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Review

Solid-phase micro-extraction of drugs from biological matrices

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Abstract

Solid phase micro-extraction was originally designed as a technique for the solvent-free analysis of volatile organic contaminants in environmental samples. However, a wide variety of applications are now being pursued, including the analysis of drugs from a variety of matrices. In this review, the analysis of drugs by SPME from biological and related matrices, including water, urine, blood, hair and saliva, is discussed. A general overview of the special problems and techniques involved in SPME from biological matrices is presented, along with specific references and discussion of the analysis of many types of drugs and metabolites. It is seen that SPME is a highly versatile and flexible technique for these analyses.

Keywords: Reviews; Biological matrices; Solid-phase microextraction; Drugs

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1. Introduction: solid phase extraction of drugs from biological matrices

The successful extraction of drugs from biological fluids and matrices presents several challenges. Biological materials, including urine, blood, saliva and hair, are much more complex than many others. They often contain proteins, salts, acids, bases and numerous organic compounds with similar chemistry to the analytes of interest. Also, within classes of drugs, such as steroids or benzodiazepines, a variety of acid–base behaviors and functional groups, which strongly affect solubility or affinity to a sorbent can be present. Thus, general extraction methods for biological matrices have either been complex, if selectivity is desired, or straightforward, but not selective, leaving the analyst with a complicated separation following the extraction. For biological matrices, selective analysis for drugs from a variety of matrices is often performed today by solid-phase extraction [1], while classical methods have employed liquid–liquid extraction. General solid-phase extraction chemistry is described in the recent texts by Fritz [2] and by Thurman and Mills [3]. For an example of the advantages and disadvantages of SPE in drug analysis, de Zeeuw presents a comprehensive drug screening method for drugs by solid-phase extraction [4]. This method exemplifies both the promise and difficulty of SPE for drug analysis, as a variety of drugs are screened by the single method, yet there are many potentially laborious steps involved. SPME can help to overcome some of the difficulties in SPE, including solvent usage, sorption and desorption kinetic effects from the flow-through design of SPE, and automation.

2. SPME: direct and headspace

Solid-phase micro-extraction was developed in the late 1980s by Arthur and Pawliszyn [5]. Initially, the extractions were of volatile organic contaminants, such as BTEX from water, with myriad other applications developing during the 1990s. A commercial SPME device was first marketed by Supelco in 1993 and an automated system has been marketed by Varian. A recent applications guide shows nearly 400 separate applications references for SPME [6] and three dedicated textbooks have been published [7–9]. Early work in SPME focused on studies of the dynamics and chemistry of the extraction process from liquid samples [10,11] which have now been thoroughly described. SPME was interfaced to HPLC in 1995 [12] and has been interfaced to CE [13], GC–ICP-MS [14] and numerous other techniques. In a text, Pawliszyn [15] extensively describes method development issues involved in direct immersion SPME. A listing of issues involved in generating reproducible SPME results is provided in Table 1.

Although originally developed for sampling from liquids, SPME has been often shown more effective, when sampling from headspace. In headspace SPME, first described in 1993 [16–18], the fiber is placed

Table 1
Factors affecting the reproducibility of SPME extraction and SPME-GC injection [7,14]

Extraction conditions	GC injection conditions
Fiber dimensions (length, film thickness)	Inlet liner diameter and geometry
Physical condition of the fiber (cracks, adsorbed material, aging)	Inlet liner deactivation
Extraction temperature	Moisture in the needle
Sample matrix effects (salt content, pH, organics, proteins, etc.)	Position of the fiber in the inlet
Agitation type and speed	Inlet temperature
Sampling time (non-equilibrium methods)	Inlet flow-rate
Sample volume	Column temperature and flows
Headspace volume	
Vial shape	
Time between extraction and analysis	
Adsorption on the glassware	

above the liquid or matrix to be sampled. Headspace SPME has been shown to have the advantages of speed, as extraction kinetics from headspace are faster than from liquid, high recoveries, as air–fiber partition coefficients are often very large with minimal fiber fouling or contamination, as the fiber does not contact the liquid sample directly. Headspace SPME is often the technique of choice if the analytes are appreciably volatile or can be made volatile with moderate heating of the sample.

3. SPME method development

When developing SPME methods for drug analysis, the analyst is faced with numerous choices. For extensive details the reader is referred to the text by Pawliszyn [7] and the chapter by Lord [19]. Several major issues related to drug analysis are presented here. These include: fiber and extraction method choice, adjustments to the matrix (pH and ionic strength), derivatization, and desorption conditions.

The choice of fibers and extraction modes is generally limited to those commercially available fibers and to headspace versus direct immersion extraction. For drug analysis, virtually all of the commercially available fiber coatings have been used. Most of the work in drug analysis has been done using direct immersion, although headspace extraction has been used for volatile drugs such as amphetamines or for volatile metabolites. Table 2 shows the commercially available fibers and coating thicknesses, with comments about the general classes of analytes preferred for each.

Often, in SPME extraction, the matrix must be adjusted to enhance the extraction recovery of analyte into the fiber. Most often, this involves adjustment of the pH or salt content of an aqueous matrix, such as urine, blood or saliva. For ionic analytes, pH adjustment is often necessary. Since the SPME fiber coatings are neutral, the pH should be adjusted such that the analyte of interest is also neutral. Recoveries of basic analytes are enhanced by basic pH; recoveries of acidic analytes are enhanced by acid pH. Salt content may also affect the recovery in an extraction. Addition of salt, such as sodium chloride, often increases recovery due to salting out effects.

Many drugs of interest are not volatile, are polar, or are strongly bound to the matrix. In these cases, derivatization may be necessary to make them amenable to thermal desorption from the SPME fiber and GC analysis. Several derivatization techniques have been used in environmental analysis, and are described in detail by Pan and Pawliszyn [20]. Derivatization can occur by adding appropriate reagents to the matrix, followed by extraction; by doping the fiber with the reagents, followed by extraction; by extracting, then exposing the fiber-

Table 2
Commercially available SPME fibers [6]

Stationary phase and film thickness	Abbreviation	General application (analyte type)
Polydimethylsiloxane (100 μm)	PDMS	Non-polar, volatile
Polydimethylsiloxane (30 μm)	PDMS	Non-polar, volatile and semi-volatile
Polydimethylsiloxane (7 μm)	PDMS	Non-polar, semi- and non-volatile
Polydimethylsiloxane–divinylbenzene (65 μm)	PDMS–DVB	Polar
Polyacrylate (85 μm)	PA	Polar, general use
Carboxen–polydimethylsiloxane (75 μm, 85 μm)	CAR–PDMS	Volatile, gaseous, trace analysis
Carbowax–divinylbenzene (65 μm, 75 μm)	CW–DVB	Polar, volatile (low temperature limit)
Carbowax–templated resin (50 μm)	CW–TPR	Polar, HPLC
Divinlybenzene–carboxen–PDMS (50/30 μm)	DVB–CAR–PDMS	Broad range of polarities from C_3 to C_{20}

containing extract to the derivatizing reagent, or by derivatizing within the GC inlet. The potential for derivatization to enhance drug analysis by SPME is great and has been explored relatively little.

GC desorption conditions also can significantly affect SPME analysis. Okeyo and Snow [21,22] and Langenfeld et al. [23] have presented general discussions of SPME-GC injections. It should be noted that SPME is not subject to the solvent effects that focus analytes in splitless and on-column injections. SPME desorption is perceived to be a slow process, although it can be made rapid by using a low thickness fiber and a narrow-bore inlet liner [23,24]. Special narrow-bore glass sleeves are currently available for the splitless inlets of most major gas chromatographs. The inlet conditions become more important as the analyte becomes more volatile; obtaining the narrowest possible injection bandwidth is critical.

In the applications described below, the ideas about extraction and injection of drugs by SPME presented above are seen in the authors' method development schemes. Due to variations in extraction recovery, which many of the authors discuss, most of the quantitative methods employed internal standards. Headspace SPME is employed wherever possible, to avoid exposure of the fibers to urine or blood matrices, although the fibers have generally proven robust in direct immersion as well. Detection limits and linear ranges are easily competitive with other techniques, and in many cases, are superior. Analysis of drugs by SPME is in its infancy and continued study of these techniques, along with the development of new applications, will significantly benefit pharmaceutical, clinical and forensic analysis.

4. Specific applications of SPME to drug analysis

There are numerous references to the SPME analysis of drugs from biological matrices. This review is organized by class or by specific drug. A summary of references is given in Table 3. Several general reviews of drug analysis by SPME have recently been presented [25–30]. A complete issue of *Journal of Chromatography B* was recently dedicated to chromatography and capillary electrophoresis in forensic and clinical toxicology [31].

Table 3
Summary of references to drug analysis by SPME

Analyte class	References
General reviews	[25–32]
Alcohol and other volatiles	[33–39]
Amphetamines	[40–50]
Analgesics and PCP	[51–53]
Anesthetics	[54–57]
Antidepressants	[58–61]
Barbiturates	[62–64]
Benzodiazepines	[65–70]
Cannabinoids	[71,72]
Cocaine	[73]
Proteins	[74]
Steroids	[75–78]
Miscellaneous substances	[79–86]

Degel provides a comparison of several new extraction technologies (SPME, cartridge SPE, disk SPE) for toxicological analysis [32]. It is noted that most work in this area is focused on solving problems in forensic analysis, although the potential for SPME in other clinical and pharmaceutical sciences cannot be overlooked. Below, each class or individual drug listed in Table 3 is considered briefly, with some detail on critical aspects of the extraction method. In many cases, there are several possible SPME methods for the same analysis.

4.1. Alcohol and volatiles

Ethanol was determined in whole blood and urine by Kumazawa et al. [33] using headspace SPME with a carbowax–divinylbenzene-coated fiber. It should be noted that they considered the PDMS fiber first, but rejected it due to low recoveries. The headspace method involved 0.5-ml samples of blood, with sodium sulfate added, heated in 4-ml vials to 70°C, and 15 min exposure of the fiber. The GC analysis required 7 min. The method then employed in the examination of ethanol in patients' blood following oral administration. Lee et al. recently showed an improved extraction method for ethanol from various body fluids by headspace SPME by using a carboxen–PDMS fiber [35]. Whole blood samples, are heated to 60°C in the presence of ammonium sulfate and sodium dithionate. A carboxen–PDMS fiber is exposed to the headspace for 15 min, followed by GC analysis. Calibration curves

were linear from 2.5 to 400 mg/l for whole blood and from 0.5 to 400 mg/l for urine. They reported a sensitivity improvement of 1–3 orders of magnitude over the previous method.

Brewer et al. developed a confirmation method for volatiles, related to traffic fatalities by headspace SPME and GC–MS [34]. They showed the determination of ethanol, methylene chloride, and volatile petroleum products from urine and gastric samples. The authors pointed out that the absence of an air or solvent peak was a tremendous benefit of using SPME for analysis of volatiles. Grote and Pawliszyn [36] provide a comprehensive discussion of the determination of ethanol, acetone and isoprene in human breath. They present extensive optimization studies, including the effect of fiber selection, relative humidity, sampling temperature and extraction time on the results. A simple modification of the classical SPME device is used for sampling breath. They found detection limits in the low nmol/l range and linear ranges approximately 10–500 μmol/l.

Additional methods for the analysis of volatiles from biological samples by SPME are provided by Iwasaki et al. [37], and Lee et al. [38]. Vergnais et al. [39] provide a detailed discussion of the analysis of volatile metabolites from staphylococci, including studies of fiber coating choices and extraction conditions.

4.2. Amphetamines

Several groups have addressed the analysis of amphetamines by SPME, and they have employed a variety of extraction and analysis methods. Krogh and co-workers employed a propyl chloroformate derivatization on a urine sample containing amphetamines, prior to SPME extraction using a PDMS fiber, followed by GC–MS analysis. The method included addition of salt and adjustment to basic pH, which enhanced the extraction [40,41]. They reported excellent results at the 4-μg/ml level. Lord and Pawliszyn presented an extensive optimization study of headspace and direct immersion SPME for amphetamine and methamphetamine from clinical urine samples [42]. They studied extraction mode, and fiber choices, sample volume, extraction temperature, GC conditions, agitation rate, salt content and the effects of several interferences. Using the optimized method, they obtained interference-free chromatograms of urine extracts that showed detection limits of 0.5 ng/ml for amphetamine and 1.1 ng/ml for methamphetamine from aqueous samples. They also demonstrated the method for several narcotic analgesics.

Centini et al. used headspace SPME for qualitative and quantitative analysis of several amphetamine-related compounds [43]. They reported improved mass chromatograms, due to reduced interferences from a urine matrix. They also reported both linear and exponential calibrations over a 100–2000-ng/ml concentration range. They used a 100-μm PDMS fiber, with a 15-min adsorption time at 90°C. One-ml spiked urine samples were adjusted to pH 9 and 1 g of NaCl was added to enhance the 'salting out' effect.

Koide et al. used headspace SPME with GC-NPD to determine amphetamine and methamphetamine in human hair [44]. Hair was prepared for SPME by mixing with an internal standard and with 6.7 *M* NaOH and heating to 75°C until all of the hair dissolved. They found that headspace extraction temperature had a strong influence on response, with the optimum temperature between 50 and 60°C. Extraction was relatively slow, requiring 20 min or more, while desorption was rapid, occurring in less than 1 min. The detection limits were 0.1 ng/mg hair for amphetamine and 0.4 ng/mg hair for methamphetamine.

A comprehensive analysis of 21 amphetamine-related compounds has been presented by Battu et al. [45]. Several additional authors [46–50] have also recently provided methods and discussion of the analysis of amphetamines by SPME.

4.3. Analgesics and phencyclidine (PCP)

Analgesics are widely used in pain relief and are some of the most readily abused drugs. A SPME method for the analysis of meperidine, which is commonly used in place of opiates and methadone, which is used in the treatment of opiate abuse, was recently described by Myung et al. [51] as an alternative to liquid–liquid or solid-phase extraction. They found greater recoveries using a 100-μm PDMS fiber versus an 85-μm polyacrylate fiber, with a maximum at a 30-min immersion time. They

presented an interesting study of pH and sample salt concentration, in which the degree of ionization of the analyte at the given pH was seen as a critical factor in recovery, and that the salt concentration can be used to enhance recovery of partially ionized species. For their final method (pH 11, 15% salt concentration, 30-min immersion), they obtained relative standard deviations of about 2–5% for samples with concentrations of 1–2 μg/ml in urine. Seno et al. have also described a method for the analysis of meperidine [52].

Phencyclidine, an analgesic developed in the 1950s, the use of which has been discontinued due to hallucinogenic effects, has been analyzed in whole blood and urine by Ishii et al. [53] using a PDMS fiber in the headspace mode at 90°C. The samples were de-proteinated and treated with NaOH prior to extraction. Detection limits were approximately 0.25 ng/ml for urine and 1.0 ng/ml for blood. Lower recoveries were noted from blood than from urine.

4.4. Anesthetics

Kumazawa et al. analyzed a mixture of 10 local anesthetics from human blood by direct immersion SPME [54,55] using a PDMS fiber. They reported detection limits in the 50–700-ng/ml range, depending upon the analyte. Samples were pre-treated with perchloric acid for deproteination, salt was added and the pH was adjusted to about 7. Equilibration was completed in 40 min. Watanabe et al. developed a method for five local anesthetics in blood using headspace SPME and GC–MS [56]. Using synthesized d_{10}-lidocaine as internal standard, samples adjusted with 5*M* NaOH and a 45-min headspace extraction with 100 μm PDMS fiber, they obtained linear calibration curves in the ranges of approximately 0.1–20 μg/g, with no interferences and an analysis time of 65 min/sample. Their method was successfully applied in a legal case.

Koster et al. employed SPME with both GC and HPLC for the analysis of lidocaine in human urine [57]. They used direct immersion of a PDMS fiber and studied the effects of time, pH, ionic strength, temperature and agitation. They obtained extraction yields of 22% in 45 min with a reproducibility of less than 5% relative standard deviation. Linear ranges were found from 5 to 1000 ng/ml for SPME-GC and from 25 to 1000 ng/ml for SPME-LC.

4.5. Antidepressants

Ulrich and Martens provide a comprehensive discussion of the SPME extraction and GC detection using NPD, of 10 antidepressant drugs and their metabolites in human plasma [58]. The extraction involved direct immersion of a 100-μm PDMS fiber into the plasma, then washing the fiber with water and methanol, followed by a typical desorption into a splitless GC inlet. They also included studies of the effects of protein content and of α-acid glycoprotein content on the extraction recoveries. Peak areas were found to increase with decreasing protein concentration. The analytes were well-separated and showed a linear range from 125 to 2000 ng/ml. Limits of quantitation were approximately 100 ng/ml.

Lee et al. used headspace SPME with a PDMS fiber and GC-NPD to detect tricyclic antidepressants in whole blood [59]. They optimized the temperature during headspace extraction and found that, while higher temperatures gave higher recoveries, the maximum practicable temperature was 100°C, due to high pressure and septum damage. Exposure times were found to be about 60 min to reach maximum signal and linear calibration curves were found from about 25 to 1000 ng of analyte in the headspace vial. Kumazawa et al. [60] also provide an additional method for the analysis of several antidepressants by SPME.

Namera et al. developed a simple method for the analysis of tetracyclic antidepressants in blood using headspace SPME and GC–MS [61]. Blood (0.5 g) was treated with 0.5 ml 1 N sodium hydroxide and an internal standard an placed into a 12-ml vial. The vial was sealed and heated to 120°C. A 100-μm PDMS fiber was exposed to the headspace for 45 min. Separation and quantitation were performed using GC–MS with selected ion monitoring. To optimize the method, they examined the effects of temperature, pre-heating time and fiber exposure time on recovery. Limits of detection were found in the low ng/g range, with linearity of 3 orders of magnitude. Relative standard deviations of quantitative results were about 5–10%.

4.6. Barbiturates

Li and Weber [62] have employed SPME-CE for the analysis of barbiturates from urine and serum. The total sample preparation and analysis time was under 30 s. They used a home-made poly(vinyl chloride) coating on a stainless steel rod. Several analyses at high parts-per billion levels are shown. Hall and Brodbelt have also examined the SPME of barbiturates, employing more traditional GC–MS analysis [63,64]. They found the most efficient extraction with a 65-μm carbowax–divinyl benzene fiber. Recoveries from urine for most of the barbiturates they studied were 83–99% of those from water. Calibration for butalbital spiked into deionized water was linear in the range of 0.063–1.5 μg/ml.

4.7. Benzodiazepines

Benzodiazepines are a large family of compounds used clinically as central nervous system tranquilizers. Jinno et al. have developed and evaluated an SPME-microcolumn LC method for the analysis of benzodiazepines from human urine [65]. They examined five fiber coatings and found that a specially designed sol–gel [66] coated SPME fiber showed the highest responses. Of the commercially available fibers, they obtained the highest response with a carbowax–divinylbenzene template fiber. They evaluated matrix pH, extraction time and desorption time, finding enhanced extraction at high pH [7,8], long extraction times (1–3 h) and lower desorption solvent temperatures (20–40°C). This is one of a few references to SPME-HPLC of drugs and provides a useful blueprint for optimizing SPME-HPLC methods.

Krogh et al. used solvent modified SPME to extract diazepam form human plasma [67]. Prior to extraction, they immobilized 1.5 μl of 1-octanol onto a polyacrylate-coated fiber. This step was carried out by exposing the fiber directly to 2 ml of 1-octanol for 2 min. The octanol-treated fiber was then exposed to plasma samples that were modified to release diazepam from proteins in the matrix and adjusted to pH 5.5. GC detection was performed using NPD. They obtained a detection limit of 0.10 nmol/ml from plasma.

Luo et al. have extracted five benzodiazepines from water, urine and serum using direct immersion SPME with a carbowax–divinylbenzene fiber and GC–MS analysis [68]. The samples were saturated with salt, buffered to pH 7 and held at 45°C during extraction. They found the method to be linear from 0.1 to 2 μg/ml with limits of detection between 0.02 and 1 μg/ml. Inter- and intra-day variability was less than 12.5%. Guan et al. have recently presented a method for the determination of benzophenones, as an indirect method for benzodiazepines, using SPME and GC-ECD [69]. An additional method is provided by Seno et al. [70].

4.8. Cannabinoids

A method for determining cannabidiol, Δ^8-THC, Δ^9-THC and cannabinol from water and human saliva using direct immersion with PDMS fibers and analysis by ion trap GC–MS was developed by Hall et al. [71]. They examined several fibers and extraction conditions, and their method compared favorably with a liquid–liquid extraction. The linear range for all four compounds was found to be 5–500 ng/ml with detection limits in the high pg/ml range. Strano-Rossi and Chiratti [72] developed a SPME method for analyzing cannabinoids from human hair. Hair samples were washed with petroleum ether, hydrolyzed with NaOH and neutralized prior to SPME analysis by direct immersion. They used 30-μm PDMS fibers and sampled for 15 min. Quantitation was by GC–MS in SIM mode. Detection limits of 0.1 ng/mg hair for cannabinol and Δ^9-THC, and 0.2 ng/mg for cannabidiol were found. Cannabinoids were detected in ranges of 0.1–14 ng/mg.

4.9. Cocaine

Cocaine, which is a naturally occurring alkaloid and stimulant, that is widely abused, has been analyzed from spiked urine samples by Kumazawa et al. using direct immersion SPME with a PDMS fiber and GC-NPD [73]. The extraction was carried out at room temperature for 30 min with NaF added to the extraction vial. Linearity was obtained in the range of 60–500 ng/ml, with a detection limit of 12 ng/ml of urine. By using a selective detector, background noise and interference were especially low.

4.10. Proteins

Liao et al. have demonstrated the SPME extraction and separation of proteins by micro-liquid chromatography [74]. The fiber coating used was polyacrylic acid, which acted as a cation exchanger. One hundred-ml liquid samples containing myoglobin, cytochrome c and lysozyme were extracted, with relatively short extraction times of 5–10 min needed for equilibration. Following the extraction, the fiber was washed with 0.02 *M* sodium phosphate, pH 6.2, to remove extraneous material. The fiber was then desorbed into a vial containing 0.5 *M* NaCl in 0.02 *M* sodium phosphate for 5 s. An aliquot from this solution was then injected on HPLC. SPME showed the potential for analysis of protein mixtures.

4.11. Steroids

Estrogens and anabolic steroids were determined in serum and urine by Okeyo and co-workers [75–77]. Since many steroids are non-volatile and have multiple polar functional groups, they employed direct immersion SPME using either polyacrylate or carbowax–divinyl benzene fibers, followed by derivatization in the headspace of pure bis-(trimethylsilyl)trifluoroacetamide (BSTFA). The fiber is then desorbed, as usual, in the splitless inlet of a GC–MS. Several steps in the process were studied, including extraction time, sample pH and salt content, derivatization temperature and GC injection conditions were studied. For mixtures of steroids, it was found that many of these cannot be optimized for all compounds, as there is too much variety of functional groups and chemistry within the family of compounds. It was also observed that the post-extraction, on-fiber derivatization was reasonably rapid (15–30 min) and produced single products, even when more than one reactive functional group was present. Detection limits for anabolic steroids were in the low pg/ml urine range and the linear range spanned about 2–3 orders of magnitude. Corticosteroids in urine were also examined by Volmer and Hui [78], employing SPME, coupled with LC–MS.

4.12. Miscellaneous substances

SPME of 13 antihistamines, which are commonly available over the counter and are moderately strongly addictive, and their analogues, was examined by Nishikawa et al. [79]. They reported high recoveries for terolidine, diphenhydramine, diphenylpyraline and orphenadrine, while little-no recovery of benactyzine and piperilate was reported. Linearity in the low μg/ml range was obtained in both blood and urine, although absolute recoveries from blood were significantly lower. Detection limits ranged from 76 to 473 ng/ml from blood and from 13 to 186 ng/ml from urine.

DeBruin et al. have shown the extraction of monocyclic amines from urine, blood and milk using headspace SPME [80]. Monocyclic amines are intermediates in the production of many compounds, including rubber, plastics, dyes, pesticides and pharmaceuticals, and are of toxicological interest. They studied and optimized the fiber selection and temperature, pH and ionic strength of the matrix for maximum adsorption of the analyte onto the SPME fiber. Using a PDMS–DVB fiber, pH greater than 13, 4.0 *M* NaCl and temperatures optimized for each individual component, they obtained detection limits of 0.40–7.7 ppb for the amines from blood. Detection limits from water, urine and milk were lower. Separation and detection of the extracts were performed using GC–MSD.

Benzophenone-3 is a common ingredient in sunscreens and other cosmetics, and can be absorbed through the skin. Because of this wide use, the study of benzophenone-3 metabolic processes and excretion is clinically interesting. Felix et al. have developed an SPME method for the determination of benzophenone-3 and its metabolites in urine, using GC–ion trap MS for separation and detection [81]. They found that a 65-mm carbowax–DVB fiber gave the best extraction efficiency and noted some desorption time-related carryover. Equilibration times were about 40 min and addition of small amounts of salt enhanced extraction from water. From urine, they found a linear range of about 10–1000 ng/ml for three benzophenones.

Urinary organic acids are characteristic in the diagnosis of many diseases. An SPME method for the screening of acid methyl esters was described by Liebich et al. [82]. The acids are derivatized directly in the urine by exposure to trimethyloxonium tetrafluoroborate. This is followed by direct immersion

extraction using an 85-μm polyacrylate fiber, with desorption into GC or GC–MS. The total preparation time was about 40 min and 29 organic acids were positively identified from a urine sample. Valproic acid, an antiepileptic agent, was determined in human plasma using equilibrium dialysis followed by SPME [83]. Following dialysis at room temperature, the samples were adjusted to pH 2.5 and extracted using a PDMS fiber for 3 min. GC-FID was used for separation and detection. They found a detection limit of 1 μg/ml of free valproic acid.

Epicuticular hydrocarbons and other volatile compounds have been extracted from insects by Moneti et al. [84]. These compounds are of wide interest in many research areas, including taxonomy, courtship, nest-mate recognition and caste recognition. Headspace SPME at 170°C, in a 2-ml capacity vial, with a 7-μm PDMS fiber was employed on the collected cuticular material from insects. The authors identified numerous compounds that are characteristic of several species of insects.

Volmer and Hui have studied the decomposition of erythromycin A, a macrolide antibiotic, using SPME, coupled to LC–tandem mass spectrometry [85]. At pH<3, they found rapid degradation into several products which were readily extracted using a PDMS–DVB fiber.

Takegawa et al. have determined cyanide, a rapid-acting poison in blood by headspace SPME and GC. Using a carbowax–divinylbenzene-coated fiber, and internal standard quantitation, they found a linear range of 0.04–4.0 μg/ml cyanide in blood. The headspace method involved equilibration for 45 min at 50°C [86].

5. Conclusions

SPME has been employed for a wide variety of drug analyses from biological fluids and matrices. It has shown low detection limits and excellent quantitation. Especially in the headspace mode, SPME extractions offer the potential for very ‘clean’ analyses, with little to no interference from non-volatile compounds. Most of the methods shown to date have involved headspace techniques for volatile drugs. Some methods have employed direct immersion and derivatization for less volatile analytes. SPME has the potential for rapid screening of field analysis of urine, blood or breath samples. Most of the applications have been related to forensic and toxicological analysis; however, they show the potential of SPME for other drug analysis, such as clinical, metabolic and pharmaceutical analysis. If HPLC or CE interfacing is available, SPME could be employed for numerous additional problems.

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