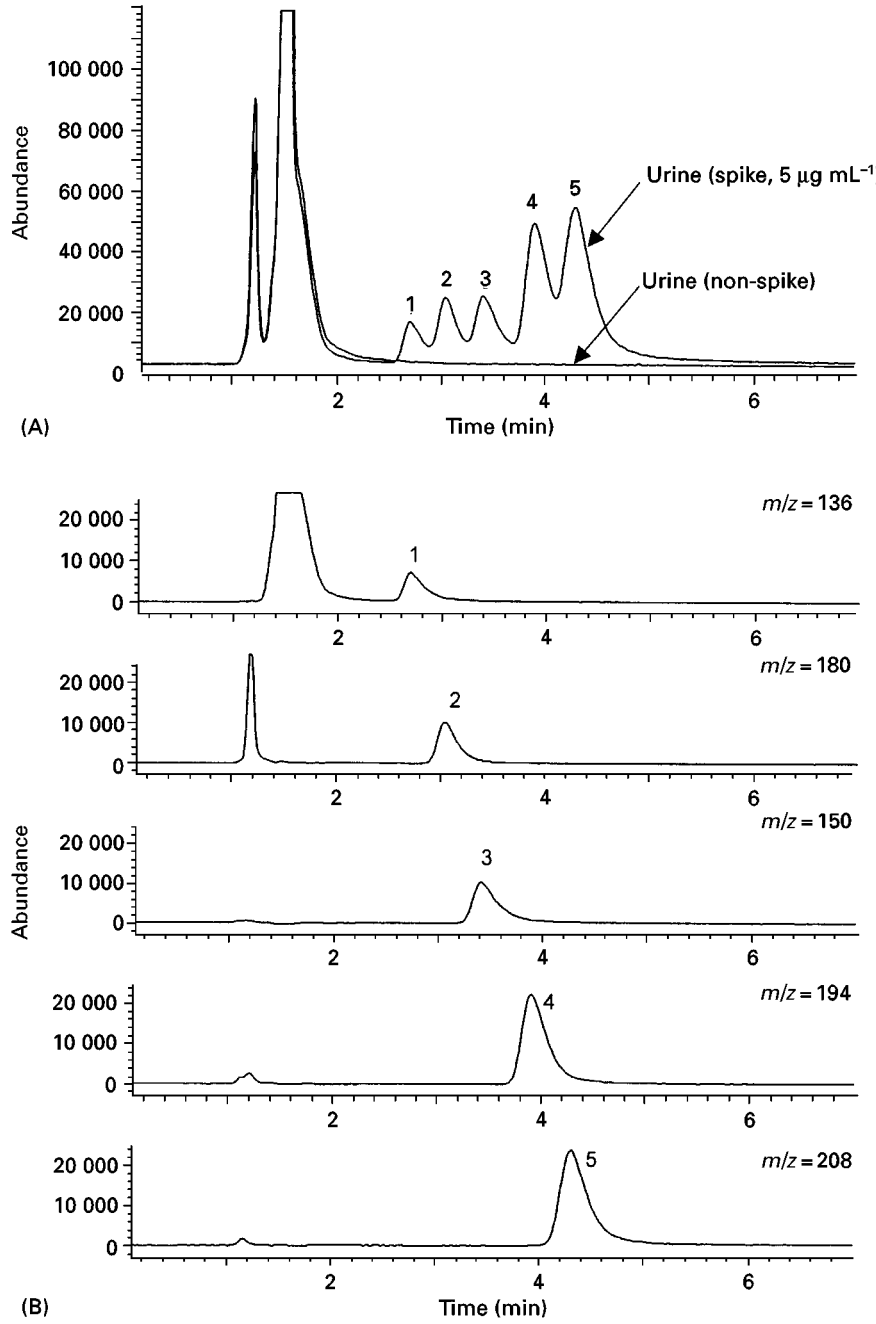



**Figure 5** Total ion and SIM chromatograms obtained from urine samples spiked with amphetamines by in-tube SPME/LC/MS. (A) Total ion chromatograms obtained from urine and spiked urine samples; (B) SIM chromatograms obtained from spiked urine sample. Urine sample (10 μL) was diluted ten times with water and used for analysis after filtration. Stimulants were spiked at a concentration of 5 mg $mL^{-1}$ urine. LC/MS conditions: column, Supelcosil LC-CN (3.3 cm × 4.6 mm i.d., 3 μm particle size); column temperature, 25°C; mobile phase, acetonitrile/50 mM ammonium acetate (15 : 85); flow-rate, 0.4 mL $min^{-1}$; fragmentor voltage, 40 V; ionization mode, positive ESI; SIM ion, $m/z = 136$ (AM), 150 (MA), 180 (MDA), 194 (MDMA) and 208 (MDEA). In-tube SPME conditions: capillary, Omegawax 250 (60 cm × 0.25 mm i.d., 0.25 μm film thickness); sample pH, 8.5; draw/eject cycles, 15; draw/eject volume, 35 μL; draw/eject flow-rate, 100 μL $min^{-1}$, desorption solvent, mobile phase. Peaks: 1, AM; 2, MDA; 3, MA; 4, MDMA; and 5, MDEA. (Reproduced with permission from Kataoka H, Lord HL and Pawliszyn J (2000) *Journal of Analytical Toxicology* 24: 263, by permission of Preston Publications, A Division of Preston Industries, Inc.)

solvent-modified SPME technique is limited by the incompatibility of the SPME coatings with most organic solvents. Luo *et al.* developed a direct immersion-SPME method for the simultaneous analysis of five benzodiazepines in urine and serum samples. These drugs were efficiently extracted from these samples with a 65-μm Carbowax/divinylbenzene (DVB) fibre under conditions of saturated salt with

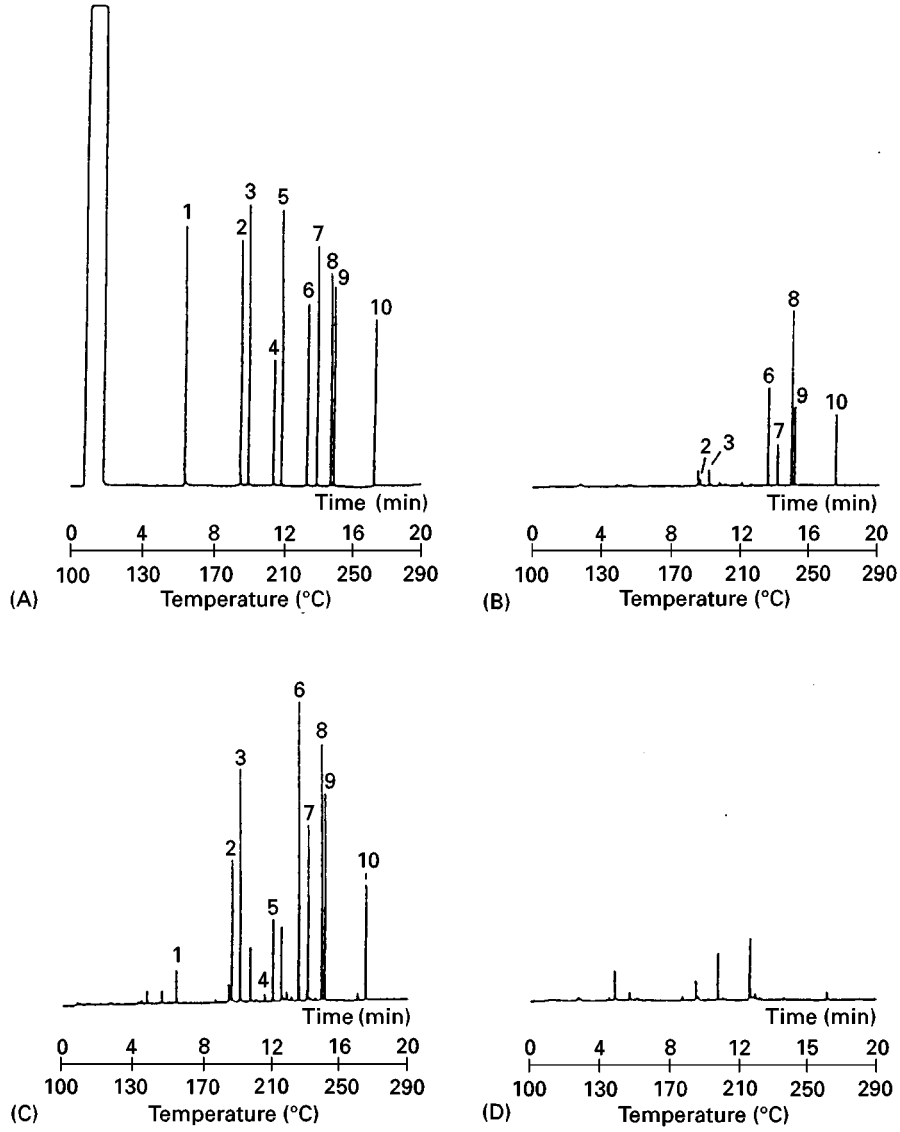



**Figure 6** Capillary GC of ten local anaesthetics extracted from human whole blood by use of direct immersion-SPME. (A) The authentic drugs (50 ng each on column); (B) a drug extract at pH 7 without salt; (C) a drug extract at pH 7 in the presence of 0.5 g NaCl; (D) a blank extract at pH 7 in the presence of 0.5 g NaCl. The mixture of ten drugs (5 μg each) was added to 1 mL of human whole blood. SPME conditions: fibre, 100 μm PDMS; extraction, at room temperature for 40 min with stirring; desorption, 1 min exposure in GC injection port. GC conditions: column, DB-17 (30 m × 0.25 mm i.d., 0.25 μm film thickness); column temperature, initially hold at 100°C for 1 min and increase to 290°C at 10°C $min^{-1}$; injector and detector temperatures, 250°C; He carrier gas flow-rate, 3 mL $min^{-1}$; injection, splitless; detector, FID. Peaks: 1, ethyl aminobenzoate; 2, prilocaine; 3, lidocaine; 4, procaine; 5, mepivacaine; 6, tetracaine; 7, bupivacaine; 8, *p*-(butylamino)benzoic acid-2-(diethylamino)ethyl ester; 9, benoximate; and 10, dibucaine. (Reproduced with permission from Kumazawa T, Sato K, Seno H, Ishii A and Suzuki O (1996) *Chromatographia* 43: 59.)

pH 7 and sampling at 45°C with agitation, and analysed by GC-MS.

Guan *et al.* analysed the metabolites of benzodiazepines from acid-hydrolysed urine samples using a direct immersion-SPME method in combination with GC-electron capture detection (ECD). The detection limits were 2–20 ng $mL^{-1}$ for most drugs tested. Jinno and Taniguchi, however, developed an SPME method coupled with HPLC for the analysis of six benzodiazepines in human urine samples. Sensitivity may be increased by the combination of saturated salt and weakly alkaline conditions in the extraction matrix. As shown in **Figure 8**, a 65-μm PA fibre was found to be more efficient in the extraction of benzodiazepines than a 100-μm PDMS fibre.

### Narcotics and Other Illicit Drugs

Kumazawa *et al.* and Makino *et al.* developed direct immersion SPME methods in combination with GC-NPD for the rapid analysis of cocaine in urine

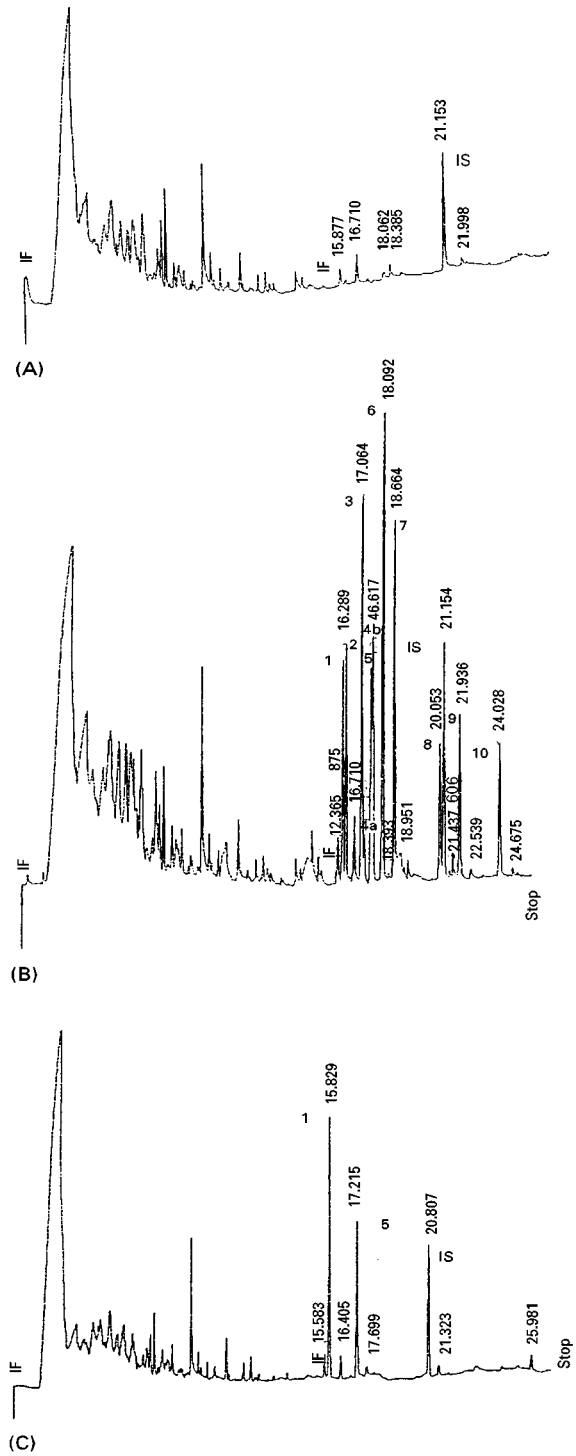



**Figure 7** Typical SPME-GLC-NPD chromatograms obtained from (A) blank plasma with internal standard, (B) plasma spiked with ten antidepressant drugs and metabolites, each 375 ng $mL^{-1}$, and (C) a sample of a patient after suicidal intoxication with amitriptyline (amitriptyline, 766 ng $mL^{-1}$; nortriptyline, 489 ng $mL^{-1}$. SPME conditions: fibre, 100-μm PDMS; extraction, shaking at 700 rpm for 10 min at 22°C; desorption, 1 min exposure in GC injection port. GC conditions: column, DB-1 (30 m × 0.32 mm i.d., 0.25 μm film thickness); column temperature, programme from 140°C to 220°C at 20°C $min^{-1}$ and from 220°C to 270°C at 2°C $min^{-1}$; injector and detector temperatures, 260°C and 300°C, respectively; $N_2$ carrier gas flow-rate, 0.7 mL $min^{-1}$; injection, splitless; detector, NPD. Peaks: 1, amitriptyline; 2, trimipramine; 3, imipramine; 4a, *cis*-doxepine; 4b, *trans*-doxepine; 5, nortriptyline; 6, mianserine; 7, desipramine; 8, maprotiline; 9, clomipramine; and 10, desmethylclomipramine. IS, internal standard (chloramitriptyline). (Reproduced with permission from Ulrich S and Martens J (1997) *Journal of Chromatography B* 696: 217. Copyright Elsevier Science.)

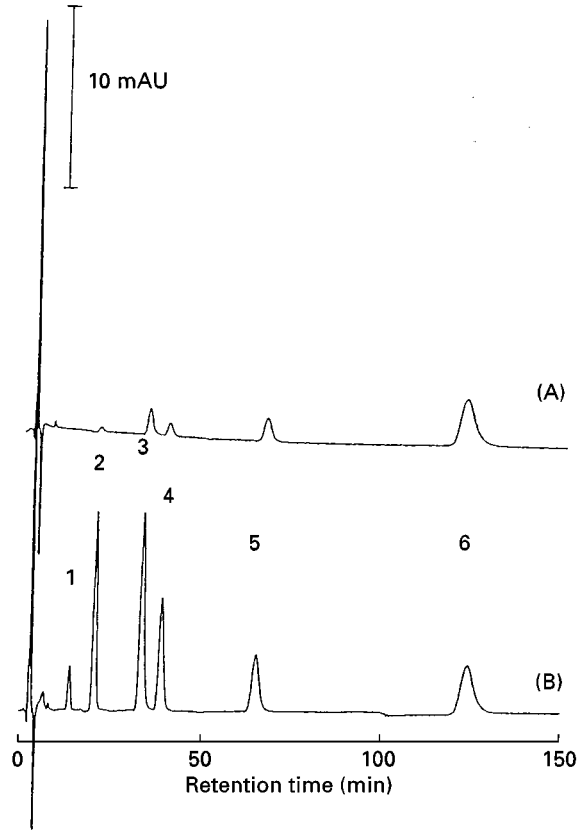



**Figure 8** Chromatograms of extracted drugs with (A) 100-μm PDMS and (B) 85-μm PA. SPME conditions: extraction, stirring at 840 rpm for 3 h at 60°C; desorption, 30 min exposure in desorption chamber. HPLC conditions: column, Siperiorex ODS (250 mm × 1.5 mm i.d.); mobile phase, acetonitrile/water; flow-rate, 100 μL $min^{-1}$; detection, UV at 220 nm. Peaks: 1, nitrazepam; 2, flunitrazepam; 3, fludiazepam; 4, diazepam; 5, clotiazepam; and 6, medazepam. (Reproduced with permission from Jinno K and Taniguchi M (1997) *Chromatography* 18: 244.)

samples. Recovery of cocaine by this technique using a 100-μm PDMS fibre was 20%, and the detection limit was about 12 ng $mL^{-1}$. Lord and Pawliszyn applied the SPME/GC-FID method developed for amphetamines to the analysis of meperidine, codeine, methadone, morphine and heroin in spiked urine samples. Furthermore, Hall *et al.* applied an immersion SPME technique to the analysis of four cannabinoids in human saliva. These drugs were extracted with a 100-μm PDMS fibre and analysed in the range from 5 to 500 ng $mL^{-1}$ by GC/MS. Using this method, $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THA) was detected in a saliva sample collected 30 min after the subject had smoked marijuana (**Figure 9**).

Strano-Rossi and Chiarotti reported an immersion SPME method using a 30-μm PDMS fibre in combination with GC/MS for the analysis of cannabinoids in alkaline hydrolysed hair samples. The method is also applied to the analysis of other drugs such as methadone, cocaine and cocaethylene in hair samples.

### Other Drugs

Yashiki *et al.* developed a simple and rapid method for the analysis of nicotine and its principal metabolite, cotinine, in urine samples using headspace SPME and GC/MS-SIM. Krogh *et al.* applied a direct immersion SPME technique to the analysis of the antiepileptic drug valproic acid in plasma samples. The drug was extracted with a 100-μm PDMS fibre after dialysis of plasma samples, and then analysed by GC-FID. Seno *et al.* developed headspace SPME methods for the simple analysis of five phenothiazine drugs and thirteen diphenylmethane antihistaminic drugs and their analogues in urine and whole blood samples. A 100-μm PDMS fibre was exposed in the headspace of the sample vial after preheating of the sample in the presence of NaOH, and the drugs extracted in the fibre were analysed by GC-FID. The recoveries from blood extracts were lower than those from urine extracts for all drugs. Hall and Brodbelt reported a direct immersion SPME method coupled with ion-trap GC/MS for the analysis of eight barbiturates in

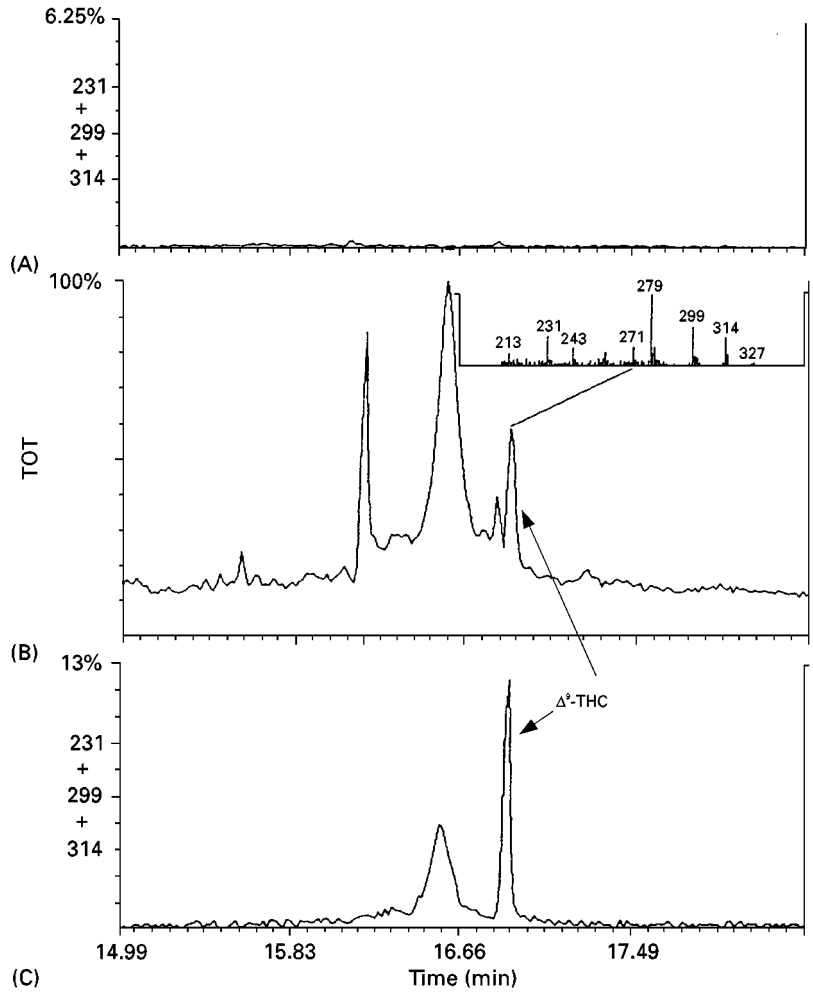



**Figure 9** Chromatograms after performing SPME on human saliva samples prior to and after marijuana smoking. (A) SIM chromatogram of saliva sample before marijuana smoking; (B) total ion chromatogram of saliva sample after marijuana smoking; (C) SIM chromatogram of saliva sample after marijuana smoking. SPME conditions: fibre, 100 μm PDMS; extraction, immersion for 10 min with stirring; desorption, exposure for 12 min in GC injection port. GC/MS conditions: column, DB-5ms (30 m × 0.25 mm i.d., 0.5 μm film thickness); oven temperature, initially hold at 50°C for 0.2 min and increase to 280°C at 15°C $min^{-1}$, and finally hold at 280°C for 2 min; transfer line temperature, 280°C; detection, ion trap (electron ionization mode); SIM ion, $\Delta^9$ -THC ($m/z = 231$, 299, 314). (Reproduced with permission from Hall BJ, Satterfield-Doerr M, Parikh AR and Brodbelt JS (1998) *Analytical Chemistry* 70: 1788. Copyright American Chemical Society.)

urine samples. A 65-μm Carbowax/DVB fibre was suitable for the extraction of these drugs. The detection limits reached 1 ng $mL^{-1}$. Okeyo *et al.* developed a straightforward method for performing derivatizing reactions of five steroids *in situ* in SPME fibres. After extraction of drugs from serum samples by direct immersion SPME, the drugs extracted on 85-μm PA fibre were derivatized in the headspace of the silylating reagent bis(trimethylsilyl)trifluoro-acetamide, and then analysed by GC/MS. With derivatization, SPME and GC analysis can be easily extended to the analysis of semi- and non-volatile compounds.

Volmer and Hui developed a SPME/LC/MS method for isolating and analysing eleven corticosteroids and two steroid conjugates from urine samples. After extraction in the vial by direct immersion SPME using 65-μm Carbowax/DVB fibre, the drugs extracted in the fibre were desorbed in the desorption chamber of the SPME/HPLC interface, and then analysed by electrospray LC/MS. As shown in **Figure 10**, several corticosteroids and steroid sulfates spiked in urine samples were selectively analysed, although a minor peak was observed in the blank control urine in the SIM trace for cortisone.

Furthermore, Kataoka *et al.* developed an automated in-tube SPME/LC/MS method for the determination of the histamine $H_2$-receptor antagonist ranitidine in urine samples. The ranitidine in urine samples was directly extracted into Omegawax 250 capillary by 10 draw/eject cycles of 30 μL of sample at pH 8.5, desorbed from the capillary with

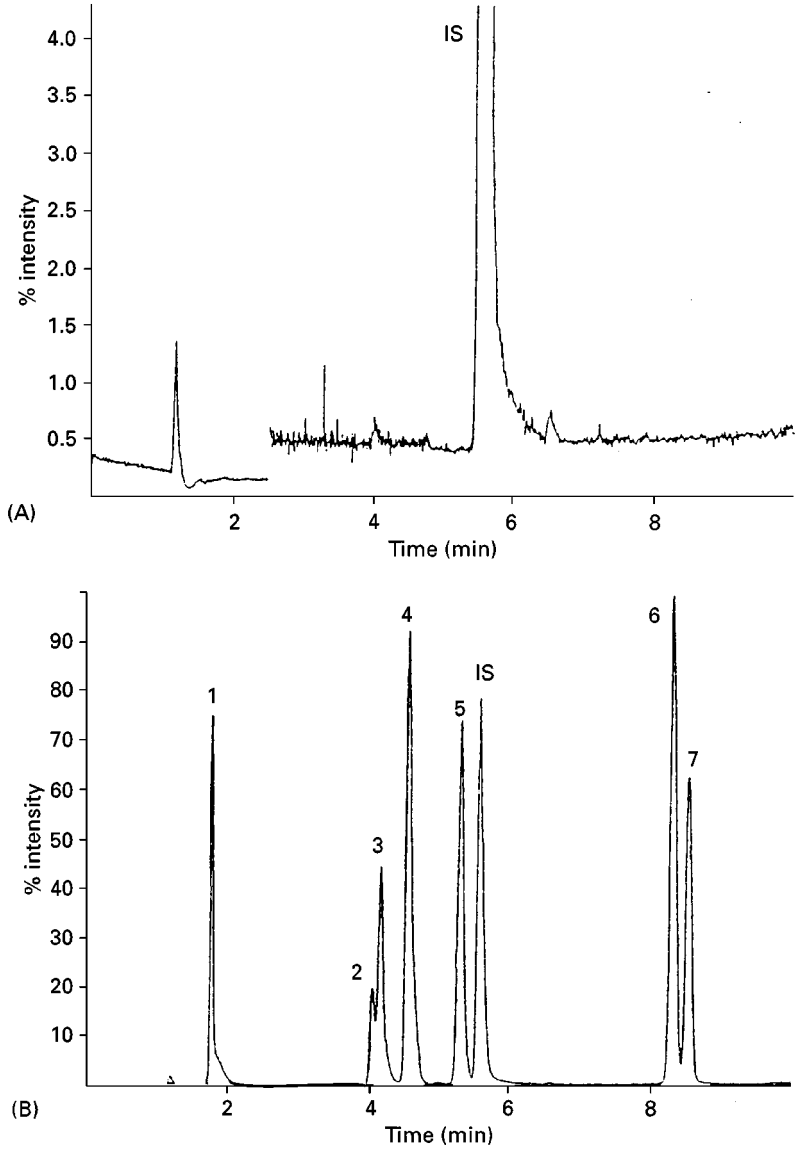



**Figure 10** SPME/LC/MS analysis of several corticosteroids and steroid conjugates by time-scheduled SIM. The original urine sample was spiked at the 20 mg $mL^{-1}$ level. (A) Blank control urine; (B) spiked urine. LC/MS conditions: column, YMC ODS-AQ (50 mm $\times$ 4.0 mm i.d., 3 μm particle size); column temperature, 25°C; mobile phase, A = 100 mM ammonium acetate and B = acetonitrile/methanol (50 : 50: + 100 mM ammonium acetate), A : B was gradient programmed from 60 : 40 to 20 : 80 in 10 min; flow-rate, 1 mL $min^{-1}$; fragmentor voltage, 40 V; ionization mode, negative ESI. SPME conditions: fibre, 65 μm carbowax/DVB; sample pH, 8.5; extraction, immersion for 15 min with stirring; desorption, methanol/water (50 : 50) for 5 min. Peaks: 1, estriol-3-sulfate; 2, cortisone; 3, fludrocortisone; 4, estrone-3-sulfate; 5,6-methylprednisolone; 6, budesonide (epimer B); 7, budesonide (epimer A); IS = internal standard (niflumic acid) at 20 μg $mL^{-1}$. (Reproduced with permission from Volmer DA and Hui JPM (1997) *Rapid Communications in Mass Spectrometry* 11: 1926. Copyright John Wiley & Sons Limited.)

methanol, and then analysed by electrospray LC/MS. Using this technique, nine beta-blockers and metabolites in urine and serum samples were also analysed. These methods were simple, rapid, selective and sensitive, and directly applied to urine samples and serum samples after ultrafiltration. Propranolol (PL) and its metabolites were successfully detected in the serum sample of a patient administrated PL (see **Figure 11**).

## Prospective of SPME in Biomedical Analysis

The main advantages of SPME are simplicity, rapidity, solvent elimination, high sensitivity, small sample volume, lower cost and simple automation. Since 1995, a number of SPME methods have been developed to extract drugs from various biological samples such as urine, serum, plasma, whole blood, saliva

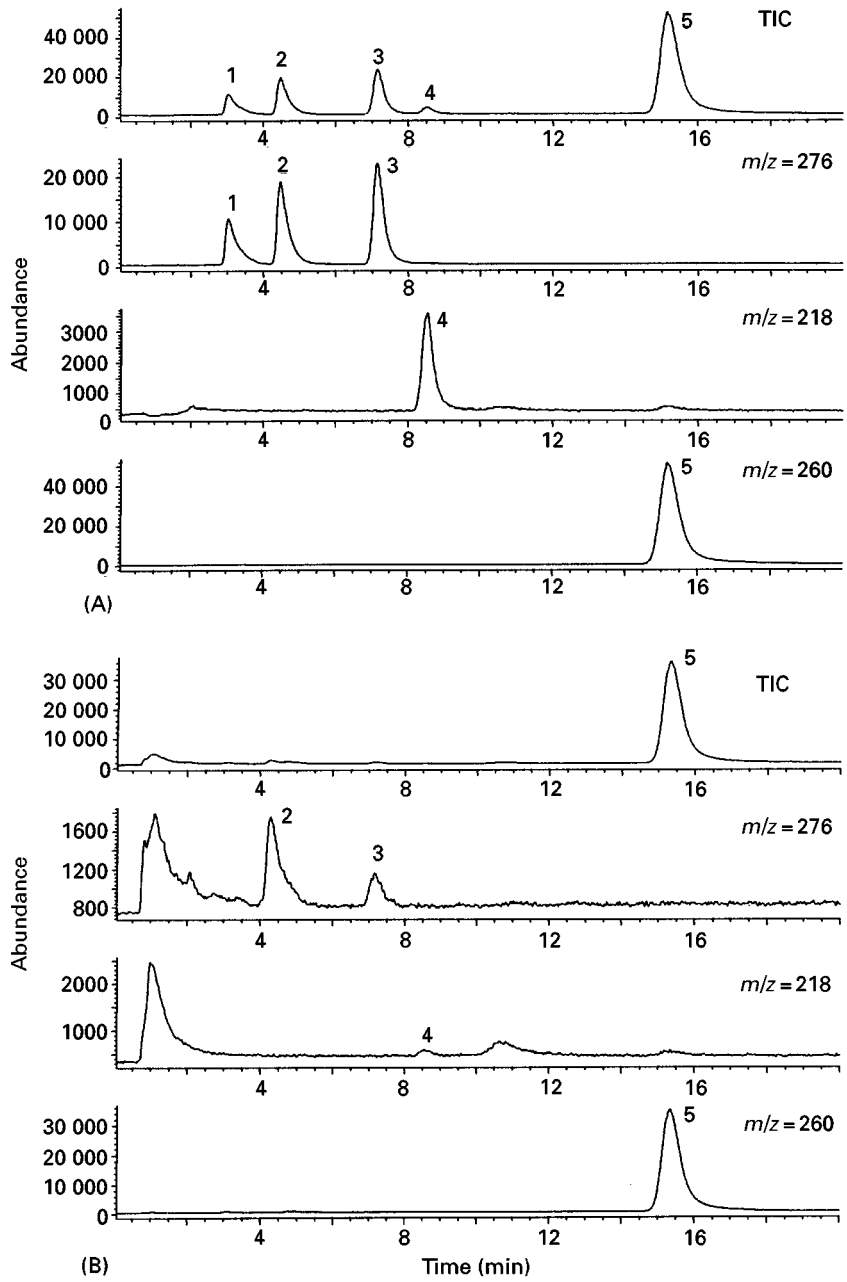



**Figure 11** Total ion and SIM chromatograms obtained from standard propranolol and its metabolites, and a clinical serum sample by in-tube SPME/LC/MS. (A) Standard solution containing 200 ng $mL^{-1}$ propranolol (PL), 50 ng $mL^{-1}$ 4-hydroxypropranolol (4-OH-PL) and 7-hydroxypropranolol (7-OH-PL), 20 ng $mL^{-1}$ 5-hydroxypropranolol (5-OH-PL) and *N*-desisopropylpropranolol (NDP). (B) Clinical serum sample (100 μL). Serum sample was diluted five times with 1% acetic acid and used for analysis after ultrafiltration. LC/MS conditions: column, Hypersil BDS $C_{18}$ (5.0 cm × 2.1 mm i.d., 3 μm particle size); column temperature, 25°C; mobile phase, acetonitrile/methanol/water/acetic acid (15 : 15 : 70 : 1); flow-rate, programme from 0.25 to 0.45 mL $min^{-1}$ for 20 min run; fragmentor voltage, 70 V; ionization mode, positive ESI; SIM ion, $m/z$ = 218 (NDP), 276 (hydroxypropranolols) and 260 (PL). In-tube SPME conditions: capillary, Omegawax 250 (60 cm × 0.25 mm i.d., 0.25 μm film thickness); sample pH, 8.5; draw/eject cycles, 15; draw/eject volume, 35 μL; draw/eject flow-rate, 100 μL $min^{-1}$, desorption solvent, mobile phase. Peaks: 1, 5-OH-PL; 2, 4-OH-PL; 3, 7-OH-PL; 4, NDP; and 5, PL. (Reproduced with permission from Kataoka H, Narimatsu S, Lord HL and Pawliszyn J (1999) *Journal of Analytical Chemistry* 71: 4237. Copyright American Chemical Society.)

and hair. The affinity of the fibre coating for an analyte is the most important factor in SPME. As shown in Table 1, fibre coatings of different polarity and thickness were selected for each drug. Most drugs in biological samples were extracted with 100-μm PDMS for nonpolar drugs and 85-μm PA for polar drugs. A solvent-modified fibre can improve selectivity and shorten extraction time. Although the theories

of fibre and in-tube SPME methods are similar, there are significant differences between these methods. The extraction of analytes is performed on the outer surface of the fibre for fibre SPME and in the inner surface of the capillary for in-tube SPME. Commercially available SPME fibres for drug analysis are limited, by GC capillary columns with a vast array of stationary phases are commercially available for in-tube SPME. Headspace fibre SPME is suitable for the extraction of drugs in gaseous, liquid and solid samples, because of the avoidance of contact with an aggressive matrix incompatible with the fibre. Direct immersion fibre SPME can be used to extract drugs from clear and cloudy liquid samples, however, in-tube SPME is limited to the extraction of clear liquid samples. The headspace SPME technique, therefore, is suitable for direct extraction from whole blood samples, while immersion fibre SPME or in-tube SPME methods require deproteinization or ultrafiltration of these samples prior to extraction. As mentioned above, the extraction efficiency of fibre SPME depends on extraction time, agitation, heating, sample pH and salt concentration. For in-tube SPME, number, volume and speed of draw/eject cycles, and sample pH are important factors for efficient extraction. On the other hand, the desorption of analyte from a fibre or capillary coating depends on the temperature of the injection port and exposure time in combination with GC or GC/MS, or component and volume of solvent when used in combination with HPLC or LC/MS. Therefore, these SPME parameters should be optimized when developing a new SPME method for drug analysis.

With further development of new coating materials, such as affinity coatings for target drugs and chiral coatings for optically active drugs, the further development of derivatization methods, further coupling with different analytical instruments, such as capillary electrophoresis, and improvement of the extraction and desorption conditions, the SPME technique is expected to be widely applied in the future for highly efficient extraction of drugs from various biological samples.

*See also:* **II/Chromatography: Gas:** Derivatization. **Extraction:** Solid-Phase Microextraction.

## Further Reading

Battu C, Marquet P, Fanconnet AL, Lacassie E and Lachatre G (1998) Screening procedure of 21 amphetamine-related compounds in urine using solid-phase microextraction and gas chromatography–mass spectrometry. *Journal of Chromatographic Science* 36: 1–7.

Degal F (1996) Comparison of new solid-phase extraction methods for chromatographic identification of drugs in clinical toxicological analysis. *Clinical Biochemistry* 29: 529–540.

Eisert R and Levsen K (1996) Solid-phase microextraction coupled to gas chromatography: a new method for the analysis of organics in water. *Journal of Chromatography A* 733: 143–157.

Junting L, Peng C and Suzuki O (1998) Solid-phase microextraction (SPME) of drugs and poisons from biological samples. *Forensic Science International* 97: 93–100.

Kataoka H, Narimatsu S, Lord HL and Pawliszyn J (1999) Development of on-line in-tube solid-phase microextraction/LC/MS system. *Chromatography* 20: 237–246.

Kroll C and Borchert HH (1998) Solid phase microextraction (SPME) for sample preparation during drug metabolism studies. *Pharmazie* 53: 172–177.

Lord HL and Pawliszyn J (1998) Recent advances in solid-phase microextraction. *LC-GC* S41–S46.

Namera A, Yashiki M, Kojima T and Fukunaga N (1998) Solid phase microextraction in forensic toxicology. *Japanese Journal of Forensic Toxicology* 16: 1–15.

Pawliszyn J (1995) New directions in sample preparation for analysis of organic compounds. *Trends in Analytical Chemistry* 14: 113–122.

Pawliszyn J (1997) *Solid Phase Microextraction*: *Theory and Practice*. New York: John Wiley.

Pawliszyn J (1999) *Applications of Solid Phase Microextraction*. Cambridge, UK: The Royal Society of Chemistry.

Penton ZE (1997) Sample preparation for gas chromatography with solid-phase extraction and solid-phase microextraction. *Advances in Chromatography* 37: 205–236.

Sporkert F and Pragst F (2000) Use of headspace solid-phase micro-extraction (HS-SPME) in hair analysis for organic compounds. *Forensic Science International* 107: 129–148.

Ulrich S and Martens J (1997) Solid-phase microextraction with capillary gas–liquid chromatography and nitrogen–phosphorus selective detection for the assay of antidepressant drugs in human plasma. *Journal of Chromatography B: Biomedical Applications* 69: 217–234.

Volmer DA and Hui JPM (1997) Rapid determination of corticosteroids in urine by combined solid-phase microextraction/liquid chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* 11: 1926–1934.

Zhang Z, Yang MJ and Pawliszyn J (1994) Solid phase microextraction: a new solvent-free alternative for sample preparation. *Analytical Chemistry* 66: 844A–853A.