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Review

Solid-phase microextraction for the analysis of biological samples

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Abstract

Solid-phase microextraction (SPME) has been introduced for the extraction of organic compounds from environmental samples. This relatively new extraction technique has now also gained a lot of interest in a broad field of analysis including food, biological and pharmaceutical samples. SPME has a number of advantages such as simplicity, low cost, compatibility with analytical systems, automation and the solvent-free extraction. The last few years, SPME has been combined with liquid chromatography and capillary electrophoresis, besides the generally used coupling to gas chromatography, and has been applied to various biological samples such as, e.g., urine, plasma and hair. The objective of the present paper is a survey of the application of SPME for the analysis of biological samples. Papers about the analysis of biologically active compounds are categorised and reviewed. The impact of SPME on various analytical fields (toxicological, forensic, clinical, biochemical, pharmaceutical, and natural products) is illustrated. The main features of SPME and its modes are briefly described and important aspects about its application for the determination of pharmaceuticals, drugs of abuse and compounds of clinical and toxicological interest are discussed. SPME is compared with other sample pretreatment techniques. The potential of SPME and its main advantages are demonstrated. Special attention is paid to new trends in applications of SPME in bioanalysis.

Keywords: Review; Solid-phase microextraction; Sample pretreatment; Bioanalysis; Biochemical analysis

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1. Introduction

Sample pretreatment is very often the most time consuming step of an analytical process. Today's practicioners ask for more efficient, selective and sensitive analytical methods. There is a continuous need for faster, robust analytical procedures leading to lower detection limits. Sample preparation methods should provide increased sample loads, decreased labour force and less exposure to chemicals, enhanced productivity and quality of data with increasing regulatory constraints and integration of information management systems [1]. Conventional extraction techniques like liquid–liquid extraction (LLE) or soxhlet extraction are laborious, time-consuming and difficult to automate. Moreover they require relatively large quantities of organic solvents (hydrocarbons, chlorinated solvents, etc.) which are often expensive, toxic, carcinogenic and hazardous to the environment. An ideal sample preparation technique should be solvent-free, simple, inexpensive, efficient, selective and compatible with a wide range of separation methods. Solid-phase extraction (SPE) meets many of the above requirements; hence it has been recognised as a major sample pretreatment technique with a vast application area. In typical SPE the sample is passed through a minicolumn filled with an appropriate extraction material. Compounds of interest are retained on column while interferences are washed away. The analytes are recovered by eluting the column with a proper solvent. An attractive feature of SPE is the availability of various extraction materials, which favour and incorporate different types of interactions, a fact that can greatly improve extraction selectivity. It can also be automated and coupled on-line to liquid chromatography (LC) and gas chromatography (GC). SPE can also be coupled directly to mass spectrometry (MS), provided that the selectivity is adequate. However SPE has also some important limitations: plugging of the cartridge or blocking of the pores by matrix components, high elution volumes and batch-to-batch variations (although the latter aspect has greatly improved during the last years). Moreover, it is a multi-step process and is therefore suspect to analyte loss. Finally SPE often involves a concentration step through solvent evaporation and in this way it is not applicable to the extraction of volatile or thermolabile compounds.

Miniaturisation can prove a solution to the above problems. An alternative that should not be ignored, is the so-called micro- or semi-micro-SPE. In this case the dimensions of the SPE sorbent are minimised in order to carry out the extraction in a disc or a packed pipette tip. In a recent report a membrane disk was packed between two supporting steel screens in the top of a syringe which served as the sample reservoir [2]. Three types of membranes were used for the extraction of 30 organic compounds from aqueous and biological samples. This interesting approach combines some of the advantages of SPE and SPME concerning elution volumes (20–50 μl), ease and extraction time. With a similar set-up verapamil, a calcium channel blocker, and its primary metabolite norverapamil were determined in urine, using a C_8 membrane-bonded phase [3].

Although SPME has been recently introduced [4] it has gained much research interest and popularity. SPME is based on the partition of the analyte between the extraction phase and the matrix. The method uses a small fused-silica fiber, coated with a suitable polymeric phase, mounted in a syringe-like

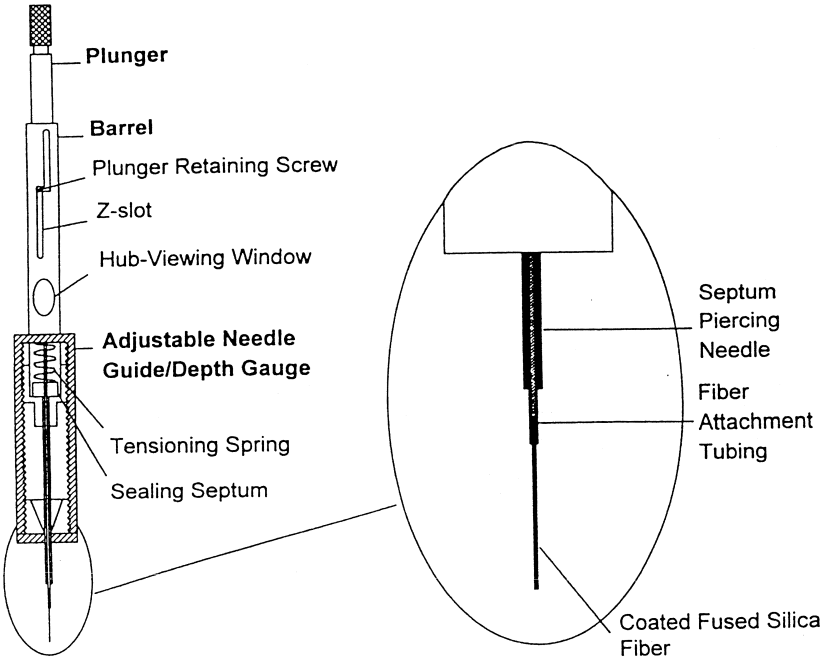

Fig. 1. Scheme of a SPME device (from Ref. [5]).

protective holder (Fig. 1). During extraction the fiber is exposed to the sample by suppressing the plunger. Sorption of the analytes on the fiber takes place in either the sample by direct-immersion or the headspace of the sample. After equilibrium or a well-defined time, the fiber is withdrawn in the septum-piercing needle and introduced into the analytical instrument where the analytes are either thermally desorbed or re-dissolved in a proper solvent for LC or capillary electrophoresis (CE). The technique was commercialised in 1993 by Supelco. Initial work was exclusively done with SPME-GC [6–8] due to the direct and convenient sample introduction into GC and the main application area was environmental analysis. Coupling to LC requires an appropriate interface and was first reported in 1995. The development of in-tube SPME enabled the automation of SPME-LC. Extraction takes place in a piece of ordinary capillary GC column hosted for protection inside a needle to pierce the septa (see Fig. 2). An aliquot (25 μl) of the sample is aspirated and dispensed several times into the capillary. Desorption of the analytes is achieved by aspiring a proper organic solvent and dispensing the eluate into the injection loop [9]. Actually the principle of in-tube SPME is close to that of SPE, because the use of a thin layer of stationary phase is not an essential difference with SPE in a cartridge.

SPME has successfully been coupled to CE [10,11] and packed column supercritical fluid chromatography (PCSFC) [12]. In the last years new devices have been developed to facilitate SPME for air monitoring, fast gas chromatography and on-site sampling. The use of SPME becomes more and more widespread as some problems observed in the first steps of its utilisation are now solved. Excellent reviews described the theory, the practice, the state of the art and the future aspects of SPME [5,13–15]; the inventor of the technique J. Pawliszyn provided a comprehensive monograph [16]. A recent report reviewed the use of SPME in forensic science, but this was unfortunately somewhat limited because only Japanese papers are mentioned [17].

The scope of the present review is to survey the papers reporting on the use of SPME for the determination of pharmaceuticals, drugs of abuse, biologically active compounds and compounds of general biological or toxicological interest in bio-

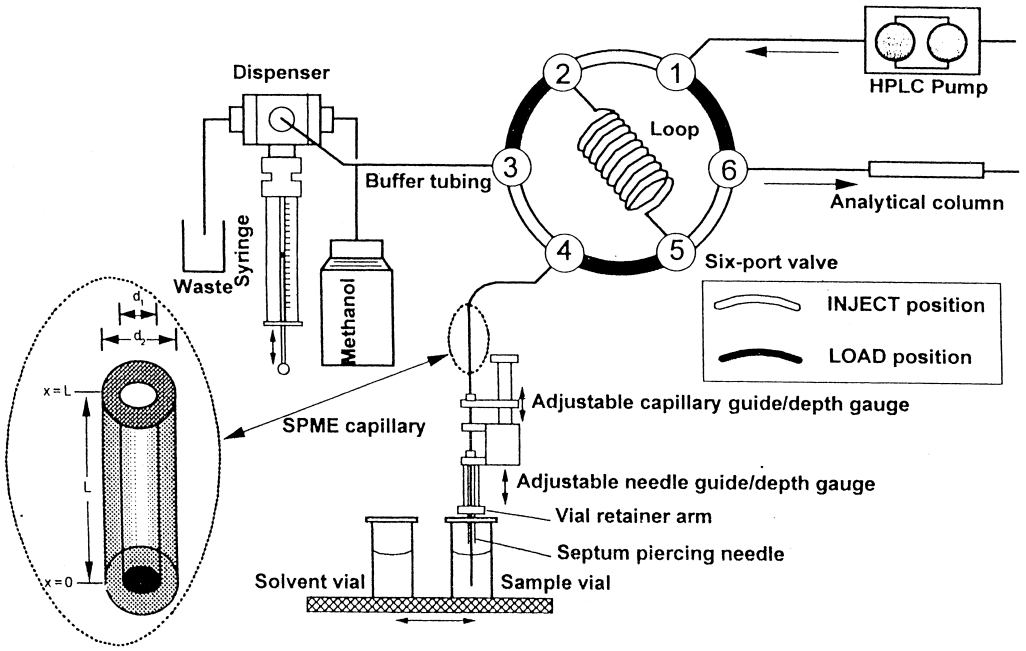

Fig. 2. Diagram of the in-tube SPME-LC interface. The sample is frequently aspirated in the SPME capillary and dispensed back to its vial by movement of the syringe (valve in INJECT position). The six-port valve is switched to LOAD and methanol is pushed into the SPME capillary. The eluate is transferred to the loop, the valve is switched to INJECT and subsequently directed by the LC elution solvent towards the analytical column. A detailed view of the in-tube SPME capillary is included in the left side of the figure (from Ref. [9]).

logical samples. The major criteria were the type of the analyte and the type of sample. The majority of the reviewed papers deals with low-molecular mass compounds, although a few examples are given which describe the potential of SPME for the determination of proteins. First a short and general description of the method and its main features is given. In the applications part, the paper is divided into eight major paragraphs with regard to the groups of analyte.

2. Solid-phase microextraction

2.1. Extraction mode

There are, in general, two extraction modes: direct sampling from the aqueous phase and headspace (HS) extraction. The main criteria for mode selection are nature of the sample matrix, analyte volatility and affinity of the analyte for the matrix. Medium volatile analytes can be extracted from clean samples by direct-immersion (DI) of the fiber into the sample. In this case the mass transfer rate is determined mainly by diffusion of the analyte in the coating provided that the sample is 'perfectly' agitated. In practice a thin layer of sample liquid is formed around the fiber, hindering the direct access of the analytes to the coating; the analytes should penetrate this layer in order to reach the coating. This layer is actually stationary and cannot be removed without vigorous agitation methods (sonication). For dirtier samples the fiber can be protected by a membrane [5].

HS-SPME was first reported in 1993 [8]. This mode is preferred for volatile compounds: volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, ethylbenzene, xylene (BTEX). HS-SPME provides cleaner extracts, greater selectivity and longer fiber life time. Three phases (coating, headspace and matrix) are involved in the extraction process; therefore the affinity of the analytes for all three phases de-

termines the extraction yield. In most cases, the time-limiting step is the transfer of the analytes from the sample to the headspace and thus extraction can be optimised by gentle heating or stirring of the sample.

2.2. Coating

The properties (physical and chemical) of the coating are crucial for the partition process. The main commercial available coatings are polydimethylsiloxane (PDMS) of different film thickness (7, 30 and 100 μm), 85 μm polyacrylate (PA), 65 and 60 μm polydimethylsiloxane–divinylbenzene (PDMS–DVB), 75 μm Carboxen–PDMS, 65 μm Carbowax–DVB (CW–DVB) and 50 μm Carbowax–templated resin (CW–TPR). Selection of the coating is mainly based on the principle 'like dissolves like'. Non-polar analytes have relatively high affinity for the apolar PDMS phases which are often first choice, since they also offer long life-time. PA is more polar and can be used for the extraction of polar compounds, such as phenols. Mixed phases are mainly used for the extraction of volatile compounds. The extraction yield of these fibers is higher compared to PDMS, but their life-time is limited. Furthermore, the sorption process of the available mixed-phase coated fibers is based on adsorption rather than absorption as is the case for PDMS- and PA-coated fibers, which means that co-extracted compounds can more easily displace or interfere with the analyte of interest. Coating thickness is selected according to the efficiency required, the extraction time and the nature of the analyte. The thinner the coating the faster the partition equilibrium can be reached. The choice of coating thickness is also related to the molecular mass of the analyte: for small-molecular mass compounds high extraction yields can be obtained with relatively thick coatings.

Recently new phases have appeared as a result of the on-going research: porous layer silica-bonded LC coatings (C_8, C_{18}) [18,19], carbon-graphitised silica [20]. Chong et al. reported on new sol–gel PDMS phases tolerating temperatures up to 320°C, which is desirable for the analysis of less volatile compounds [21]. Further discussion on the sol–gel phases is given in Section 3.2.2.

2.3. Extraction conditions

Extraction yield can be optimised by altering sample conditions such as pH, salt concentration, volume, temperature and extraction time. Salt concentration and pH affect SPME in the same way as in most extraction procedures (SPE or LLE). Salt addition can improve the extraction yield of compounds of interest; salts like NaCl, $(NH_4)_2SO_4$, Na_2CO_3 are often added to the sample. Adjustment of pH may improve the extraction yield for compounds that can be protonated. In most of the cases pH is adjusted in order to obtain the analyte in its neutral form, to enhance the extraction yield in combination with the addition of salt. Care has to be taken when direct-immersion SPME is used, since extreme pH values (lower than 2 and higher than 10) can damage the coating and thus it is difficult to implement large pH changes. Sample volume selection should be based on the estimated partition constant K_{fs}. If available large sample volumes ($\geq$10 ml) should be used for compounds with high K_{fs} values. Small sample volumes can only be used, if is taken into account that the sample is depleted by extraction. On the other hand for very large sample volumes the amount of the analyte extracted is no longer related to the sample volume [22,23]. For headspace extraction the gaseous phase volume should be minimised in order to increase the yield.

Agitation of the sample is used in order to enhance the extraction recovery with time or to reduce the equilibrium time. Agitation methods used include magnetic stirring, sonication, fiber vibration and flow through cells. Vigorous or harsh agitation modes such as sonication may affect the coating, thus they should be used with caution.

An increase in temperature can increase the extraction yield in non-equilibrium situations, but may also decrease the distribution constant. Extraction time in most of the reviewed papers varies from 1 to 60 min. SPME is an equilibrium process, but very often extraction is ended in a fixed time before reaching equilibrium. Equilibrium time is governed by mass transport between sample and coating, and therefore affected by coating thickness, agitation method, temperature, etc. The presence of headspace in the sample vial can also influence equilibrium time and yield in both DI and HS-SPME.

Fig. 3 shows the effect of some of the above mentioned parameters on the extraction efficiency of benzodiazepines with a PA-coated fiber. In this example SPME is coupled with semi-microcolumn LC for the analysis of urine samples. Fig. 3a depicts the time-sorption profiles which reflect the effect of extraction time. Fig. 3b shows that an increasing temperature increases the extraction yield due to a faster mass transfer, i.e., if equilibrium has not been reached, the extraction yield at a certain extraction time can be increased by the faster mass transport at elevated temperatures. However, the authors state that the compounds decompose at temperatures above 60°C, a decrease in yield can also be observed by the fact that K_{fs} values decrease with increasing temperature. Fig. 3c shows that by 'salting-out' extraction efficiency can be improved. Because the extraction yield is influenced by pH (as shown in Fig. 3d) and the pH of the sample was not adjusted after adding salt, the increase in yield by 'salting-out' could even be higher. Accuracy and precision of SPME can be easily affected in a negative way by the influence of various parameters on the extraction yield. More detail about the theory and the principles

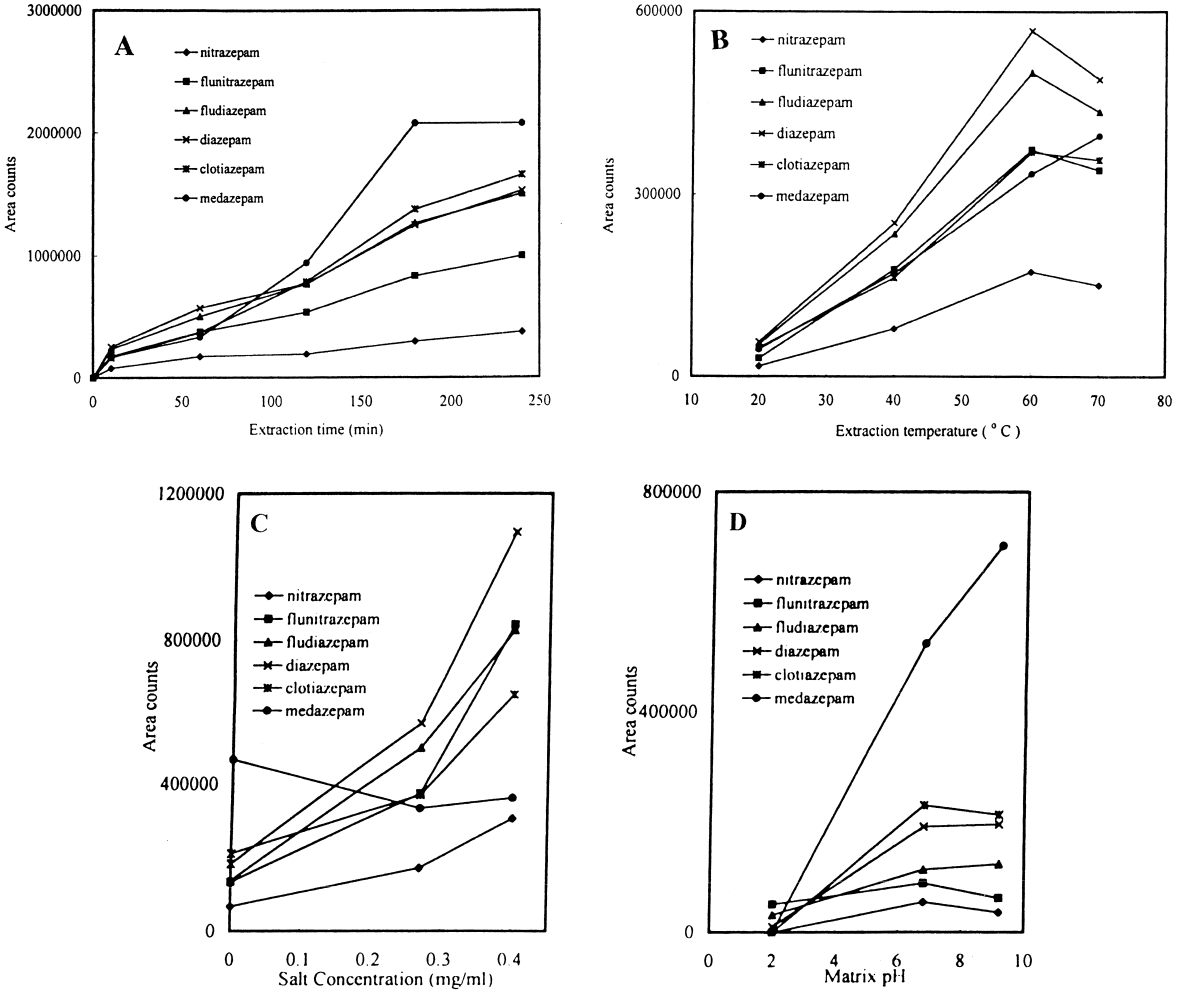

Fig. 3. Effect of extraction parameters on the extraction efficiency of benzodiazepines with a PA-coated fiber. (A) Extraction time: 60°C, 0.27 g/ml salt, pH of matrix, 30 min desorption in 30 μl acetonitrile, drug concentration 100 ppb. (B) Extraction temperature: extraction time unknown, 0.27 g/ml salt, pH of matrix, 30 min desorption in 30 μl acetonitrile, drug concentration 100 ppb. (C) Salt concentration: extraction time 60 min, 60°C, pH of matrix, 30 min desorption in 30 μl acetonitrile, drug concentration 100 ppb. (D) Matrix pH: extraction time 60 min, 30 min desorption in 30 μl acetonitrile, drug concentration 100 ppb (from Ref. [24]).

of SPME is not within the scope of this review. For a deeper insight the reader should look to the numerous publications on this topic, e.g., the monograph of J. Pawliszyn [16].

2.4. Desorption

When SPME is coupled to GC analyte desorption from the fiber is straightforward. The septum-piercing needle of the SPME device is introduced into the GC injector where the fiber is exposed to the heated chamber and the analytes are thermally desorbed. A narrow bore insert is required for fast desorption. Hot on-column injection with the highest possible temperature can be used. Split–splitless injection can be used in order to eliminate carry-over. In this case desorption of the analytes from the fiber occurs in splitless mode, so that the main part of the desorbed amount of analyte is introduced in the GC column, where it can be cryo focused. During the analysis the injector is operated in the split mode, so possible carry-over could be thermally desorbed without entering the column.

For the coupling with LC the fiber is placed into a small desorption chamber with three ports in T-configuration (sometimes a piece of PEEK tubing). The chamber is mounted in the injection loop position of a typical six-port injection valve. By switching the valve, the chamber (and therefore the fiber) is flushed by the mobile phase, which desorbs the analytes. Static desorption of the fiber depends on time and the composition of the desorption liquid. Accordingly dynamic desorption is governed by the eluent (most cases the mobile phase) and the selection of flow-rate [25], and may cause peak broadening. In automated in-tube SPME desorption of the analytes is achieved by repeated aspiration and dispension of an aliquot of an organic solvent into the injection loop. This method enhances full automation and can be performed with typical LC autosamplers. Moreover the in-tube desorption was reported to be quantitative with no carry-over effects.

2.5. New trends in SPME

The nature of SPME offers attractive aspects for innovative modifications and applications. Thus, many researchers adopted the concept of SPME to their needs and adjusted the device accordingly. A first approach to in-tube microextraction was named inside needle capillary adsorption trap device (INCAT) and was reported for the headspace extraction of VOCs [26]. The device utilised a hollow needle encircling either a short length of GC capillary or an internal carbon coating that was used as sorbent.

Direct coupling of SPME to MS is a substantial goal, but is perhaps hindered by interfacing problems. Recently, SPME has been directly coupled to ion mobility spectrometry (IMS) [27]. Sample introduction was made through a hole drilled in the IMS sample ticket holder. Coupling with infrared (IR) spectroscopy has been reported for the determination of 10 VOCs (benzene, toluene, chloroform, etc.) in water [28]. In this report a small square of parafilm served as the extraction phase. VOCs were detected directly in the parafilm by IR spectroscopy.

SPME has been applied to a wide variety of research fields, e.g., the study of the sonochemical degradation of ethylbenzene in aqueous solutions [29]. An interesting combination is microwave-assisted SPME for the extraction of organic components in foods. The water present in the food absorbed the microwave energy and ‘pushed’ the target compounds out of solid matrixes [30]. SPME has also been used for the extraction of inorganic ions, combined with atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES). Methylcyclopentadienyl manganese, a gasoline antiknocking agent, was determined in beverages by means of SPME-GC–AAS [31]. The coupling of SPME with GC–inductively coupled plasma-MS enabled the simultaneous determination of organometallic compounds (Hg, Sn, Pd) after their in situ derivatisation with sodium tetraethyl borate [32]. Recently SPME combined with electrochemistry was used to extract inorganic mercury and organo mercury compounds from aqueous solutions and mercury vapours from gas. A carbon steel wire coated with 10-μm gold was employed as the working electrode and SPME fiber. A platinum wire was used as counter electrode and an standard Ag/AgCl electrode was used as reference. Analysis was performed by ion-trap GC–MS after a capacitive discharge desorption of the fiber [33]. Another SPME-electrodeposition device [34] is described in Section 3.4.

3. SPME in bioanalysis

SPME was initially applied to the analysis of organic compounds from rather clean samples (air, water) [4,35]. The majority of SPME reports are still on the field of environmental analysis. Until the time of the last literature search in Chemical Abstracts and Current Contents (July 1999) a total of 475 publications utilising SPME had been indexed. Apart from environmental analysis, numerous papers were on the topic of flavour–aroma and food analysis. Recently, SPME is increasingly used in bioanalysis. Successful coupling with LC and CE enables the analysis of proteins, polar alkaloids, pharmaceuticals and surfactants that cannot be analysed by GC. Fig. 4 depicts the distribution of number of published papers with publication year and type of application. The number of papers published in 1999 are omitted from the graph as they would give a wrong impression of the observed trend.

The literature was categorised in eight main groups according to the type of analyte. Hence the review is divided into eight major paragraphs describing the application of SPME in the following areas: toxicological and forensic analysis, drugs of abuse, clinical chemistry, analysis of pharmaceuticals in biological samples, biochemical analysis, semiochemical analysis, and analysis of natural products. Within these paragraphs further divisions were made in order to highlight either compounds of high interest and therefore a large number of papers published on these compounds, or a specific field where SPME offers substantial advantages. It should be stressed that categorising such a large number of applications from various research groups was not an easy task and some choices are arbitrary. An overview of the applications together with the used analytical system, some experimental conditions and important data is given in Table 1.

3.1. Toxicological analysis

Toxicological analysis is a field where routine and research are integrated to a great extent. Hence new methods are often rapidly implemented and improve the usual heavy tasks of toxicological laboratories. SPME offers great advantages to toxicological analy-

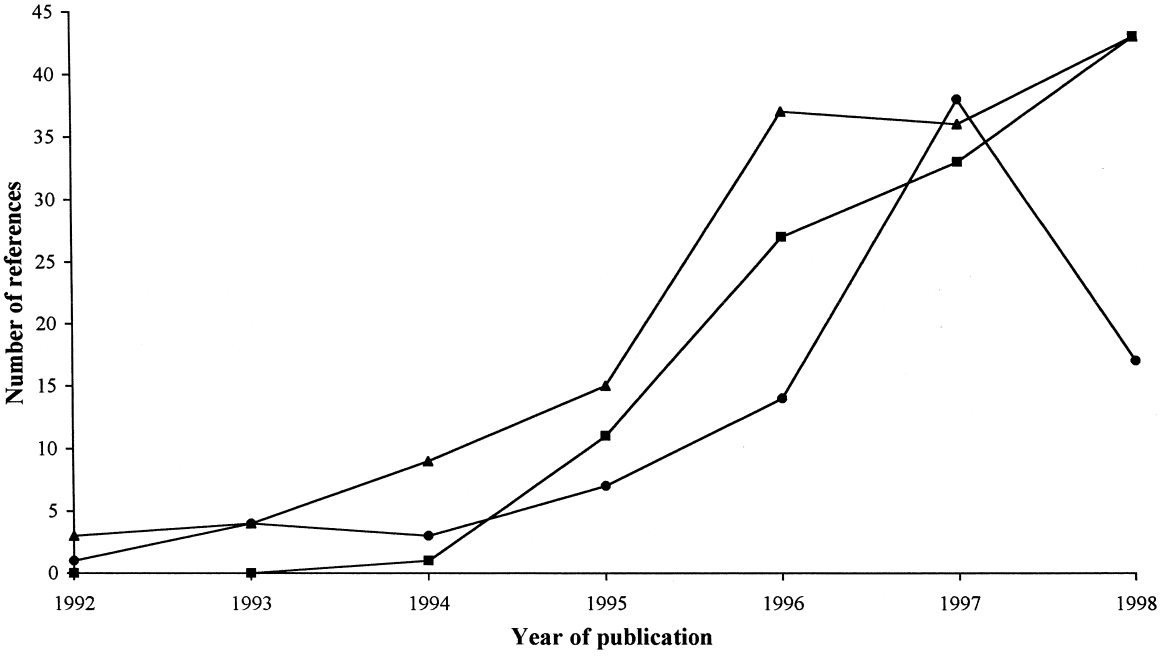

Fig. 4. Distribution of papers published on SPME according to type of application (general (●), environmental (▲), bio-analysis (■)) and year of publication.

Table 1
Application of SPME in bioanalysis

Analyte	Sample	Method	Fiber coating (thickness μm)	Analytical system (LOD)	Remarks	Refs.
Alkyl carnitines	Urine	DI	CW (65) PDMS (100)	LC–ESI-MS	Direct SPME-ESI-MS	[110]
Alkyl nitrites	Blood	HS	PDMS (100) PA (85)	GC–FID (1 ng/ml)	Application with some theoretical aspects	[81]
Amino acids	Blood, urine	DI	PDMS (100) PA (85)	GC–MS	Homocysteine, cysteine, methionine determination	[111]
Amphethamine Methamphetamine	Urine	DI	PDMS (100)	GC	Use of high pH (12) buffers.	[66]
Amphethamines	Biosamples	HS	PDMS (100)	GC–MS		[58]
Amphethamines	Urine	HS	PDMS (100)	GC–MS	20 times higher sensitivity compared to HS	[62]
Amphetamine	Urine	DI Automated	PDMS (100)	GC–NPD, GC–MS (50 ng/ml)	Derivatisation in sample before extraction	[63,64]
Amphetamine-related compounds	Urine	HS	PDMS (100)	GC–MS (1-50 ng/ml)	Optimisation for 21 compounds	[57]
Amphetamines	Urine	DI HS	PDMS–DVB (65) PDMS (100)	GC–FID	Optimisation of extraction parameters	[60]
Amphetamines	Blood	HS	PDMS (100)	GC–MS	Derivatisation in GC-injector during desorption	[61]
Amphetamines	Hair	HS	PDMS (100)	GC–NPD (0.1–0.4 ng/ml)	Determination of drug of abuse in hair	[59]
Amphetamines	Urine	DI	PDMS (100)	GC–MS (1–10 ng/ml)	Optimisation	[65]
Anaesthetics	Blood	DI	PDMS (100)	GC–FID (54–158 ng/ml)	Extraction after deproteinisation	[82,83]
Anaesthetics	Blood	HS	PDMS (100)	GC–MS (0.05–0.5 μg/ml)	Applied to a medico-legal case	[44,45]
Aniline, phenols, nitrobenzenes	Plasma	DI	PA (85) 1 mm length	GC–MS	Protein binding study, determination of free concentrations	[124]
Anilines, phenols, substituted benzene	Cell cultures	DI	PA (85) 1 mm length	GC–FID, GC–ECD	Determination of membrane–water partition coefficient and free concentration	[125]
Anorectic compounds	Urine	DI	PDMS (30)	GC–MS		[117]
Antidepressants	Blood	HS	PDMS (100)	GC–FID (16–25 ng/ml)		[90]
Antidepressants	Plasma	DI	PDMS (100)	GC–NPD, GC–MS (100 ng/ml)	Theoretical model for influence of proteins	[113]
Antihistaminics	Urine blood	HS	PDMS (100)	GC–FID (76–472 ng/ml)		[91]
Aromatic hydrocarbons	Urine	HS	PA (85)	GC–MS	On-fiber derivatisation with BSTFA	[53]
Aromatic amines	Urine blood	HS	PDMS (100) PA (85) PDMS–DVB (65) CW–DVB (65) CX–PDMS	GC–FID, GC–MS (0.4–7.7 ng/ml)		[106,107]
Attractants to flies	Air	HS	PDMS (100)	GC–MS	Attractants to Mexican flies	[138,139]
Barbiturates, benzodiazepines	Urine	DI	PA (85)	CE–UV (MEKC)	SPME–MEKC method for toxic drugs	[74,75]

Barbiturates	Buffer, urine serum	DI	Home made PVC on steel	CE–UV	Coupling method for SPME-CE	[77]
Barbiturates	Urine	DI	CW–DVB (65) PA (85) PDMS (30)	GC–MS	Optimisation, determination of distribution coefficients	[76]
Benzodiazepines	Urine	DI	PA (85) CW–TPR (50) Sol–gel PDMS (50)	(Semi) micro LC–UV		[24,72] [73]
Benzodiazepines	Plasma	DI	PA (85) PDMS (7,100)	GC–FID	1-Octanol modified fiber, pre-treated plasma	[69,70]
Benzodiazepines	Urine serum	DI	CW–DVB (65) PA (85) PDMS (100) PDMS–DVB (65)	GC–FID, GC–MS (0.02–0.1 μg^{-}/ml)		[71]
Benzodiazepines	Urine	DI	PDMS–DVB (65)	GC–FID (10–150 ng/ml)		[67]
Benzodiazepines	Urine	DI	PDMS (100)	GC–ECD (2–20 ng/ml)	Hydrolysis of the compounds before extraction, comparison with LLE	[68]
Benzophenone-3 and metabolites	Urine	DI	PDMS (30) PA (85) CW–DVB (65)	GC–MS (260 ng/ml)	Comprehensive optimisation	[104]
Cannabinoids	Saliva buffer	DI	PDMS (7,30,100) CW–DVB (65) PA (85)	GC–MS (10 ng/ml)	Optimisation, comparison with LLE	[79]
Cannabinoids	Hair	DI	PDMS (30)	GC–MS (0.1 ng/ml)		[80]
Carbamate pesticides	Blood urine	HS	PDMS (100)	GC–FID (0.01–0.5 μg^{-}/ml)		[46]
Chlorophenols	Urine	DI	PA (85)	GC–MS (1–98 ng/l)	Application in sawmill workers samples	[50]
Chlorophenols	Blood	HS	PA (85)	GC–ECD ng/ml levels		[51]
Cocaine, heroine	Buffer	HS	PA (85) CX (65)	IMS	Analysis of drug vapors by direct coupling of SPME-IMS	[27]
Cocaine	Urine	DI	PDMS (100)	GC–NPD (12 ng/ml)		[84]
Corticosteroids	Urine	DI	PDMS–DVB (60) PA (85) CW–DVB (65) CW–TPR (50)	LC–MS (4–30 ng/ml)	Short column LC	[118]
Cresol isomers, phenol	Blood	HS	PA (85)	GC–FID (140–200 ng/ml)		[85]
Cyanide	Blood	HS	CW–DVB (65)	GC–NPD (0.02 μg/ml)		[42]
Dinitroaniline herbicides	Blood urine	HS	PDMS (100)	GC–ECD 1 ng/ml blood, 0.1 ng/ml urine	Application in rat blood	[48]
Drugs–poisons	Bio samples				Review	[17]
Drugs	Bio samples	DI	PDMS (100)	GC	Comparison of different extraction modes for clinical analysis	[101]
Ethanol, methanol	Blood urine	HS	CW–DVB (65)	GC–MS (10–20 mg/l)		[37,38]
Ethanol	Blood	HS	CX–PDMS (75)	GC–FID	Determination after drinking beer	[40]

	urine			(0.2–0.5 mg/l)		
Ethanol, methylene chloride	Urine	HS	PDMS (100) PA (85)	GC–MS	Toxicological analysis of traffic victims	[36]
Fatty acids	Insect glands	HS	CW–DVB	GC–MS	Study of the effect of different lines, deactivated silica pre- and post-columns	[137]
Hydrocarbons	Blood	HS		GC–MS (0.1–1 mg/g)	Inflammable substances from fire victims	[97]
Hg, alkylated Hg, PB, Sn	Biological fluids	HS	PDMS (100)	GC–MS–MS (7–22 ng/ml)	Derivatisation with tetraethylborate	[56]
Hg, methylated Hg	Urine	HS	PDMS (100)	GC–MS–MS	Derivatisation with sodium tetraethylborate	[55]
Hg, methylHg	Biological fluids	HS	Silica fiber modified in HF	GC–AAS (26 ng)	Hydride derivatisation with potassium tetrahydroborate	[54]
Lidocaine	Urine	DI	PDMS (100)	GC–FID, LC–UV (5–25 ng/ml)	Model compound for optimisation, some theoretical aspects	[120]
Malathion	Blood	HS	PDMS (100)	GC–MS	Application to a forensic case	[41]
Methadone	Urine	DI	PDMS (100)	GC–MS		[78]
Methylxanthines	Human fluids	DI	PDMS (100) PDMS–DVB (65) PA (85) CW–DVB (65)	GC–MS 0.2–0.9 μg/ml blood 0.06–0.7 μg/ml urine		[112]
Nereistoxin	Human serum	HS	PDMS (100) PDMS–DVB (65) PA (85) CW–DVB (65)	GC–MS (0.005–0.5 μg/ml)	Application to a suicide case	[43]
Organic acids	Urine	DI	PA (85)	GC–MS	Derivatisation in sample before extraction	[109]
Organic solvents	Pharmaceuticals	HS	PDMS (100), PDMS–DVB (65), CW–DVB (65)	GC–MS, (5 pg/ml–2 ng/ml)	Residual organic solvents in pharmaceuticals	[121–123]
Organophospate pesticides	Blood urine	HS	PDMS 100	GC–NPD (1–80 ng/ml)		[47]
Organochlorine	Blood			GC–ECD (μg/ml)	Derivatisation	[52]
Pentachlorophenol	Urine	DI	PA (85)	GC–MS (0.4 μg/l)	HCl hydrolysis prior to extraction	[49]
Phencyclidine	Blood urine	HS	PDMS (100)	GC–SID (0.25–1 ng/ml)	Extraction after deproteinisation	[89]
Phenothiazines	Blood urine	HS	PDMS (100)	GC–FID (0.01–0.2 μg/ml)		[87]
Phenylethylamine	Urine	HS	PDMS–DVB (65)	GC–NPD (20 ng/ml)		[88]
Pheromones	Insects		PDMS (7, 100)	GC–FID	Extraction by rubbing the fiber on the gland	[131,132]
Pheromones	Culture medium	DI	PDMS (100)	GC	Analysis of biological signal compounds	[130]
Proteins		DI	C_{18}	CE–MS–MS	Analysis of yeast protein	[10]

Steroids	Serum	DI	PA (85)	GC–MS	In situ derivatisation after extraction	[115,116]
Tetramethyl-piperidine-1-oxyl	Keratinocytes	HS	PDMS (7,100)	GC–FID	Comparison with LLE and SPE	[103]
Thinner compounds	Blood urine	HS	PDMS (100)	GC–FID (2–5 ng/ml)		[92]
Trimethylamine	Urine	HS	PDMS (100) CX–PDMS (75)	GC–MS	Use of deuterated TMA as internal standard	[108]
Valproic acid	Plasma	DI	PDMS	GC–FID (1 μg/ml)	Free concentration in plasma dialysate	[114]
VOCs	Living organism				*Phyllonorycter sylvella* moths	[136]
VOCs-BTEX	Urine	DI	PDMS (100)	GC–MS		[96]
VOCs-BTEX	Blood	HS	CX–PDMS	GC–MS (5–14 ng/l)	Human fluid from environmental polluted urban areas	[98]
VOCs	Human breath	DI	PDMS (100) PA (85) PDMS–DVB (65) CW/DVB (65)	GC–MS	Device for breath analysis, optimisation	[105]
VOCs	Staphylococci	HS	PA (85) PDMS (100)	GC–FID		[144]
VOCs	Penicillium	HS	PA (85) PDMS (95)	GC–MS	Analysis of biogenic VOCs in a chemotaxonomic study	[142]
VOCs	Living organism	HS	PDMS (100)	GC–IR	Direct deposition infrared spectrometry	[135]
VOCs	Whey protein	HS		GC–MS	Whey protein concentrates	[128,129]
VOCs	Blood urine	HS	Home made carbon black	GC–FID, GC–MS (10 pg/ml)	Samples from employees in dry-cleaning establishments	[20]
Warfare agents	Water	DI	PDMS, PA, CW–DVB, PDMS–DVB	GC–SIM GC–FID GC–MS ng/ml level	Comparison with LLE	[99,100]

sis in both research and routine analysis. Headspace SPME-GC–MS has proved a very powerful tool in toxicological analysis. The preconcentration of the analytes obtained on PDMS and PA fibers offered great advantages compared to conventional headspace GC–MS. HS-SPME enabled the determination of VOCs in the investigation of two traffic fatalities. Ethanol and methylene chloride were determined in human urine; a series of alkanes were identified in a gastric sample and in the contents of a drinking glass [36]. SPME finds extensive use in the analysis of light alcohols (methanol and ethanol) in biological samples [37–40], e.g., SPME has been used for the determination of ethanol in blood and urine of car drivers. SPME was superior to the normally used static headspace sampling with regard to needed equipment, costs and carry-over, and provided wide linearity and excellent precision. Extraction recovery on a polar CW–DVB fiber was enhanced with the addition of $(NH_4)_2SO_4$. Recently Lee et al. reported on an improved method for the extraction of ethanol utilising a CX–PDMS fiber [38].

SPME has also been used in the analysis of poison agents like malathion and cyanide. Extraction recovery of malathion from the headspace of human blood was enhanced with the addition of $(NH_4)_2SO_4$ and H_2SO_4. Malathion proved to be stable in blood although it decomposes at excessive temperature [41]. Cyanide, one of the most powerful and rapidly acting poisons, showed low recovery from rat blood samples. Despite this fact, HS-SPME-GC provided superior sensitivity compared to the existing analytical methods. Moreover excellent quantitation and good precision were achieved [42].

Nereistoxin, a compound first isolated from a marine annelid, forms the basis for the production of widespread pesticides. Recently, Namera et al. [43] reported on the HS-SPME-GC–MS analysis of nereistoxin and metabolites in human serum. Various parameters were investigated, i.e., fiber type, exposure time, salt addition and pH. Preheating the sample prior to HS-SPME was not found necessary, which is in agreement with previous findings of the same authors for the extraction of other types of semi-volatiles [44,45]. HS-SPME combined with GC has been applied in the analysis of carbamate pesticides [46] and organophosphoric pesticides in blood and urine. Fig. 5 depicts the influence of some parameters on the extraction of several pesticides from human blood [47]. For a further validation, the method was successfully applied to the analysis of blood from a rat fed orally with a representative herbicide [48].

Pentachlorophenol is a widely used industrial preservative, biocide and pesticide and is a possible carcinogenic agent. Chlorophenols in general are considered as a priority pollutant, thus biomonitoring of these compounds is used as an indication of occupational exposure or environmental contamination. Urinary pentachlorophenol was hydrolysed with HCl and extracted on a PA fiber and consequently analysed by GC–MS [49–51]. Analysis in selected ion monitoring (SIM) resulted in limit of detection (LOD) in the low ng/l range for the five analysed chlorophenols in the urine of industrial workers. The authors claim much higher sensitivity (up to 700-fold higher) compared to conventional LLE used by the USA Environmental Protection Agency (EPA) for the determination of chlorophenols in water (see Table 2). However the EPA protocol employs less sensitive detection modes: FID or MS in full scan. The determination of 20 persistent organochlorine in blood was accomplished by SPME-GC–ECD [52]. Polar substances as tri-, tetra- and pentachlorophenols were analysed simultaneously with less polar compounds such as hexachlorobenzene (HCB), α-, β- and γ-hexachlorocyclohexane, DDT and its derivatives and with some polychlorinated biphenyls (PCBs). Compared to conventional procedures the proposed method was fast, reproducible and cheap. Moreover there was no derivatisation needed, in contrast with other extraction procedures.

Another potent pollutant is the group of PAHs, a well-known group of environmental carcinogens. A useful approach to assess human exposure and PAH uptake, is to measure PAH metabolites in urine. Naphthalenes, phenanthrenes and pyrenes were determined by GC–MS after extraction and in situ (on-fiber) derivatisation with bis(trimethylsilyl)trifluoroacetamide (BSTFA) or hydrolysis. GC analysis of polar organic compounds is mostly performed after derivatisation, which is often necessary in order to enhance analyte volatility. Derivatisation may require additional time, concentration and drying steps. In situ derivatisation on the SPME fiber can prove a very efficient and advantageous approach. A

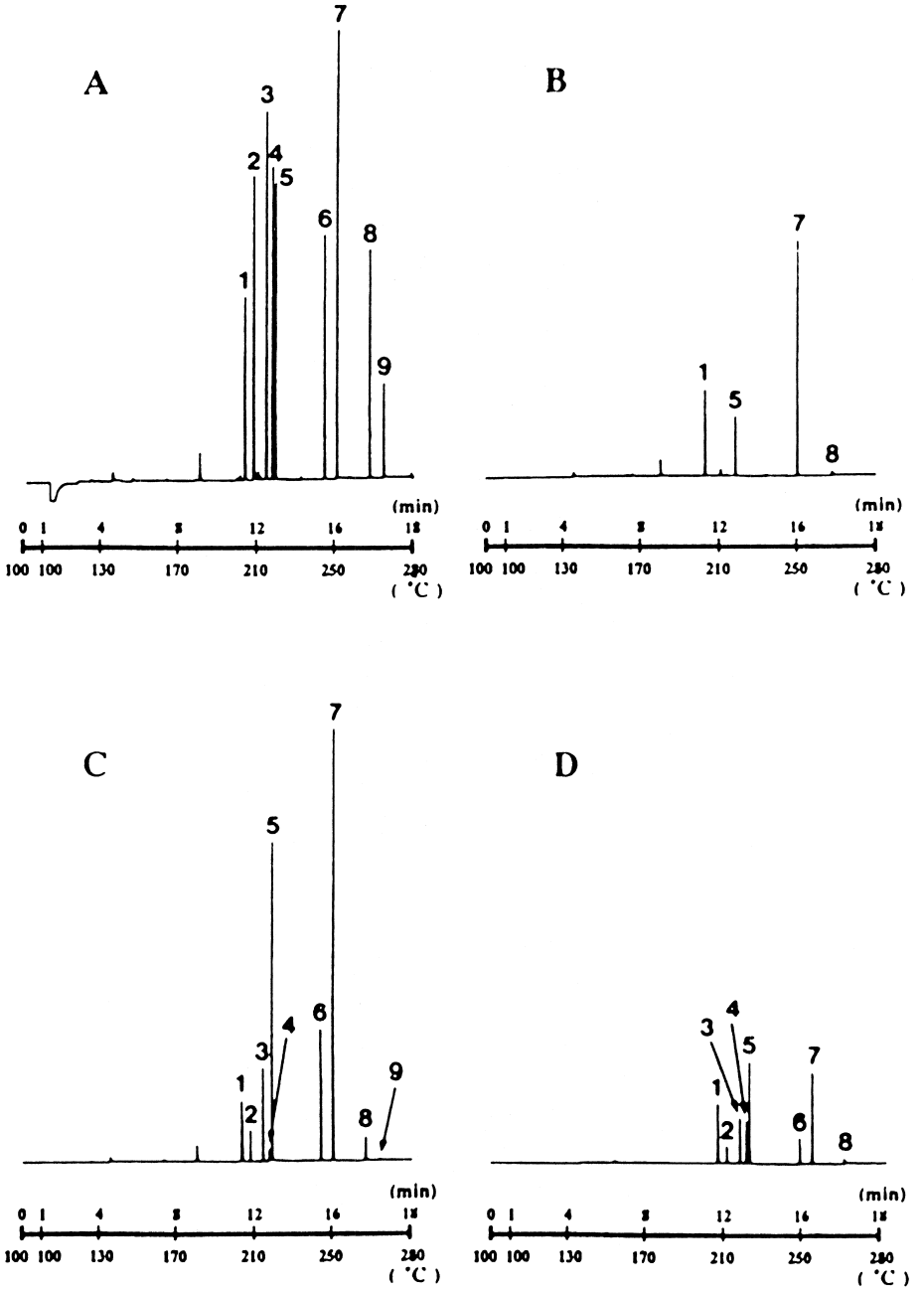

Fig. 5. GC–NPD of nine organophosphate pesticides extracted from human whole blood (0.5 ml) by use of HS-SPME. (A) Pesticides (7.5 ng on column) without extraction. (B) Extraction in the presence of 0.5 ml distilled water. (C) Extraction in the presence of 0.5 ml distilled water–100 μl 6 *M* HCl. (D) Extraction in the presence of 0.5 ml distilled water–100 μl 6 *M* HCl–0.4 g $(NH_4)_2SO_4$–0.4 g NaCl. Peak identities: (1) IBP, (2) methyl parathion, (3) fenitrothion, (4) malathion, (5) fenthion, (6) isoxathion, (7) ethion, (8) EPN, (9) phosalone. Blood (0.5 ml) was spiked with a mixture of pesticides (200 ng each) (from Ref. [47]).

Table 2
Comparison of LOD (ng/ml) of chlorophenols obtained by GC–MS after SPME of urine and the EPA method (LLE) for water analysis (from Ref. [51])

Analyte	SPME		EPA	
	EI[a]	NCI[a]	FID	MS (full scan)
2-Chlorophenol	41	98	310	3300
2,4-Dichlorophenol	6	2	390	2700
2,4,6-Trichlorophenol	9	0,03	640	2700
2,3, 4,6-Tetrachlorophenol	7	6		
Pentachlorophenol	9	8	7400	3600

[a] EI, electron impact, NCI, negative chemical ionisation.

PA fiber was immersed into a 5-ml sample for 45 min. Following extraction the fiber was placed for 45 min in the headspace of 10 μl of a BSTFA solution. The method was tested for its applicability to metabolite profile analysis using a smoker's urine. The authors reported satisfactory performance in spite of the not yet optimised method [53].

Bioanalysis of mercury species is of great importance to monitor accumulation via the food chain in biological organisms. It is mostly conducted after derivatisation of organomercury species with borate agents. Methylmercury was determined by AAS in biological samples (mink hair and skin) following hydride derivatisation with KBH_4 and HS-SPME [54]. The authors did not use a polymeric-coated fiber, since they found unsatisfactory sensitivity. Instead they modified a silica fiber by immersion for 3.5 h in concentrated hydrofluoric acid and consequent heating at 200°C for 3 h. Determination of urinary Hg and methyl Hg was conducted by SPME-GC–MS following in situ ethylation with sodium tetraethyl borate [55]. SPME-GC–MS–MS was also used for the determination of Hg(II) and alkyl Hg, Pb and Sn species in human urine after derivatisation with sodium tetraethylborate. According to the authors the proposed method offers discrete advantages when compared to ICP-MS: (a) the species could be directly identified via their precursor and daughter ions; (b) analysis could be performed with a commercially available hyphenated technique at moderate costs without an additional interface; (c) the capability of a real multi-element/multi-species determination with low detection limits and a minimum of sample preparation [56].

3.2. Drugs of abuse

Analysis of drugs of abuse (DOA) represents one of the major tasks in analytical toxicology laboratories. It is no surprise that this was the bioanalytical field where SPME was first applied and used extensively for the extraction of many types of drugs of abuse from biological fluids. SPME is often used for the determination of some types of DOA (amphetamines, benzodiazepines and barbiturates). Hence the applicability of SPME on these groups is described in separate sections. It should be noted that many of these compounds can also be used as normal drugs (Section 3.5).

3.2.1. Amphetamines

In the last decade, abuse of amphetamines and derivatives increased dramatically as a result of new tendencies among the youth, such as pep pills (XTC) and its anorectic properties. Thus analysis of amphetamines becomes of increased interest in toxicology, occupational medicine and law enforcement. Amphetamines in their basic form are semi-volatile compounds, and thus from the 10 papers that have been reported so far on the SPME of amphetamines, six utilise headspace sampling [57–62]. Compared to conventional headspace sampling, HS-SPME enhances the sensitivity up to 20 times for the analysis of urine samples of amphetamine abusers [62]. Lord and Pawliszyn [60] in an exhaustive optimisation study described the influence of extraction temperature, agitation, sample volume, fiber coating type, calibration method, base buffer and salt additives in urine samples. As a compromise between the decreasing K_{fs} value (lower recovery) and the reduction of the sampling time between sample and headspace (shorter extraction times), they used 60°C as the extraction temperature.

In a recent report HS-SPME was used for the extraction of amphetamines from human hair [59]. Human hair analysis is gaining interest in the analysis of drugs of abuse, since it offers attractive features: easy and 'unlimited' sampling and, as the most important aspect, the possibility to measure the drug after months of use. Drugs are incorporated into hair and remain there for several months. Thus, long-term abuse and also the history of the abuse can be ascertained. Hair was alkalinised with NaOH and

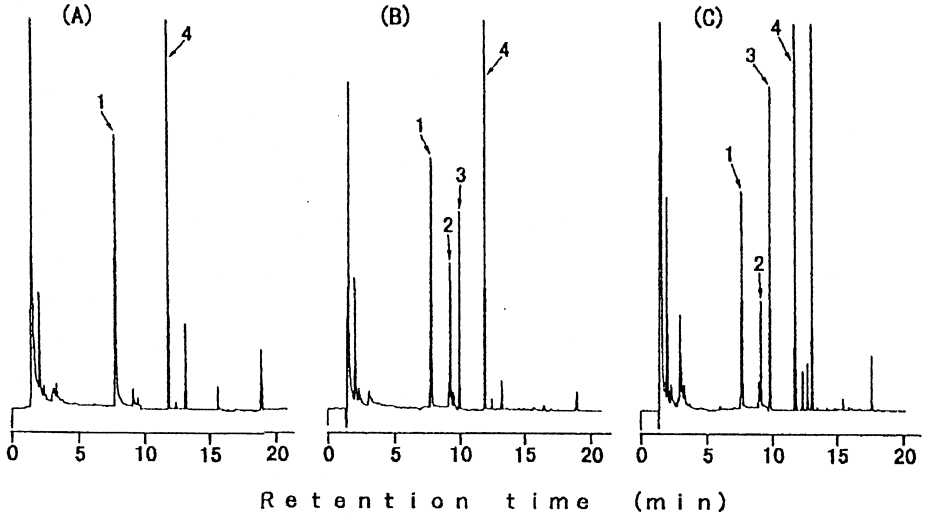

Fig. 6. Analysis of amphetamines by GC–NPD following HS-SPME extraction from human hair. (A) Normal hair. (B) Normal hair after addition of amphetamine (1.5 ng) and methamphetamine (16.1 ng). (C) Hair of an amphetamine abuser. Peak identities: (1) α-phenethylamine (internal standard), (2) amphetamine, (3) methamphetamine, (4) *N*-propyl-β-phenethylamine (from Ref. [59]).

heated to 55°C. Adsorption from the headspace lasted 20 min and analysis was performed by GC–NPD. Fig. 6 depicts the potential of the method for the identification of amphetamine abuse.

Although amphetamines are mostly GC analysed as free bases, Ugland et al. [63] reported an alkylchloroformate derivatisation scheme converting the amphetamines to their carbamate derivatives. They claimed higher recoveries (up to 100%) compared to underivatised extraction. However, it should be noted that SPME is an equilibrium process, which means that a yield of 100% cannot be obtained. Some authors prefer to compare the extraction yield obtained from a sample to that of a standard solution which can result in a recovery of 100% or even higher depending on the composition of the sample. The paper [63] also described the automation capability of direct-immersion extraction, while HS-SPME was not compatible with the autosampler. Very recently the same group reported on the automated determination of 'Ecstasy' and the so-called 'designer drugs' (amphetamine derivatives) in urine utilising a similar experimental protocol [64].

Myung et al. [65] optimised the direct-immersion extraction of three amphetamines and four other stimulants from human urine by studying the effect of ionic strength and pH value of the sample. PDMS fibers showed higher affinity for the stimulants compared to PA fibers. Constant ionic strength was crucial in order to achieve reproducible recoveries. Addition of NaCl and KOH to reach a pH of 10 increased extraction recovery by a factor of 2.4–61.8, depending on conditions and analyte. Ameno et al. [66] developed an even harsher experimental method for the determination of amphetamine and methamphetamine in urine. The samples were adjusted to pH 12 with the addition of 10 N NaOH. A PDMS fiber was immersed in the samples for 20 min and subsequently washed with NaOH–H_3BO_4 buffer (pH 12) before introduction into GC.

3.2.2. Benzodiazepines

The first report came from Suzuki's group and utilised DI-SPME-GC–FID for the analysis of 13 benzodiazepines in urine [67]. Very recently the same group reported a modification of the method employing hydrolysis of benzodiazepines to form benzophenones prior to extraction [68]. Krogh et al. used another approach in order to improve extraction recovery [69]. They proposed a solvent-modified extraction scheme that employs the modification of a PA fiber by sorption of 1-octanol before its direct immersion in blood plasma samples. The amount of diazepam extracted this way was twice as high

compared to the amount extracted without the use of 1-octanol. The method was further optimised in a recent publication [70]. Parameters, which were found to affect analyte recovery, were studied in a factorial design and response surface methodology. Luo et al. [71] optimised the extraction of five benzodiazepines from aqueous solutions and biological fluids. The authors state that the extraction of oxazepam and lorazepam from unmodified urine and serum samples results in much lower extraction yields than those obtained from aqueous solutions, which shows that the biological matrix interferes with the sorption process.

Jinno's group demonstrated the potential of coupling SPME with capillary liquid separation techniques for the determination of benzodiazepines and barbiturates (see also Section 3.2.3). SPME was coupled to semi-micro-LC [24,72], micro-LC [73] and micellar electrokinetic chromatography (MEKC) [74,75]. Micro-LC offered low organic solvent consumption. Coupling to MEKC provided an attractive alternative for the simultaneous analysis of benzodiazepines and barbiturates and proved an appropriate method for trace analysis. The methods based on SPME could be used in order to analyse benzodiazepines without the tedious and complex pretreatment protocols often reported. Relatively long equilibrium times were observed for some of the analytes, as already shown in Fig. 3. Desorption in the mobile phase took place in a house built interface and lasted 30 min. Urine samples were saturated with salt to improve the extraction yield and to standardise low random salt concentrations in human biological fluids. Three SPME fibers (PA, CW–TPR and sol–gel PDMS with a C_{11} functional group) were evaluated for urine extraction. Sol–gel coatings enhance surface area and thermal stability compared to typical PDMS coatings. They also contain free hydroxyl groups, so they are suitable for the extraction of more polar compounds. This coating gave the highest recovery for the three benzodiazepines, however the CW–TPR coating was chosen for faster extraction, since it required half the time to reach equilibrium.

3.2.3. Barbiturates

For the extraction of barbiturates a polar CW–DVB coating gave the best results [76]. Salt addition improved extraction efficiency for all the eight analysed barbiturates, but a salt content above 50% of the saturated solution gave a negative effect for the extraction of phenobarbital. However, because the authors failed to adjust the pH of the samples, the negative effect could also be due to a change in pH of the sample. Analysis by gas chromatography–ion-trap MS gave detection limits of about 1 ng/ml. In order to minimise carry-over effects, following analyte desorption the fiber was cooled, treated with methanol–water (2:8) for 3 min and subsequently placed back to the injector for 4 min.

SPME has also been coupled to CE for the determination of barbiturates [74,77]. Li and Weber reported an off-line SPME-CE coupling [77], utilising plasticised PVC-coating around stainless steel rods (O.D. 1.1 mm) as the extraction coating (3 cm length). Fifty μl of the barbiturate solution to be extracted were injected in a Teflon tube (I.D. 1.5 mm). The extraction needle was inserted in the Teflon tube and was left horizontally for 4 min. Next the needle was inserted in another Teflon tube (I.D. 1.2 mm) containing 5 μl of the back extraction solution. The rod was removed and the back extraction solution was transferred to an injection vial. The back extraction process was repeated until there was no analyte evident in the extract, usually this required 9 min for the whole procedure. The method is selective, since alkaline and neutral compounds are not to be extracted and back extracted, respectively. With this simple device the authors solved the technical problem of handling the very small volumes employed in CE injection (nl) and back extraction (μl), but still only a small aliquot can be injected. Porous phosphate triester gave the best performance as a plasticiser for the PVC coating. Fig. 7 depicts the CE analysis of blank and spiked urine. The figure also demonstrates the effect of extraction time on extraction recovery from real samples.

3.2.4. Other drugs of abuse

The use of SPME for the extraction of methadone from urine was one of the first applications of SPME in bio-analysis [78]. Urine was adjusted to pH 7.7 and a PDMS fiber was dipped in the sample for 15 min. GC–MS rounded the total analytical procedure time to about 20 min. Analysis of contraband drug

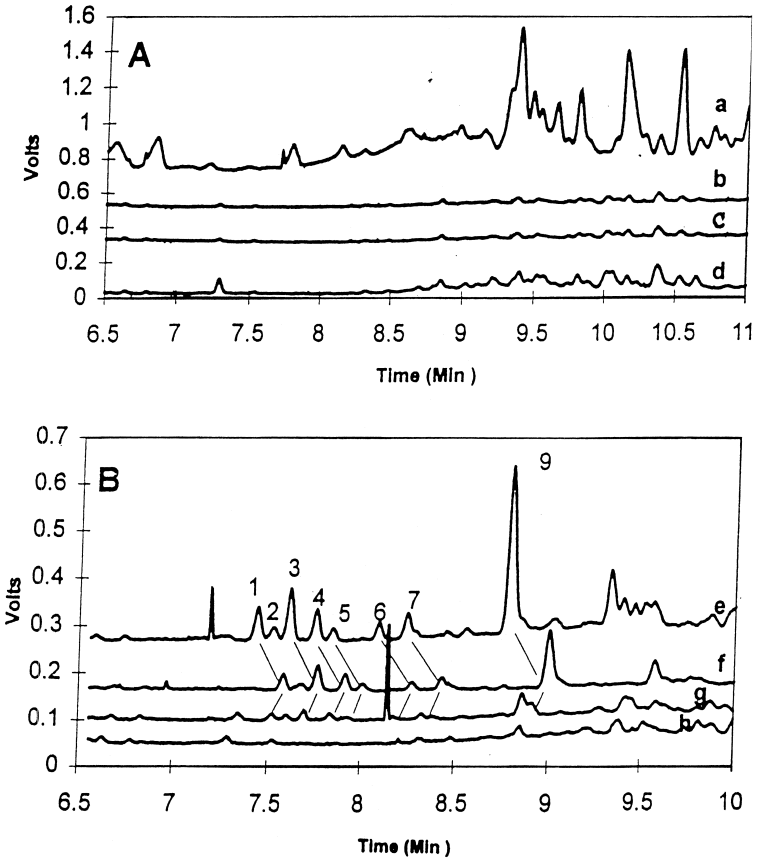

Fig. 7. CE analysis of blank urine and urine spiked with barbiturates after DI-SPME with a home-made PVC-coated fiber. (A) Blank urine sample directly injected (a) and extracted for 5 (b), 10 (c) and 30 min (d). (B) Barbiturate-spiked sample extracted for 30 (e) and 5 min (f,g). Blank urine extracted for 5 min (h). Peak identities and concentrations (in e and f): (1) pentobarbital, 0.6 ppm; (2) butabarbital, 0.55 ppm; (3) secobarbital, 0.76 ppm; (4) amobarbital, 0.53 ppm; aprobarbital, 0.64 ppm; (6) mephobarbital, 0.15 ppm; (7) butalbital, 0.73 ppm; (9) thiopental, 1 ppm. Concentration in (g) is 0.3 times that of (e) and (f) (from Ref. [77]).

vapours was accomplished by headspace SPME-GC–MS and SPME-ion mobility spectrometry [27]. The method enabled the detection of cocaine and heroine vapours and their decomposition products in vapour state. Thus it can prove a valuable addition to the existing methods of analysis (GC, LC), since it is handy and suitable for on-site sampling in confined spaces (e.g., cargo containers). SPME has recently been applied to the determination of cannabinoids in water and human saliva [79] and human hair [80]. Cannabis is by far the most widespread used psychotropic drug; thus cannabinoid analysis is a usual task in analytical toxicology laboratories. Many techniques have been reported for the analysis of cannabinoids with immunoassays and GC–MS offering the best choices. SPME was compared to LLE of saliva from samples of marihuana smokers. Saliva offers an attractive biological sample for many reasons such as low protein and salt content, easy sample collection, etc. The sample was acidified and extracted with five commercial fibers. All the fibers extracted the cannabinoids efficiently, but the CW–DVB showed carry-over effects, which were attributed to the poor desorption of the lipophilic cannabinoids. The three PDMS fibers were chosen to be further used, since they could withstand elevated desorption temperatures (270°C). Addition of acetic acid improved recovery up to 7-fold, but Δ^9-THC, the main cannabinoid of interest, was the only alkaloid detected at a significant level. Fig. 8 depicts

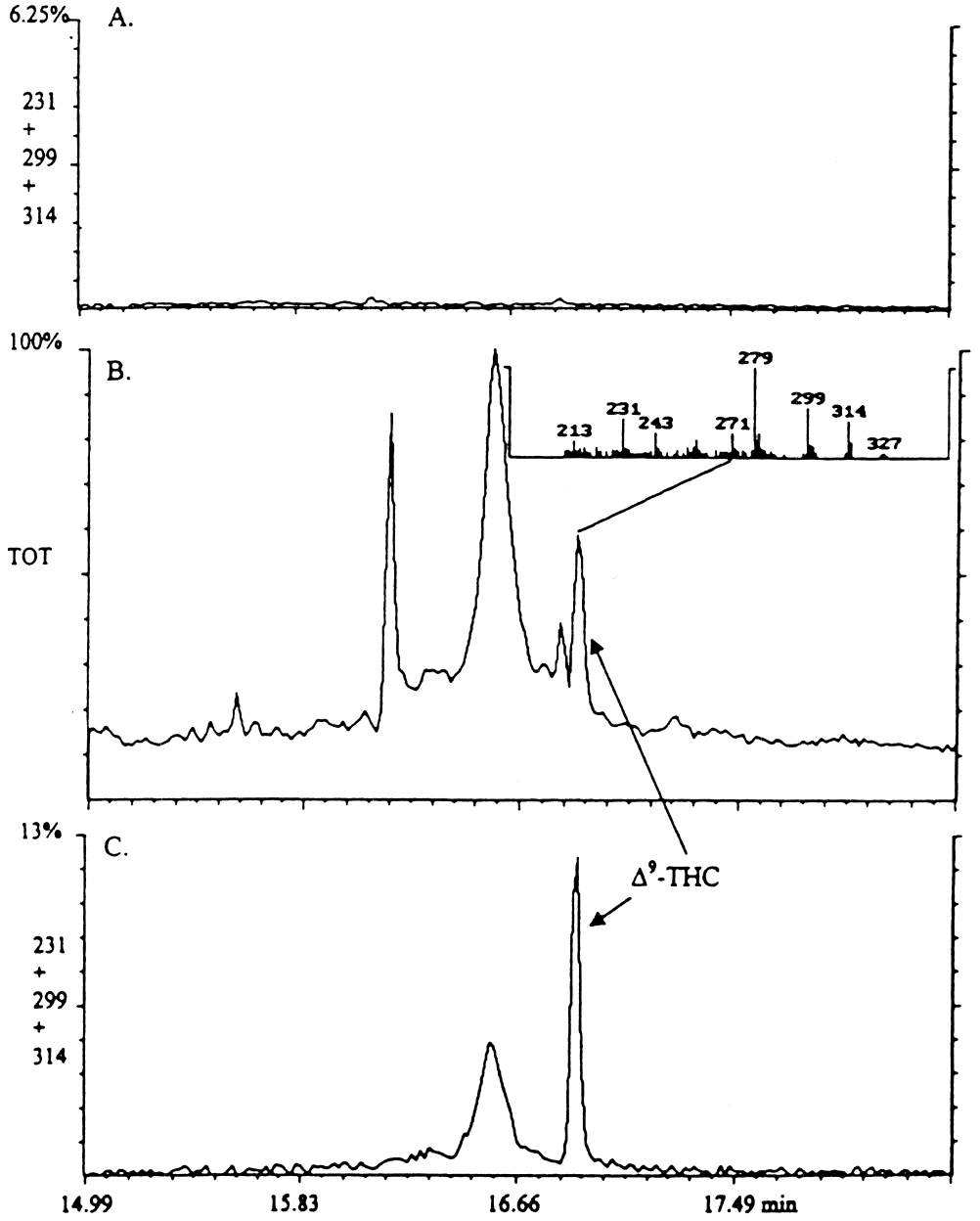

Fig. 8. SPME-GC–MS analysis of saliva, prior to (A) and after cannabis smoking (B). The peak at 16.9 min is corresponding to Δ^9-tetrahydrocannabinol (Δ^9-THC). (C) A blow up of Δ^9-THC in (B). (A) Relative abundance of selected ion monitoring (231, 299, 314 m/z) for the quantitation of (Δ^9-THC). (B) Full scan 120–350 m/z. (C) Selected ion monitoring (231, 299, 314 m/z) for the quantitation of (Δ^9-THC) (from Ref. [79]).

the results for the analysis of saliva prior and after marihuana smoking.

Alkyl nitrites have become popular as inhalant DOA due to their aphrodisiac effect. In aqueous environment they hydrolyse rapidly to alcohol and nitrite ion. Tytgat and Deanens [81] described the

headspace extraction of *n*-butyl nitrite from blood. PA fibers proved to be more efficient for extraction of polar nitrites than PDMS fibers, but required longer equilibrium time. Conditioning of the fibers for 30 min at 150°C improved extraction recovery. Higher conditioning temperatures (up to 240°C) did not result to any significant differences in performance.

Suzuki's group has extensively used SPME employing a more or less universal experimental protocol for the analysis of biological fluids and applied it to various compounds such as benzodiazepines [67], local anaesthetics [82,83], cocaine [84], cresol isomers and phenol [85], meperidine [86], phenothiazines [87], 1-phenylethylamine [88], phenylcyclidine [89], tricyclic antidepressants [90], diphenylmethane antihistaminics [91] and thinner components [92], of which some already have been described in the previous sections. The SPME fiber was pre-treated by heating at 250°C for 1 h in order to remove contaminants. The authors reported that severely contaminated fibers could be cleaned by thermal desorption at 280°C for 1–2 h.

3.3. Forensic analysis

Inflammable substances (toluene, xylenes and hydrocarbons) have been determined in the blood of a fire victim with HS-SPME-GC–MS [93]. Recently HS-SPME-MS has been extensively used in the monitoring of biological fluids from humans exposed to airborne BTEX [94–97]. The interferences of the matrix in the analysis of benzene in urine were studied by Perbellini et al. [98]. Urinary benzene concentrations reported by different investigators vary considerably even when environmental levels are comparable. The authors attributed these variations to varying sampling and analytical methodologies. They also assumed that part of the benzene in urine is sorbed onto sediment, bound to specific proteins and is released with pH modification or by heating. Early reports utilised SPME in the determination of chlorinated hydrocarbons [20] and thinner components [92] (toluene, benzene, *n*-butanol, *n*-butyl acetate and *n*-isoamyl acetate) in human blood and urine.

Chemical warfare agents (nerve agents) were detected at ppb and sub ppb level with SPME-GC–MS and SPME-GC–NPD [99]. Four SPME fibers were evaluated for the extraction of sarin, soman, tabun, *O*-ethyl-*S*-2(diisopropylamino) ethyl-methylphosphonothiolate in natural water samples. A CW–DVB fiber showed low uptake of the nerve agents. Moreover peak shape was poor, a fact attributed to either the absorption of water and the inevitable injection of water in the GC, or either to the difficulty in desorbing the polar substances from the fiber. For PDMS and PA fibers extraction yield was greatly increased by salt addition. With the PDMS fiber soman had a much higher uptake (70 ng) compared to the other nerve analytes (1–4 ng) due to its hydrophobic character. The PDMS–DVB fiber gave the highest uptake of the substances and the least differences of yield between soman and the other substances, so it was easier to monitor all the compounds together. Compared to LLE with dichloromethane, SPME recoveries were higher in most of the cases, i.e., with SPME higher concentrations of analyte were found in the same samples compared with LLE. In a later paper the same group utilised in situ derivatisation and optimised extraction efficiency by studying several parameters: fiber selection, pH, salt content, derivatisation temperature, extraction and derivatisation order [100].

3.4. Clinical chemistry

SPME has proven a useful tool in clinical chemistry. Compared to existing techniques it shows benefits and offers a good alternative to conventional methods [17,101,102].

Drug metabolism in human keratinocyte cells was studied by HS-SPME-GC–FID [103]. The stable nitroxyl radical 2,2,6,6-tetramethylpiperidin-1-oxyl and its apolar metabolite 2,2,6,6-tetramethylpiperidine were best extracted on a 7-μm PDMS fiber. SPME was compared to SPE and LLE and showed superior results with regard to recovery and precision.

Benzophenone is a common ingredient in sunscreens and other products [104]. The compound may be absorbed by the body, so there is a need for monitoring its accumulation, metabolism and excretion. SPE and SPME of benzophenone and metabolites from water and human urine was evaluated and

optimised concerning salt addition, sorption and desorption time, solvent and carry-over effects. Determination was performed with GC–ion-trap MS.

An attractive proposal is the construction of a SPME-electrodeposition device for the determination of putrescine and cadaverine [34]. The three-electrode system consisted of a Ag/AgCl reference electrode, a stainless steel mesh counter electrode, which surrounded a pencil lead; the latter served as both the SPME device and the working electrode. The pencil lead was immersed in a pH 8 borate buffer, and −1.70 V potential versus the reference electrode was applied, resulting in an electrochemical reduction of buffer solution protons. Subsequently, diamines present in the solution are converted into their free-base form and retained on the electrode which is used as the SPME fiber. The device was then transferred to a capillary GC equipped with a thermionic detector.

Determination of breath compounds attracts an increasing interest in clinical and toxicological analysis. More than 100 VOCs have been identified in normal human breath by GC–MS. The main methods currently utilised for preconcentration of these compounds are chemical interaction, adsorptive binding and cold trapping, and are tedious procedures, that require complex devices and suffer from particular problems (e.g., excess of water from the breath). SPME offers an alternative that can overcome such limitations [105]. The fiber was directly exposed in the mouth of the subject. An inert tubing was added to the device, in order to protect the fiber from the subject's tongue. Four fibers were evaluated by analysing a standard sample of ethanol, acetone and isoprene with a relative humidity of 99% four times with each fiber. The method demonstrated numerous advantages compared to the existing extraction techniques, requiring only 1–3 min for sampling. The technique proved to be sensitive enough with detection limits in the low nmol/l range.

HS-SPME of monocyclic aromatic amines was first optimised in aqueous samples and then applied to biological fluids [106,107]. Treatment of whole milk and blood with alkaline solutions salt and heat resulted in saponification of the fats, thus requiring an extra centrifugation step. In general the amount of anilines extracted form the samples was smaller than the amount extracted from water. The complexity of the matrix affected both the amount extracted and the LOD (Table 3). Urine which is the least complex matrix gave values close to those in water. Excellent reproducibility and low detection limits were obtained, providing a fast and sensitive method for biomonitoring hazardous amines and possible metabolites in urine, blood and breast milk. Mills et al. determined trimethylamine in urine by quantitative stable isotope dilution GC–MS following HS-SPME on CX–PDMS. The method was reported useful in screening for trimethylaminouria (fish odour syndrome) [108].

Table 3
LOD (ng/ml) of monocyclic aromatic amines in various matrixes after extraction by SPME (from Ref. [106])[a]

Analyte	Water	Urine	Milk	Blood
Aniline	3.17	3.39	5.33	7.71
o-Toluidine	1.55	1.88	3.47	6.25
2-Chloroaniline	0.88	1.05	2.01	4.72
2,6-Dimethylaniline	0.70	0.81	1.67	4.09
2,4,6-Trimethylaniline	0.18	0.40	6.60	4.58

[a] Water and urine were spiked with 20 ppb aniline, 10 ppb *o*-toluidine, 5 ppb 2-chloroaniline, 5 ppb 2,6-dimethylaniline and 2 ppb 2,4,6-trimethylaniline. Milk and blood were spiked with 20 ppb of all analytes.

The analysis of urinary organic acids can be of great importance to diagnose certain diseases. Derivatisation is absolutely necessary due to the wide range in structure and polarity of the organic acids. Existing preparation techniques require laborious processes of extraction and isolation with organic solvents. Liebich et al. proposed a much simpler alternative, utilising derivatisation with trimethyloxonium tetrafluoroborate (TMO) and subsequent DI-SPME on a PA fiber. The esterification of the acids with TMO occurred in aqueous environment, thus enabling the direct derivatisation in urine in only 15 min. Fig. 9 demonstrates the GC–MS analysis of the urinary acids methyl esters. Up to 29 acids could be identified with no severe interference problems [109].

Determination of carnitine, an essential factor in the fatty acid metabolism of organisms, is of significant clinical interest. The analysis is rather problematic due to carnitines betaine structure, which is a hindrance for the detection in biological samples.

Recently the determination of acylcarnitines in urine was described with the use of SPME-LC–ESI-MS [110]. CW-coated fibers were selected for their higher recovery, although they required long equilibrium time (more than 15 h). The hydrophobic properties of the analyte caused low affinity towards the SPME fiber and long extraction time. Furthermore poor mass spectra were observed. However the method was applied successfully to the analysis of urine from patients with cardiac disorders.

Homocysteine, cysteine and methionine were determined by GC–MS after alkylformate derivatisation and SPME on an 85-μm PA fiber [111]. The most frequently used method for the assay of these compounds has been high-performance liquid chromatography with fluorescence detection after fluorescent tagging. The authors studied the aqueous derivatisation with several *N*(*O*,*S*)-alkoxycarbonyl alkyl esters by using both SPME and LLE, as some of the reagents caused a degradation of the fiber coating. SPME has also been used for the determination of four methyloxanthines in human whole blood and urine after ingestion of cocoa and coffee [112].

3.5. Pharmaceuticals

Modern analytical and extraction techniques often have strong impact in applied analytical fields like the determination of pharmaceuticals in biological samples. SPME's automation capabilities enhance the development of fully controlled protocols which are necessary in pharmaceutical industry. Hence the interest in SPME has been immense, although modern bioanalysis of pharmaceuticals is mainly focused to liquid chromatographic techniques.

For the extraction of 10 antidepressants, 2 ml of human plasma were alkalinised with NaOH and a PDMS fiber was immersed in the sample for 10 min [113]. Extraction recovery from plasma was 50 times lower compared to the extraction recovery from water, a fact indicating strong protein binding. Protein precipitation with perchloric or uranyl acetate did not increase the recovery, but addition of water to the sample proved an easy way to circumvent this problem and increase sensitivity as the protein concentration was lowered by dilution. GC–MS with EI showed better sensitivity for the tertiary amines than for the secondary amines. The authors provided a detailed and useful discussion on the influence of plasma proteins in analysis. Salt addition did not affect recovery for the extraction of tricyclic antidepressants [90]. In contrast, the yield increased dramatically after blood alkalinisation with NaOH. The same was observed for the HS extraction of 13 diphenylmethane antihistaminics from whole blood and urine [91]. Protein precipitation did not improve extraction from blood and the low recovery was attributed to protein or membrane lipid binding of the drug.

Valproic acid (an antiepileptic agent) was also reported to be highly (over 90%) bound to plasma proteins. Krogh et al. [114] used automated equilibrium dialysis on an automated sequential trace enrichment of dialysate (ASTED) system in order to ensure the determination of the non-bound drug and to remove proteins and other contaminants prior to the introduction of the SPME fiber. The system utilised a modified flat-bed dialyser and a Cuprophane membrane with a molecular mass cut-off of 15 000 Da. A PDMS fiber was inserted in the collected dialysate for 3 min and subsequently inserted in the GC–FID system for analysis. Heating at 250°C in a second GC at the beginning of each day was an efficient means to clean the fiber. A special column (Nucol) was employed in GC separation, thus enabling the direct analysis of acidic analytes.

Okeyo et al. [115] reported the extraction of seven steroids by immersion of a PDMS fiber in human serum. Silylation of the steroids occurred in situ with the exposition of the fiber in the headspace of a BSTFA solution and incubation at 60°C for 1 h. Special attention was paid to avoid the disastrous contact of the fiber with the BSTFA solution. Analysis of the silylated compounds was performed by GC–MS. In a follow-up the same group analysed estrogens and anabolic steroids in human urine [116]. It was shown that parameters like extraction time, incubation temperature, pH and ionic strength greatly affect both extraction and derivatisation process. Each analyte has a separate optimum; therefore, for the analysis of mixtures, a compromise seems necessary. However the method reached low LODs.

Five anorectic agents were determined in human

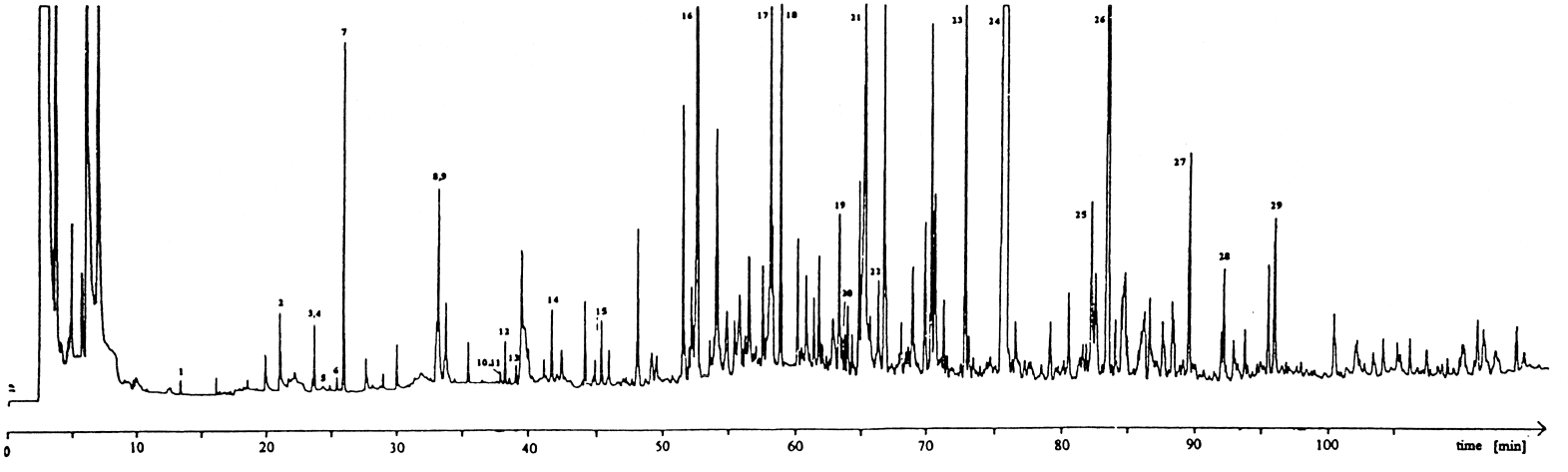

Fig. 9. GC–MS analysis of urinary acids methyl esters after derivatisation with trimethyloxonium tetrafluoroborate and SPME with PA coated fiber. Peak identities: (1) malonic acid; (2) phosphoric acid; (3) succinic acid; (4) ethylmalonic acid; (5) maleinic acid; (6) methylsuccinic acid; (7) benzoic acid; (8) phenylacetic acid; (9) 3-methylglutaric acid; (10) 3-methylglutaconic acid; (11) methoxysuccinic acid; (12) 3-hydroxy-3-methylglutaric acid; (13) adipic acid; (14) 3-methyladipic acid; (15) 3-4-methyleneadipic acid; (16) methoxyphenylacetic acid; (17) citric acid; (18) azelaic acid; (19) fyroylglycine; (20) hydroxymandelic acid; (21) 4-hydroxyphenylacetic acid; (22) homovanillic acid; (23) 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; (24) hippuric acid; (25) 3-carboxy-4-methyl-5-phenyl-2-furanpropionic acid; (26) 3-indoleacetic acid; (27) methoxyhippuric acid; (28) isomer to (27); (29) methoxyindoloacetic (from Ref. [109]).

urine by SPME-GC–MS [117]. Compounds analysed were fenfluramine (Isomeride®), phendimetrazine (Plegine®), norfenfluramine, phenmetrazine and proadifen. From the tested fibers the 30-μm PDMS fiber gave the best results. However, some differences in extraction performance were observed for different fibers from the same batch.

A combination of SPME with fast short-column LC–MS was published very recently for the determination of corticosteroids in urine [118]. Several SPME parameters were investigated, including fiber polarity, extraction time and ionic strength. The influence of salt concentration was demonstrated: the yield of ionised compounds increased up to 23 times by addition of salt in the sample. The method could analyse 11 corticosteroids and two steroid conjugates. Compared to conventional pretreatment methods, SPME offered similar performance but was much easier to use and faster to perform. The same authors, using SPME and LC–ESI-MS–MS, studied the decomposition of erythromycin-A in aqueous solutions [119]. Erythromycin-A, a macrolide antibiotic extensively used against bacterial infections, has been shown to undergo dehydration in vivo under acidic conditions when administered orally. Degradation experiments were conducted at varying pH at room temperature. LC–MS identified anhydroerythromycin as the major degradation product. Using SPME on PDMS–DVB and LC–MS–MS on a triple quadrupole mass spectrometer, 14 degradation products were identified. However the use of a soft ionisation technique (ESI) and low energy level collision-induced dissociation, thus hindering identification.

For the determination of anesthetics in human biological fluids, SPME in both DI and HS mode combined with GC and LC has been employed. Suzuki and co-workers reported low yields for the HS extraction of 10 local anesthetics from human whole blood [82]. In a follow-up [83] using DI extraction, they achieved a 2–6-fold increase in recovery for six of the 10 drugs. Two of the 10 drugs were extracted with the same efficiency in both methods, while another two drugs were best extracted from headspace. Lidocaine a local anesthetic agent was analysed in human urine by SMPE-GC and SPME-LC [120]. The paper describes the optimisation of the DI extraction procedure and pays special attention to the desorption in the LC interface. Fig. 10 depicts a chromatogram of the SPME-LC analysis of lidocaine in urine.

SPME has also been used for the determination of residual solvents in pharmaceutical preparations [121–123]. Compared with static headspace analysis, HS-SPME gave lower LODs for the volatile compounds. Three fibers with different polymer films

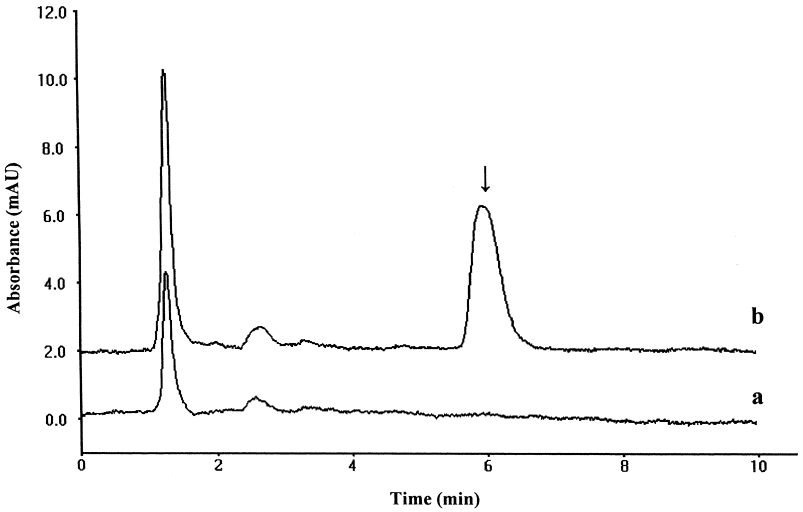

Fig. 10. SPME-LC analysis of five-times diluted blank urine (a) and 5-times diluted urine spiked with 0.5 μg/ml lidocaine (b). Peak indicated by arrow is lidocaine (from Ref. [120]).

were compared and the PDMS–DVB-coated fiber was found to give the highest yield for the analysed analytes. Besides the normal HS-SPME mode, the authors [121] describe also a so-called gas-tight SPME mode, which utilises a gas-tight syringe (normally used for static headspace sampling) in which a SPME fiber is mounted. By pulling up the plunger not only the SPME fiber is withdrawn into the syringe needle, but also a certain volume of headspace is withdrawn into the gas-tight syringe. With this new approach lower LODs could be obtained compared with 'normal' HS-SPME, although the relative standard deviation for the latter one is superior.

3.6. Biochemical analysis

In exhaustive extractions with a solvent or on a solid-phase the equilibrium between matrix compounds and drug is disturbed, leading to a shift towards the freely dissolved fraction, which means that not only the free dissolved amount is determined. The amount of drug available for SPME is only the freely dissolved fraction of the compound. Therefore extraction of a small amount does not necessarily perturb its equilibrium with the matrix. Vaes et al. [124] used SPME in order to measure the protein binding of four polar drugs (aniline, nitrobenzene, 4-chlor-3-methylphenol, 4-*n*-pentylphenol). Drug binding to bovine serum albumin (BSA), usually measured by equilibrium dialysis, was determined by SPME. Protein binding determined with SPME (PA-coated fibers) gave comparable results to equilibrium dialysis. Calibration curves of free drug were measured with SPME. It was shown that an increasing hydrophobicity is related to an increase in affinity for BSA. In a follow-up the same group studied the membrane–water partition coefficients and free concentration in in vitro systems [125]. Compared to the typical *n*-water–octanol partitioning, the phospholipid–water partition coefficient can prove a more suitable parameter in modelling the kinetic behaviour of organic chemicals. The authors determined phospholipid–water partition coefficients for 19 organic compounds using a PA fiber. SPME fibers were cut to a length of 1 mm to accomplish negligible depletion of the extracted compounds. Free concentration was determined for four of the chosen compounds in postmitochondrial (9000 *g*) and microsomal (10 000 *g*) centrifugation fractions of trout liver homogenates and rat hepatocytes. The same group used SPME also to investigate the quantitative structure–activity relationships for the toxicity of narcotic pollutants against water flea, guppy and pond snail [126]. In order to display narcosis, models were developed to describe the partition process, taking into account the composition of biomembranes. The results were in agreement with the membrane–water coefficients, and this supported the hypothesis that toxicity is directly related to accumulation in biological membranes.

A method employing microextraction-CE–MS–MS for rapid protein identification was reported by Figeys et al. [10]. The extraction-CE device was developed in-house by gluing two CE capillaries in a Teflon sleeve containing a small amount of C_{18} material. Identification of proteins was enabled by correlation of tandem mass spectra with protein sequence database. LODs were in the low nanogram level for yeast proteins separated by high-resolution two-dimensional CE. The authors use the term SPME in the title of the paper, but the term SPE in text. The fact is that some authors use the term SPME in experiments describing actually miniatur-

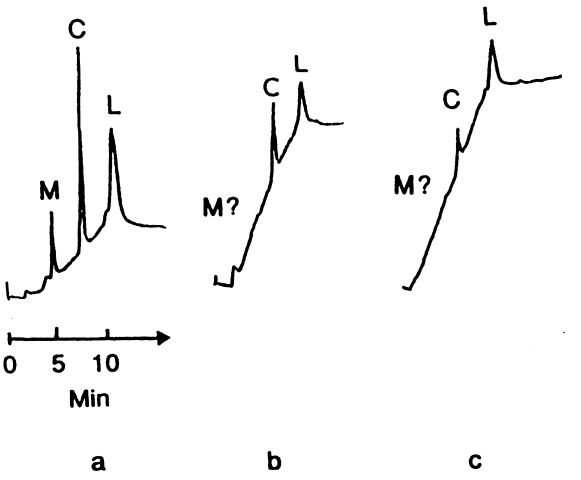

Fig. 11. Cation-exchange microchromatography of a mixture of model proteins. Samples: (a) the original sample consisting of myoglobin (M), cytochrome c (C) and lysozyme (L); (b,c) proteins adsorbed onto and then released from a home-made polyacrylic acid-coated fiber with extraction times of 5 and 240 s, respectively (from Ref. [127]).

ised SPE (MSPE). Therefore dimensions are not a safe borderline between the two extraction techniques, the nature of the extraction process could be used instead. In typical SPE, trapping of the analytes on the solid-phase occurs, while in SPME, partition of the analytes between the sample and solid-phase is the major mechanism. In this context, the paper describes miniaturised SPE and not SPME.

For the extraction of proteins, SPME was coupled to micro-LC using columns based on a new continuous polymer bed technology. Very short extraction time (a few seconds) was used to ensure that the capacity of the home-made polyacrylic acid-coated fiber was sufficient. Because of the low protein binding capacity, the amount of basic proteins adsorbed onto the fiber was found to be proportional to the concentration of the protein [127]. Proportionality was also obtained for longer extraction times, provided that the protein content does not exceed the binding capacity; otherwise the extraction of strongly absorbed proteins was favoured. Fig. 11 shows chromatograms of the analysis proteins obtained with the micro-LC system with and without SPME. Because myoglobulin was almost completely in its neutral form at the used extraction conditions, it was not or only slightly adsorbed on the cation exchanger-coated fiber. Besides the selectivity, Fig. 11 also shows that cytochrome c is displaced by lysozyme during extraction, i.e., at longer extraction time (compare Fig. 11B,C) the amount of lysozyme is increased as the amount of cytochrome c is decreased.

For the determination of VOCs in protein-containing solutions, SPME gave superior extraction efficiency compared to LLE. SPME recovered almost three times as many compounds as obtained with LLE after solvent evaporation [128,129].

3.7. In vivo and semiochemical analysis

In vivo analysis is a special application area where SPME is gaining ground due to its unique characteristics: on-site sampling, easy extraction of volatiles, analysis of the whole extracted amount. Analysis of sex pheromones may greatly profit from the above advantages of SPME. Pheromones are produced in low quantities and are often a multicomponent blend dominated by a main compound. Therefore it is difficult to determine their composition or to detect minor components. SPME of the pheromones of the *Laminaria digitata* spermatozoid and the subsequent GC analysis on a fused-silica column covered with a cyclodextrin enabled the detection of four diastereoisomers of lamoxirine secreted by the algae [130]. For the extraction, 20 ml of medium from a culture with released eggs were decanted into a small flask and stirred while the PDMS fiber was immersed for 30 min. Lepidoptera produce pheromones in an epithelial gland located on the female abdominal tip. One of the mainly used extraction techniques is soaking or washing the glands in organic solvent, but in this way the blends obtained contain the pheromones of both gland cells and the gland surface which is believed not to be identical with the pheromone release. Active carbon coal or glass capillary tubes were also used to trap low quantities of pheromone, but the low release rate hindered the determination of the real amounts of emitted pheromone. Currently SPME has gained interest. The gland of *Sesamia nonagrioides* was extruded from the insect and a 7-μm PDMS fiber was gently rubbed on the tenument of the glandular area for 5 min. SPME was validated by rubbing experiments on an aluminium foil over an area where a reference pheromone solution was deposited. Compared to gland washing experiments, SPME gave higher yields for the three detected pheromones and satisfactory reproducibility [131]. The airborne pheromones of *Metamasius hemipterus* (coleoptera) were sampled by exposing the fibers in the jars with the insects. Compared to the typical pheromone analytical methodologies (gland rinsing, air trapping) SPME was much faster, cheaper, easier and more reproducible. As a consequence it enabled frequent sampling from individual species [132]. Analysis of cuticular hydrocarbons from ants [133] and wasps [134] was accomplished with both SPME and LLE using either pentane or hexane. SPME sampling of signalling chemicals from ants altered the actual profile obtained with LLE, especially with regard to long-chain hydrocarbons. Nevertheless SPME-GC offered adequate precision and accuracy and allowed multiple experiments and extraction of a special part of the insect's body.

The use of SPME enabled the use of GC–direct deposition-infrared spectroscopy (GC–DD-IR) in the

analysis of volatiles from living organisms [135]. GC–IR coupling is a powerful alternative to GC–MS, as similar sensitivity can be obtained and the technique is capable of identifying unknown natural compounds at the picogram level. However the method suffers from the presence of the ice that is formed from water coming from the organisms. The authors investigated various isolation methods: trapping on absorbent, cryo-trapping and extraction, thermal desorption and SPME. The use of HS-SPME led to a rigorous absence of water enabling a rapid and sensitive sampling. Thus, the volatiles from a male asparagus fly were collected within 1 min and subsequently analysed by GC–EI-MS and GC–DD-IR (Fig. 12), illustrating the potential of the method for following the kinetics of pheromonal emission from individual insects 'on-line'. The structure proposed for the unknown pheromone was 1-hydroxyethyl cyclopropyl ketone. SPME combined with GC–MS enhanced sensitivity for the determination of semiochemicals released from *Phyllorycter sylvella* moth [136]. SPME provided superior extraction efficiency compared to gland washing, since the amount collected with a PDMS fiber from one calling female was as large as the amount extracted from the glands of 20 females after washing.

SPME and a solid injection technique were evaluated for the GC–MS analysis of long-chain fatty acids from insect exocrine glands [137]. Both methods were found to be more suitable and offered more representative results than liquid extraction. HS-SPME with a CW–DVB fiber gave higher yields with sample heating at 140°C.

Robacker et al. extensively used SPME to investigate the association of bacteria with fruit flies. Volatile chemicals from the headspace of tryptic soy broth culture of *Staphylococcus aureus* [138] and *Klebsiella pneumoniae* [139] were collected on a 100-μm PDMS fiber and analysed by GC–FID, GC–FTD and GC–MS. The experimental results were somewhat in contradiction with existing methods for semiochemical analysis: many chemicals (alcohols, ketones and pyrazines) were detected in lower concentrations; on the other hand several amines were detected in bacterial emissions. The latter was a critical since ammonia, 1-pyrrolidine and 2,3,4,5-tetrahydropyridine were found to be the most important compounds in attracting flies. In addition, bioassays showed that artificial mixtures of the identified chemicals reached 89% [138] and 73–87% [139] effectiveness in attracting flies. The authors used the developed method for the analysis of ammonia and water-soluble amines (methylamine, triethylamine, dimethylpyrazine and putrescine) emanating from lures for the Mexican fruit fly (*Anastrepha ludens*) [140,141].

It can be concluded that HS-SPME-GC has a great potential in the analysis of biogenic VOC emissions, e.g., it can be very useful for the fast detection of unwanted fungi growth. It can also be used in chemotaxonomic studies, e.g., the classification of an organism on the basis of emission patterns. Hence many investigations of varying perspective have been reported recently: volatiles emitted from the *Penicillium* fungi species [142], fragrance emission from human skin [143], volatile metabolites from staphylococci [144], VOCs from buffalo gourd root powder [145], green leaf VOCs [146], volatiles of bracket fungi *Fomitopsis pinicola* and *Fomes fomentarius* [147]. For the determination of VOCs from *Penicillium* fungi a special device was developed for the removal of the carbon dioxide formed by fungi cultures. The results were compared with those obtained by Tenax adsorption, a method which requires diffusion for 14 days. The method was able to determine characteristic metabolites (isopentyl alcohol, 1-octene-2ol, 3-octenone, 3-octanol, 2-methylisoborneol, geosmin) and identified several sesquiterpene hydrocarbons, and alcohols. The real benefit of SPME was the possibility to identify metabolites which were not previously reported from *Penicillium* species [142].

SPME was also used for the sampling of air volatiles from various sources: single chemicals, slow release formulations, mixtures of chemicals or emissions from living organisms (Coleoptera and microbial cultures) [148]. A versatile moving-air system was developed for delivering the volatiles in a wind tunnel or other bioassay device. Sampling by SPME occurred just before the wind tunnel, and was followed by analysis on GC. A problem that should be considered in the analysis of air samples is the difficulty in calibration procedures. Gaseous phase samples are not easy to operate and the preparation of reference standards of varying concentrations is difficult. Matz et al. described a hyphenated SPME-

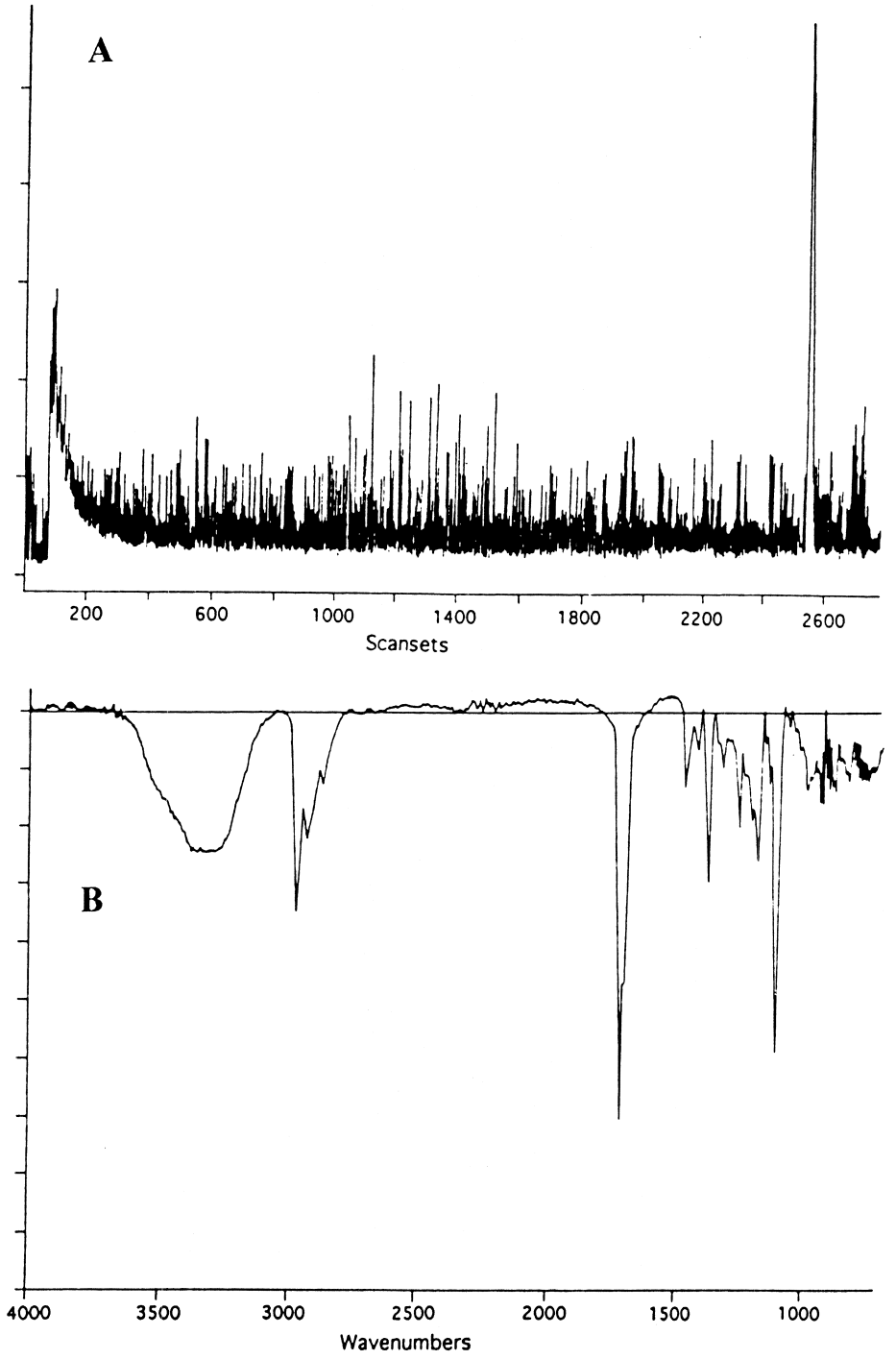

Fig. 12. Identification of unknown pheromone collected within 1 min by SPME from one individual emission of male *Platyparea poeciloptera*. (A) Gran Schmidt reconstructed chromatogram obtained by GC–DD-IR (Digilab Tracer). (B) IR spectrum of the pheromone (from Ref. [135]).

GC–MS system to be used for process control in bioreactors [149]. By incorporating the principles of SPME and membrane extraction with sorbent interface (MESI), they developed a thermal membrane desorption application (TMDA). A polymeric hollow fiber membrane (15 cm length, 700 μm I.D.) is housed in a stainless steel tube (Fig. 13), and connected to the GC capillary column. The fiber membrane was flushed with a sample from the bioreactor and solutes migrated into the membrane depending on their hydrophilicity. Next the fiber was flushed with water and nitrogen. Thermal desorption of the solutes trapped on the fiber occurred by heating the steel tube with a coaxial heater mounted on its outer surface. Full system automation and computer manipulation resulted in good reproducibilities and analysis cycles of 5–10 min. However, analysis time strongly depended on sampling time and GC carrier gas flow-rate used during desorption. Both parameters had also significant impact on the LOD. Several types of membranes were tested; PDMS demonstrating the best results for the analysis of VOCs and semi-volatiles (toluene, phenol, cresol, indole and naphthalene).

3.8. Analysis of natural products

Development of sampling and pretreatment methods for plant material is of the utmost importance in the search for new bioactive compounds. SPME offers attractive features for screening purposes, such as enabling sampling in remote locations.

HS-SPME proved very useful for the GC–MS analysis of volatiles in herbal medicines and herbal extracts/formulations [150]. A PDMS fiber extracted up to 17 terpenoids of interest from the headspace of herbal drop formulations.

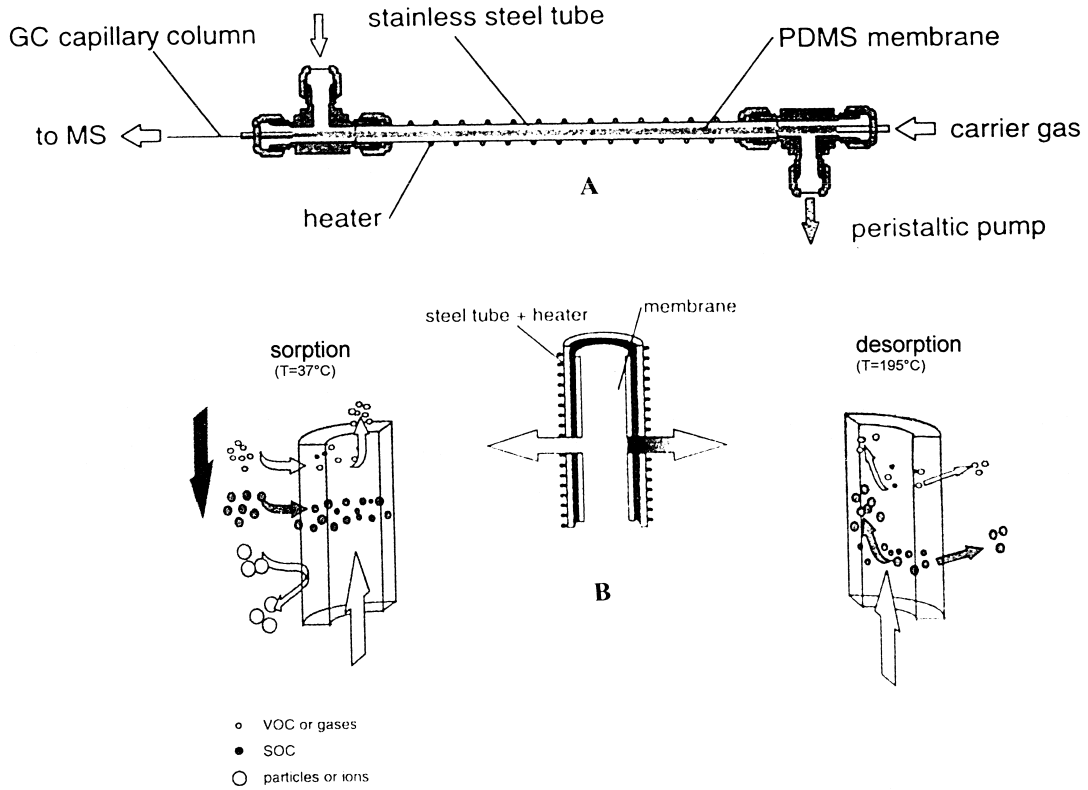

Fig. 13. (A) Schematic representation of the thermal membrane desorption application for process control in bioreactors. (B) A detailed view of the probe in sorption (left) and desorption (right) mode (from Ref. [149]).

Sampling approaches of SPME were evaluated in the GC–NPD analysis of tobacco alkaloids [151]. Ground tobacco was alkalinised and subsequently sonicated in a water bath. The solution was filtered and transferred into a GC autosampler vial, where a 100-μm PDMS fiber was directly immersed in the sample for 12 min. Fig. 14 shows a chromatogram of tobacco alkaloids using DI-SPME and GC–NPD. Sampling conditions were investigated thoroughly. Direct-immersion proved superior over headspace sampling. Alkalinisation of tobacco samples was tested with triethanolamine, triethylamine, KOH and NH_4OH solutions with nicotine as the model compound. The chosen base (NH_4OH) gave both high recovery and minimum damage of the extraction fiber. Usage of thinner fibers (7 μm PDMS) resulted in peak tailing, which was attributed to interactions with the uncovered silica surface on the core. The method was considered as semi-quantitative due to matrix effect and fiber ageing problems.

The applicability of HS-SPME for the analysis of monoterpenes from conifer needles has also been investigated [152]. SPME enrichment was optimised by studying the influence of fiber coating thickness, exposure time and exposure temperature. Four types of pine needles were analysed and revealed typical terpene patterns. HS-SPME was found attractive due to better handling and possibilities of sample enrichment in comparison with the normally used static headspace sampling with a gas-tight syringe. However, for the quantification of multi-component mixtures of terpenes having a wide boiling range, the

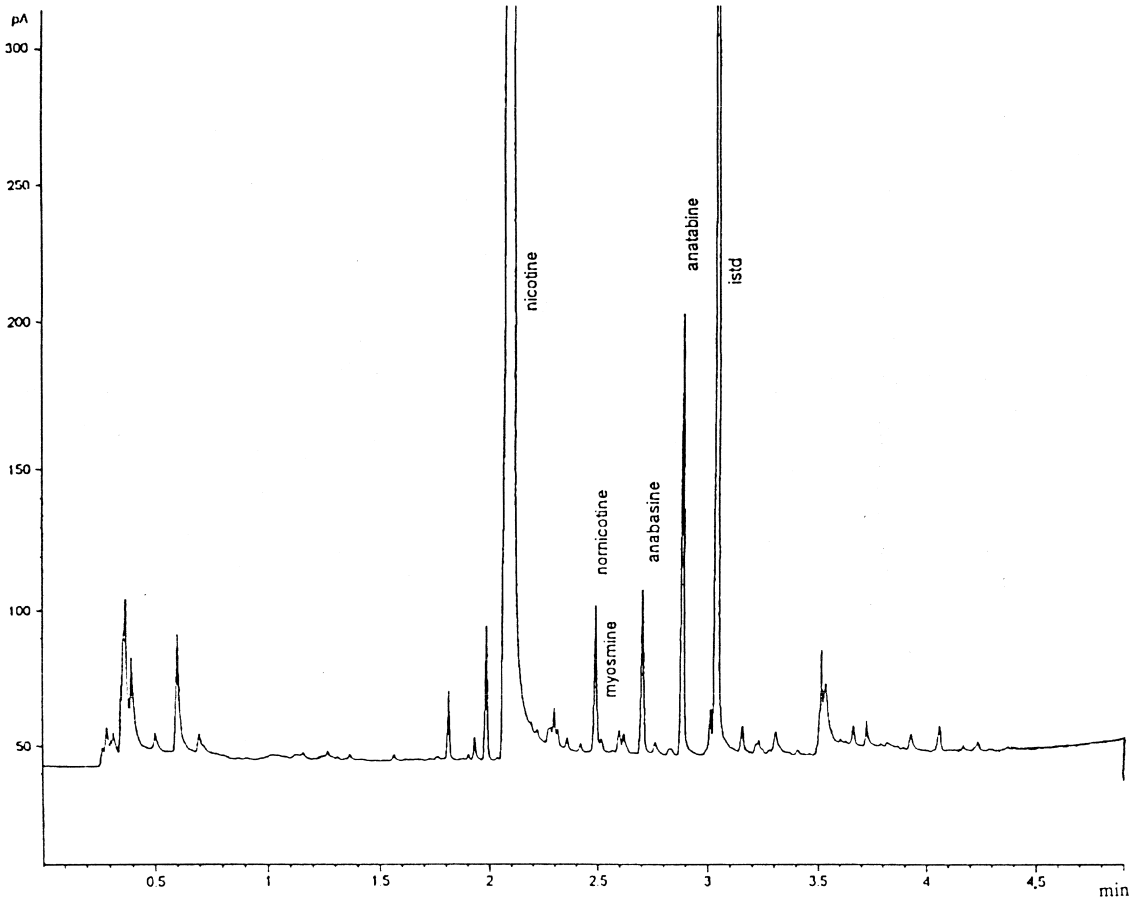

Fig. 14. Chromatogram of tobacco alkaloids analysis using SPME-GC–NPD with a 100-μm PDMS-coated fiber (from Ref. [151]).

very different distribution constants between gas phase and PDMS fibers should be taken into account.

4. Conclusions

SPME has evolved rapidly as a major sample pretreatment technique with a wide application area. There is a continuously growing interest in the technique from various fields. SPME was originally introduced for the GC analysis of volatiles in environmental samples. Since then, SPME has also proven useful and beneficial to food quality control, flavour chemistry, petroleum industry, toxicological and forensic analysis, clinical chemistry, determination of pharmaceuticals in biological samples, biochemical analysis and analysis of natural products. The use of SPME will undoubtedly increase during the following years, as the technique is further optimised, evaluated and validated by many researchers. As shown in Fig. 4, utilisation of SPME increases relatively fast in bioanalysis and related fields. The method has a broad future in routine analysis of pharmaceuticals in biological samples, toxicological analysis and also in conjunction with high-throughput screening. Implementation of automated SPME procedures in these fields would have a large impact with regard to human effort, cost and consumption of organic solvents. Moreover, utilisation of fully integrated methods holds a strong promise for the increase of accuracy and precision.

SPME offers promising features that are advantageous for specific applications: on-site sampling, compatibility with portable GC, etc. Coupling with liquid separation methods has opened an even wider perspective, especially in the field of bioanalysis. For example, SPME shows advantages for the determination of the protein-free amount of drug in biological fluids. However, despite the numerous advantages the method should not be seen as a panacea, a substitute or an opponent of the existing standard methods as SPE. It should instead be considered as a complementary technique, which offers an attractive alternative to more conventional systems. Generally, SPME is still relatively slow and/or yields are relatively low, but significant improvements are being made nowadays. Research effort is currently directed towards the development of new SPME fiber coatings in the search for new selectivities. Incorporation of other principles as, for instance, membrane technologies, antibodies, receptors and molecular imprinted polymers could greatly enhance the development of special fibers and further promote future applications. The combination of SPME with micro- and nano-separation techniques also seems very interesting.

5. Nomenclature

AAS	atomic absorption spectroscopy
AES	atomic emission spectrometry
ASTED	automated sequential trace enrichment of dialysate
BSTFA	bis(trimethylsilyl)trifluoroacetamide
BTEX	benzene, toluene, ethylbenzene, xylene
CE	capillary electrophoresis
CX–PDMS	carboxen–polydimethylsiloxane
CW–DVB	carbowax–divinylbenzene
CW–TPR	carbowax–templated resin
DOA	drugs of abuse
DI	direct immersion
ECD	electron capture detection
EI	electron impact ionisation
EPA	US Environmental Protection Agency
ESI	electron spray ionisation
FID	flame ionisation detection
FTD	flame thermionic detection
HS	head-space
IMS	ion mobility spectrometry
INCAT	inside needle capillary adsorption trap
LLE	liquid–liquid extraction
LOD	limit of detection
LOQ	limit of quantitation
MEKC	micellar electrokinetic chromatography
MESI	membrane extraction with sorbent interface
MSPE	micro solid-phase extraction
NPD	nitrogen–phosphorus detection
PA	polyacrylate
PAH	polycyclic aromatic hydrocarbon
PCSFC	packed column supercritical fluid chromatography
PDMS	polydimethylsiloxane

PDMS–DVB	polydimethylsiloxane–divinylbenzene
PEEK	poly ether ether ketone
RSD	relative standard deviation
SID	surface ionisation detection
SIM	selected-ion monitoring
SPME	solid-phase microextraction
SPE	solid-phase extraction
TMDA	thermal membrane desorption application
TMO	trimethyloxonium tetrafluoroborate
VOCs	volatile organic compounds
XTC	Ecstasy

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