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Review

Headspace solid-phase microextraction procedures for gas chromatographic analysis of biological fluids and materials

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Abstract

Solid-phase microextraction (SPME) is a new solventless sample preparation technique that is finding wide usage. This review provides updated information on headspace SPME with gas chromatographic separation for the extraction and measurement of volatile and semivolatile analytes in biological fluids and materials. Firstly the background to the technique is given in terms of apparatus, fibres used, extraction conditions and derivatisation procedures. Then the different matrices, urine, blood, faeces, breast milk, hair, breath and saliva are considered separately. For each, methods appropriate for the analysis of drugs and metabolites, solvents and chemicals, anaesthetics, pesticides, organometallics and endogenous compounds are reviewed and the main experimental conditions outlined with specific examples. Then finally, the future potential of SPME for the analysis of biological samples in terms of the development of new devices and fibre chemistries and its coupling with high-performance liquid chromatography is discussed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Reviews; Headspace analysis; Solid-phase microextraction; Sample preparation

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1. Introduction

Current sample preparation procedures using solvents are time-consuming, labour intensive, multistage operations. Each step, especially concentration (solvent evaporation), can introduce errors and losses especially when analysing volatile compounds. Additionally, waste solvent has to be disposed of, adding to the expense of the procedure. Many of the limitations of classical LLE methods have been reduced by SPE using cartridges, discs and microwell plates. SPE needs less solvent, but is still time consuming, and often requires a concentration stage which may result in loss of volatile compounds. Adsorption of analytes onto the walls of the extraction devices can occur and trace impurities in the extraction solvent can simultaneously become concentrated. SPME was invented by Pawlisyzn and co-workers [1,2] in late 1989 in an attempt to redress limitations inherent in these methods of sample preparation. SPME integrates sampling, extraction, concentration and sample introduction into a single solvent-free step.

1.1. Solid-phase microextraction apparatus

SPME uses a short length of narrow diameter fused-silica optical fibre externally coated with a thin film polymeric (e.g. Carbowax, DVB, PDMS, PA) stationary phase or a mixture of polymers blended with a porous carbon-based solid material (e.g. PDMS–Carboxen) [3]. The coated fibre is immersed directly into the sample, where analytes preferentially partition by adsorption or absorption (depending on type of fibre) from the solution to the stationary phase and are concentrated. After equilibrium is reached (from a few minutes to several hours depending on the properties of the analyte measured) or after a defined time, the fibre is withdrawn and transferred to either a GC injection port [3] or a modified HPLC rheodyne valve [4]. The fibre is exposed and the analytes desorbed, either thermally in the hot GC injector or, in the case of HPLC, eluted by the mobile phase, and subsequently conventionally chromatographed. With ‘dirty’ matrices such as sludges and biological fluids, or using solid samples, the technique can be operated in the HS mode with the fibre directly exposed to the gas above the sample in a heated sealed vial. In both sampling modes agitation (e.g. stirring or sonication) of the sample matrix improves transport of analytes from the bulk sample phase to the vicinity of the fibre.

The commercially available (from Supelco) SPME unit, consists of a short-length (1 or 2 cm) narrow diameter fused-silica fibre coated with a stationary phase attached to a stainless steel guide rod. This is housed in a hollow septum-piercing needle into which the fibre can be withdrawn for protection when not in use. The whole needle/fibre assembly is contained in a holder, adjustable to allow for variable depth of fibre exposure either during sampling or desorption. A modified assembly has recently become available to enable sampling in the field [5,6].

SPME extraction is a complex multiphase equilibrium process. An extraction can be considered complete when the concentration of analytes has reached distribution equilibrium between the sample and coating. This means that once equilibrium is achieved the amount extracted is independent of further increases in extraction time. The higher the

distribution constant of a compound the higher the affinity of that compound for the SPME fibre coating. The theory of the thermodynamic, kinetic and mass transfer processes underlying direct immersion and HS-SPME has been extensively discussed by Pawliszyn [3]. How factors such as sample volume, extraction time and agitation conditions affect equilibrium are accounted for. Models to quantitatively describe the mass transfer in non-equilibrium sampling from a condensed matrix and in the HS are available [7,8]. Three textbooks [3,9,10] and research papers [11–16] by Pawliszyn and co-workers provide details of the mathematical expressions describing the physicochemical processes involved.

1.2. Solid-phase microextraction fibres

Several fibre coatings are commercially available (Table 1) for the extraction of volatile and semivolatile compounds and the list is growing, extending the range of applications. Both PDMS and PA phases extract via absorption with analytes dissolving and diffusing into the bulk of the coating. The remaining types (Carbowax–DVB, Carbowax–TPR, PDMS–Carboxen, PDMS–DVB) are mixed coatings and extract via adsorption with analytes staying on the surface (as a monolayer) of the fibre [3]. The PDMS–Carboxen coating is a special case comprising a mixed carbon (Carboxen 1006 adsorbent, surface area approximately 1000 m^2/g) phase with small micropores. As two different physicochemical mechanisms operate, the mathematical theory underpinning the extraction processes needs to be modified accordingly [14]. The type of fibre used affects the selectivity of extraction (in general, polar fibres are used for polar analytes and non-polar types for non-polar analytes as with conventional GC stationary phases). For example, the bipolar porous PDMS–Carboxen fibre is designed to 'retain' highly volatile solvents and gases. Some phases have different thicknesses (e.g. 7, 30 and 100 μm) and this affects both equilibrium time and sensitivity of the method. Usually the thinnest acceptable film is employed to reduce extraction times. Different methods (bonded, non-bonded, cross-linked) are used to attach the coating to the fused-silica core. Most polymer films are coated directly (non-bonded types) or partially cross-linked. These can be damaged if exposed to high levels of organic analyte or strong acid or alkali. All fibres require initial conditioning (0.5–4 h) prior to use and have a maximum desorption temperature, similar to GC

Table 1
SPME fibres currently available commercially

Fibre coating	Film thickness (μm)	Polarity	Coating stability	Maximum temperature (°C)	Analytical application	Recommended uses
Polydimethylsiloxane	100	Non-polar	Non-bonded	280	GC/HPLC	Volatiles
(PDMS)	30	Non-polar	Non-bonded	280	GC/HPLC	Non-polar semivolatiles
	7	Non-polar	Bonded	340	GC/HPLC	Mid- to non-polar semivolatiles
PDMS–divinylbenzene	65	Bi-polar	Cross-linked	270	GC	Polar volatiles
(DVB)	60	Bi-polar	Cross-linked	270	HPLC	General purpose
(StableFlex fibre)	65	Bi-polar	Cross-linked	270	GC	Polar volatiles
Polyacrylate (PA)	85	Polar	Cross-linked	320	GC/HPLC	Polar semivolatiles (phenols)
Carboxen–PDMS	75	Bi-polar	Cross-linked	320	GC	Gases and volatiles
(StableFlex fibre)	85	Bi-polar	Cross-linked	320	GC	Gases and volatiles
Carbowax/DVB	65	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
(StableFlex fibre)	70	Polar	Cross-linked	265	GC	Polar analytes (alcohols)
Carbowax/templated resin (TPR)	50	Polar	Cross-linked	240	HPLC	Surfactants
DVB–PDMS–Carboxen[a]	50/30	Bi-polar	Cross-linked	270	GC	Odours and flavours

[a] Stableflex design on a special 2 cm length fibre.

stationary phases. High purity carrier gases are essential as some phases can easily become oxidised by trace levels of oxygen. Fibres can be reused several times (e.g. up to 50 or more) depending on the sample matrix.

1.3. Extraction and desorption conditions

Extraction and equilibrium processes can be varied and enhanced in a number of ways. When extracting semivolatile compounds from an aqueous matrix the fibre is usually immersed directly into the sample. If the sample is agitated with a magnetic stirrer or ultrasonically the time to reach equilibrium is lowered. Dedicated apparatus for this purpose is available (Supelco). Time to equilibrium is a function of the analyte and conditions used (e.g. fibre chemistry and thickness) and this is usually measured experimentally for a given set of conditions. HS sampling is generally used for more volatile compounds and has the advantage of faster equilibrium times and the selectivity for the analytes of interest is improved. Non-equilibrium sampling can be employed in both sampling modes. Extraction efficiency can be improved by modifying matrix, target analytes and the SPME device itself. To maintain precision and reproducibility these conditions and others such as incubation temperature, sample agitation, sample pH and ionic strength, sample volume, extraction and desorption times must be kept constant [3]. The effects of temperature, pH, change of activity coefficient by salting out (e.g. adding K_2CO_3, NaCl, Na_2SO_4, $(NH_4)_2SO_4$) are similar to those encountered in conventional HS sampling [17]. In addition, saturation with salt can help normalise random salt concentrations found in biological matrices. To prevent losses deactivation of glassware and vials before use by silanisation is recommended [18–20]. Wercinski [9] gives a comprehensive practical guide to SPME method development procedures.

For GC desorption, a narrow bore (0.75 mm I.D.) unpacked injection liner is required to ensure a high linear carrier gas flow, reduce desorption time and prevent peak broadening. As no solvent is used, injections are carried out in the splitless mode to ensure a complete transfer of analyte and to increase sensitivity. Both time and temperature used for desorption influence recovery and these need to be optimised. The position of the fibre inside the injector is important as temperature varies along its length. Septa can easily become damaged with the large (24 gauge) SPME guide needle: the use of a Merlin Microseal septumless system (23 gauge SPME needle required) or JADE valve is recommended. These valves also stop contamination of the liner with septa material. To prevent carryover the fibres may also be further desorbed between analytical runs in a separate hot GC injector. A dedicated unit for this purpose is available.

When using SPME for quantitative analysis the same criteria apply for the selection and use of internal standards as with other forms of sample preparation and instrumental analysis. HS-SPME involves multiphase equilibrium processes and careful consideration must be given to the physicochemical properties of the candidate compounds. For complex heterogeneous matrices, calibration using standard additions is advised. GC–MS is the optimal quantitation technique as it allows isotopically labelled (deuterium or ^{13}C) analogues to be spiked into the sample. The behaviour of these compounds closely mimics the target analytes.

1.4. Derivatisation methods

Derivatisation can increase the volatility and/or reduce the polarity of some analytes and therefore can improve extraction efficiency, selectivity and subsequent GC detection. Three procedures are currently used: direct, derivatisation on the SPME fibre and derivatisation in the GC injector port [3]. In the direct technique the derivatisation reagent is added into the sample matrix; the SPME fibre then extracts the derivatised analytes either in solution or HS and delivers them to the GC. This approach has been used with phenols in water by converting them to acetates with acetic anhydride [21]. Trimethyloxonium tetrafluoroborate has been used to form methyl esters of urinary organic acids [22], methanolic HCl to form esters of organic acids in tobacco [23] and propyl chloroformate to derivatise the amino group on amphetamines in urine [24,25]. Other reagents include pentafluorobenzaldehyde for primary amines [26], sodium tetraethylborate (including its deuterated analogue) [27–31] and

thioglycol methylate [32] for in situ derivatisation of organometallics.

On-fibre derivatisation (e.g. with diazomethane) can be employed after the extraction procedure. Extracted compounds on the fibre are exposed (e.g. in a heated sealed HS vial) to the derivatising reagent in the vapour phase for a given time. Damage to the coating is prevented by HS derivatisation. This has been employed for serum steroids [33], urinary organic acids [34] and urinary hydroxyl metabolites of polycyclic aromatic hydrocarbons (naphthalene, phenanthrene, pyrene) [35]. Silylation with bis(trimethylsilyl)trifluoroacetamide (BSTFA) at 60°C for 45–60 min is effective for all these analytes. Simultaneous derivatisation and extraction can be carried out. Prior to extraction the fibre is doped with reagent and on sampling the analytes are extracted and converted to derivatives that have a high affinity for the coating. This is not an equilibrium process as the analytes are converted as soon as they are extracted onto the fibre for as long as the extraction process continues. 1-Pyrenyldiazomethane has been used for the simultaneous HS extraction and derivatisation of fatty acids (by forming pyrenylmethyl esters) [18–20]. Loss of reagent was minimal as it had a low vapour pressure and a high affinity for the coating. The esters were completely desorbed and the fibre could be reused. Recently *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride has been used in a similar manner for monitoring formaldehyde in air [36]. Derivatisation can be carried out on the SPME fibre in a GC injector port [19]. Nagasawa et al. [37] made elegant use of this approach to measure amphetamines, which after extraction were derivatised in the liner by injection of heptafluorobutyric anhydride to form amide derivatives.

2. Headspace solid-phase microextraction analysis of biological fluids and materials

Since its invention there has been a rapid growth in the number of applications of SPME, evidenced by the growing number of published papers. Originally it was confined to analysis of pollutants in environmental matrices (e.g. pesticide and aromatic hydrocarbon contamination of water) [3,38]. The potential of the technique was soon recognised for measuring volatile and semivolatile components in beverages, flavourings, foodstuffs, forensic specimens and pharmaceutical products and a tool for determining physicochemical properties of organic compounds. Many of these applications are discussed in a recent book [10]. However, it was not until 1994 that the first substantive uses of SPME with clinical and toxicological specimens were reported [39,40]. The aim of this review is to provide updated information on the use of HS-SPME for the GC analysis of biological fluids and materials. It should be recognised that direct immersion SPME interfaced with either GC or HPLC can also be used to measure a range of biologically relevant compounds. The method selected depends on the extraction properties (primarily volatility and polarity) of the analyte and the type of material being handled. Preliminary experiments using an aqueous solution of the compound are useful to address this question. Direct immersion will only be discussed where pertinent to the review. Some of these other methods are covered in another paper in this issue.

HS-SPME is ideal for the analysis of biological specimens as interference from high-molecular-mass components (e.g. proteins) in the matrix is reduced, yielding cleaner extracts. Although HS-SPME is an equilibrium rather than an exhaustive (e.g. LLE) extraction method, by careful adjustment of the extraction conditions (agitation, pH, salting out, temperature, time) significant enhancements in sensitivity can be achieved to enable the detection of even semivolatile analytes. Derivatisation of target compounds by acylating, alkylating and silylating reagents can also improve sensitivity. As only the HS gas is sampled, more aggressive (e.g. strong acid or alkali) sample preparation and derivatisation regimes can be used compared to direct immersion where fibre damage might occur. However, high levels of non-polar organic solvents in, or added to, the matrix can cause the fibre to swell. As complex interactions occur between the different phases in HS sampling, appropriate internal standards (preferably isotopically labelled), are essential for quantitative analysis.

The remaining sections discuss the application of HS-SPME with different biological fluids and ma-

terials used for investigations in clinical, forensic and toxicology laboratories.

2.1. Headspace solid-phase microextraction analysis of urine

Urine is a relatively simple biological fluid to collect and is frequently used for drug screening, forensic purposes, monitoring workplace exposure to chemicals and other investigations as it can contain the target analyte together with diagnostic metabolites. In urine, excreted compounds can become concentrated by the kidney. Early SPME applications focused on very volatile compounds such as ethanol and solvents of abuse in urine: today, a variety (amphetamines, antihistamines, tricyclic antidepressants) of drugs, organometallics, pesticides and industrial chemicals can be measured. Many methods have been pioneered by the research groups of Kojima, Namera and Yashiki in Hiroshima and Lee, Kumazawa, Sato and Suzuki and their co-workers in Tokyo.

2.1.1. Drugs and their metabolites

HS-SPME is suitable for the measurement of drugs in urine as matrix effects are minimal and sample preparation is simple. By the use of high incubation temperatures even semivolatile compounds can be measured; some drugs may be extracted from steam in the vial at temperatures above 100°C. Unlike very volatile compounds (see Section 2.1.2.), semivolatiles only transfer into the HS slowly and a preheating period is not always necessary before SPME sampling. With analysis of semivolatile drugs long equilibrium times (20–60 min) are often required. Once equilibrium has been achieved the amount of analyte extracted by the fibre theoretically becomes constant with time. However, Yashiki et al. [41] found significant decreases can occur after equilibrium has been reached for amphetamines and tricyclic antidepressants. A suggested cause was a decrease in the fibre–HS partition coefficient over time. Careful optimisation of sample pH, ionic strength and derivatisation procedures can improve sensitivity, reproducibility and subsequent chromatography for many drugs.

A summary of published HS-SPME methods for the analysis of a range of classes of drug is shown in Table 2. Direct immersion SPME has also been used effectively for drugs and can extend the application of the technique to other types of analyte including, barbiturates [55] benzodiazepines [56,57] and neuroleptics [58]. Better detection limits are often obtained with direct immersion.

The fibre type can have an importance influence on extraction. Lord and Pawlisyzn [44] systematically investigated the effect on fibre chemistry in terms of extraction efficiency and equilibrium time for the extraction of amphetamines. Consideration must also be given to the ruggedness of the fibre in withstanding the extraction medium. However, most drugs can be satisfactorily extracted on a thick (100 μm) film PDMS fibre. Detection limits vary according to the class of drug and detector used, typically 1–100 ng/ml. These limits compare favourably with other sample preparation methods.

2.1.2. Alcohols, solvents and other chemicals

Volatile solvents and chemicals are measured in body fluids either for forensic purposes or to monitor workplace exposure. HS-SPME is particularly apt for the analysis of these substances, having better sensitivities than conventional HS and is easier to operate than purge-and-trap methods. Also when using MS detection the absence of an air peak with HS-SPME can be useful in the identification of very volatile unknown substances. HS-SPME extraction of volatile substances is particularly affected by fibre chemistry and type of matrix modification used and these must be optimised to ensure good recoveries. Shirley [59] evaluated a number of these variables for the analysis of 11 volatile (molecular mass less than 90) compounds with varying properties. Fig. 1 clearly shows the effect of fibre chemistry on extraction efficiency. Although this was performed by direct immersion into an aqueous solution, similar effects would be expected with HS sampling. The carbon-based PDMS–Carboxen fibre, was the most sensitive (in some cases 200 times greater) for all analytes except isopropylamine. Popp and Paschke [60] found similar findings with this fibre, with extraction efficiencies up to 90% and detection limits in the range ng/l for non-polar solvents. The PDMS–Carboxen fibre has only been available commercially since 1997. Many earlier reports of analysis of volatile substances in biological fluids

Table 2
Summary of published methods for HS-SPME–GC of drugs in urine and blood

Drug	Specimen	Matrix additive	Vial temp. (°C)	Preheat time (min)	Extraction time (min)	Fibre type	Detector	Ref.
Amphetamines								
Amphetamine, methamphetamine,	Urine	K_2CO_3	80	20	5	100 μm PDMS	MS	[41]
Fenfluramine,	Blood	NaOH	80	20	5	100 μm PDMS	MS	[42]
3,4-Methylenedioxyamphetamine,	Urine	NaCl	75	30	15	100 μm PDMS	MS	[43]
3,4-Methylenedioxymethamphetamine	Urine	NaCl	60	–	15	65 μm PDMS–DVB, 100 μm PDMS	FID	[44]
	Urine	NaOH, NaCl	100	20	10	100 μm PDMS	MS	[45]
	Blood	NaOH	70	–	15	100 μm PDMS	MS	[46]
Antidepressants								
Amitriptylene, imipramine,	Urine	NaOH	100	30	15	100 μm PDMS	FID	[47]
trimipramine, chlorimipramine,	Blood	NaOH	100	30	60	100 μm PDMS	FID	[48]
setiptiline, maprotiline, mianserin	Blood	NaOH	120	–	45	100 μm PDMS	MS	[49]
Alkaloids								
Nicotine, cotinine	Urine	K_2CO_3	80	20	5	100 μm PDMS	MS	[50]
Antihistaminics	Urine	NaOH	98	10	10	100 μm PDMS	FID	[51]
	Blood	NaOH	98	10	10	100 μm PDMS	FID	[51]
Phenothiazines	Urine	NaOH	140	10	40	100 μm PDMS	FID	[52]
	Blood	NaOH	140	10	40	100 μm PDMS	FID	[52]
Phencyclidine	Urine	NaOH, K_2CO_3	90	10	30	100 μm PDMS	SID	[53]
	Blood	NaOH, K_2CO_3	90	10	30	100 μm PDMS	SID	[53]
Meperidine (pethidine)	Urine	NaOH, NaCl	100	10	30	100 μm PDMS	FID	[54]
	Blood	NaOH, NaCl	100	10	30	100 μm PDMS	FID	[54]

therefore have poorer detection limits than can be achieved today with this fibre chemistry [61]. However, with this fibre the trapped compounds can condense deep within its porous capillary structure and rigorous desorption conditions are needed to ensure no carryover of analytes. This can also influence the extraction capacity (dynamic range) [10] and reproducibility [60,62] and should be borne in mind during method development.

The measurement of solvents and similar chemicals is straightforward. Typically urine (2–10 ml) is saturated with a salt [NaCl or $(NH_4)_2SO_4$] and mixing at 40–60°C and the HS sampled (10–15 min) using the appropriate fibre. Once the vial is sealed the liquid and gaseous phases should be allowed to equilibrate (30–60 min) at the required temperature before sampling. Generally a thick fibre coating is used to ensure high recoveries. Guidotti and Vitali (in Ref. [10]) provide details of extraction and GC conditions for HS-SPME of a range of solvents: these and other methods are summarised in Table 3. A linear response over several orders of magnitude (e.g. 0.05–500 μg/l) is usually found for these volatile analytes. Lower detection limits are possible with the more non-polar solvents compared to water-soluble analytes such as ethanol and methanol. Some compounds (e.g. pentachlorophenol) are also excreted as conjugates and these must be hydrolysed before analysis. Simple in-vial procedures can be used for this purpose to avoid the loss of volatile analytes [70]. Care must be taken to ensure complete desorption and that the fibre does not become cross-contaminated from solvent vapours in the laboratory atmosphere. Contamination can also arise from septa, tubing and disposable syringes. Blank tests should always be run in parallel.

Quantitation is usually achieved by external calibration using spiked urine samples collected from donors not exposed to the chemicals being measured. For a number of compounds high purity deuterated analogues are available. Using these as internal standards provides the best precision. Fustinoni et al.

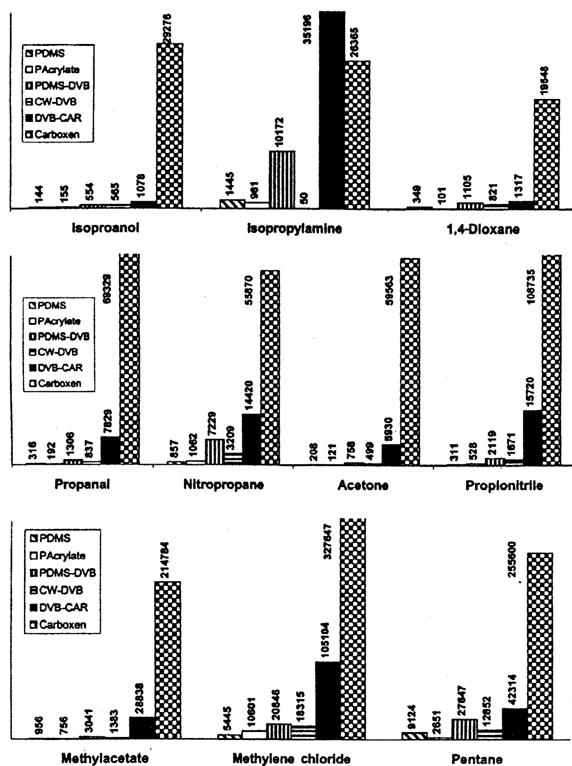

Fig. 1. Area responses for ten volatile analytes extracted by direct immersion with six different SPME fibre chemistries. The absolute responses have been adjusted for FID discrimination. PDMS, 100 μm polydimethylsiloxane; Pacrylate, 85 μm polyacrylate; PDMS–DVB, PDMS–divinylbenzene; CW–DVB, Carbowax–DVB StableFlex; Carboxen, Carboxen–PDMS StableFlex; DVB–CAR, DVB–Carboxen–PDMS StableFlex. From Ref. [59].

[68] used this approach to measure low levels of benzene, toluene, ethylbenzene and xylenes in urine, with [$^2H_6$]benzene, [$^2H_8$]toluene and [$^2H_{10}$]p-xylene as internal standards. The HS equilibrium kinetics of the internal standards were comparable to those of the corresponding aromatic compounds (Fig. 2). Fast equilibrium times were achieved, with time to reach equilibrium longer for the higher boiling point compounds. Using this technique the reproducibility (coefficient of variation: 2–7%) was excellent across a range of analyte concentrations. For the analysis of less volatile chemicals direct immersion SPME can be used effectively, and this coupled with HS derivatisation procedures can extend the range of applications of the technique.

2.1.3. Anaesthetics

Urine is used to monitor occupational exposure of hospital staff to inhalation anaesthetics (e.g. nitrous oxide, halothane and isoflurane). There is one report of the use of HS-SPME with GC–MS detection for this purpose [71]. Urine samples (10 ml) were acidified with H_2SO_4, 10% NaCl added and analysed at room temperature. The performances of the microporous 1 cm PDMS–Carboxen and the recently introduced 2 cm DVB–Carboxen–PDMS fibres were compared (Fig. 3). Although similar equilibrium times (about 15 min) were found, as expected the longer fibre had a much higher extraction efficiency. Linearity extended over four orders of magnitude with detection limits less than 100 ng/l for nitrous oxide and 30 ng/l for halogenated compounds. Compared to static HS, these limits were 10-fold lower for nitrous oxide and 100-fold lower for the halides.

2.1.4. Metals and organometallics

The potential of SPME with GC–FID or GC–MS detection to measure metallic and organometallic species in biological fluids is beginning to be explored. Methods have been reported for inorganic lead [31], inorganic mercury [72,73] and alkylated species of lead [72], mercury [72,73] and tin [72]. Samples are digested and decomplexed using established methods and then derivatised in-situ with sodium tetraethylborate (pH 4–5) to increase the volatility of the analytes. After derivatisation (typically 10 min) the SPME fibre is exposed to the HS at room temperature. A 100 μm PDMS fibre gave the highest extraction efficiencies for the ethylated compounds for most of the reported methods. Detection limits were in the ng/l range and depended on the specific detector used. Using this technique Dunemann et al. [72] demonstrated the differences in urinary excretion of inorganic mercury between subjects with and without mercury amalgam teeth fillings. Recently Mester and Pawlisyzn [32] using direct immersion SPME have extended the approach to the speciation of arsenic (as monomethylarsonic acid and dimethylarsinic acid) in urine. Thioglycol methylate was used as the derivatisation reagent. The hyphenation of SPME to other instrumental methods (e.g. GC–ICP-MS and GC–AAF) offers potential to

Table 3
Summary of HS-SPME methods used for the measurement of alcohols, solvents and chemicals in urine

Compound	SPME fibre	Additive	Vial temp. (°C)	Extraction time (min)	Detection	Detection limit	Ref.
Benzene	PDMS	NaCl	60	15	MS	0.128 μg/l	[10]
Toluene	PDMS	NaCl	60	15	MS	0.061 μg/l	
Ethylbenzene	PDMS	NaCl	60	15	MS	0.044 μg/l	
Xylenes	PDMS	NaCl	60	15	MS	0.039 μg/l	
Styrene	PDMS	NaCl	50	10	MS	0.046 μg/l	
Methylene chloride	PDMS	NaCl	50	10	MS		
Trichloroethylene	PDMS	NaCl	50	10	MS		
Tetrachloroethane	PDMS	NaCl	50	10	MS		
Methyl ethyl ketone	PDMS–DVB	NaCl	50	10	MS	33.5 μg/l	
Methanol	PDMS–Carboxen	NaCl	50	10	MS	422 μg/l	
Toluene/benzene/ isoamyl acetate/ *n*-Butanol/n-butyl acetate	PDMS		80	5	FID	1.1–2.4 μg/l	[63]
Toluene/xylenes	PDMS	NaCl	25	5	FID	1.0 μg/l	[64]
Ethanol	Carbowax–DVB	$(NH_4)_2SO_4$	70	15	FID	10–20 μg/ml	[65]
	PDMS–Carboxen	$(NH_4)_2SO_4$	60	15	FID	0.2–0.5 μg/ml	[61]
Methyl ethyl ketone	PDMS–Carboxen	$(NH_4)_2SO_4$	50	15	FID	21.6 μg/l	[66]
Methylene chloride/ chloroform	PDMS–Carboxen		30	20	FID	0.2 μg/l	[67]
Benzene/toluene/ xylenes	PDMS	NaCl	40	15	MS	12–34 ng/l	[68]
Methanol/formic acid	PDMS–Carboxen	$(NH_4)_2SO_4$	60	10	FID	0.1–0.6 μg/0.5 ml	[69]

extend the range of analytes measured and lower detection limits.

2.1.5. Pesticides

Pesticides are occasionally measured in urine and other fluids. Samples arise from accidental exposure or cases of suicide. Organophosphate [74] (e.g. ethion, fenthion, isoxathion, malathion) and carbamate [75] (e.g. fenobucarb, isoprocarb, propoxur, xylylcarb) classes of pesticide have been extracted and detected using GC–NPD and GC–FID, respectively. Urine (0.5–1.0 ml) was extracted with a 100 μm PDMS fibre under acidic (organophosphates) or neutral (carbamates) conditions at high temperature (70–100°C) for approximately 30 min. All pesticides gave linear calibration curves with low detection limits (0.8–12 ng/0.5 ml, organophosphates and 10–50 ng/ml carbamates). Dinitroaniline herbicides [76] (e.g. benfluralin, ethalfluralin, isopropalin, profluralin) have also been measured using a similar approach but with GC–ECD.

2.1.6. Endogenous compounds and their metabolites

The extraction of endogenous compounds in urine using SPME is still relatively unexplored [10,22,33,77,78]. Mills et al. [77] used HS-SPME with stable isotope dilution GC–MS to quantitatively determine trimethylamine in urine. Excretion of trimethylamine is increased in the rare inherited disorder trimethylaminuria (fish odour syndrome) and can be used to diagnose the condition. The highly volatile analyte was extracted using basic conditions (pH 14) with either PDMS or PDMS–Carboxen fibres, the latter being approximately 12 times more sensitive. The results obtained were comparable to other methods such as nuclear magnetic resonance spectroscopy and conventional HS. Mills and Walker [70] also used HS-SPME with GC–MS to profile other volatile urinary compounds from both normal control patients and those with a range of diseases in order to assess its potential value for diagnostic metabolic clinical laboratories. The pH

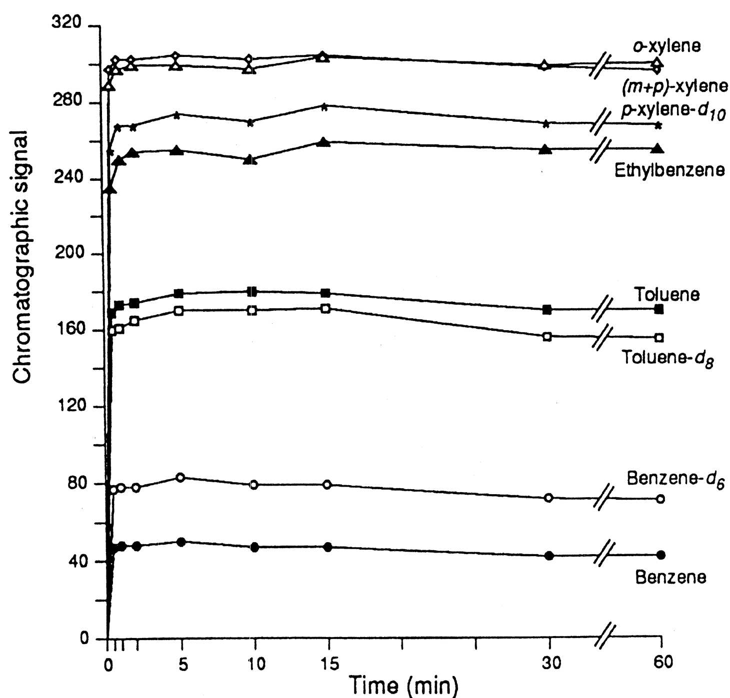

Fig. 2. HS equilibrium kinetics of benzene, ethylbenzene, toluene, xylenes and deuterated analogues spiked (1250 ng/l) into urine (2 ml), saturated with NaCl (1 g) and sampled with a 100 μm PDMS fibre at 40°C. GC–MS was used for detection. Measurements taken at 30 s, 1, 2, 5, 10, 15, 30 and 60 min. From Ref. [68].

(i.e. highly acidic or basic) used for extraction had a significant effect on compounds found in the HS from normal control specimens. The fibre chemistry also influence the profile with the PDMS–Carboxen type proving to be the most useful for extracting the range (e.g. alcohols, aldehydes, amides, ketones, N- and O-heterocyclics and sulphur containing compounds) of analytes found. A number of compounds derived from food additives and plasticisers were always evident. Abnormal profiles were demonstrated in samples from patients with severe ketosis, and the inborn errors of metabolism, homocystinuria, medium-chain acyl-CoA dehydrogenase deficiency and multiple acyl-CoA dehydrogenase deficiency (glutaric aciduria type II). Using a simple in-vial hydrolysis procedure the presence of possible glycine and carnitine conjugates of *n*-hexanoic and *n*-octanoic acids was shown in medium-chain acyl-CoA dehydrogenase deficiency. HS-SPME profiling of urinary volatiles may prove a useful method to supplement other diagnostic procedures that involve the screening of non-volatile compounds (e.g. amino and organic acids) [79].

2.2. Headspace solid-phase microextraction analysis of blood

The direct analysis of whole blood is problematic due to clot formation during heating of the HS vial. This affects the stirring rate and release of components into the HS gas leading to non-reproducible results. Deproteinisation pretreatments can be used (e.g. addition of a strong acid followed by centrifugation) however this can lead to loss of very volatile compounds. In most applications this step is dispensed with. The addition of strong alkali to the sample causes haemolysis and thus prevents clot formation: NaOH is often used. Compared to LLE and SPE, absolute recoveries of analytes from whole blood are often low (0.05–10%), however, extracts are very clean and give few background interferences which enhance detection limits. The use of specific

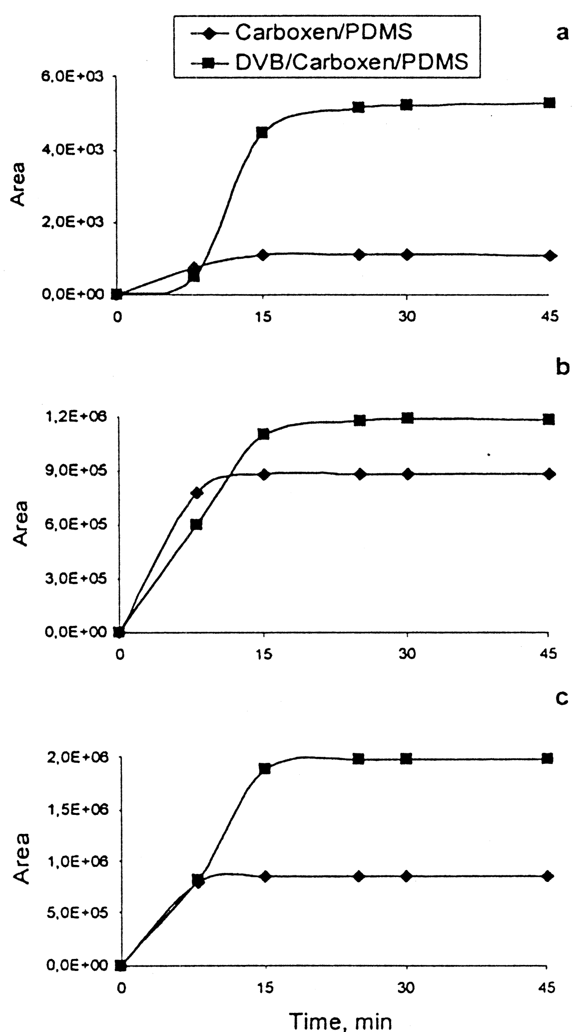

Fig. 3. Exposure time profile of nitrous oxide (a), isoflurane (b) and halothane (c) using Carboxen–PDMS and DVB–Carboxen–PDMS fibres at room temperature. From Ref. [71].

GC detectors can also be advantageous. Many of the methods developed for the analysis of urine can be directly applied to whole blood.

2.2.1. Drugs and their metabolites

A summary of HS-SPME methods for the analysis of whole blood is shown in Table 2, and these are similar to urine. Again sample pretreatments are simple. Detection limits are usually poorer (e.g. phenothiazines: urine, 10–20 ng/ml; whole blood, 100–200 ng/ml [52]). However, detection limits are below therapeutic plasma concentrations for most measured drugs. Care is needed with the addition of certain salts to the matrix as this can promote clot formation in whole blood leading to lower extraction efficiencies (Table 4) [46].

For some drugs derivatisation can be used to improve reproducibility and chromatographic separation. Namera et al. [46] recently developed a stable isotope dilution GC–MS procedure for amphetamines in whole blood using heptafluorobutyric anhydride derivatisation. The method simultaneously analysed amphetamine, fenfluramine and methamphetamine using $[^2H]_5$methamphetamine as internal standard (Fig. 4). Extraction was with a PDMS fibre at 70°C for 15 min. Prior to GC–MS analysis 1 μl of heptafluorobutyric anhydride was injected into the liner followed by the SPME fibre. Simultaneous derivatisation and desorption occurred inside the injector. Detection limits were 5–10 ng/g with intra- and inter-day RSDs between 1.0 and 9.2%. Derivatisation with trifluoroacetic anhydride is also effective for this class of drug [45].

Although outside the scope of this review there is the possibility to increase the range (e.g. antiepileptic drugs, β-blocking agents [80]) of analytes measured

Table 4
Recovery of fenfluramine and amphetamines in the presence of NaOH, K_2CO_3, NaCl, or $(NH_4)_2SO_4$. From Ref. [46]

Composition of mixture	Recovery of drugs and standard deviation (n=5) (%)		
	Fenfluramine	Amphetamine	Methamphetamine
0.5 g Blood+0.5 ml NaOH	6.45±0.29	1.94±0.03	6.24±.09
0.5 g Blood+0.5 ml K_2CO_3	5.36±0.30	2.29±0.25	5.46±0.13
0.5 g Blood+0.5 ml water+ 0.5 g NaCl	0.45[a]	0.22[a]	0.31[a]
0.5 g Blood+0.5 ml water+ 0.5 g $(NH_4)_2SO_4$	N.D.[b]	0.31[a]	N.D.[b]

[a] Values are mean of duplicates.
[b] N.D., not detected.

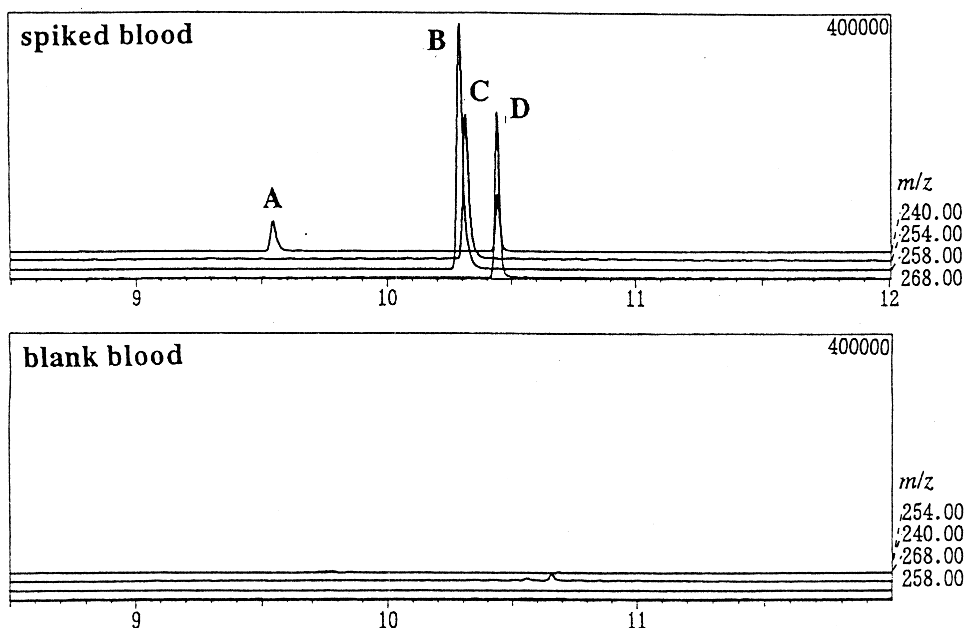

Fig. 4. Typical GC–MS single ion monitoring chromatogram of heptafluorobutyrated amphetamines (0.5 μg/g) in blood. Peaks (A) heptafluorobutyrated amphetamine, (B) heptafluorobutyrated $[^{2}H]_{5}$methamphetamine, (C) heptafluorobutyrated methamphetamine, (D) heptafluorobutyrated fenfluramine. From Ref. [46].

by using plasma or serum and direct immersion SPME coupled to HPLC desorption. The small capacity of the fibre can limit sensitivity and the problem of matrix interferences needs careful consideration for routine drug screening applications.

2.2.2. Alcohols, solvents and other chemicals

The procedures described for measurement of solvents and chemicals in urine are also appropriate for blood (Table 3). Liu et al. [81] also reported a technique for extracting *o*-, *m*- and *p*-dichlorobenzenes and Takekawa et al. [82] a method for cyanide in blood. For all methods, whole blood (0.2–1 ml) is used, as volatile compounds are lost in deproteinisation procedures. Better detection limits are usually obtained with urine, as matrix effects can interfere with the release of analytes into the HS with blood. The technique is particularly suitable for monitoring blood ethanol concentrations. Penton [83] automated the procedure. The 65 μm Carbowax–DVB fibre gave similar results to the routine HS method with a detection limit of 20 μg/ml. Better sensitivities (0.5 ng/ml) are possible with the PDMS–Carboxen phase [61]. Sodium dithionite can be added to prevent oxidation of ethanol to acetaldehyde during analysis [61]. HS-SPME can also be used to detect volatiles in tissue samples [84] and on skin for forensic analyses [9].

Recently HS-SPME has been applied to monitor volatile organic compounds in the blood of persons exposed to environmental levels of pollutants (e.g. benzene, toluene, xylenes) [85–87]. Cardinali et al. [87] with GC–MS and multiple single-ion monitoring achieved detection limits of less than 50 pg/ml for 8 solvents extracted from 5 ml of blood. Toluene and methylene chloride could not be measured to these levels due to contamination problems from the laboratory air. It has been suggested these methods could be used in epidemiological studies to assess the effects of pollution on human health [85–87].

2.2.3. Anaesthetics

Kumazawa et al. [88] first described a HS-SPME method for local anaesthetics in deproteinised blood. This has subsequently been refined by Watanabe et

al. [89] for measurement of bupivacaine, dibucaine, lidocaine, mepivacaine and prilocaine for forensic purposes. Whole blood (0.2 ml) with $[^{2}H]_{10}$lidocaine as internal standard was alkalinised with 5 *M* NaOH and heated in a vial at 120°C. The HS was sampled with a 100 μm PDMS fibre for 45 min. With GC–MS, detection limits ranged from 0.05 to 0.5 μg/g. Ester-type (benoxinate, procaine, tetracaine) local anaesthetics could not be analysed as they were hydrolysed by the strong alkaline conditions [89].

2.2.4. Pesticides

Pesticides have been extracted and measured in whole blood and serum using similar analytical conditions to those described for urine [74–76,90,91]. Detection limits were poorer (about ten times less sensitive) in blood due to matrix effects but were better than many SPE methods [76]. The choice of salt used influenced recoveries when using whole blood [76]. Precipitation of pesticides can occur with NaCl; ammonium citrate is often preferred [10].

Namera et al. [92] recently measured a class of arylamide herbicides (butachlor, diphenamide, propanil, propyzamide) only in serum with GC–MS detection. Samples were saturated with NaCl and heated to 90°C for 45 min. The method was used to detect propanil (1.15–17.1 μg/g) in serum samples from a patient who attempted suicide. With these relatively non-volatile herbicides high extraction temperatures were required; even at 110°C the absorption of diphenamide was still increasing (Fig. 5). Many agricultural chemicals (e.g. carbaryl) decompose at high temperature and are unstable in harsh (strong acid or alkali) conditions and this must be considered when developing SPME methods for their analysis [92]. Organophosphorous pesticides can also degrade during refrigerated storage [93].

2.3. Headspace solid-phase microextraction analysis of faeces

There are a few applications of HS-SPME for direct analysis of faeces. Faecal material contains a complex mixture of compounds derived from end point metabolism and components from the diet such

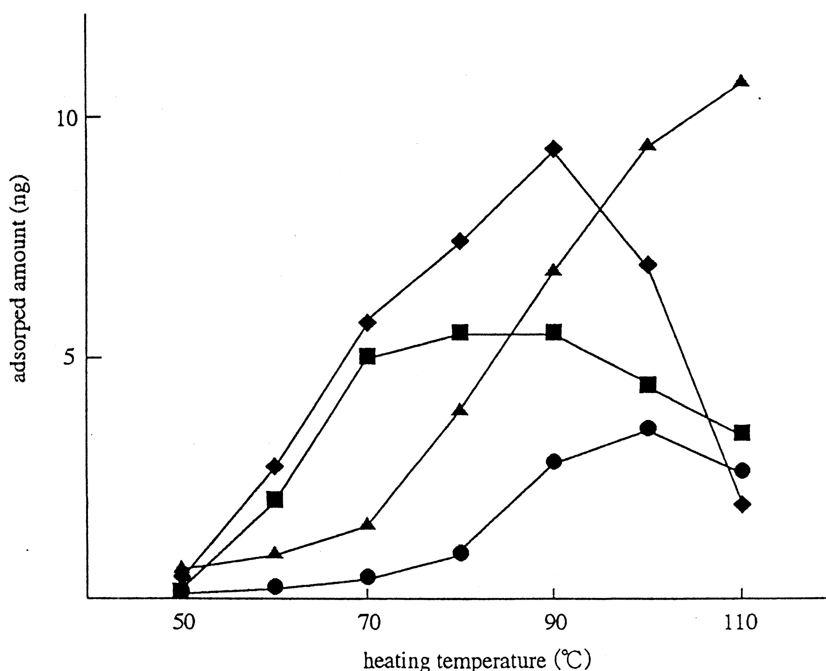

Fig. 5. Effect of temperature on the amount of different arylamine herbicides extracted from spiked (5.0 μg/ml) serum (0.2 ml) with a 100 μm PDMS fibre sampling for 45 min. ●, propanil; ■, propyzamide; ▲, diphenamide; ♦, butachlor. From Ref. [92].

as food additives. Faecal short-chain fatty acids (C_2–C_6) are often measured as they reflect colonic fermentation and can be diagnostic in the investigation of disturbances in metabolism through malabsorption or antibiotic therapy. Pan et al. [18,19] used a PA fibre with a novel derivatisation procedure to measure fatty acids (C_2–C_{10}) in sewage sludge and milk. The derivatisation step was included to overcome problems of peak tailing and ghosting with the highly polar acids. Before extraction the fibre was impregnated with a *n*-hexane solution of 1-pyrenyldiazomethane (PDAM), a non-volatile derivatisation reagent, and then exposed to the HS vapour. The short-chain fatty acids were derivatised in situ to pyrenylmethyl esters and then desorbed in a hot GC injection port. Mills et al. [20] adopted this procedure to measure faecal short-chain fatty acids (C_1–C_6) but with incorporation of several deuterated analogues to enable accurate quantitation by MS with single-ion monitoring (Fig. 6). The method had good linearity, recovery and precision and was used to show differences in the profile of fatty acids excreted in cystic fibrosis and in a sample of ileostomy fluid.

HS-SPME has also been used for the analysis of short-chain fatty acids directly without derivatisation in cheese [94,95] and in wastewater [96]. A range of fibre chemistries was employed. We recently used a PDMS–Carboxen fibre for the profiling of volatile components released into the HS at 50°C from acidified (pH 1–2) faeces (Fig. 7). In addition to the main short-chain fatty acids, compounds derived from food additives and end products (e.g. dimethyl sulphide, 4-methylphenol) of metabolism were present. Good peak chromatographic shapes were found for all of the fatty acids even without derivatisation using the porous carbon phase. Carry over occurred if the fibre was not thoroughly desorbed between analyses. For accurate quantification of fatty acids, the inclusion of a derivatisation step is recommended, as this significantly increases their molecular mass and enables the use of more selective ions for MS detection. There is also some improvement in resolution of isomers (e.g. 2-methylbutyric acid from isovaleric acid). We are exploring the potential of both approaches to investigate the fatty acid signatures from faecal bacteria in patients with different disease states and undergoing antibiotic therapy.

2.4. Headspace solid-phase microextraction analysis of breast milk

Breast milk is a useful matrix for the non-invasive

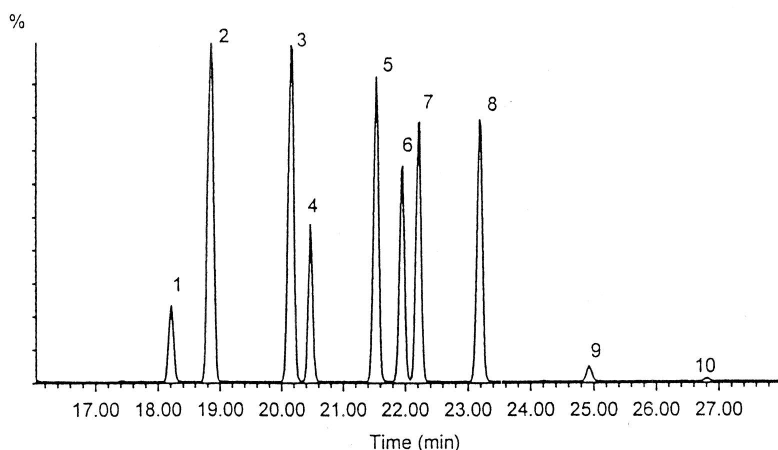

Fig. 6. Faecal short-chain fatty acid profile of a normal adult on a normal diet. 0.193 g of dry faeces was analysed using an 85 μm PA SPME fibre loaded with PDAM derivatising agent for 15 min at room temperature. The loaded fibre was exposed to the HS vapour for 30 min at 50°C and desorbed in a GC injector at 260°C for 4 min. Detection was by MS operated in the single ion monitoring mode. Key to PDAM derivatised acids: (1) formic, (2) acetic, (3) propionic, (4) isobutyric, (5) *n*-butyric, (6) 2-methylbutyric, (7) isovaleric, (8) *n*-valeric, (9) isocaproic, (10) *n*-hexanoic. From Ref. [20].

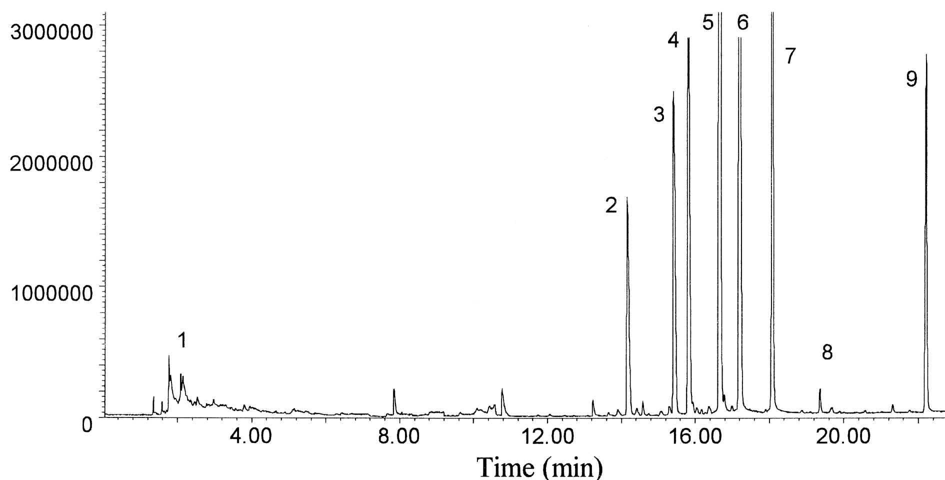

Fig. 7. Profile of volatile compounds in the HS of faeces from a normal adult on a normal diet. 0.327 g of dry faeces was acidified (pH 1–2), saturated with NaCl and analysed using a 75 μm PDMS–Carboxen fibre. The fibre was exposed for 30 min at 50°C and desorbed in a GC injector at 250°C for 2 min. Key: (1) dimethylsulphide, (2) acetic acid, (3) propionic acid, (4) isobutyric acid, (5) *n*-butyric acid, (6) 2-methylbutyric and isovaleric acids, (7), *n*-valeric acid (8), isocaproic acid (9) 4-methylphenol.

biomonitoring of environmental, medical or occupational exposure to chemicals. Milk has been used to estimate environmental exposure levels of highly lipophilic compounds such as chlorinated pesticides and polychlorinated biphenyls. A SPME–GC–ECD method for the rapid analysis of these compounds involving direct insertion of the fibre into the milk matrix has been reported [97]. DeBruin et al. [98] demonstrated the potential of HS-SPME to measure monocyclic amines (aniline, *o*-toluidine, 2-chloroaniline, 2,6-dimethylaniline, 2,4,6-trimethylaniline) in spiked breast milk. A PDMS–DVB fibre was used under highly basic (pH 13) conditions at 45°C. Detection limits were in the ppb range with a 15 min sampling time. Elevated levels of these potentially carcinogenic aromatic amines were found in the breast milk of a woman who smoked cigarettes [3]. As experienced with whole blood, milk lipids can cause poor extraction efficiencies and poor chromatography. Their removal prior to analysis is recommended.

2.5. Headspace solid-phase microextraction analysis of hair

The analysis of hair can be used for forensic purposes and to monitor drug compliance and abuse. As with biological fluids, drugs and their metabolites are expressed in hair. Measurements along a strand of hair can provide a record of drug usage. Before analysis the hair matrix must be either digested enzymatically (e.g. with a protease) or more usually with strong alkali (e.g. 1 *M* NaOH). SPME has been used to detect cannabinoids, cocaine, methadone and its metabolites [99,100] by direct immersion of the fibre in the solution remaining after digestion. However with highly basic conditions damage to the polymer coating of the fibre can occur leading to variable results.

As discussed, HS-SPME can measure a number of semivolatile drugs in body fluids with high sensitivity. Koide et al. [101] first attempted this method with a 100 μm PA fibre to extract amphetamine and methamphetamine in hair using specific GC–NPD for detection. Using 1 mg of hair the detection limits were 0.1–0.4 ng/mg. This approach has been extended by Sporkert and Pragst [102] who used HS-SPME combined with GC–MS to quantitatively determine a range of basic lipophilic drugs (Fig. 8). A 10 mg amount of hair was digested for 30 min at 70–90°C with 1 ml of 1 *M* NaOH and 0.5 g Na_2SO_4 together with an internal standard. The HS was sampled with the appropriate fibre (Table 5) and analytes desorbed for 5 min at either 250 or 290°C.

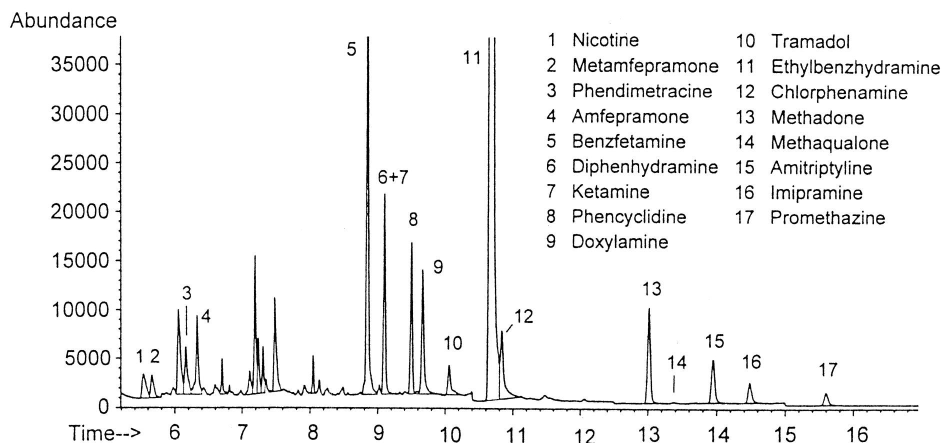

Fig. 8. GC–MS single ion monitoring chromatogram of a 10 mg hair sample (non-smoker) spiked with 16 drugs after HS-SPME sample preparation. Concentrations: 1 ng/mg, ethylbenzhydramine (peak 11, internal standard) 10 ng/ml, nicotine not added. Fibre 85 μm PA. Adsorption: 15 min at 70°C. Single-ion monitoring measurements in nine time windows using specific ions characteristic of the drugs. From Ref. [102].

Detection limits were between 0.05 and 1.0 ng/mg with absolute recoveries of the analytes between 0.04 and 5.7%. These recoveries agreed with Watanabe et al. [89] for local anaesthetics (0.6–8.5%) and Namera et al. [49] for tetracyclic antidepressants (0.12–0.53%) for HS-SPME analysis of blood and urine, but are much lower than those of Koide et al. [101] for amphetamines in hair (48–62%). The method was not suitable for cocaine or heroin as these ester-type drugs are hydrolysed under basic conditions. For determination of acidic drugs, such as cannabinoids, the pH of the alkaline digest was reduced before sampling. Care must be taken in all aspects of sample preparation as varying the amounts of hair used in the HS vial can lead to highly variable recoveries (Fig. 9). These effects were thought to arise from an increase in drug solubility in the aqueous phase or to elevated viscosity of the matrix due to the presence of more dissolved hair proteins. At present the method is limited to a relatively small range of semivolatile lipophilic drugs however there is potential to extend this by the use of either pre- or post-extraction derivatisation techniques [103].

Using similar hydrolysis and extraction conditions Pragst et al. [104] used HS-SPME to profile the

Table 5
Methods used for the HS-SPME analysis of drugs in hair. 10 mg of hair in 1 ml 4% NaOH plus 0.5 g Na_2SO_4 was analysed by GC–MS with single ion monitoring; adapted from Ref. [102]

Drug	Internal standard	HS-SPME conditions	LOD/LOQ[a] (ng/mg)
Amitriptyline	Dimetacrine	PA, 90°C, 20 min	0.05/0.15
Clomethiazole	*N,N*-Diethylaniline	PMDS/DVB 60°C, 15 min	0.5/1.7
Diphenhydramine	Ethylbenzhydramine	PA, 80°C, 20 min	0.05/0.15
Doxepine	Dimetacrine	PA, 90°C, 20 min	0.2/0.7
Lidocaine	Etidocine	Carbowax–DVB 70°C, 15 min	0.1/0.4
Methadone	[$^2H_9$]Methadone	PA, 80°C, 20 min	0.1/0.4
Nicotine	*N,N*-Diethylaniline	PA, 60°C, 15 min	1/3.5
Tramadol	Ethylbenzhydramine	PA, 90°C, 20 min	0.1/0.4
Trimipramine	Dimetacrine	PA, 90°C, 20 min	0.2/0.7

[a] LOD, limit of detection; LOQ, limit of quantification.

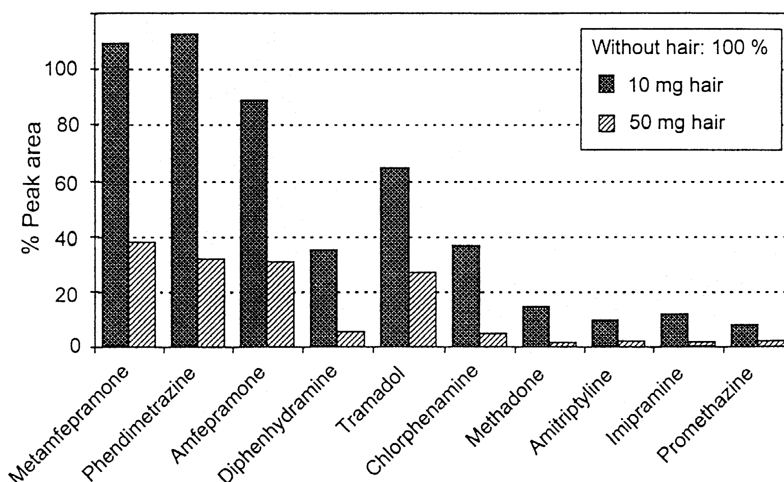

Fig. 9. Effects of the amount of hair sample on the GC–MS peak areas of some drugs after HS-SPME from 1 ml 4% NaOH and 0.5 g Na_2SO_4 solution. All samples were spiked with 50 ng of each drug. 100% is the peak area in absence of hair. HS-SPME conditions: 85 μm PA, adsorption 15 min at 70°C. From Ref. [102].

volatile components released from hair. The aim of the study was to isolate metabolic markers indicative of chronically elevated alcohol consumption. High levels of the fatty acids ethyl palmitate, ethyl stearate and ethyl oleate were found in samples from three alcoholics and these have been suggested as potential markers; further investigations to corroborate these findings are taking place [103].

2.6. Solid-phase microextraction analysis of expired breath and saliva

Recently there has been increased interest in the determination of compounds in breath for clinical diagnosis and toxicological purposes. Over one hundred volatile compounds have been identified in human breath using GC–MS [105]. SPME allows for the direct non-invasive sampling of expired air. Grote and Pawlisyzn [106] modified a commercially available SPME device by covering the fibre needle with a tube with a small opening to allow the patient's breath to pass over the exposed fibre. The shield prevented damage to the delicate fibre during sampling. The effects of fibre chemistry were examined and the 65 μm PDMS–DVB phase was found to be effective for isoprene and acetone. The sampling process took less than 1 min with detection limits of 5.8 n*M* ethanol, 1.8 n*M* acetone and 0.3 n*M* isoprene. However the more polar PDMS–DVB phase was particularly sensitive to changes in humidity levels compared to the non-polar PDMS. The volatile analytes were stable on the fibre for over 8 h allowing for breath sampling remote from the laboratory. Hyspler et al. [107] used a different indirect approach with the expired air first collected into an inert 8 l Tedlar bag which was subsequently sampled through a septum with a PDMS–Carboxen fibre at 40°C for 10 min. Similar detection limits (0.25 n*M*) were found for isoprene, a marker for body cholesterol synthesis. At present SPME is limited to the detection of compounds with relatively high concentration in human breath however improvement in design of the sampling device and new fibre chemistries should allow for its increased application in the future to other diagnostic analytes. There is also potential to use fibres preloaded with specific derivatisation reagents for highly polar and volatile compounds contained in breath. This was demonstrated by Martos and Pawlisyzn [36] who used a PDMS–DVB fibre impregnated with *o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride (PFBHA) for the sensitive measurement of ambient formaldehyde. This involved the formation of a PFBHA-oxime derivative of the volatile analyte.

The analysis of drugs in saliva is attractive as it easy to collect and quantitative measurements may

reflect the non-protein fraction of the drug in plasma. To date there have been two reports of direct immersion SPME for analysis of saliva for cannabinoids [108] and methadone and one of its metabolites [109].

3. Conclusions and future potential

It is now established that HS-SPME is a powerful method for sample preparation and is finding increased application in many laboratories involved in analysis of biological fluids and materials. It affords a number of advantages in simplifying sample preparation, increasing reliability, selectivity and sensitivity. The physicochemical principles and parameters underlying the SPME process are being described and these allow for improvements in calibration and quantitation under different sampling conditions. SPME itself may be used to measure physicochemical constants and coefficients in complex biological systems [110]. Its versatility is enhanced by the possibility of using direct insertion into the sample matrix for less volatile components and there are significant benefits to be gained through careful manipulation of the extraction conditions. Novel derivatisation procedures may extend further the utility of the technique.

The advantages of using in-tube SPME are just beginning to be explored for the extraction of a range of environmental pollutants, drugs and metabolites and other analytes [111–116]. Here the short externally coated SPME fibre is replaced with a length (50–100 cm) of internally coated fused-silica GC capillary column. The sample is slowly passed through the tube where analytes are selectively adsorbed according to their affinity for the stationary phase [e.g. PDMS, poly(ethylene)glycol] selected. The compounds are then desorbed into a GC or HPLC system for analysis with a small volume of wash solution. Due to its selectivity and speed of analysis it may have potential uses in combinatorial synthesis and screening and other areas of routine drug monitoring [117].

SPME can now be interfaced with a number of other instrumental techniques such as HPLC–MS, CE [118–120] and ICP-MS which further widens its application as an extraction procedure. Autosamplers that permit both temperature controlled HS and agitated direct immersion SPME sampling have recently become commercially (MPS-2 from Gerstel and Combi PAL from Varian) available for use with a range of GC instruments. This will further enhance the reliability and reproducibility of the technique and allow higher throughput as has become routine with other sample preparation methods such as SPE using cartridges and microwell plates. The availability of an autosampler for use with conventional HPLC–SPME is still awaited, but this may become unnecessary with the current advances being made with automated in-tube HPLC–SPME methods. Here the stainless steel Rheodyne loop is replaced with a length of coated GC column to serve as an active in-line selective extraction device and with minor changes to solvent flow it can be configured as a conventional HPLC system [121].

The development of a wider range and more selective and sensitive fibre chemistries remains an active research area [122–124]. Since the technique was introduced there has been a gradual increase in the number of phases available and there are now fibres of different lengths to increase extraction efficiency. Mixed bed coatings (e.g. PDMS–Carboxen–PDMS–DVB) and coatings of differing layers offer the potential to extract a range of analytes simultaneously as the fibres have a spectrum of selectivities. Wu et al. [112] recently demonstrated the improvements obtained for the extraction of a series of β-blocking drugs in urine and serum by the use of novel polypyrrole polymers compared to a conventional Omegawax GC phase with in-tube SPME. Further customised coatings such as selective Carboxens, chirally active phases, various derivatised cyclodextrins, ion exchangers [125], HPLC stationary phase particles [126,127] and sol–gel porous silicas [128] are expected to become available in future. As evidenced with SPE, there are exciting possibilities for incorporating antibodies or proteins onto the fibre for specific molecule interactions and the development of molecularly imprinted polymer fibres with artificial receptors [129] for target analytes (e.g. specific drugs) as the fibre-bonding technology matures. Attention must also be given to the quality control procedures used in the manufacture of the fibres. It has recently been shown that some reproducibility problems experienced during analysis can originate from variable surface properties of different fibres [130].

Changes to the design of the device to allow for remote sampling of the aquatic environment, industrial atmosphere or even niche applications such as expired breath are beginning to take place. Most analytes once trapped on the fibre are sufficiently stable to allow their transport from the field or point of care in a hospital to the laboratory or they may be analysed in situ by portable micro-GC equipment. With the development of more sensitive fibre phases it may be possible to further miniaturise the technique. The fragility of the fibre assembly still remains a drawback. It should be possible to make fibres with a stainless steel, tungsten or other metal core in place of the fused-silica to increase their mechanical strength. Reuse of the fibres after they have been immersed in dirty matrices, such as biological fluids containing high-molecular-mass contaminants can lead to non-reproducible results. This problem may be overcome by protecting the fibre during sampling with a diffusion limiting membrane sheath with a specific molecular mass cut-off [131]. As the market for SPME increases in future this could lead to the introduction of disposable low-cost 'one shot' extraction fibres (e.g. in the form of a carousel) or tubes such as in other areas of sample preparation e.g. SPE multiwell plates.

A more radical approach to the design and concept of SPME has been recently proposed (Twister, available from Gerstel) [132]. Rather than a fibre, a coated (with similar types of phase but as a thick 0.3–1.0 mm layer) magnetic stirring bar is used and this is compatible with both GC and HPLC desorption procedures. The technique, known as stir bar sorptive extraction, gives 500 times improved sensitivity compared to a 100 μm PDMS fibre for certain applications due to the increased (20–350 μl) amount of phase available for sorption. The method has been used for the enrichment of a range of volatile and semivolatile compounds in aqueous samples. Its application to biological fluids is awaited with interest.

SPME is barely a decade old. The past 10 years have seen the technique grow from a few environmental uses for extraction of volatile compounds in contaminated water using GC detection to a multitude of applications involving an array of different detectors that we have today. SPME has already displaced established preparation methods such as conventional HS and LLE and is becoming the routine method of choice in many laboratories involved in analysis of biological fluids. There is no reason to doubt that these exciting developments will continue in the future. However, continually making SPME more complicated in order to extend its range of uses may be counterproductive as its main advantages of simplicity and speed would be lost.

4. Nomenclature

AAF	atomic absorption fluorescence
BSTFA	bis(trimethylsilyl)trifluoroacetamide
CE	capillary electrophoresis
CoA	coenzyme A
DVB	divinylbenzene
ECD	electron capture detection
FID	flame ionisation detection
GC	gas chromatograph/ic/y
HPLC	high-performance liquid chromatograph/ic/y
HS	headspace
ICP	inductively coupled plasma
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantification
MS	mass spectrometry/ic
NPD	nitrogen–phosphorous detection
PA	polyacrylate
PDMS	polydimethylsiloxane
PDAM	1-pyrenyldiazomethane
PFBHA	*o*-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride
RSD	relative standard deviation
SID	surface ionisation detection
SPE	solid-phase extraction
SPME	solid-phase microextraction
TPR	templated resin

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