

Recent Advances in Solid-Phase Microextraction and Related Techniques for Pharmaceutical and Biomedical Analysis

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Abstract: Sample preparation is essential for isolating desired components from complex matrices and greatly influences their reliable and accurate analysis. Solid-phase microextraction (SPME) is a new and effective sample preparation technique. Fibers and capillary tubes coated with an appropriate stationary phase are usually used for SPME, but alternative microextraction techniques, including solid-phase dynamic extraction using an internal coated needle, microextraction in a packed syringe and stir-bar-sorptive extraction using a coated magnetic stir bar, have been developed recently. These techniques, in combination with gas chromatography (GC), GC-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC), LC-MS or capillary electrophoresis, can be used for analysis for complex mixtures. These microextraction techniques save preparation time, as well as solvent purchase and disposal costs. This review summarizes recent advances in SPME and related microextraction techniques and their applications in pharmaceutical and biomedical analysis.

Keywords: Sample preparation for drug analysis, Solid-phase microextraction, In-tube solid-phase microextraction, Solid-phase dynamic extraction, Microextraction in a packed syringe, Stir-bar-sorptive extraction.

1. INTRODUCTION

Analysis of drugs in pharmaceutical products and biological samples is growing in importance, both in the development of more selective and effective drugs and in understanding their therapeutic and toxic effects. Knowledge of drug levels in body fluids, such as serum and urine, allows the optimization of pharmacotherapy and provides a basis for studies of patient compliance, bioavailability, pharmacokinetics and the influences of co-medications. The quantitative and qualitative analysis of drugs and their metabolites has been applied extensively in pharmacokinetic studies, since pharmacokinetic variables, such as time to reach maximum plasma concentration, clearance and bioavailability, have to be known for a new drug to be approved. In addition, therapeutic drug monitoring (TDM) is used to improve drug therapy. In contrast, drugs of abuse, illicit drugs, intoxicating drugs and poisons are analyzed in clinical and forensic toxicology. The screening of drugs of abuse in body fluids is also important for identifying and treating users of these drugs and for monitoring drug addicts following withdrawal from therapy.

Pharmaceutical products and biological materials are complex and may contain acids, bases, salts, proteins and other organic compounds with similar properties to the analytes of interest. In addition, these analytes are often present at low concentrations in biological samples. Drug analyses have been performed using various analytical instruments under many circumstances, including clinical control for diagnosis and treatment of diseases, doping

control, forensic analysis and toxicology. However, despite the development of highly efficient analytical instrumentation for the endpoint determination of analytes in pharmaceutical products and biological samples, sample pretreatment is usually necessary to extract, isolate and concentrate the analytes of interest from complex matrices, because most analytical instruments cannot handle the sample matrices directly. In general, sampling and sample preparation steps constitute over 80% of the total analysis time, and these steps are important in determining the success of analyzing compounds of interest in complex matrices such as biological samples. Therefore, it is not an exaggeration to say that the choice of an appropriate sample preparation method greatly influences the reliability and accuracy of the analysis.

In this context, the main objectives of sample preparation are the removal of unwanted macromolecular contaminants and materials that would affect chromatographic and electrophoretic separation; the solubilization of analytes to enable injection under the initial chromatographic and electrophoretic conditions; dilution to reduce solvent strength or avoid solvent incompatibility; concentration of the analytes so that they are within the detection limits of the analytical instrument; and derivatization of the analytes for enhancement of sensitivity and selectivity. In developing an analytical method, it is important to determine how much time and effort is necessary for sample preparation. For example, TDM usually requires specificity to distinguish the drug to be monitored from similar compounds, metabolites or co-administered drugs. In contrast, a pharmacokinetic study of a potential drug candidate requires a specific and sensitive analytical method. Therefore, in carrying out efficient sample preparation, it is important that: sample loss be kept at a minimum, so that the analyte of interest can be

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recovered in good yield; coexisting components are efficiently removed; problems do not occur in the chromatography and electrophoresis systems; the procedure can be performed conveniently and quickly; and that the cost of analysis be kept to a minimum.

Although many sample preparation techniques, including liquid-liquid extraction (LLE) and solid-phase extraction (SPE), have been used for drug analysis, these methods have various drawbacks, such as complicated and time-consuming procedures, the requirement for large amounts of sample and organic solvent, and difficulty in automating these procedures. For example, LLE requires time-consuming, labor-intensive and multi-stage operations, and each step can introduce errors and losses. Furthermore, use of harmful chemicals and large amounts of solvents cause environmental pollution and health hazards to laboratory personnel, as well as additional operational costs for waste treatment. SPE using cartridges or disc and microwell plates has reduced many of the limitations of traditional LLE methods, but SPE is still a time-consuming multi-step process that often requires concentration by solvent evaporation. Long sample preparation times limit the number of samples, and multi-step procedures are prone to loss of analytes. In addition, clotting, channeling and percolation are typical problems encountered in the laboratory during SPE. LLE and SPE are usually performed off-line, since their automation is complex. Although automated systems are available, these did not lead to a breakthrough in the economics of sample preparation.

These drawbacks can be avoided by use of solid-phase microextraction (SPME), a solvent-free extraction technique invented by Pawliszyn and co-workers in 1990 [1]. SPME enables simultaneous extraction and pre-concentration of analytes from gaseous, liquid and solid samples. A polymer-coated fiber is used as an extraction device, and the analytes extracted can be directly analyzed by gas chromatography (GC), GC-mass spectrometry (GC-MS), high performance liquid chromatography (HPLC) and LC-MS. The SPME method reduces sample preparation times and disposal costs, as well as improving detection limits. A new SPME technique, known as in-tube SPME, which uses fused-silica GC capillary columns for SPME, has recently been developed. In-tube SPME is suitable for automation, which not only shortens analysis times but increases accuracy and precision relative to manual techniques. Further development of new SPME coating materials, such as molecularly imprinted polymers (MIP), and further hyphenation with capillary electrophoresis (CE) have been reported. Thus, the significance of SPME has been rapidly recognized, and this technique has been widely used, not only in clinical and pharmaceutical analyses, [2-10] but in environmental [11-14] and food [15] analyses. The number of publications on SPME applications increased exponentially year on year, such that there have been about 1500 publications since SPME was first developed [16]. The details of SPME have been summarized in several recent reviews [16-26] and books [27-30].

In this article, I review the advances in SPME and related microextraction techniques that have occurred during the past five years. In the first part (Section 2), we describe the

general aspects of SPME and related techniques used to select appropriate extraction devices. In the second part (Section 3), I describe applications of these techniques in pharmaceutical and biomedical analysis.

2. SPME AND RELATED MICROEXTRACTION TECHNIQUES

Microextraction techniques have been regarded as the most attractive for the pretreatment of complex sample matrices prior to chromatographic and capillary electrophoretic processes because they enable rapid analysis at low operating costs and with no environmental pollution. The recent trend in sample preparation processes focuses on how to miniaturize the process and which medium to use for the extraction and pre-concentration of sample components. In this section, I review in detail fiber SPME, in-tube SPME (or capillary microextraction), solid-phase dynamic extraction (SPDE), microextraction in a packed syringe (MEPS) and stir-bar-sorptive extraction (SBSE), all of which have been widely used for forensic, clinical and pharmaceutical analysis. Fiber SPME is the most widely used technique. In-tube SPME was developed primarily to extend SPME to high-throughput applications and automated instrumentation. SBSE was developed to increase the sensitivity of SPME. Various new affinity SMPE sorbents, including immunosorbents and MIPs, have been used for the specific preparation of samples and are also described in this section. Other new microextraction techniques, such as liquid-liquid microextraction (liquid-phase [31-33] or single-drop [34] microextraction), solvent bar microextraction [35] and liquid membrane microextraction, [36-38] are not reviewed here, since they have been recently reviewed [39,40].

2.1. Fiber Solid-Phase Microextraction (Fiber SPME)

Fiber SPME [1] is a new sample preparation technique using a fused-silica fiber coated on the outside with an appropriate stationary phase. The fiber SPME device consists of a fiber holder and fiber assembly with built-in fiber inside a needle and looks like a modified syringe. The fiber holder, which consists of a plunger, a stainless steel barrel and an adjustable depth gauge with a needle, was designed to be used with reusable and replaceable fiber assemblies. The fused-silica fiber is coated with relatively thin films of several polymeric stationary phases, which are conventionally used as coating materials in chromatography. This film acts like a "sponge", concentrating the organic analytes during absorption or adsorption from the sample matrix. The commercially available fiber used many times in one device. Stationary phases are immobilized as non-bonded, bonded, partial crosslinked or highly crosslinked films. Non-bonded phases are stable with some water-miscible organic solvents, but slight swelling may occur when used with nonpolar solvents. Bonded phases are stable with all organic solvents except for some nonpolar solvents. Partially crosslinked phases are stable in most water-miscible organic solvents and some nonpolar solvents. Highly crosslinked phases are equivalent to partially crosslinked phases, except that some bonding to the core may occur. The advantages of these phases for SPME applications are similar to their advantages as GC stationary phases. The affinity of the fiber coating for an analyte is the most important factor

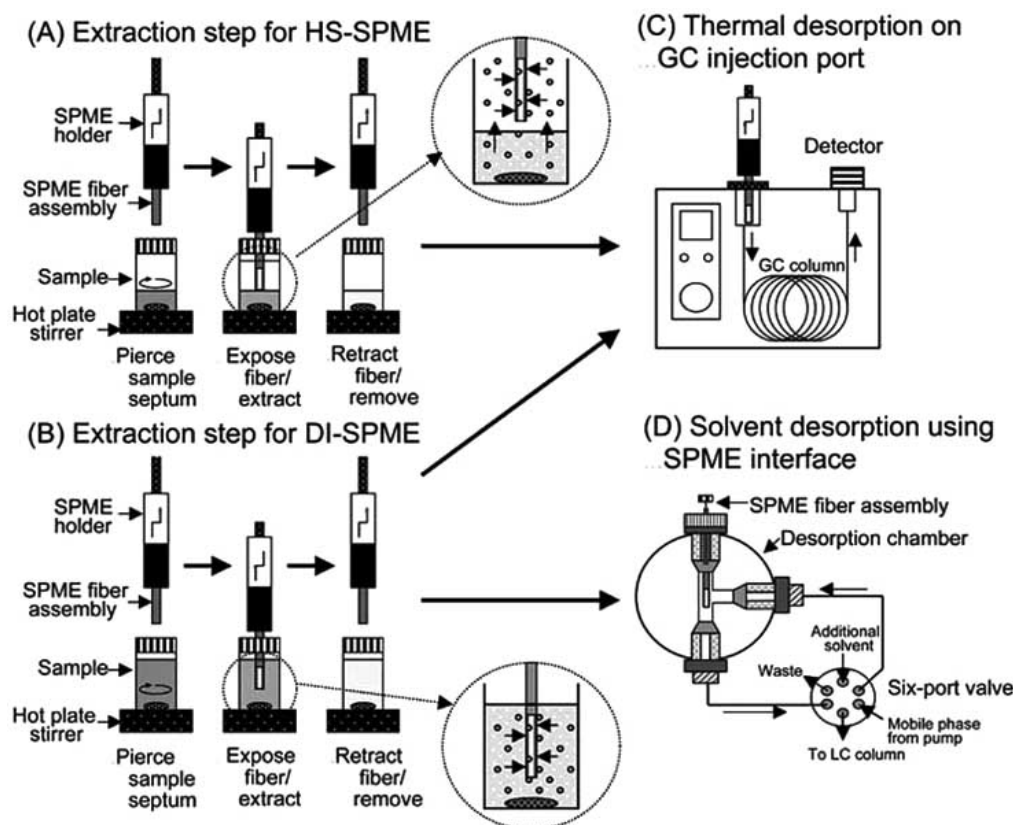


Fig. (1). Extraction process by HS and DI fiber SPME, and desorption systems for GC and HPLC analyses.

in SPME. A suitable polarity and thickness of the fiber coating can be selected for the drug under investigation. Besides commercial fibers, unique coating fibers, such as polypyrrole (PPY), [41] immunoaffinity, [42] alkyl-diol-silica (ADS) [43] and sol-gel porous silica, [44] have been developed.

The process of fiber SPME is illustrated in Fig. 1. In fiber SPME, analytes are extracted directly from the sample onto a polymeric stationary phase coated onto the fiber. When the fiber is inserted into the sample, the target analytes partition from the sample matrix into the stationary phase until equilibrium is reached. Two types of fiber SPME techniques can be used to extract analytes: headspace (HS)-SPME (Fig. 1A) and direct immersion (DI)-SPME (Fig. 1B). At first, a sample is placed into a vial, which is sealed with a septum-type cap. The fiber should be cleaned before analyzing any sample in order to remove contaminants that give a high background in the chromatogram. In HS-SPME, the fiber is exposed in the vapor phase above a gaseous, liquid or solid sample. In DI-SPME, the fiber is directly immersed in liquid samples. The sample is often agitated with a small stirring bar to increase the rate of equilibration. After a suitable extraction time, the fiber is withdrawn into the needle, and the needle is removed from the septum and transferred to an instrument for desorption, which is followed by separation and quantitation. SPME has been used routinely in combination with GC and GC/MS, and successfully applied to a wide variety of compounds in gaseous, liquid and solid samples, especially for the extraction of volatile and semi-volatile organic compounds. SPME was also directly coupled with HPLC and LC/MS to

analyze weakly volatile or thermally labile compounds not amenable to GC or GC/MS. The analyte is desorbed from the fiber coating by heating the fiber in the injection port of a GC or GC-MS (Fig. 1C), or by loading solvent into the desorption chamber of the SPME/HPLC interface (Fig. 1D).

Fiber SPME can be applied to gaseous, liquid, semi-liquid and solid samples. It is not necessary to remove particles in the samples before extraction because they can be removed by washing the fiber with water prior to insertion into the GC injection port and desorption chamber of the SPME/HPLC interface. However, the fibers should be carefully handled, because they are fragile and can be easily broken, and the fiber coating can be damaged during insertion and agitation. Furthermore, high-molecular weight compounds such as proteins may adsorb irreversibly onto the fiber, thus changing the properties of the stationary phase and rendering it unusable. The fiber SPME/HPLC method also has the advantage of eliminating the solvent front peak from the chromatogram, but peak broadening is sometimes observed because analytes can be slowly desorbed from the fiber.

A detailed procedure should be optimized for each compound to be analyzed and for each matrix to be sampled experimentally. The choice of SPME fibers is the first step. The amount of analyte extracted onto the fiber depends on the polarity and thickness of the stationary phase coating on the fiber, the extraction time and the concentration of analyte in a sample. In general, volatile compounds require a thick polymer coat, whereas semi-volatile compounds are effectively extracted using a thin coat. A thicker fiber

requires a longer extraction time, but recoveries are generally higher. Extraction of analyte may also be improved by adding salt to the sample, changing the pH or temperature, agitating the sample. Salting out by addition of salts such as sodium chloride and sodium sulfate increases extraction efficiency. The partitioning of targeted analytes between sample and fiber is also strongly affected by pH because compounds have different pKa values. For example, a sample may be acidified for extracting acidic compounds or made alkaline for extracting basic compounds. A sample may be agitated by stirring to enhance the extraction efficiency in non-equilibrium situations. Effects of temperature are different in HS- and DI-SPME and depend on the volatility of the analytes. 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 vial size and sample volume consistent. In general, immersion SPME is more sensitive than headspace SPME for analytes predominantly present in liquid form, whereas headspace SPME is more suitable for extracting more volatile compounds. In extracting compounds from biological samples, headspace SPME has a lower background than immersion SPME. Because headspace and immersion SPME differ in kinetics, both approaches should be evaluated in optimizing fiber SPME conditions for specific analytes. The combination of fiber SPME and GC or GC/MS is not suitable, however, for the extraction of less volatile or thermally labile compounds. Thus, to extract polar compounds from biological samples, derivatization approaches are frequently used. There are four types of derivatization technique that are usually used in combination with SPME. 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 fiber doping method, simultaneous derivatization and extraction are performed in the fiber coating by a two-step process: doping the fiber with a derivatizing agent and exposing the doped fiber to the sample for extraction. This technique can be used for polar volatile compounds. Another in-coating derivatization technique is also performed by a two-step process: doping the fiber with the sample for extraction and exposing the doped fiber in the headspace to the 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. These derivatization methods for SPME have been recently reviewed in detail [45].

Efficient thermal desorption of the analyte from the fiber coating in a GC injection port is dependent on injection depth, injector temperature and exposure time. A narrow bore GC injector insert is required to ensure high linear flow, and the fiber should be exposed immediately after the needle is introduced into the insert. Needle exposure depth should be adjusted to place the fiber in the center of the hot injector zone. Desorption time is determined by the injector temperature and the linear flow rate around the fiber. The HPLC interface consists of a six-port injection valve and a special desorption chamber, and requires desorption of analyte with specific desorption solvent or mobile phase

prior to HPLC or LC-MS analysis. The desorption chamber is placed in the position of the injection loop. After sample extraction, the fiber is inserted into the desorption chamber at the "load" position under ambient pressure. When the injector is changed to the "inject" position, the mobile phase comes into contact with the fiber, desorbs the analytes, and delivers them to the HPLC column for separation. The two desorption techniques that can be used to remove the analytes from the fiber are dynamic and static desorption. In dynamic desorption, the analytes can be removed by a moving stream of the mobile phase. When the analytes are more strongly adsorbed to the fiber, the fiber can be soaked in the mobile phase or another strong solvent for a specified time for static desorption before injection onto the HPLC column. For each of these techniques, rapid and complete desorption of analytes using minimal solvent is important for optimizing the SPME/HPLC or SPME/LC/MS methods. The combination of these desorption techniques with HPLC and LC-MS has been applied to the analysis of various polar compounds such as drugs and pesticides.

2.2. In-Tube Solid-Phase Microextraction (In-Tube SPME)

In-tube SPME [46] is a new sample preparation technique that uses an open tubular capillary as an SPME device and can be coupled on-line with HPLC or LC/MS. Although the technique using a GC capillary tube is also known as open-tubular trapping, [21] it is coupled on-line with GC. Capillary microextraction (CME) [47-49] is basically identical to these techniques and can be coupled with GC and HPLC. In-tube SPME is suitable for automation, and extraction, desorption and injection can be done continuously using a standard autosampler. Automated sample handling procedures not only shorten the total analysis time, but they are more accurate and precise than manual techniques. Using in-tube SPME, organic compounds in aqueous samples are directly extracted from the sample into the internally coated stationary phase of a capillary. These compounds are then desorbed by introducing a stream of mobile phase, or by using a static desorption solvent when the analytes are more strongly adsorbed to the capillary coating. The desorbed compounds are subsequently injected into the LC column for analysis. It is therefore necessary to prevent plugging of the capillary column and flow lines by filtering the sample solution before extraction. Although the extraction yields are generally low, these compounds may be extracted reproducibly using an autosampler, and introduce all of the extracts may be introduced into the LC column after in-tube SPME.

A schematic diagram of an automated in-tube SPME/LC-MS system using an Agilent 1100 series LC/MSD is illustrated in Fig. 2. The standard autosampler is suitable for construction of an on-line in-tube SPME/LC-MS system. In addition, an in-tube SPME system has been constructed using two Microfeeder MF-2 pumps equipped with MS-GAN microsyringes [23]. The capillaries selected have coatings similar to those of commercially available SPME fibers. The capillary is placed between the injection loop and the injection needle of the HPLC autosampler. The capillary connections are facilitated by using a 2.5-cm long sleeve of

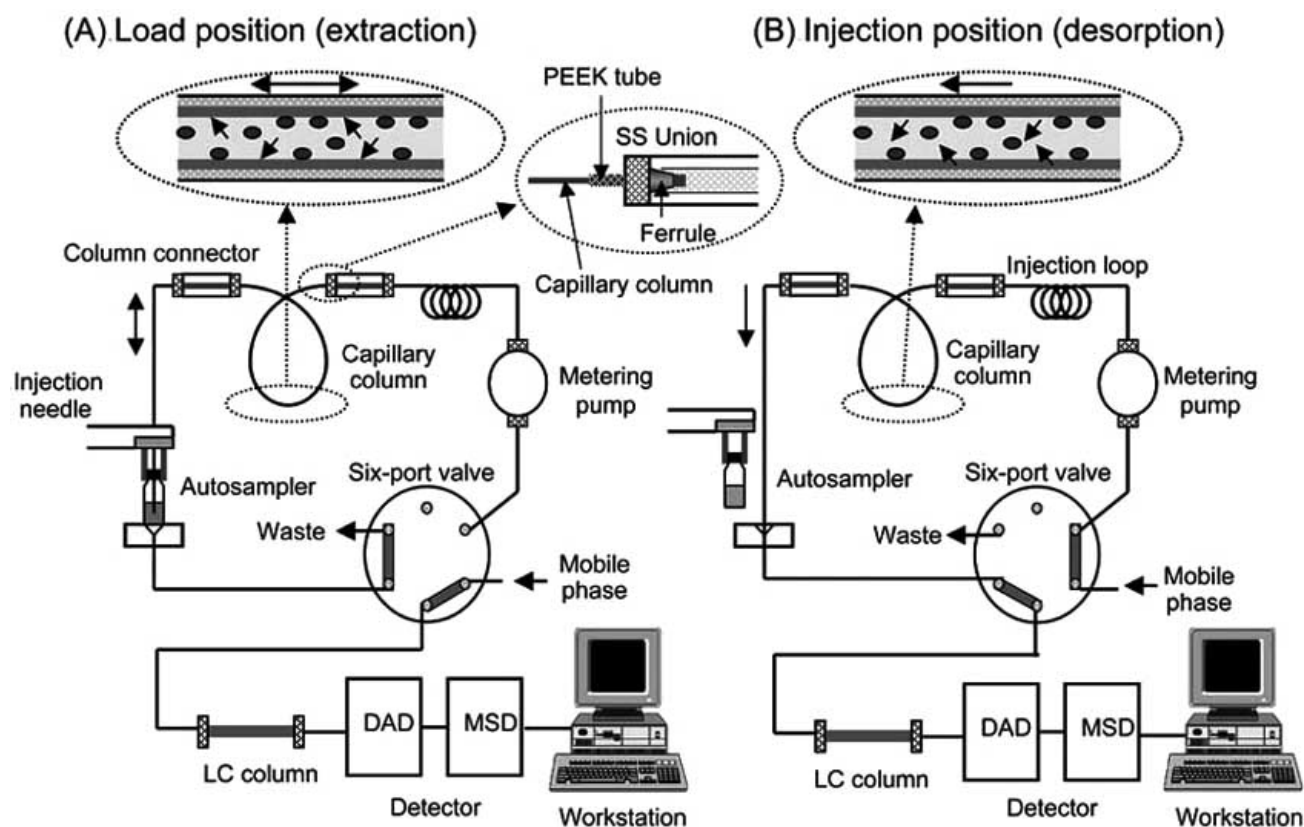


Fig. (2). Schematic diagrams of an in-tube SPME/LC/MS system. (A) Load position (extraction), (B) injection position (desorption).

1/16 inch polyether ether ketone (PEEK) tubing at each end of the capillary, fixed with 1/16-inch SS unions (0.25 mm bore stainless steel nuts) and ferrules. An injection loop is installed to prevent contamination of the metering pump by the sample. By building in UV, diode array or fluorescence detectors between the HPLC and the MSD, multidimensional and simultaneous multidetections are also possible, improving analyte identification. As shown in Fig. 2A, the injection syringe repeatedly draws and ejects sample from the vial under computer control, while the analytes partition from the sample matrix into the stationary phase until equilibrium is almost reached. Subsequently, the extracted analytes are directly desorbed from the capillary coating by mobile phase flow or by an aspirated desorption solvent after switching the six-port valve (Fig. 2B). Therefore, it is necessary to prevent plugging of the capillary column and flow lines during extraction with the in-tube SPME, and particles must be removed from samples by filtration before extraction. The desorbed analytes are transported to the HPLC column for separation and detected using a UV or mass selective detector (MSD). Drawing and ejection of the sample solution, switching of the valves, control of peripheral equipment such as the HPLC and MSD, and analytical data processing are all computer-controlled. Therefore, labor can be reduced and high precision can be realized. Furthermore, by combining in-tube SPME with LC-MS, compounds of a wide variety of molecular weight and volatility can be handled. In addition, a large number of samples can be automatically processed by the autosampler without carryover, because the injection needle and capillary column are washed in methanol and the mobile phase before the sample is extracted.

In-tube SPME is an extraction method whereby the analyte is transferred and depends on the distribution coefficient of the analyte as well as its affinity for the fiber SPME. It is therefore important to raise the distribution factor in the stationary phase to optimize the rapidity and efficiency of extraction. In in-tube SPME, the amount of analyte extracted into the stationary phase of the capillary column depends on the polarity of the capillary coating, the number and volume of draw/eject cycles and the pH of the sample. Several commercially available capillary columns, which differ according to the selectivity of the stationary phase, internal diameter, length and film thickness, have been used. For example, a low polarity column with a methyl silicon liquid phase selectively retains hydrophobic compounds, whereas a high polarity column with a polyethylene glycol liquid phase selectively retains hydrophilic compounds. Since the internal diameter, length and film thickness of the column and other dimensions affect the amount of sample that can be loaded and the amount of compound that can be extracted, these parameters should be chosen carefully. If the dimensions are increased, the load and amount extracted may increase, but the extension of the sample bandwidth may cause peak broadening and tailing. In addition, if the film thickness of the stationary phase is large, large amounts of compound can be extracted, but its quantitative desorption from the capillary column may be difficult. 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. Although capillary columns with a chemically bonded or cross-linked liquid phase are very stable for water and organic solvents, they readily deteriorate in the presence of strong inorganic

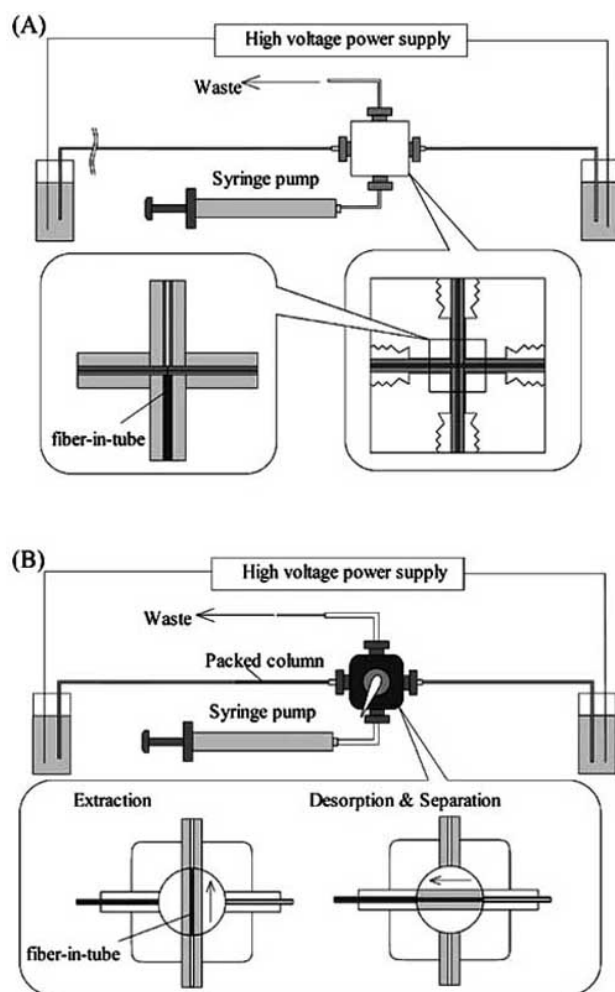


Fig. (3). Schematic diagrams of on-line coupling of miniaturized fiber-in-tube SPE with electrodriven separation system. (A) CE, (B) CEC. Reprinted with permission from authors in reference [23].

acids or strong alkalis. However, capillary columns are generally stable for the mobile phase usually used in HPLC.

Although commercially available open tubular capillary columns have mainly been used as extraction devices for in-tube SPME, some unique phases and technical solutions have been developed to improve extraction efficiency and selectivity. Extraction phases better suited to extracting of relatively polar compounds from aqueous samples have been found to enhance the sensitivity and overall utility of this method. For example, in a new capillary column, the inner wall of a commercially fused silica capillary was coated with PPY polymer [41]. In addition, an MIP was synthesized for use as an in-tube SPME adsorbent, in which a capillary packed with MIP particles in a PEEK tube was used for the selective analysis of propranolol, [50] and a fused silica capillary coated with β -estradiol MIP was used for in-tube SPME [22]. Furthermore, a highly biocompatible SPME capillary packed with alkyl-diol-silica (ADS) particles was developed as a restricted access material (RAM), in which the bifunctionality of the ADS extraction phase prevented fouling of the capillary by protein adsorption while simultaneously trapping the analytes in the hydrophobic porous interior [51]. An alternative approach consists of an

in-tube SPME technique using a monolithic capillary column comprised of one piece of organic polymer or silica with a unique flow-through double-pore structure [52]. Other techniques include wire-in-tube or fiber-in-tube SPME, which improve extraction efficiency while extending the method to microscale applications [23,24]. Modified capillary columns inserted with stainless-steel wire and PEEK tubes packed with fibrous rigid-rod heterocyclic polymers are used for in-tube SPME. By inserting a stainless-steel wire or several hundred fine filaments of polymer into the extraction tube, the internal volume of the tube can be significantly reduced, and the extraction can be optimized. In the fiber-in-tube SPME, the polymer filaments can be employed as the extraction medium. The short tube inserted fiber is also used as an on-line interface between the fiber-in-tube SPME and CE [23,24]. Fig. 3 shows the on-line systems coupling miniaturized fiber-in-tube SPME with electrodriven separation methods. Two types of interface devices have been designed especially for the hyphenation with this technique. In the CE application using a commercially available cross connector (Fig. 3A), the separation capillary and precapillary are connected in a face-to-face configuration, and the end of the fiber-in-tube SPME cartridge is placed to face the gap between these capillaries. Another device is designed for coupling with electrochromatography (CEC), in which a commercially available small four-port two-way valve is employed as an interface housing (Fig. 3B). The polytetrafluoroethylene (PTFE) tube packed longitudinally with polymer filaments is incorporated into the PTFE rotor of the valve and used as an fiber-in-tube SPME cartridge. In addition, a simple SPME device has been fabricated for use in on-line immunoaffinity CE [53].

In in-tube SPME, complete equilibrium extraction is generally not obtained for any of the analytes, because the analytes are partially desorbed into the mobile phase during the ejection step. Although an increase in the number and volume of draw/eject cycles can enhance the extraction efficiency, the bandwidth may widen, and peak broadening is often observed. The draw/eject volume and the number of sample solutions affect the extracted amount and are dependent on the capacity of the column. In our experiments, the optimum draw/eject volume in the case of a capillary column 60 cm long and having an internal diameter of 0.25 mm was 30-40 μ L for tested drugs. Under these conditions, the extraction efficiency did not increase even at high volumes. In addition, the draw/eject speed corresponds to the agitation speed of fiber SPME, and the extraction efficiency increases with speed. The optimal flow rate of draw/eject cycles was 50-100 μ L/min. Below this level, extraction requires an inconveniently long time, and above this level, bubbles form inside the capillary and extraction efficiency is reduced. Under ideal conditions, draw/eject of the sample solution should be performed until the compound reaches distribution equilibrium, thus maximizing the amount extracted. However, it is possible to stop the extraction before equilibrium to reduce the analysis time, if sufficient sensitivity is obtained. The extraction time depends on the volume, speed and number of cycles of the draw/eject, and these conditions must be fixed in order to obtain a quantitative reproducibility.

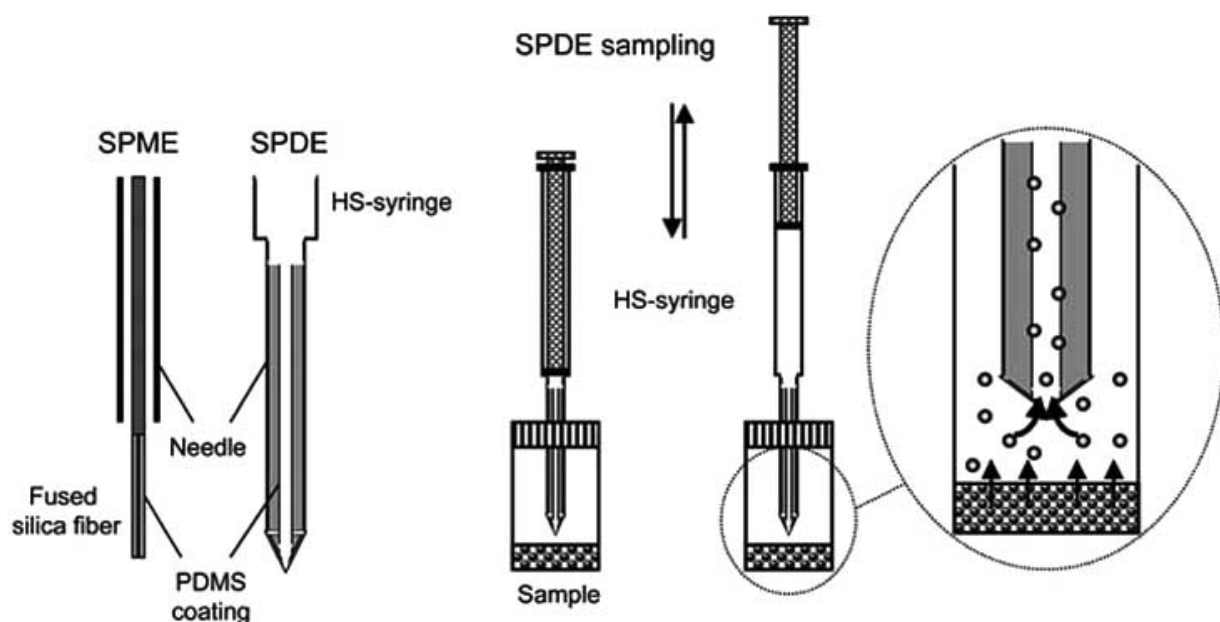


Fig. (4). Schematic representation of SPME and SPDE devices, and extraction process by SPDE.

Generally, it is possible to increase the extraction efficiency of analyte into a stationary phase in SPME by changing the pH and salt level of the sample solution. Acidic and basic compounds can be effectively extracted from acidic and alkaline sample solutions, respectively. However, the stability of each compound at the pH of the sample solution must be checked beforehand. Although salting out increases the extraction efficiency in fiber SPME, the salt deposits can clog up the column during in-tube SPME. Furthermore, the presence of a hydrophilic solvent such as methanol in the sample decreases the extraction efficiency because it increases the solubility of the compound in the sample, but methanol concentrations of 5% or less have little effect on the extraction efficiency. The amount of compound extracted into the stationary phase is dependent on the concentration of the compound in the sample.

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 the dynamic method, which desorbs into the flowing mobile phase, or the static method, which desorbs into a solvent aspirated from the outside. Static desorption is preferable when the analytes are more strongly adsorbed to the capillary coating. In each case, it is necessary to perform a quick and perfect desorption with a minimum volume of solvent. In the case of a capillary column 60 cm and having an internal diameter of 0.25 mm, desorption is usually carried out by aspirating 40 μL , depending on the capacity of the column. For the static method, it is also necessary to consider the solubility of the compound and its miscibility with the mobile phase. For the dynamic method, it is possible to directly desorb into the flowing mobile phase after switching the six-port valve. Although carryover may be observed after analyzing highly concentrated samples, the injection needle and capillary column may be washed by draw/eject of methanol and the mobile phase several times prior to the next analysis. Thus, during in-tube SPME carryover is lower or eliminated, in contrast to fiber SPME.

Furthermore, extraction and desorption operations may be performed automatically using an overall injection program.

2.3. Solid-Phase Dynamic Extraction (SPDE) and Microextraction in a Packed Syringe (MEPS)

Solid-phase dynamic extraction (SPDE) [54,55] is an inside-needle technique for vapor and liquid sampling. Stainless steel needles (8 cm) coated with a 50- μm film of polydimethylsiloxane (PDMS) and 10% activated carbon are used. A diagram of an SPDE device is compared with an SPME fiber in Fig. 4. Dynamic sampling is performed by passing the headspace through the tube using a syringe. The volume of the stationary phase of the SPDE needle is approximately 5.99 mm^3 , whereas a 100- μm PDMS SPME fiber has a volume of 0.94 mm^3 . In SPDE, the analytes are concentrated onto PDMS and activated carbon coated onto the inside wall of the stainless steel needle of a 2.5-mL gas tight syringe. When used for HS-SPDE, an amount of analyte sufficient for a reliable GC or GC-MS analysis is accumulated in the polymer coating of the inside needle wall by pulling in and pushing out a fixed volume of the headspace of sample for an appropriate number of times. Thus, SPDE sampling permits operation under dynamic conditions while keeping the headspace volume constant. The trapped analytes are then recovered by heat desorption directly into a GC injector body. A great advantage of SPDE over SPME is the robustness of the capillary. In contrast to the fragile SPME fibers, the SPDE device is nearly impossible to damage mechanically. SPDE has been successfully applied to the analysis of volatile compounds, pesticides and some drugs. However, SPDE may have carryover, because the analytes tend to remain in the inside needle wall after heat desorption in GC injection port.

Microextraction in a packed syringe (MEPS) [94] is a newly developed technique for sample preparation on-line with LC and GC-MS. This form of miniaturized SPE uses a procedure similar to in-tube SPME and SPDE. In MEPS, approximately 1 mg of solid packing material is inserted

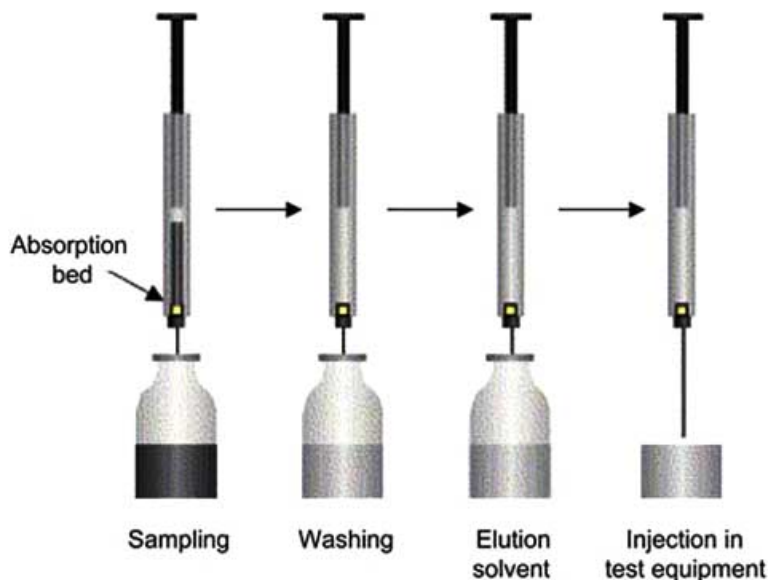


Fig. (5). Automated MEPS process. Reprinted with permission from reference [94]. (© 2004 Elsevier Science).

into a syringe (100-250 μL) as a plug with a filter from both sides, and sample preparation takes place on the packed bed. Fig. 5 shows an MEPS process. After conditioning with methanol and water, sample solution is drawn through the syringe three times (50 μL each) by the autosampler, which pumps the sample up and down three times. After washing with water to remove any interfering materials such as proteins, the extracted analytes are eluted with an organic solvent or LC mobile phase directly into the GC or LC injector. The process is fully automated. Any absorption materials such as silica based (C2, C8, C18), RAM or MIPs can be used.

SPDE and MEPS are new technique using a microsyringe as an extraction device and have not yet been extensively applied.

2.4. Stir Bar Sorptive Extraction (SBSE)

The stir bar sorptive extraction (SBSE) [21,56,57] is a sample preparation technique that is very promising for enriching solutes from aqueous samples. This technique uses a magnetic stir bar coated with PDMS phase, similar to SPME but in a thicker layer (0.3-1.0 mm). PDMS-coated stir bars are now commercially available as Twister[®] stir bars (Gerstel, Mülheim a/d Ruhr, Germany) of lengths 10 and 40 mm, coated with 55 and 219 μL of PDMS liquid phase, respectively. The 10-mm stir bars are best suited for stirring samples of 10 mL to 50 mL whereas 40-mm stir bars are better for sample volumes up to 250 mL.

The principle of SBSE is shown in Fig. 6. Sample is poured into a 20-mL headspace vial, which is stirred with a PDMS-coated stir bar for 60 min at 1000 rpm (Fig. 6A). After sampling, the stir bar is removed with tweezers, briefly applied to clean paper tissue to remove residual water droplets, and placed in an empty glass tube 187 mm long, 6 mm o.d., and 4 mm i.d. (Fig. 6B) for thermal desorption (Fig. 6C). In SBSE, sample volume and stirring speed influence extraction efficiency, and typical stirring times for equilibration are between 30 and 60 min.

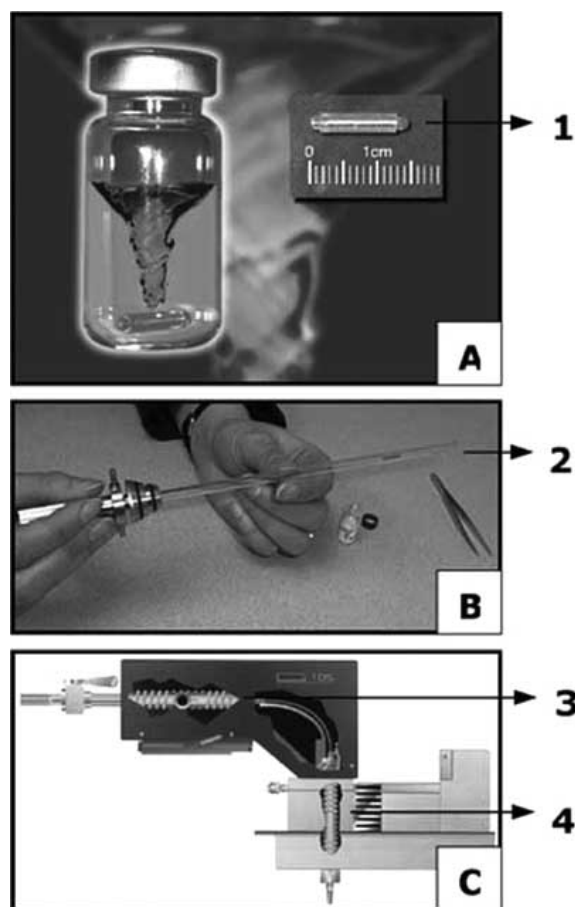


Fig. (6). Principle of SBSE. 1. Twister, 2. transfer to the thermal desorption tube, 3. thermal desorption unit, 4. programmed-temperature-vaporization injector. Reprinted with permission from reference [57]. (© 2002 Springer-Verlag).

Although the extraction mechanism is similar to that of SPME based on PDMS sorption, SBSE uses a much higher

mass of PDMS, resulting in higher recoveries and higher sample capacity. The apolar PDMS phase, however, is not suitable for extracting polar compounds. Derivatization in aqueous phase prior to SBSE sampling improves both chromatographic analysis and sample enrichment in the PDMS phase. In addition, derivatization expands the possibility of SBSE sampling for polar compounds. This technique is compatible with both GC and HPLC desorption procedures. The combination of SBSE with liquid desorption has extended the range of applications towards the analysis of high molecular mass compounds and thermolabile solutes. Theoretically, it may be more sensitive than SPME fibers for certain applications, but it requires a special desorption unit and is difficult to automate.

3. APPLICATIONS IN PHARMACEUTICAL AND BIOMEDICAL ANALYSIS

The main advantages of SPME are its simplicity, rapidity, solvent elimination, high sensitivity, small sample volume, relatively low cost and simple automation. Since 1995, a number of SPME methods have been developed to extract drugs from various biological samples, including urine, serum, plasma, whole blood, saliva and hair. The number of publications reporting pharmaceutical and biomedical applications of SPME has increased exponentially, and there have been over 150 reports during the last five years. A summary of recent applications of SPME and related microextraction techniques for the analysis of drugs in biological fluids and matrices is shown in Table 1. Although most of these applications are related to forensic and toxicological analyses, they demonstrate the versatility of this method and the potential of its use for analyzing other drugs, including clinical, metabolic and pharmaceutical applications. Most of the methods shown to date have involved HS techniques for volatile analytes or DI and derivatization techniques for less volatile analytes using a fiber coating with a polymeric phase of suitable polarity and thickness. Most nonpolar drugs in biological samples are extracted with 100 μm PDMS, 85 μm polyacrylate (PA) or a porous polymer divinylbenzene (DVB) fiber, and analyzed in combination with GC-MS. This method has low limits of detection and excellent quantitation. Especially in the HS mode, SPME extractions offer the potential for very clean analyses, with little to no interference from non-volatile compounds. Because of the relatively low partition coefficients between polar drugs and the commercially available HPLC/SPME fibers, the application of SPME to low volatility drugs and metabolites in plasma may be limited to those with high therapeutic concentrations, in the range of 1 to 100 $\mu\text{g}/\text{mL}$. The situation can be improved by using current tandem quadruple LC/MS instrumentation, allowing the analysis of compounds at concentrations approaching 1 ng/mL in plasma. The in-tube SPME technique can also be applied to polar and non-polar drugs in liquid samples using a commercially available GC capillary column, and can be easily coupled with various analytical methods such as HPLC and LC-MS. Its application to the analysis of biological samples increases. In addition, it is possible to improve extraction efficiency and selectivity by developing capillaries coated or packed with new materials, such as PPY, [66,115,117] ADS, [102] MIP [117] and

Zylon[®] fiber [111]. Similar to in-tube SPME, an automated SPDE technique using a hollow needle with an internal coating of PDMS instead of a capillary column may be suitable for headspace extraction coupled with GC-MS, and can be used for the determination of illicit drugs in hair samples [60,63,82]. On the other hand, the SBSE technique, which uses a magnetic stir bar covered with a thick layer of PDMS, can be applied to some drugs in biological samples by combination with GC-MS [57,129].

As shown in Table 1, various biological specimens, particularly plasma, serum and urine, have been analyzed using SPME and related microextraction techniques. Although blood (plasma, serum) is most useful for simultaneous screening and quantification of drugs, blood samples usually need deproteinization and, if necessary, cleavage of conjugates of the drugs and their metabolites, prior to sample pretreatment and chromatography or electrophoresis. Urine may also be used for the comprehensive screening and identification of unknown drugs and their metabolites, because of the relatively high concentrations of drugs in urine. Although urine collection is easy, it is necessary to prevent the contamination and degradation of drugs during storage. Saliva, sweat and hair analyses have recently come to be considered useful adjuncts to conventional drug testing. In particular, hair analysis is frequently used for the long-term monitoring of drug and alcohol users. The use of milk, tissue and breath samples for drug analysis are unusual in clinical applications. In this section, several applications of SPME and related techniques in analyzing biological samples are described. The details of other SPME methods used prior to 1998 are summarized in several books and reviews [7,9,10,19,28,39].

3.1. Applications in Forensic and Toxicological Analysis

Amphetamine and methamphetamine are powerful stimulants of the central nervous system and are frequently abused by athletes, drug addicts, and recreational users. Their methylenedioxy analogues, including 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxy-methamphetamine (MDMA), and 3,4-methylenedioxyethamphetamine (MDEA), are also abused to enhance sociability and liberate inhibitions, allowing the user to experience feelings of euphoria. Amphetamine, methamphetamine, and their analogues, termed 'designer drugs', are classified as controlled or illicit drugs in many countries. Due to the recent increase in abuse of these drugs, the number of requests for routine tests has increased, necessitating a convenient and rapid method for quantitating these compounds. Analysis of amphetamines in urine samples is one of the most popular applications of SPME, and HS- and DI-SPME techniques using PDMS fiber are widely used [70,71,73-76]. A home assembled desorption chamber (inner volume ca 3 μL), designed for direct coupling of SPME with ESI-high field asymmetric waveform ion mobility spectrometry (FAIMS)-MS, has been used to analyze amphetamine, methamphetamine, and their analogues in urine [74]. The method was found to be sensitive and selective without requiring chromatographic separation, and results can be obtained in less than 20 min after a urine sample arrives at the laboratory. It is feasible that SPME sampling could be done on-site, thereby requiring only the

Table 1. Applications for drug analysis using SPME and related microextraction techniques.

Analyte	Matrix	Extraction device ^a	Extraction mode ^b	Hyphenated analysis ^c	Reference
Cannabinoids	Hair	PDMS-fiber	DI	GC-MS	58
Cannabinoids	Hair	PDMS-fiber	HS + OFD	GC-MS	59
Cannabinoids	Hair	PDMS-coated syringe	HS-SPDE	GC-MS	60
Cannabinoids	Saliva	PDMS-fiber	DI	GC-MS	61
Amphetamines	Hair	PDMS-fiber	D + HS	GC-MS	62
Amphetamines, designer drugs	Hair	PDMS-coated syringe	HS-SPDE	GC-MS	63
Amphetamines, designer drugs	Hair	PDMS-fiber	HS + OFD	GC-MS	64
Amphetamine-like drugs	Hair, saliva	PDMS-fiber	HS	GC-MS	65
Amphetamines, MDA, MDMA, MDEA	Hair, urine	PPY-coated	IT	LC-MS	66
Amphetamine, fenfluramines	Blood	PDMS-fiber	D + DI	GC-MS	67
Amphetamines	Blood	PDMS-fiber	HS	GC-MS	68
Amphetamines, MDA, MDMA, MDEA	Serum	PDMS-fiber	D + HS	GC-MS	69
Amphetamines, MDMA	Urine	PDMS-fiber	DI	GC-MS	70
Amphetamines, MDA, MDMA, MDEA	Urine	PDMS-fiber	HS	GC-MS	71
Amphetamines, MDA, MDMA, MDEA	Urine	Omegawax 250 capillary	IT	LC-MS	72
Amphetamine-related drugs	Urine	PDMS-fiber	D + HS	GC-MS	73
Amphetamines, MDA, MDMA, MDEA	Urine	PDMS-fiber	D + DI	FAI-MS-MS	74
Amphetamines	Urine	PDMS-fiber	D + HS	GC-MS	75
Amphetamines	Urine	PDMS-fiber	HS	GC-FID	76
Amphetamine, MDMA	Urine	CW/TPR-fiber	DI + OFD D + DI	HPLC-FLD	77
Amphetamines	Drugs	PDMS-fiber	DI + OFD	GC-MS	78
Recreational drugs	Hair	PDMS-fiber	HS	GC-MS	79
Cocaine, benzoylecgonine	Hair	PDMS-fiber	DI	GC-MS	80
Cocaine, Cocaethylene	Sweat	PDMS-fiber	DI	GC-MS	81
Illicit drugs	Hair	PDMS-coated syringe	HS-SPDE	GC-MS	82
Illicit drugs	Saliva	PDMS-fiber	HS / DI	GC-MS	83
Illicit drugs	Serum, urine	PA-fiber	HS	GC-MS	84
Methadone	Hair	PDMS-fiber	DI	GC-MS	85
Methadone, metabolite	Hair	PDMS/DVB-fiber	HS	GC-MS	86
Methadone	Saliva	PDMS-fiber	DI	GC-MS	87
Methadone, metabolite	Plasma	PDMS-fiber	DI	GC-MS	88

Table 1. (contd....)

Nitrous oxide, isoflurane, halothane	Urine	DVB- carboxen / PDMS-fiber	HS	GC-MS	89
Halothane	Blood	PDMS-fiber	HS	GC-MS	90
Lidocaine	Hair	CW/DVB-fiber	HS	GC-MS	91
Lidocaine	Plasma	PDMS-fiber	DI	GC-FID	92
Lidocaine	Urine	PDMS-fiber	DI	MS-MS	93
Local anesthetics	Plasma	Silica C2-packed syringe	MEPS	GC-MS	94
Thiopental, pentobarbital	Hair	CW/TPR-fiber	DI	GC-MS-MS	95
Brabitrates	Blood, urine	PA-fiber	DI	GC-MS	96
Midazolam	Plasma	PA-fiber	DI	GC-MS	97
Lamotrigine, drugs	Plasma	CW/DVB-fiber	DI	GC-TSD	98
Clozapine	Plasma	PDMS-fiber	DI	GC-NPD	99
Delorazepam	Urine	CW/TPR, PDMS/DVB-fiber	DI	HPLC-UV	100
Benzodiazepines	Serum	Supel-Q plot capillary	IT	LC-MS	101
Benzodiazepines	Serum	ADS-packed capillary	IT	HPLC-UV	102
Benzodiazepines	Blood	ADS- fiber	DI	HPLC-UV	103
Benzodiazepine & metabolites	Urine	PDMS, PDMS/DVB-fiber	DI	GC-ECD	104
Benzodiazepines	Urine	ADS-fiber	DI	HPLC-UV	105
Carphedon	Urine	CW/DVB-fiber	DI	GC-FID	106
Phenothiazines	Blood, urine	PA-fiber	DI	LC-MS-MS	107
Levomepromazine	Plasma	PDMS-fiber	DI	GC-NPD	108
Amitriptyline	Urine	PDMS-fiber	DI	HPLC-UV	109
Tricyclic antidepressants	Urine	Wire-packed DB-1 capillary	IT	HPLC-UV	110
Tricyclic antidepressants	Urine	Zylon DB-1 capillary	IT	CE-UV	111
Amitraz	Plasma	PDMS-fiber	DI	GC-TSD	112
Sufentanil	Plasma	PDMS/DVB-fiber	DI	GC-MS	113
-Blockers	Serum, urine	Omegawax 250 capillary	IT	LC-MS	114
-Blockers	Serum, urine	PPY-coated capillary	IT	LC-MS	115
Propranolol	Serum	MIP-packed capillary	IT	HPLC-UV	116
Verapamil	Cell culture	PPY-coated capillary	IT	LC-MS	117
Ranitidine	Urine, tablet	Omegawax 250 capillary	IT	LC-MS	118
Rivastigmine	Plasma	PDMS/DVB-fiber	HS	GC-MS	119
Thymol	Plasma	PDMS/DVB-fiber	HS	GC-FID	120
Menthol	Plasma, urine	PDMS/DVB-fiber	HS	GC-MS	121
Methylxanthines	Blood, urine	CW/DVB-fiber	DI	GC-NPD	122
Xanthines	Serum	Monolithic capillary	IT	HPLC-UV	123
Busulphan	Plasma	CW/DVB-fiber	DI	GC-MS	124
Tetracyclines	Milk	CW/TPR-fiber	DI	LC-MS	125
Mycophenolic acid	Serum	CW/TPR-fiber	DI	HPLC-UV	126

Table 1. (contd....)

Anti-inflammatory drugs	Drugs	PA-fiber	HS-OFD	GC-MS	127
Drugs	Serum, urine	Omegawax 250 capillary	IT	LC-MS	128
Pharmaceuticals	Urine	PDMS-fiber	SBSE	GC-MS	129
Pharmaceuticals	Urine	PDMS-, PA-fiber	DI	HPLC-FLD	130
Residual solvents	Pharmaceuticals	PDMS-fiber	HS	GC-FID	131
Residual solvents	Pharmaceuticals	DVB/PDMS-, CRB/PDMS/DVB-fiber	HS	GC-FID	132
Residual solvents	Pharmaceuticals	CRB/PDMS-fiber	HS	GC-FID	133
Residual solvents	Pharmaceuticals	CRB/PDMS/DVB-fiber	HS	GC-FID	134
Phthalates	Liquid medicines	Supel-Q plot capillary	IT	HPLC-UV	135, 136
Acrolein	Urine	CW/DVB-fiber	HS	GC-MS	137
Acetone	Breath, plasma	PDMS/DVB-fiber	HS-OFD	GC-MS	138, 139
Volatiles	Blood	CRB/PDMS-fiber	HS	GC-MS	140

^a PDMS: poly(dimethylsiloxane); CRB: carboxen; PA: polyacrylate; CW: carbowax; DVB: divinylbenzene; TRP: templated resin; PPY: polypyrrole; MIP: molecularly imprinted polymer; ADS: alkyl-diol-silica. ^b HS: headspace fiber SPME; DI: Direct immersion fiber SPME; IT: in-tube SPME; SPDE: solid-phase dynamic extraction; MEPS: microextraction in a packed syringe; SBSE: Stir-bar sorptive extraction; D: derivatization; OFD: on-fiber derivatization. ^c GC-MS: gas chromatography-mass spectrometry; LC-MS: liquid chromatography-mass spectrometry; HPLC: high performance liquid chromatography; CE: capillary electrophoresis; FID: flame ionization detection; NPD: nitrogen-phosphorus detection; TSD: thermionic specific detection; ECD: electron capture detection; FLD: fluorescence detection; FAI: field asymmetric waveform ion mobility spectrometry.

fiber to be sent to a laboratory and eliminating the need to ship biohazardous material, such as urine. In addition, an automated in-tube SPME coupled with LC-ESI-MS (positive ion mode, SIM) to analyze amphetamine, methamphetamine, and their analogues has been developed [72]. The drugs are extracted in an Omegawax 250 capillary and easily desorbed with a mobile phase by a dynamic desorption technique. This method had a limit of detection (LOD) of 0.2-0.8 ng/mL (S/N=3), suggesting it can be directly applied to a diluted urine sample without interference. Recoveries of drugs added to urine samples were over 80%, with a relative standard deviation (RSD) of 7.9% or less. The extraction efficiency of this method was improved by using a PPY-coated capillary; coupled with LC-ESI-MS, it was successfully applied to the analysis of urine and hair samples [66].

As described above, hair analysis has proved a reliable tool for the retrospective detection of chronic drugs of abuse in clinical and forensic toxicology. Amphetamines in hair samples are analyzed using several microextraction techniques [62-66,79,82]. In addition, a rapid screening procedure based on the HS-SPME GC-MS has been used to detect many recreational drugs in 20-mg hair samples with no derivatization [79]. After a short acid extraction with 1 M HCl, the fiber was exposed to a 5-min absorption at 90°C, followed by thermal desorption at 250°C for 3 min. Two fully automated procedures have been used to detect amphetamines and designer drugs in human hair samples [63,64]. One, an HS-SPME on-fiber derivatization approach, [64] consists of alkaline hydrolysis and HS-SPME of hair sample, followed by placing the fiber directly into the headspace of a second vial containing N-methyl-bis(trifluoroacetamide) (MBTFA) as derivatizing reagent before GC-MS analysis. Using this method, amphetamines

and designer drugs could be analyzed in only 10 mg of hair, with an LOD of 0.01-0.17 ng/mg of hair. The main disadvantages of fiber SPME, however, are the fragility of the fused silica and the unprotected stationary phase coating on the outer surface of the fiber when extended through the syringe needle. To reduce these problems while maintaining the advantages of SPME, a new SPDE technique using internally coated needle (Fig. 4) was developed by Chromtech (Idstein, Germany). In addition, an automated headspace procedure was developed for detecting amphetamines and designer drugs in human hair using this technique [63]. After alkaline hydrolysis of 10 mg of hair, the SPDE needle was inserted into the headspace of the sample vial through the septum, and the plunger was moved up and down at 200 μ L/sec 50 times to extract the analyte dynamically. For on-coating derivatization, the syringe was positioned above a second vial containing MBTFA, and the plunger was moved up and down 6 times. The extracted analytes were desorbed from the coating by rapid heating of the needle in the GC injection port. Compared with the conventional SPME method, SPDE had a higher extraction rate, as well as a faster automated operation.

Techniques using HS-SPME on-fiber and HS-SPDE on-coating derivatization have also been applied to the analysis of several illicit drugs, such as amphetamines, cannabinoids and methadone, in hair samples [59,60,82]. Fig. 7 shows typical chromatograms obtained by HS-SPDE GC-MS in selected ion monitoring (SIM) mode (Fig. 7a and b) and HS-SPDE GC-MS-MS in multiple reaction monitoring (MRM) mode (Fig. 7c and d). Obviously, the MRM mode had a higher sensitivity and selectivity than the SIM mode for measuring drugs in hair samples, with the former having an LOD of 6-52 pg/mg hair and an RSD of 0.4-7.8% using an internal standard. In addition, a high-temperature (100-

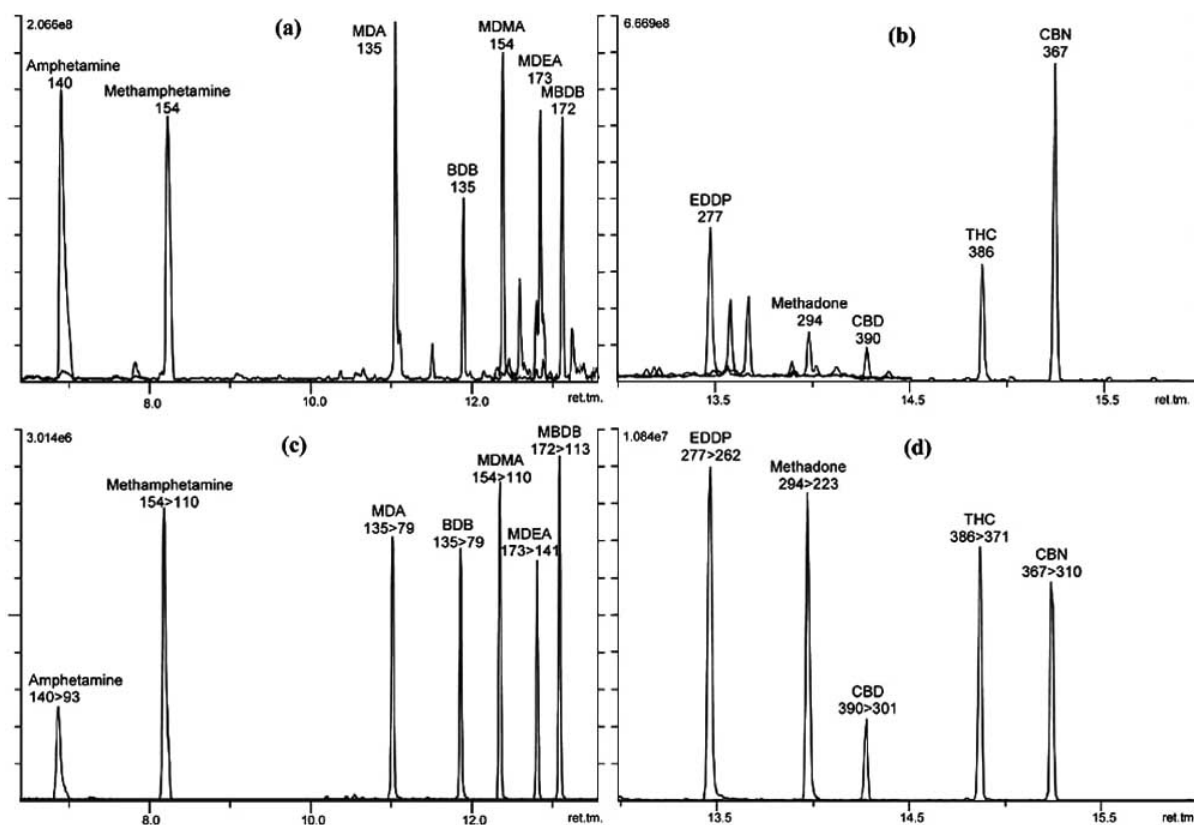


Fig. (7). Positive ion HS-SPDE GC-MS SIM chromatograms (a, b) and corresponding HS-SPDE GC-MS-MS MRM chromatograms (c, d) of identical spiked hair samples containing 1 ng/mg of the analytes. Reprinted with permission from reference [82]. (© 2003 John Wiley & Sons).

200°C, depending on the analyte) HS-SPME with simultaneous *in situ* derivatization method has been developed for screening illicit drugs in dried urine, with confirmation of the suspected substances in dried serum using GC-MS [84].

A toxicological analysis of the local anesthetic lidocaine in biological fluids, obtained from a suspected victim of lidocaine poisoning, was performed using the SPME method [93]. The SPME was coupled directly to an atmospheric pressure chemical ionization (APCI)-MS/ion trap for rapid analysis of the drug in urine samples, hereby applying MS/MS [fragmentation of $[M + H]^+$ (m/z 235) to a fragment with m/z 86]. Throughput of samples was increased using non-equilibrium SPME with PDMS fibers, and desorption was performed with a home-made chamber allowing thermostating. Only 1 min sorption and 1 min desorption were performed, after which MS detection took place, resulting in a total analysis time of 3 min. An LOD below 1 ng/mL could be obtained despite yields of only 2.1% and 1.5% for 100- and 30-m PDMS-coated fibers, respectively. A new on-line sample preparation technique MEPS has been developed to analyze the local anesthetics lidocaine, prilocaine, ropivacaine and mepivacaine in human plasma [94]. Compared with traditional LLE and SPE, MEPS reduced sample preparation time and organic solvent consumption. In addition, MEPS can treat small samples, requiring only one minute for each sample. MEPS is more easily automated than SPE and more rugged than SPME. Compared with SPME, MEPS is more stable and has a

higher recovery. Although the sampling fibers used in SPME are very sensitive to sample matrix, the new technique can be used without difficulty for complex matrices, including plasma, urine and organic solvents. In addition, this method has a much higher extraction recovery rate (60–90%) compared with SPME (1–10%).

3.2. Applications in Clinical and Pharmaceutical Analysis

Benzodiazepines are frequently used in clinical practice as tranquilizers, sleep inducers, antiepileptic hypnotics, anticonvulsants, and muscle relaxants. These drugs, however, can cause intoxication due to accidental overdose or intentional abuse. Benzodiazepines in plasma and urine can be analyzed in combination with DI-SPME HPLC-UV using PDMS/DVB and CW/TPR fibers. For example, when urine samples spiked with delorazepam were analyzed using a PDMS/DVB fiber following short extraction (30 min) and desorption (3 min) times without dilution, the LOD was 5 ng/mL [100]. As a new approach, a biocompatible SPME fiber coated with an alkyl-diol-silica (ADS) RAM for direct sampling in biological matrices by SPME has been developed [103,105]. A new ADS fiber was prepared by immobilizing ADS particles on a cleaned silica fiber with Locktite 349 adhesive. The main feature of this material is its "restricted access" property, which enables extraction of low-molecular-weight compounds such as the analyte, whereas higher-molecular-weight molecules such as proteins are size-excluded. This fiber can simultaneously fractionate

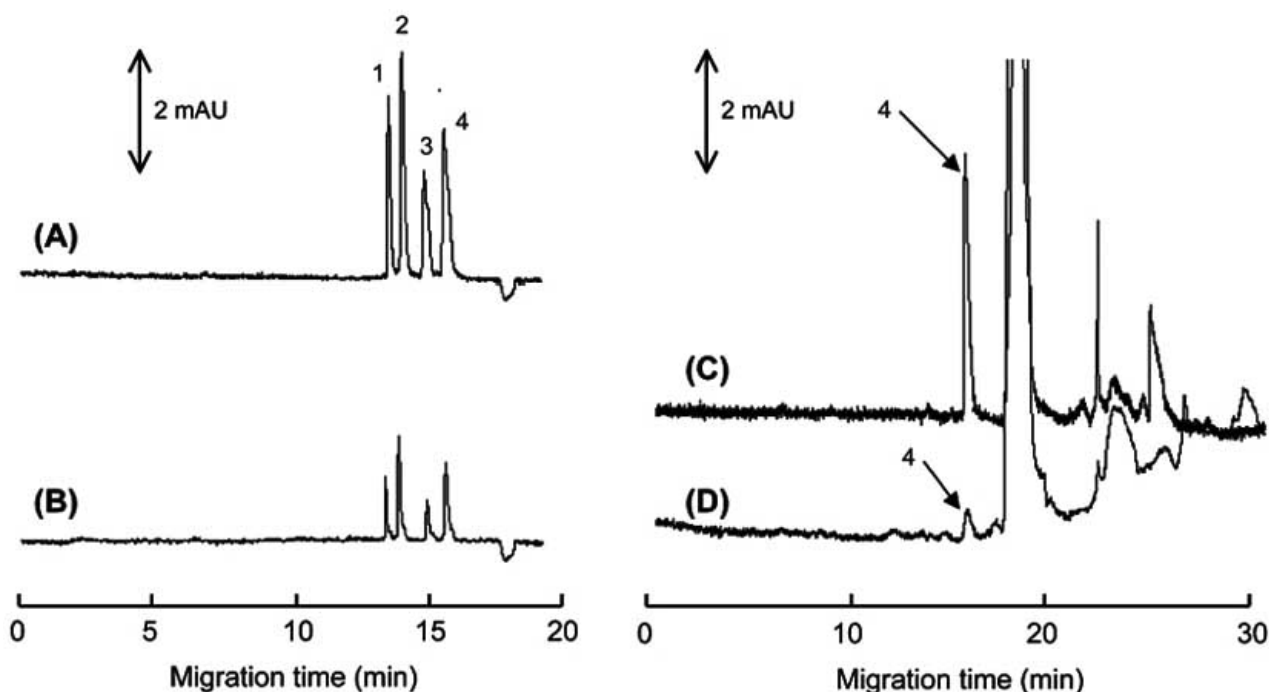


Fig. (8). Electropherograms of tricyclic antidepressant drugs. (A) Fiber-in-tube SPME/CE analysis of standard mixture (0.5 µg/mL each), (B) direct CE analysis of standard mixture (50 µg/mL each), (C) fiber-in-tube SPME/CE analysis of an actual patient's urine, (D) fiber-in-tube SPME/CE analysis of a controlled-urine spiked by 5 µg/mL amitriptyline. SPME conditions: extraction medium, HM/DB-5; packing density, 52%; extraction flow rate and time, 80 µL/min × 12.5 min (1.0 mL); desorption flow rate and time, 4.0 µL/min × 0.45 min (1.8 µL); desorption solvent, acetonitrile. Peaks: 1=desipramine, 2=nortriptyline, 3=imipramine, 4=amitriptyline. Reprinted with permission from references [26] and [111]. © 2003 Elsevier Science and © 2001 Wiley-VCH Verlag GmbH.

the protein component from a biological sample while directly measuring several benzodiazepines. The SPME-HPLC interface was slightly modified, in that the through-hole of the tee was enlarged to facilitate insertion of a large-diameter fiber and the volume of the desorption chamber was reduced to approximately 5 µL. A mixture of benzodiazepines was selected as target compounds to demonstrate the potential of this new fiber. This method was highly selective and sensitive using an LC-MS. The LODs of diazepam and related benzodiazepines were 46-750 ng/mL urine and 20-35 ng/mL blood. In contrast, an automated in-tube SPME coupled with LC-ESI-MS (positive ion mode, SIM) was used to assay seven benzodiazepines [101]. The benzodiazepines were extracted in a Supel-Q plot capillary and easily desorbed by the mobile phase during dynamic desorption, and this method was directly applied to urine and serum samples without interference. Recently, an automated biocompatible in-tube SPME method was developed using a PEEK capillary packed with ADS particles and applied to the direct extraction of several benzodiazepines in human serum [102]. The bifunctionality of the ADS extraction phase prevented fouling of the capillary by protein adsorption while simultaneously trapping the analytes in the hydrophobic porous interior. This approach required a more simplified apparatus compared with existing RAM column switching procedures, as well as overcoming the problem that in-tube SPME requires ultrafiltration or another deproteinization step prior to handling biological samples, therefore further minimizing the sample preparation requirements.

SPME-HPLC has been applied to the determination of other psychoactive drugs. For example, a method was developed for assaying the antidepressant amitriptyline in human urine by coupling SPME with microcolumn liquid chromatography, [109] and a DB-1 capillary was used for the in-tube SPME of tricyclic antidepressants in urine, with the extraction efficiency raised by insertion of stainless steel wire into the capillary [110]. In an interesting innovation for the latter, a fine wire is incorporated into the lumen of the extraction capillary, effectively increasing the surface-to-volume ratio for analysis, which limits extraction efficiency. Using this wire-in-tube SPME method coupled with microcolumn HPLC, four tricyclic antidepressants (amitriptyline, imipramine, nortriptyline, and desipramine) in urine samples were measured in the 5-500 ng/mL range by UV detection. The pre-concentration factors for desipramine, nortriptyline, imipramine, and amitriptyline were about 14.7, 17.5, 52.5, and 110, respectively, in comparison with direct injection. In addition, an on-line interface between the fiber-in-tube SPME and CE was developed, and the pre-concentration and separation of the above four tricyclic antidepressant drugs were performed with the hyphenated system [111]. As shown in Fig. 8, this technique was applied to the analysis of human urine, and amitriptyline was measured in a patient's urine.

-Adrenoceptor blocking agents (α-blockers) are used therapeutically for circulatory system diseases such as hypertension, angina pectoris, and arrhythmia. These drugs have been banned by the International Olympic Committee as doping agents. A simple and rapid analytical method is

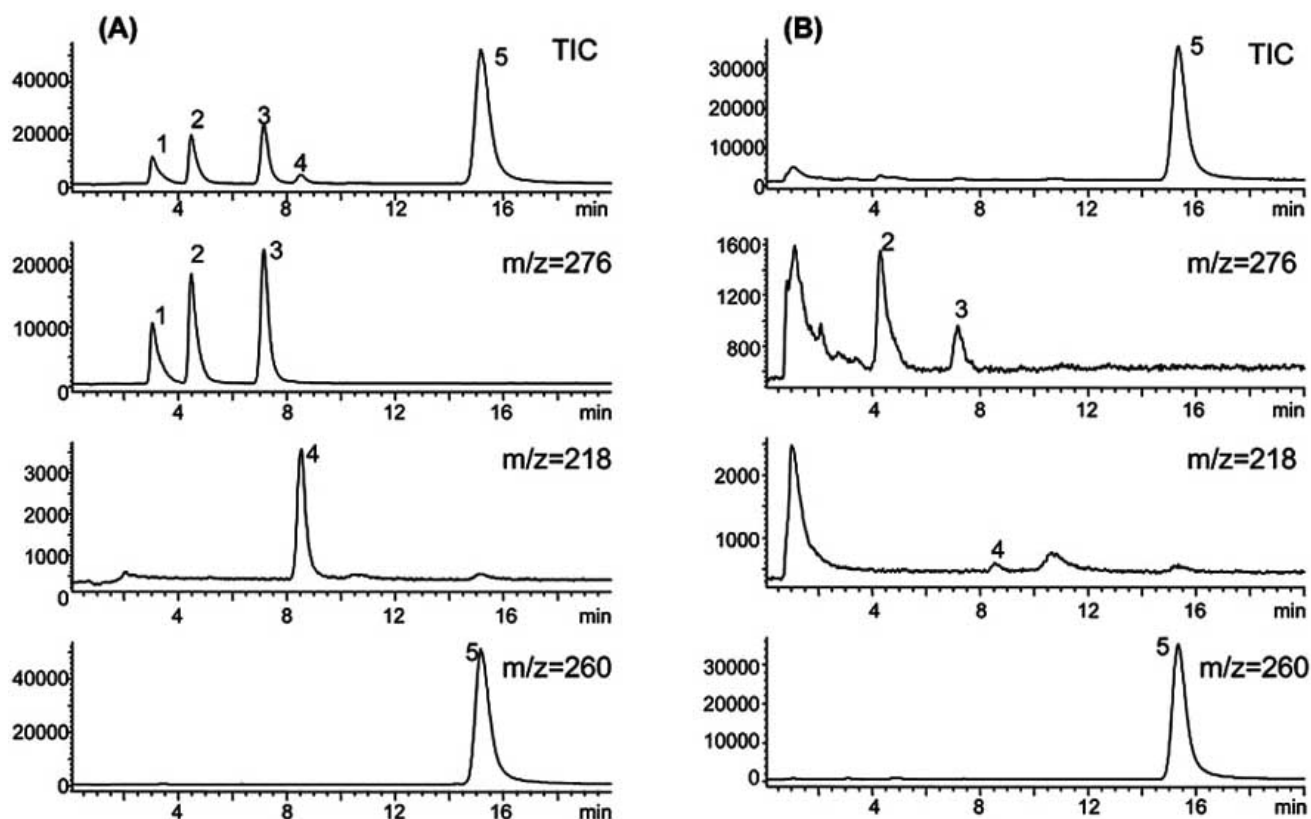


Fig. (9). Total ion and SIM chromatograms obtained from propranolol and its metabolites, and a clinical serum sample by in-tube SPME/LC/MS-SIM. (A) Standard solution containing 200 ng/mL propranolol, 50 ng/mL 4-hydroxypropranolol and 7-hydroxypropranolol, 20 ng/mL 5-hydroxypropranolol and N-desisopropylpropranolol. (B) Clinical serum sample (100 μ L). LC conditions: column, Hypersil BDS C₁₈ (5.0 cm x 2.1 mm i.d., 3 μ m particle size); mobile phase, acetonitrile/methanol/water/acetic acid (15:15:70:1); flow-rate, program from 0.25 to 0.45 mL/min for a 20 min run. ESI⁺-MS conditions: neblizer gas, N₂ (40 psi); drying gas N₂ (10 L/min, 350°C); fragmentor voltage, 70 V; capillary voltage, 3500 V; Peaks: 1=5-hydroxypropranolol (m/z 276), 2=4-hydroxypropranolol (m/z 276), 3=7-hydroxypropranolol (m/z 276), 4=N-desisopropylpropranolol (m/z 218), 5=propranolol (m/z 260). Reprinted with permission from references [26] and [114]. (© 2003 Elsevier Science and © 1999 American Chemical Society).

desired for clinical control, doping inspection, and forensic chemistry, because the toxicity of these drugs is strong, and the threshold value between a therapeutic and toxic dose is narrow. An automated in-tube SPME coupled with LC-ESI-MS (positive ion mode, SIM) was developed for β -blockers using an Omegawax 250 capillary [114]. The β -blockers extracted in the capillary were easily desorbed into the mobile phase by the dynamic desorption technique. This method resulted in an LOD of 0.1-1.2 ng/mL (S/N=3), with a linearity in the 2-100 ng/mL range. This method can be directly applied to diluted urine and ultrafiltered serum without interference. As shown in Fig. 9, this method was successful in detecting propranolol and its metabolites in the serum of patients administered propranolol. Furthermore, a PPY-coated capillary was used successfully to measure β -blockers in serum and urine samples [115]. In comparison with findings using the Omegawax 250 capillary column, the PPY-coated capillary column gave better extraction efficiency and lower detection limits for most drugs. A new in-tube SPME technique was recently developed using a PEEK capillary packed with propranolol MIP particles, in

which MIP was extensively cross-linked to polymer materials containing synthetic cavities or recognition sites with a predetermined selectivity [116]. In addition to its inherent selectivity and chemical and physical robustness, the MIP material was demonstrated to be an effective stationary phase material for in-tube SPME. Using a propranolol MIP-packed capillary, an automated on-line in-tube SPME/HPLC system was developed for the selective analysis of propranolol in serum samples.

An automated in-tube SPME technique was applied to the analysis of verapamil, a common calcium antagonist with antianginal, antihypertensive and antiarrhythmic properties, [117]. a histamine H₂ receptor antagonist, used to treat duodenal and stomach ulcers, [118] and xanthine derivatives [123]. More recently, an in-tube SPME method using a monolithic capillary column as extraction device was developed [123]. Hydrophobic main chains and acidic pendant groups of poly(methacrylic acid-ethylene glycol dimethacrylate) make it a superior material for extracting basic analytes from an aqueous matrix. An on-line

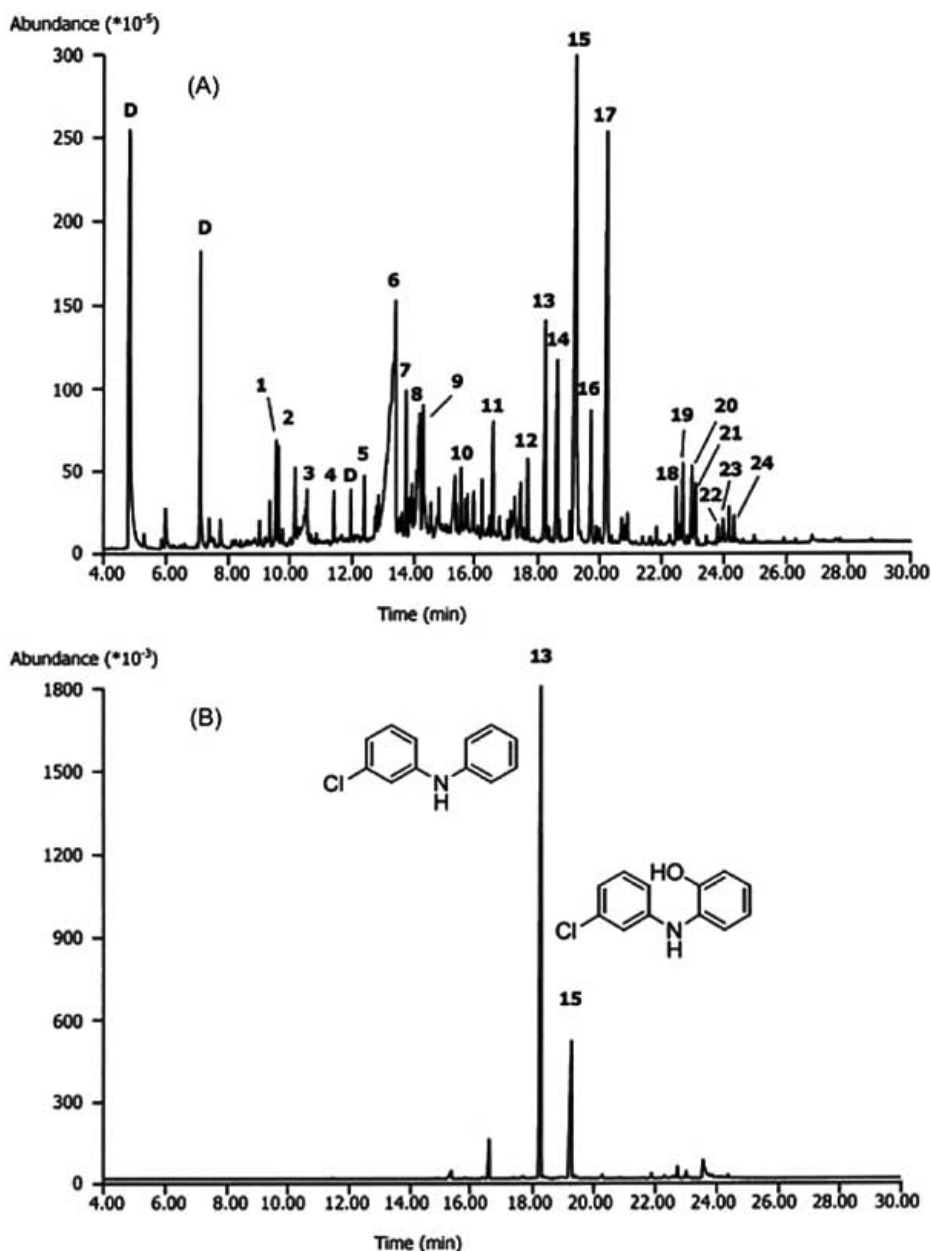


Fig. (10). SBSE thermal desorption CGC-MS analysis of 5 mL of urine from a patient being treated with drugs. (A) Total ion chromatogram, (B) extracted ion chromatogram at m/z 229 of clomipramine metabolites. GC conditions: capillary column, a 30 m \times 0.25 mm I.D., 0.25 μ m d; HP-5MS column (Agilent Technologies); oven temperature, programmed from 50 to 320°C at a rate of 10°C/min. MS conditions: Agilent 5973 mass spectrometric detector operated in the scan mode (m/z 40–500). Peaks: 1=4-vinyl-2-methoxyphenol, 2=eugenol, 3=decanoic acid, 4=cis-eugenol, 5=ethyl 4-ethoxybenzoate, 6=dodecanoic acid, 7=cadinene, 8=junipene, 9=bulnesol, 10=citronilide, 11=benzylsalicylate, 12=cannabichromene, 13=clomipramine metabolite I, 14=methadone metabolite I, 15=clomipramine metabolite II, 16=methadone, 17=androsthenol, 18=diazepam, 19=androsterone, 20=epiandrosterone, 21=nordiazepam, 22=temazepam, 23=O-methylxazepam, 24=allopregnanediol, 25=bromazepam. Reprinted with permission from reference [129]. (© 2003 Elsevier Science).

monolithic capillary in-tube SPME method was developed for the determination of theobromine, theophylline and caffeine in serum samples. This method had a high extraction efficiency for all analytes, with an LOD of 6.5–12 ng/mL.

SPME-HPLC-ESI-MS-MS using a CW/TPR fiber was shown to be suitable for the analysis of seven tetracyclines at low ng/mL levels [125]. This method was slightly modified for analysis of milk samples, because addition of potassium

chloride, which increased the recovery from aqueous samples, precipitated the protein in real samples. Because of the absence of potassium chloride and other matrix effects, tetracycline in milk had a higher LOD (100 ng/mL).

SBSE in combination with thermal desorption on-line coupled to capillary GC-MS was recently developed for the analysis of pharmaceutical drugs and metabolites in urine samples [129]. SBSE implies stirring of aqueous samples (e.g. urine or blood) with a glass stir bar coated with a thick

layer (24 μL) of PDMS for sorptive enrichment of the analytes of interest. This technique is very versatile and sensitive for analyzing a wide range of drug substances. Moreover, the relatively high enrichment efficiencies of SBSE permitted the use of mass spectrometric detection in the full scan mode. Fig. 10A shows the total ion chromatogram (TIC, m/z 40–500) of the SBSE thermal desorption GC–MS analysis of a urine sample (5 mL) of an adult multi-drug user. More than 200 compounds could be enriched and detected at the same time, resulting in a complete profile of GC-amenable compounds in the urine sample. Urine mainly reflects the food consumption of the patient, since several terpenes (Fig. 10A, peaks 1, 2, 4, 7–10) originating from the digestion of vegetables are highly abundant. The major pharmaceuticals in the urine sample are the clomipramine metabolites, 5-chloro-3-phenyl-2,1-benzisoxazole (Fig. 10A, peak 13) and 8-hydroxy-5-chloro-3-phenyl-2,1-benzisoxazole (Fig. 10A, peak 15). These compounds can be screened out more clearly by the extracted ion chromatogram at m/z 229 (Fig. 10B).

3.3. Applications to Contamination of Pharmaceuticals and Diagnoses of Diseases

In manufacturing drug substances, residual solvents arising from the final purification by recrystallization or distillation, or from one or more steps during the synthetic process, can be retained in the end products. Often these solvents, referred to as organic volatile impurities, are transferred to the pharmaceutical preparation, making their measurement mandatory. Volatile impurities are required to be tightly controlled at much lower levels (ppm to ppb levels) due to their relatively higher levels of toxicity. The HS-SPME technique was applied to the analysis of residual solvents in pharmaceutical products [131–134]. PDMS, PDMS/DVB and CRB/PDMS/DVB fibers were used for the extraction of solvents such as ethanol, cyclohexane, toluene, benzyl chloride and triethylamine, and the impurities extracted were successfully analyzed in combination with GC-FID.

Disposable plastic products are widely used for medical devices such as infusion solution bags, administration sets and containers of liquid medicines because of their flexibility, transparency and low cost compared with glassware. However, the additives in plastics have recently attracted a great deal of public attention, as several of these materials may possess endocrine disrupting properties. In investigating the contamination of alkyl phenols and phthalates in liquid medicines and intravenous injection solutions using in-tube SPME coupled with HPLC, the LODs of these compounds were found to be 1–10 ng/mL, and di-n-butylphthalate was eluted from the adhesive used to affix the paper labels to the solution by passing through the plastic [135,136].

Volatile compounds in biological samples have been used as important disease markers. For example, breath acetone is higher in diabetic patients than in controls, and analysis of breath acetone is used as a diagnostic tool for diabetes. However, it is very difficult to accurately measure the concentration of acetone in human breath due to its volatility and activity. To overcome this problem, a new

method was developed using HS-SPME with on-fiber derivatization and GC-MS, [139] and breath gas from controls and diabetic patients was collected in 3-L Tedlar bags. After absorption of *O*-2,3,4,5,6-(pentafluorobenzyl) hydroxylamine hydrochloride on PDMS/DVB fibers, the fiber was exposed to exhaled gas in the Tedlar bag at 40°C for 4 min. Finally, the formed acetone oxime on the fiber was desorbed and analyzed by GC-MS, showing that this method is simple, rapid, sensitive and solvent-free. The HS-SPME GC-MS method was also applied to the determination of acetone and other volatile biomarkers in human serum. For example, to identify biomarkers of lung cancer, the HS-SPME method was used to analyze volatile compounds in the blood of lung cancer patients, showing that the concentrations of hexanal and heptanal in the blood of these patients were much higher than in control blood [140].

In addition, SPME and related microextraction techniques have been widely used for the analysis of various contaminants, such as pesticides, [141–148] organometallic compounds [149–152] and volatile organic compounds, [153–175] in biological samples. These methods are useful for biomonitoring in toxicology and environmental chemistry.

4. CONCLUSION

Drug analysis in biological samples and pharmaceutical products is very important for TDM, pharmacokinetic studies, screening of illicit drugs and development of new medicines. The choice of an analytical method depends on the presence of the target analytes in samples at low parts per billion or less, and the variety and complexity of the sample. Therefore, sample preparation methods for complex matrices are necessary to isolate the components of interest from a sample matrix, because most analytical instruments cannot handle the matrix directly. This greatly influences the reliability and accuracy of the analytical method, but it has always been a somewhat neglected problem in analytical chemistry. Suitable sample preparation is an important prerequisite for analysis of biological samples. In general, sample preparation is still regarded as "low tech" and in many laboratories is assigned to the least trained staff, individuals who may often be reluctant to accept new technologies. This is one of the main reasons for the slow implementation of new sample preparation techniques, making sample preparation in many cases to be still the most time-consuming and often rate-limiting step of the total analytical procedure. SPME and the related microextraction techniques described in this review are very effective sample preparation techniques for qualitative and quantitative analyses of biological and pharmaceutical samples. As extraction and concentration are combined, all of the analyte extracted is directly introduced into the analytical system.

SPME is a technique for extraction of organic compounds from gaseous, aqueous, and solid matrices. It is rapid and simple, ideal for automation and for in situ measurements, and no harmful solvents are needed. The principle of SPME involves equilibration of the analytes between the sample matrix and an organic polymeric phase coated on a fused-silica fiber. SPME can be combined with

GC, and this combination has proved sensitive, accurate, and precise for quantitative analysis of different classes of volatile compounds. SPME can also be coupled with HPLC to widen its range of applications to non-volatile and thermally unstable compounds. In-tube SPME is useful for the construction of on-line automated systems. The main advantages of in-tube SPME are its simplicity, rapidity, solvent elimination, high sensitivity, small sample volume, lower cost and simple automation. The in-tube SPME technique can be successfully used to measure polar and non-polar compounds in liquid samples, and can be easily coupled with various analytical methods, such as HPLC, LC-MS and CE. In addition, it is possible to improve the extraction efficiency and selectivity by further development of new capillaries coated or packed with new materials, and the range of applications may increase after further combination with different analytical instruments. A more radical approach to the design and concept of SPME has been recently proposed. SPDE and MEPS are new microextraction techniques using a needle internally coated with PDMS or packed with adsorption materials. These miniaturized techniques are rapid and easy to automate, and can be easily coupled with various analytical methods such as GC-MS and LC-MS. SBSE using a coated magnetic stirring bar, with similar types of phase but a thicker layer, is compatible with both GC and HPLC desorption procedures, and gives 500 times improved sensitivity compared with a 100 μm PDMS fiber for certain applications, owing to the increased amount of phase available for sorption.

There is a clear trend towards the simplification of sample preparation, towards an increase in its reliability and precision, and towards the elimination of the clean-up step by using more selective extraction procedures. In the future, additional customized coatings, including chirally active phases, various derivatized cyclodextrins, ion exchangers, HPLC stationary phase particles and sol-gel porous silicas, are expected to become available. As evidenced with SPE, immunoaffinity sorbents, RAMs and MIPs are also expected to be used as new coating materials for the highly efficient extraction of drugs from various biological samples. With the development of more sensitive and selective phases it may be possible to further miniaturize the technique. Furthermore, there is an increasing interest in automating sample preparation, thus speeding these procedures, and improving precision and cost-effectiveness. As the market for SPME increases in the future, this could lead to the introduction of disposable low-cost extraction fibers (e.g. in the form of a carousel) or tubes such as in other areas of sample preparation, such as SPE multiwell plates. The key attractive features of automated sample preparation techniques include miniaturization, high throughput, reproducibility and traceability. In the last decade, new concepts have been developed, which allow the on-line coupling of sample preparation devices to separation and detection systems, and which are specially designed for automation. In the future, better integration of sampling/sample preparation and instrumental analysis will allow wider use of automated on-line analysis in forensic, clinical and pharmaceutical analysis. Last, I hope that this review will serve as a guide to choosing the most effective

sample preparation techniques for pharmaceutical and biomedical analysis.

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