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Review

Recent advances in SPME techniques in biomedical analysis

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ABSTRACT

Biomedical analyses of drugs, metabolites, poisons, environmental and occupational pollutants, disease biomarkers and endogenous substances in body fluids and tissues are important in the development of new drugs, therapeutic monitoring, forensic toxicology, patient diagnosis, and biomonitoring of human exposure to hazardous chemicals. In these analyses, sample preparation is essential for isolation of desired components from complex biological matrices and greatly influences their reliable and accurate determination. Solid-phase microextraction (SPME) is an effective sample preparation technique that has enabled miniaturization, automation and high-throughput performance. The use of SPME has reduced assay times, as well as the costs of solvents and disposal. This review focuses on recent advances in novel SPME techniques, including fiber SPME and in-tube SPME, in biomedical analysis. We also summarize the applications of these techniques to pharmacotherapeutic, forensic, and diagnostic studies, and to determinations of environmental and occupational exposure.

Contents

1. Introduction

Biomedical analysis of low molecular mass compounds primarily includes the analysis of drugs, metabolites, poisons, environmental and occupational toxicants, disease biomarkers, and endogenous substances in body fluids and tissues. Simple and rapid analytical methods are desired for clinical control, doping inspection, and forensic chemistry, because these drugs are highly toxic and because the threshold between therapeutic and toxic concentrations can be narrow. The quantitative analysis of therapeutic drugs and their metabolites has been utilized extensively in pharmacokinetic, pharmacodynamic and metabolic studies, and

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is becoming more important owing to the need to understand the therapeutic and toxic effects of drugs and the continuing efforts to discover and develop more selective and effective drugs [1–6]. Increasing knowledge of drug levels in body fluids, such as serum and urine, can optimize pharmacotherapy and provide the basis for studies of patient compliance, bioavailability, pharmacokinetics, genetics, organ function, and the effects of co-medication with other agents. In addition, approval of a new drug may require knowledge of pharmacokinetic variables such as time to reach maximal concentration in plasma, clearance and bioavailability [5–8]. Furthermore, therapeutic drug monitoring (TDM), based on measurements of drug concentrations in blood, can be used to improve patient care [5,9,10]. In contrast, the screening and confirmation of drugs of abuse in body fluids can be used to identify potential users of illegal drugs and to monitor drug addicts following withdrawal therapy. Reliable qualitative and quantitative toxicological analysis is the basis for competent toxicological judgment and consultation in doping control, and for clinical and forensic toxicology [11–16]. In addition, biological and clinical research may involve the analysis of endogenous substances, such as neurotransmitters, hormones and various diagnostic or prognostic biomarkers [17–20]. Biomarkers are especially useful for population screening, disease diagnosis, monitoring of therapy, and prediction of therapeutic response. Furthermore, the concentrations of environmental pollutants, such as heavy metals, pesticides, herbicides, volatile organic compounds (VOCs) and polycyclic aromatic hydrocarbons (PAHs), in biological samples are analyzed in biomonitoring and investigation of environmental and occupational exposure to these hazardous substances [20–24].

Biological materials consist of solid, particulate or mixed organic compounds in aqueous solution, and often contain proteins, salts, acids, bases and numerous organic compounds that may be similar to the analyte of interest. Determination of selected analytes in these complex biological matrices cannot usually be performed without appropriate sample preparation, even when using powerful analytical instruments, such as liquid chromatography–tandem mass spectrometry (LC–MS–MS). In addition, the analytes in these complex biological matrices are often present at low concentrations. Despite the development of highly sensitive and specific analytical instrumentation for the endpoint determination of analytes in biological samples, sample pretreatment is usually necessary to extract, isolate, fractionate, and/or concentrate the analytes of interest from complex matrices, and these pretreatment methods may greatly influence the reliable and accurate analysis of these analytes [1,2]. Furthermore, over 80% of the total analysis time is generally spent on these sampling and sample preparation steps [25]. Thus sample preparation is the most labor-intensive and time-consuming step of sample analysis, as well as being the most error-prone part of the process, being responsible for about 30% of the sources of errors. Therefore, sample preparation is critical in the method development, especially when analyzing trace components in biological samples. Effective sample preparation helps analytical chemists to cope with increasing demands in the laboratory. An ideal sample preparation technique should be simple, rapid, selective, efficient, solvent-free, inexpensive, and give reproducible and high recoveries without the possibility of degradation of the analyte. Sample preparation should also be amenable to automation, compatible with a wide range of separation methods and applications, and include the simultaneous separation and concentration of both hydrophilic and hydrophobic compounds from aqueous media.

Traditional liquid–liquid extraction (LLE) and conventional solid-phase extraction (SPE) methods have been widely used to prepare biological samples [1–5,10–15,19,26–32]. These sample preparation techniques, however, have various drawbacks, including complicated and time-consuming operations and their requirement for relatively large amounts of sample and organic solvents, as well as being difficult to automate. Therefore, the number of samples may be limited and multi-step procedures may be prone to loss of analytes. Furthermore, using harmful chemicals and large amounts of solvent can result in environmental pollution and health hazards for analytic personnel, as well as extra operational costs for waste treatment. Recent trends in sample preparation are clearly towards miniaturization, automation, high-throughput performance, on-line coupling with analytical instruments and cost-effectiveness through extremely low or no solvent consumption [26,33–37]. Minimizing sample preparation steps is effective, not only in reducing sources of error but in reducing time and cost. Minimizing the number of sample preparation steps is also particularly advantageous for measuring trace and ultra-trace analytes in complex matrices. Samples can be prepared for chromatography using off-line or on-line systems. Off-line procedures are good for small numbers of samples, because there is usually no need for an automated method. These procedures, however, are increasingly recognized as error-prone, tedious and labor-intensive, as well as being the time-limiting step in the analytical process. In contrast, direct on-line procedures offer the advantages of reducing sample preparation steps and enabling effective pre-concentration and clean-up of biological samples. Furthermore, on-line methods are preferable when the analytes are labile, the amount of sample is limited, and/or very high sensitivity is required. On-line procedures can be automated, thereby reducing the requirements for handling potentially infectious biomaterials. Moreover, these procedures improve precision, increase sample throughput, and minimize sample manipulations, potential contamination and cost. On-line coupled sample preparation techniques can be combined with gas chromatography (GC), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE).

Solid-phase microextraction (SPME), first introduced in the early 1990s, is a new and effective sampling and sample preparation method [38]. SPME can be classified roughly into static in-vessel microextraction and dynamic in-flow microextraction. SPME is usually performed using fibers and capillary tubes coated with an appropriate stationary phase. The most widely used technique is fiber SPME, in which a fiber-coated polymeric stationary phase is used as an extraction device, and absorption or adsorption of analytes occurs on the outer surface of the fiber. In-tube SPME was developed primarily to extend SPME to high-throughput applications and automated instrumentation. In this method, analytes are absorbed by or adsorb to the inner surface of a capillary tube. Similar microextraction techniques have been developed using a microsyringe (in-needle SPME) [34,39–43] and pipette tip (in-tip SPME) [42,43]. In-needle SPME methods include solid-phase dynamic extraction using an internal coated needle [34,39,40,43], microextraction in a packed syringe [41–43] and fiber-packed needle microextraction [36]. Fiber and in-tube SPME devices are shown in Fig. 1. These SPME techniques have the above advantages over traditional LLE and conventional SPE, as well as being useful for the pretreatment of complex sample matrices prior to chromatographic or capillary electrophoretic processes because they enable rapid analysis at low operating costs and with no environmental pollution. These SPME techniques have been widely applied to biological, environmental and food analyses, with thousands of articles published to date. In recent years, a number of review articles on SPME have been published [39,40,43–79], addressing topics including biomedical analysis [39,44–64]. Two major recent fundamental advances in biomedical applications of SPME include the development of *in vivo* SPME [60–63] and the development of high-throughput SPME using multi-well plate technology [40,42,62,63,68,82]. The details of sample preparation using microextraction techniques in biomedical analysis have also been described in books [1–5,11,83,84] and well-

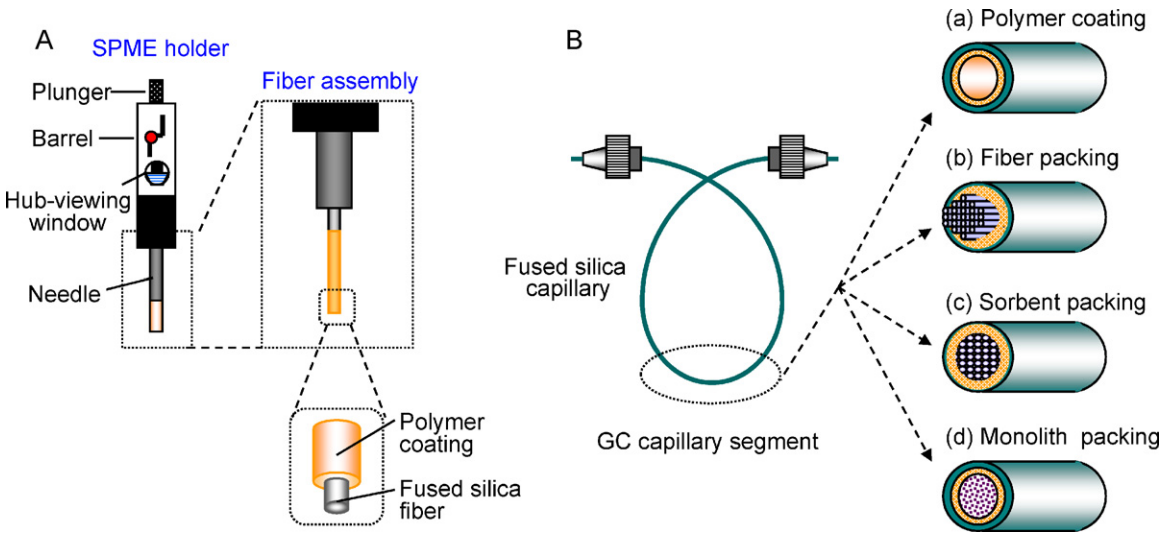

Fig. 1. Configurations of extraction devices for (A) fiber SPME and (B) in-tube SPME.

documented reviews [19,26–39,41,42,44–64,85–96]. This review focuses on new developments and directions in SPME techniques, and their recent applications in pharmacotherapeutic, forensic, clinical diagnostic, and environmental and occupational exposure studies (Tables 1 and 2).

2. Fiber SPME techniques

2.1. Conventional fiber SPME method

Fiber SPME is a convenient sample preparation technique that uses fused-silica fibers coated on the outside with an appropriate stationary phase. The fundamental processes and techniques have been reviewed [25]. The fiber SPME device consists of a fiber assembly with a built-in extraction fiber protected inside a needle and an assembly holder (Fig. 1A). Retractable SPME fibers containing fused silica (1 or 2 cm long) are now commercially available. In addition, a new generation of metal fiber assemblies has been developed; to enhance durability, shape memory and perform more robustly. When the fiber is inserted into the sample, the target analytes partition from the sample matrix into the polymeric stationary phase coated onto the surface of the fiber until equilibrium is reached. In contrast to conventional SPE with packed-bed columns and micro or non-micro columns, this arrangement combines all the steps of sample preparation into one step. Fiber SPME has been used routinely in combination with GC and GC–MS, and it has been successfully applied to a wide variety of compounds in gas, liquid and solid samples. It is used especially to extract volatile and semi-volatile organic compounds from pharmaceutical, biological, environmental and food samples. Fiber SPME has also been coupled directly with HPLC and LC–MS to analyze weakly volatile or thermally labile compounds not amenable to GC or GC–MS. In place of thermal desorption in the injection port of a GC, an SPME/HPLC interface equipped with a special desorption chamber is utilized for solvent desorption prior to HPLC analysis or off-line desorption. Fiber SPME has also been successfully combined with CE in the off-line or on-line mode to analyze different types of compounds in several matrices. The main advantages of fiber SPME are simplicity, rapidity, solvent elimination, high sensitivity, small sample volume, relatively low cost and easy automation. However, these advantages are of use only in some areas of biomedical analysis. Thus, the matrix and volatility of the target analyte must be taken into consideration.

The process of fiber SPME is illustrated in Fig. 2. Fibers should be cleaned before analyzing any sample in order to remove contaminants that may give a high background in the chromatogram. Fibers can be cleaned by insertion into a heated auxiliary injection port or a syringe cleaner and immersed in a flow of clean gas. A sample is placed into a vial, which is sealed with a septum-type cap. When the SPME needle pierces the septum and the fiber is extended through the needle into the sample, the target analytes partition from the sample matrix into the stationary phase. Although SPME has a maximum sensitivity at the partition equilibrium, there is a proportional relationship between the amount of analyte adsorbed by the SPME fiber and its initial concentration in the sample matrix before partition equilibrium. Therefore, full equilibration is not necessary for quantitative analysis by SPME. Two types of fiber SPME techniques have been used to extract analytes: headspace (HS) and direct immersion (DI) SPME (Fig. 2A and B). The sample is often stirred with a small stirring bar to increase the rate of extraction and shorten equilibration time. After a suitable extraction time, the fiber is withdrawn into the needle, and the needle is removed from the septum and inserted directly into a GC injection port or a desorption chamber at the SPME/HPLC interface. In general, HS- and DI-SPME techniques can be performed manually or automatically and used in combination with any GC, GC–MS, HPLC, LC–MS or CE system. The analyte is usually desorbed from the fiber coating by heating the fiber in the injection port of a GC or GC–MS (Fig. 2C), or by loading solvent into the desorption chamber at the SPME/HPLC interface (Fig. 2E). All of the extracted analyte is then transferred directly to the column for analysis. The HPLC interface consists of a six-port injection valve and a special desorption chamber, and requires analyte desorption with a specific desorption solvent or mobile phase prior to HPLC or LC–MS analysis. The desorption chamber is placed in the position of the injection loop. After sample extraction, the fiber is inserted into the desorption chamber at the “load” position under ambient pressure. When the injector is changed to the “inject” position, the mobile phase comes into contact with the fiber, desorbs the analytes, and delivers them to the HPLC column for separation. In contrast, the analyte can be desorbed from the fiber coating by off-line solvent desorption. The solvent solution including the desorbed analytes is then injected into the GC, HPLC or CE system (Fig. 2D). However, off-line desorption results in poorer sensitivity, because it is generally not possible to inject all of the desorbed analyte. An important advantage of off-line desorption is the possibility of using multiple SPME fibers for extraction, which improves through-

Table 1
Selected applications of fiber SPME techniques in biomedical analysis.

Analyte	Matrix	Extraction device[a]	SPME mode[b]	Extraction conditions	Analytical method[c]	LOD	Remarks	Ref.
Pharmacotherapeutic analysis								
Anesthetics, analgesics	Urine	100 μm PDMS	HS	Na_2CO_3, 100 °C, 30 min	GC–NPD	0.01–1.5 ng/mL		[97]
Propofol	Blood, tissues	100 μm PDMS	HS	100 °C, 25 min	GC–MS	–	Postmortem examination	[98]
Propofol	Blood, breath	100 μm PDMS	HS	40 °C, 5 min	GC–MS	Blood 72.20 nmol/L, breath 0.006 nmol/L	Anesthesia	[99]
Propofol	Breath	85 μm PA	HS	60 °C, 25 min	GC–MS	3.6 pg/mL	Anesthesia	[100]
Valproic acid	Plasma	100 μm PDMS	HS + OFD	NaCl, 80 °C, 20 min	GC–MS	300 ng/mL	Derivatized with IBCF/ethanol	[101]
Valproic acid	Serum	Hollow-fiber coated wire	HS	pH 1.5, NaCl, 70 °C, 10 min, 900 rpm	GC–FID	85 ng/mL		[102]
7-Aminoflunitrazepam	Urine	Immunoaffinity-fiber	DI	Shaking on the rotary shaker at 100 rpm for 30 min	LC–MS–MS	0.018 ng/mL		[103]
Benzodiazepines	Serum	Immunoaffinity-glass rods	DI	30 min with shaking on a rotary shaker	LC–MS–MS	0.001–0.015 ng/mL		[104]
Diazepam, nordiazepam, oxazepam, dextromethorphan, pseudoephdrine	Blood	PDMS, Coated silica (C18, RP-amide C16) particle	DI	30 min, 850 rpm	LC–MS–MS	0.8–3 ng/mL	Multi-fiber, 96-well plate, Concept 96 system, drug–protein binding study	[105]
Diazepam and metabolites	Blood	PPY (<10 μm)	DI	Direct contact 30 min	LC–MS–MS	3–7 ng/mL	*In vivo* sampling of beagle vein	[106]
Diazepam and metabolites	Blood	Hydrophilic PPY	DI	Direct contact with circulating blood using sampling interface, 2 min, 20–40 s	LC–MS–MS	3 ng/mL	*In vivo* sampling of rats	[107]
Diazepam and metabolites	Blood	Hydrophilic PPY	DI	Direct contact 30 s	LC–MS–MS	3 ng/mL	*In vivo* sampling of beagle vein	[108]
Diazepam and metabolites	Blood	PEG, PEG/C18-bonded silica	DI	Direct contact 0.5–2 min	LC–MS–MS	–	*In vivo* sampling of beagle vein	[108–110]
Oxazepam, diazepam and nordiazepam	Blood	PEG-C18	DI	Direct contact 1–2 min	LC–MS–MS	–	*In vivo* sampling of animal vein	[111]
Fenoterol and methoxyfenoterol	Blood	Reverse phase amide	DI	Direct contact 4 min or 1 h	LC–MS–MS	–	Animal circulatory system	[112]
Velapamil, loperamide, diazepam, nordiazepam, warfarin	Plasma	Polyacrylonitrile coated wire	DI	Expose to undiluted plasma at 37 °C for 1 h	HPLC–UV, LC–MS	–	Drug plasma protein binding study	[113]
Propranolol	Urine	Sol–gel-fiber	HS & DI	HS: 2 mol/L NaOH in urine, 100 °C, 50 min; DI: pH 12, 90 °C, 50 min	GC–FID	HS: 0.275 ng/mL; DI: 0.193 ng/mL		[114]
Propranolol enantiomers	Urine	Sol–gel-fiber	HS	90 °C (water bath), 30 min, 4 M NaOH	CEZ-DAD	8–10 ng/mL		[115]
β-Blockers	Urine, plasma	MIP-coated fiber	DI	750 rpm, 30 min	HPLC–UV	3.8–6.9 ng/mL	Propranolol (template)	[116]
Adrenolytic drugs	Plasma	PPY-coated wire	DI	10 min	LC–MS	0.11–0.18 ng/mL		[117]
Verapamil	Urine	PPY-coated wire	DI	2 min	IMS	2 μg/mL	surface enhanced laser desorption	[118]
Captopril	Plasma	PPY-coated wire	DI	pH 4, NaCl, 45 °C, 15 min	IMS	6.3–7.5 ng/mL		[119]
Clenbuterol	Serum, urine	65 μm PDMS/DVB	DI	pH 11.7, 50 °C, 60 min	HPLC–UV	5–9 ng/mL	β-Adrenergic drug	[120]
Tricyclic antidepressants, anticonvulsants	Plasma	50 μm CW/TPR	DI	pH 5, NaCl, 85 °C, 30 min	HPLC-DAD	2000 ng/mL	Spiked sample	[121]
Tricyclic antidepressants	Plasma	65 μm PDMS/DVB	DI	pH 11.0, 30 °C, 30 min	LC–MS	0.1 ng/mL		[122]
Antidepressants	Plasma	PPY-coated wire	DI	pH 7, 25 °C, 40 min	HPLC–UV	16–25 ng/mL		[123]
Selective serotonin reuptake inhibitor antidepressants	Urine	65 μm PDMS/DVB	DI	$NaHCO_3$, NaCl, 100 °C, 30 min	GC–MS	0.4 ng/mL	Patient, derivatized with acetic anhydride	[124]

Table 1 (*Continued*)

Analyte	Matrix	Extraction device[a]	SPME mode[b]	Extraction conditions	Analytical method[c]	LOD	Remarks	Ref.
Fluoxetine, norfluoxetine	Plasma	65 μm PDMS/DVB	DI	pH 9, 50 °C, 30 min	HPLC–UV	5–10 ng/mL	Spiked sample	[125]
Naproxen & metabolites	Urine	100 μm CW/TPR	DI	pH 3, NaCl, 20 °C, 30 min	HPLC–UV	30 ng/mL	Patient sample	[126]
Ibuprofen & glucronide	Urine	65 μm PDMS/DVB	DI	pH 3.8, NaCl, RT, 30 min	HPLC–UV	250 ng/mL	Patient sample	[127]
Anethole	Serum	PDMS/DVB-fiber	HS	Na_2SO_4, 50 °C, 30 min	GC–MS	3.6 ng/mL	Characteristic marker for the consumption of aniseed spirits	[128]
Chloramphenicol	Urine	50 μm CW/TPR	DI	pH 5.0, 50 °C, 30 min	HPLC–UV	37 ng/mL		[129]
Antibiotic drugs	Plasma	PPY-coated wire	DI	20 °C, 10 min	HPLC–UV	1–3 ng/L	Off-line, on-line (artificial vein system)	[130]
Antibiotic drugs	Blood	PPY-coated wire	DI	Direct contact 5 min	HPLC–UV		*In vivo* sampling of circulating blood	[131]
Linezolid, daptomycin	Blood	Polythiophene, PPY	DI	RT, 10 min	HPLC–UV	25–46 ng/L	Multi-resistant antibiotics	[132]
Drugs	Plasma, urine	Coated silica (C18, RP-amide C16, cyano) particle	DI	RT, 2–5 min	LC–MS–MS	<1 ng/mL	Biocompatible, *in vivo* and *in vitro* assays	[133]
Forensic analysis								
Amphetamine, MDMA	Urine	50 μm CW/TPR	DI + OFD; D + DI		HPLC-FLD			[134]
Methamphetamine, amphetamine	Urine	1-Ethoxyethyl-3-methylimidazolium bis(trifluoromethane) sulfonylimide ion liquid	HS	NaOH, NaCl, 50 °C, 30 min	GC–MS	0.1–0.5 ng/mL		[135]
Methamphetamines	Serum	PPY-coated	HS	30 min	HPLC-FLD	100–250 ng/mL	OPA derivatization	[136]
Methamphetamine	Saliva	Pencil lead fiber (60 mm × 0.35 mm)	HS	NaCl, 80 °C, 50 min, 600 rpm	GC–MS	27 ng/mL		[137]
Club drugs	Urine	PDMS-fiber	HS + OFD	NaCl, 55 °C, 60 min	IMS	5–8 ng/mL		[138]
Selegiline, norselegiline	Blood, urine	PDMS/CAR/DVB	HS	NaOH, NaCl, 90 °C, 25 min	GC–MS	0.01–0.05 ng/mL	Parkinson disease patient	[139]
Recreational drugs	Hair	100 μm PDMS	HS	10 mg hair, acid hydrolysis, 90 °C, 10 min	GC–MS	0.01–0.12 ng/mg		[140]
Cocaine, morphine, 6-monoacetylmorphine	Hair	100 μm PDMS	HS	125 °C, 25 min	GC–MS	2–5 pg/mg	Opiate analysis	[141]
Cocaine, cocaethylene	Hair	100 μm PDMS	DI	pH 8.5, NaCl, 25 min	GC–MS	0.02–0.08 ng/mg		[142]
Cocaine, cocaethylene	Plasma	100 μm PDMS	DI	pH 9, NaCl, RT, 25 min	GC–MS	11–19 ng/mL	Drug abuse	[143]
Cocaine, cocaethylene	Urine	100 μm PDMS	DI	pH 8–10, RT, 20 min	GC–MS	5 ng/mL	Patient	[144]
Tramadol	Plasma	65 μm PDMS/DVB	HS	NaOH, 100 °C, 30 min	GC–MS	0.2 ng/mL	Healthy volunteers	[145]
Fentanyl	Plasma	PDMS, own preparation	HS	pH 12, 85 °C, 30 min	GC–MS	0.01 ng/mL	Patch treatment	[146]
Ethyl glucronide	Hair	85 μm CAR/PDMS	HS	90 °C, 10 min	GC–MS–MS	0.6 pg/mg	Derivatization	[64]
Strychnine	Blood	65 μm CW/DVB	DI	Dilution (1:10 H_2O), RT, 20 min	GC–MS	7 ng/mL	Poisoned individuals	[147]
Δ-Tetrahydrocannabinol, cannabinol, cannabidiol	Hair	100 μm PDMS	HS + OFD	125 °C, 20 min	GC–MS	0.01 ng/mg	Derivarized with BSTFA/TMCS	[148,149]
Δ-Tetrahydrocannabinol, cannabinol, cannabidiol	Hair	100 μm PDMS	HS	NaOH, Na_2CO_3, 90 °C, 40 min	GC-ITMS-MS	0.007–0.031 ng/mg	THC-D3 (internal standard)	[150]
Biomarkers and clinical analysis								
Sterols	Serum	C18 coating	DI + OFD	KCl, 90 °C, 90 min	GC–FID	250–1100 ng/mL	On-fiber derivatized with BSTFA	[151]
Angiogenesis modulator (β-Estradiol, 2-methoxyestradiol)	Culture media	85 μm PA	DI + OFD	40 °C, 45 min	GC–MS	0.03–0.2 ng/mL	On-fiber derivatized with TFA	[152]
Angiotensin I and II	Blood	Cation-exchange diol silica (XDS) RAM	DI	60 min	LC–MS	8.5 pM		[153]
Fatty acids (C_{12}-C_{24})	Sputum	Sol–gel derived butyl methacrylate/hydroxy-terminated silicone	DI + OFD	pH 2.6, NaCl, 80 °C, 2 h	GC–MS	1.68–150.4 ng/mL	Patients with pulmonary tuberculosis	[154]

Fatty acid ethyl esters	Meconium	65 μm PDMS/DVB	HS	90 °C, 40 min, 250 rpm	GC–MS	0.05–0.16 nmol/g		[155]
Malondialdehyde	Blood	65 μm PDMS/DVB	HS	50 °C, 40 min	GC–MS	0.04 ng/mL	Oxidative stress biomarker, derivatization	[156]
2-Pentene-one (aldehyde adduct)	Urine, plasma	65 μm PDMS/DVB	HS	25 °C, 10 min	GC–MS	1–33 μmol/L	Biomarker, in-fiber derivatization	[157]
Aldehydes	Breath	65 μm PDMS/DVB	HS + OFD	60 °C, 8 min	GC–MS	0.001–0.056 ng/mL	Biomarker, lung cancer	[158]
Metaldehyde	Serum	100 μm PDMS	HS	70 °C, 25 min	GC–MS	250 ng/mL		[159]
PCB 126 and 153	Tissue	85 μm PA	HS	100 °C, 40 min	GC–MS	2 ng/g brain, 0.2 ng/mL serum	Rat brain and serum, oral exposure	[160]
Hydrocarbons	Breath	100 μm PDMS	HS	Expose into Tedlar bag 25 °C, 30 min	GC-TOF-MS	0.04–8.0 ng/mL	Biomarkers in lung disorders	[161]
Volatile sulfur compounds	Breath	85 μm CAR/PDMS	HS	30 °C, 10 min	GC–MS	0.04–0.18 ppbv	Strage polymer bags	[162]
Volatile sulfur compounds	Breath	85 μm CAR/PDMS	HS	30 °C, 10 min	GC–MS, GC-FPD	0.15–2.3 ppb	Healthy volunteers	[163]
2-Pentylfuran	Breath	DVB/ CAR/PDMS	HS	Expose into collection tedlar bag for 48 h	GC–MS		Respiratory pathogen	[164]
Flavorr compounds	Breath	100 μm PDMS	HS	Direct sampling, 8–60 s	GC–MS		Human nose	[165]
Volatile organic emanations	Skin	65 μm PDMS/DVB	HS	Direct sampling, 0.5 h	GC–MS		Human skin (arms)	[166]
Alcohol and aldehyde	Skin	85 μm CAR/PDMS	HS	60 °C, 20 min	GC–MS	0.15–1.01 ng/mL	Alcohol metabolism	
Nonenal	Skin	65 μm PDMS/DVB	HS	40 °C, 20 min	GC–MS	0.02–0.04 ng/mL	Body odor	
Sulfur compounds	Breath, skin	65 μm PDMS/DVB	HS	50 °C, 20 min	GC–MS	0.5–0.7 ng/mL	Oral odor, body odor	
Flavoring agent eugenol	Serum	65 μm PDMS/DVB	HS	Na_2SO_4, 50 °C, 30 min	GC–MS	3.2 ng/mL	Beverage drinking	[167]
Volatiles	Skin cell cultures	65 μm PDMS/DVB	HS	60 °C, 60 min	GC–MS			[168]
Volatile organic compounds (1-butanol, 3-hydroxy-2-butanone)	Breath	75 μm CAR/PDMS	HS	Expose into Tedlar bag RT, 30 min	GC–MS	ng/L level	Lung cancer patients	[169]
Volatile organic compounds	Breath	75 μm CAR/PDMS	HS	Expose into Tedlar bag 37 °C, 10 min	GC–MS	0.7–17.2 ppb	Lung cancer patients	[170]
Volatile organic compounds	Breath	75 μm CAR/PDMS	HS	Expose into Tedlar bag RT, 15 min	GC–MS		Smokers, passive smokers, non-smokers	[171]
Volatile biomarkers	Cancer cell lines	65 μm PDMS/DVB	HS	250 °C, 30 min	GC–MS	0.08–0.23 ng/mL	Lung cancer cell lines; Biomarkers	[172]
Volatile biomarkers	Blood	75 μm CAR/PDMS	HS	60 °C, 15 min	GC–MS		Liver cancer patients	[173]
Environmental and occupational health analysis								
Hazardous solvent biomarkers	Saliva	75 μm CAR/PDMS	HS	60 °C, 180 min, 1000 rpm	GC–MS	3–100 ng/mL	Synthetic leather workers	[174]
Neurotoxicity of toluene	Brain	85 μm StableFlex PDMS/DVB	DI	Direct contact with brain-hippocampus, 2 min	GC–MS		Toluene exposure mice	[175]
Volatile organic compounds	Breath	65 μm PDMS/DVB	HS	10 min breath (0.7–2.5 mL)	GC–MS		Disposable collection device	[176]
Volatile organic compounds	Blood, urine	65 μm PDMS/DVB	HS	NaCl, 2 mL	GC–MS		Occupational exposure, biomarkers	[177]
Volatiles	Blood	75 μm CAR/PDMS	HS	NaF, Na_2CO_3, 40 °C, 6 min	GC–MS	0.005–0.12 ng/mL	Spiked sample	[178]
Chloroform, toluene, xylenes	Blood	100 μm PDMS	HS	$(NH_4)_2SO_4$, 100 °C, 15 min	GC–MS	–	Sniffing fatality	[179]
2,5-Hexanedione (hexane metabolite)	Urine	75 μm CAR/PDMS	HS	50 °C, 20 min	GC–FID	25 ng/mL	Occupational exposure, biomarkers	[180]
Dichloromethane, perchloroethylene	Urine	75 μm CAR/PDMS	HS	NaCl, 22 °C, 30 min	GC–MS	0.005 ng/mL	Workplace occupation	[181]
Tetramine	Urine	85 μm PA	DI	NaCl, 30 min	GC-FTD	188 ng/mm^2	Cannabinoids	[182]
Organophosphorous pesticides	Blood	85 μm PA	HS	NaCl, 70 °C, 20 min	GC–NPD	2–55 ng/mL	Postmortem sample	[183]

Table 1 (*Continued*)

Analyte	Matrix	Extraction device[a]	SPME mode[b]	Extraction conditions	Analytical method[c]	LOD	Remarks	Ref.
Parathion, paraxon	Blood, urine	65 μm CW/DVB	DI	Dilution (1:10 H_2O), 70 °C, 20 min	GC–MS	3–25 ng/mL	Poisoning cases	[184]
Quinalphos	Blood, urine	65 μm CW/DVB	DI	Dilution (1:10 H_2O), 60 °C, 60 min	GC–MS	2–10 ng/mL		[185]
Selenium metabolites	Urine	75 μm CAR/PDMS	HS	22 °C, 5–40 min	GC-ICP-MS	0.13–0.26 pg/g		[186]
Selenite	Urine saliva, milk	100 μm PDMS	HS, DI	25 °C, 20 min	GC–MS	0.03–0.08 ng/mL		[187]
Cyanide	Blood	75 μm CAR/PDMS	HS	H_3PO_4, Na_2SO_4, 100 °C, 15 min	GC–MS	10 ng/mL	Fire victim	[188]
Cyanide, alkylnitriles	Blood	75 μm CAR/PDMS	HS	90 °C, 10 min	GC–NPD	2.8 ng/mL		[189]
Ocratoxin A	Urine	Multi carbon-tape fiber	DI	pH 3, ambient temperature, 1 h	LC–MS–MS	0.3 ng/mL	Multi-fiber, 96-well plate, Concept 96 system	[190]

[a] PDMS: poly(dimethylsiloxane); CAR: carboxen; PA: polyacrylate; CW: carbowax; DVB: divinylbenzene; TRP: templated resin; PEG: polyethylene glycol; PPY: polypyrrole; MIP: molecularly imprinted polymer; ADS: alkyl-diol-silica; RAM: restricted access materials.

[b] HS: headspace fiber; DI: Direct immersion; D: derivatization; OFD: on-fiber derivatization.

[c] GC–MS: gas chromatography–mass spectrometry; LC–MS: liquid chromatography–mass spectrometry; HPLC: high-performance liquid chromatography; CZE: capillary zone electrophoresis; IMS: ion mobility spectrometry; ITMS: ion trap mass spectrometry; TOF: time of flight; ICP: inductively coupled plasma; FID: flame ionization detection; NPD: nitrogen-phosphorus detection; FTD: flame thermionic detector; TSD: thermionic specific detection; FLD: fluorescence detection; DAD: photodiode array detector.

put, particularly for on-site applications using prototype multi-well plates.

HS–SPME is used to extract volatile and semivolatile analytes from the vapor phase above gaseous, liquid or solid samples. In addition to the efficient separation from nonvolatile matrix components and the enrichment of substances on the fiber, this method provides a further advantage, in that fiber damage by aggressive or irreversibly adsorbed matrix components is minimized because the fiber is not in direct contact with the sample. Therefore, more than 100 samples can generally be analyzed using the same fiber. In particular, metal fibers can extend this to 500 samples. In DI–SPME, which is used to extract non-volatile analytes or those with very low volatility, the fiber is directly immersed in liquid samples. Thermodynamically, the same principles as in HS-SPME must be taken into account for good extraction recovery. A hollow-fiber membrane may be used to protect the SPME fiber from insoluble components in the sample. Derivatization in combination with SPME is important if the extract is analyzed by GC, in that derivatization enhances extraction or chromatography. SPME-based pre- and post-extraction derivatization and simultaneous extraction–derivatization are useful for the analysis of highly polar or labile analytes. The details of derivatization for SPME have also been reviewed [75,81].

2.2. New SPME fiber coating devices

Commercially available SPME fibers are coated with a liquid polymer and/or a porous solid sorbent by immobilization of fused silica fibers as non-bonded, bonded, partially cross-linked or highly cross-linked films. Non-bonded phases are stable when used with some water-miscible organic solvents, but slight swelling may occur when used with nonpolar solvents. Bonded phases are stable with all organic solvents, except for some nonpolar solvents. Partially cross-linked phases are stable in most water-miscible organic solvents and some nonpolar solvents. Highly cross-linked phases are equivalent to partially cross-linked phases, except that some bonding to the core may occur. The most important factor in fiber SPME is the affinity of the fiber coating for an analyte. Depending on the substance being analyzed, a suitable polarity and thickness of the fiber coating can be selected. Among the commercial fibers, the most widely used coating is apolar polydimethylsiloxane (PDMS), which has high affinity for the extraction of non-polar compounds. The more polar polyacrylate (PA) is more suitable for extraction of polar compounds. Both phases, however, have linear structure and lack specific surface area. In contrast, mixed coatings blended with porous solid particles polydivinylbenzene (DVB), carboxen (CAR; carbon molecular sieve) or templated resin (TPR), such as PDMS/DVB, carbowax (CW; polyethylene glycol)/DVB (CW/DVB), PDMS/CAR, CW/TPR, and DVB/CAR/PDMS, have large specific surface areas and can be used to extract volatile low molecular-mass and polar analytes. These coatings increase retention capacity due to the mutually potentiating effects of adsorption and distribution to the stationary phase. In the mixed coatings, PDMS and CW are used as the glue (binder) to hold the particles in place. Although fibers coated with thicker films require a longer time to achieve extraction equilibrium, they may be more sensitive since they can extract greater amounts of analytes. In general, volatile compounds require a thick polymer coat, whereas semi-volatile compounds require a thinner coat. Most nonpolar drugs in biological samples can be extracted with PDMS, PA, DVB or PDMS/DVB fiber, and can be analyzed in combination with GC–MS. The StableFlex type of fiber has a flexible fused-silica core and is less breakable. The recent development and commercialization of metal fiber assemblies has provided users with fibers having enhanced durability, shape memory and robust performance.

Table 2
Selected applications of in-tube SPME techniques in biomedical analysis.

Analyte	Matrix	Extraction device[a]	Extraction conditions[b]	Analytical method[c]	LOD	Remarks	Ref.
Pharmacotherapeutic analysis							
Butyrophenones (moperone, floropipamide, haloperidol, spiroperidol, bromperidol, pimozide)	Plasma	DB-17 (60 cm × 0.32 mm i.d., 0.25 μm)	pH 8.5, 30 μL, D/E = 20, 0.25 mL/min	On-line, LC–MS–MS	0.03–0.2 ng/mL	Neuroleptic drugs	[191]
Mirtazapin, citalopam, paroxetine, duloxetine, fluoxetine, sertraline	Plasma	OV-1701 (80 cm × 0.25 mm i.d)	pH 9.0, 100 μL, D/E = 15, 315 μL/min	On-line, HPLC–UV	5–20 ng/mL	Nontricyclic antidepressants	[192]
Dexepin, clozapine, imipramine	Urine	Poly(N-isopropylacryl-amide-co-ethylene dimethacrylate) monolith (15 cm × 0.25 mm i.d.)	pH 10, 0.02 mL/min (10 min);	On-line, HPLC–UV	12.5–71.5 ng/mL	Tricyclic antidepressants	[193]
Theobromine (TB), theophylline (TP), caffeine CA), propranolol enanthiomers	Urine	Poly(methacrylate-co-ethylene dimethacrylate) monolith (15 cm × 0.25 mm i.d.)	TB, TP, CA: pH 3, 240 μL, 0.04 mL/min (6 min); Propranolol: pH 4, 800 μL, 0.04 mL/min (20 min)	On-line, CEC-UV	Propranolol: 4–7 ng/mL		[194]
Candesartan, Losartan, Irbesartan, Calsartan, Telmisartan	Plasma, serum	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (15 cm × 0.25 mm i.d.)	pH 2, 400 μL, 0.04 mL/min (10 min)	On-line, HPLC-FLD	0.1–15.3 ng/mL;	Angiotensin II receptor antagonists	[195]
Candesartan, Losartan, Irbesartan, Telmisartan	Urine	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (2 cm × 0.53 mm i.d.)	pH 2, 2 mL, 0.2 mL/min (10 min)	Off-line, CZE-UV	15–20 ng/mL	Angiotensin II receptor antagonists	[196]
Telmisartan	Tissues (heart, liver, kidney)	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (15 cm × 0.25 mm i.d.)	pH 2, 400 μL, 0.04 mL/min (10 min)	On-line, HPLC-FLD	0.24–1.8 ng/g	Nonpeptide antagonist of the angiotensin II type-1 receptor	[197]
Clenbuterol	Urine	Hydroxylated poly(glycidyl methacrylic acid-co-ethylene dimethacrylate) monolith (2 cm × 0.53 mm i.d.)	pH 5.5–8.0, 0.5 mL, 0.07 mL/min	Off-line, HPLC–UV	2.3–7.7 ng/mL	β_2-Adrenergic receptor agonist	[198]
Ephedrine, pseudoephedrine	Plasma, urine	Poly(methacrylic acid-co-ethylene glycol dimethacrylate) monolith (2 cm × 0.53 mm i.d.)	pH 7.5, 2 mL, 0.2 mL/min (10 min)	Off-line, CZE-UV	5.3–8.4 ng/mL		[199]
Ketoprofen, fenbufen, ibuprofen	Urine	β-Cyclodextrin coated (60 cm × 0.25 mm i.d.)	250 μL, 0.3 mL/min	On-line, HPLC–UV	1.7–10 ng/mL	Non-steroidal anti-inflammatory drugs	[200]
Caffeine, paracetamol, acetylsalicylic acid	Plasma	RAM polymeric monolith (1 cm × 50 μm)	4 bar for 1 min	In-line, CZE-UV	0.3–1.9 ng/mL		[201]
Fluoxetine	Serum	Antibody-immobilized fused silica (70 cm × 0.25 mm i.d.)	50 μL, D/E = 20, 0.4 mL/min	On-line, LC–MS	5 ng/mL	Immunoaffinity capillary	[202]
Fluoxetine and norfluoxetine enantiomers	Plasma	PPY capillary (60 cm × 0.25 mm i.d.)	pH 9, 100 μL, D/E = 20, 0.315 mL/min	On-line, HPLC-FLD	5 ng/mL	Serotonin reuptake inhibitors	[203]
Forensic analysis							
Amphetamine, methamphetamine, their methylenedioxy derivatives	Urine	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (15 cm × 0.25 mm i.d.)	pH 6, 400 μL, 0.04 mL/min (10 min)	Off-line, CZE-UV	25–34 ng/mL	Abused drugs	[204]

Table 2 (*Continued*)

Analyte	Matrix	Extraction device[a]	Extraction conditions[b]	Analytical method[c]	LOD	Remarks	Ref.
Amphetamine, methamphetamine, their methylenedioxy derivatives	Urine	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (15 cm × 0.25 mm i.d.)	pH 5, 400 μL, 0.04 mL/min (10 min)	On-line, HPLC–UV	1.4–4.0 ng/mL	Abused drugs	[205]
Heroin, 6-monoacetylmorphine, morphine, codeine, papaverine, and narcotine	Urine	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (3 cm × 0.53 mm i.d.)	pH 4.5, 2 mL, 0.2 mL/min (10 min)	Off-line, CZE-UV	6.6–19.5 ng/mL	Opiates	[206]
Camptothecin, 10-hydroxycamptothecin	Plasma	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (15 cm × 0.25 mm i.d.)	pH 3, 640 μL, 0.04 mL/min (16 min)	On-line, HPLC-DAD, FLD	1.79–2.62 ng/mL	Alkaloids	[207]
Boldenon, nandrolone, testosterone, methyltestosterone,, stanozolol epiandrosterone, androsterone)	Urine	Supel-Q PLOT (60 cm × 0.32 mm i.d., 17 μm)	40 μL, D/E = 20, 0.15 mL/min	On-line, LC–MS	9–182 pg/mL	Anabolic steroids, doping agents	[208]
Biomarkers and clinical analysis							
Cortisol	Saliva	Supel-Q PLOT (60 cm × 0.32 mm i.d., 17 μm)	40 μL, D/E = 20, 0.15 mL/min	On-line, LC–MS	5.0 pg/mL	Stress biomarker	[209]
Nicotine, cotinine, related alkaloids	Urine, saliva	CP-Pora PLOT amine (60 cm × 0.32 mm i.d., 10 μm)	pH 5.0, 40 μL, D/E = 25, 0.15 mL/min	On-line, LC–MS	15–40 pg/mL	Tobacco smoking	[210]
Dopamine	Urine	Poly(vinylphenylboronic acid-co-ethylene glycol dimethacrylate (30 cm)	pH 9.0, 0.1 mL/min, 10 min	On-line, LC–MS–MS	1.2 ng/mL	Cis-diol biomolecule	[211]
8-Hydroxy-2′-deoxyguanisine	Urine	MIP capillary (4.8 cm × 0.53 mm i.d.), guanosine template	1 mL, 0.03 mL/min with syringe infusion pump	Off-line, HPLC–UV	3.2 pmol/mL	Biomarker of *in vivo* oxidative DNA damage	[212]
Environmental and occupational health analysis							
Hexanal, heptanal	Plasma	Poly(methacrylic acid-ethylene glycol dimethacrylate) monolith (20 cm × 0.53 mm i.d.)	pH 2.2, 1 mL, 0.15 mL/min	Off-line, HPLC-DAD	2.4–3.6 nmol/mL	In situ derivatization	[213]
Co, Ni, Cd	Urine	Porous Al_2O_3-coated fused-silica (40 cm × 0.32 mm i.d.)	pH 9.0, 1 mL, 0.2 mL/min	On-line, ICP-MS	0.33–1.5 pg/mL		[214]
Cu, Hg, Pb	Hair	Sol–gel 3-mercaptopropyl-trimethoxysilane modified silica (40 cm × 0.32 mm i.d.)	pH 6.0, 10 mL, 0.25 mL/min	On-line, ICP-AES	0.17–0.52 ng/mL		[215]

[a] PPY: polypyrrole; MIP: molecularly imprinted polymer; RAM: restricted access material.

[b] D/E: draw/eject cycle.

[c] LC–MS: liquid chromatography-mass spectrometry; HPLC: high-performance liquid chromatography; CZE: capillary zone electrophoresis; CEC: capillary electrochromatography; FLD: fluorescence detection; DAD: photodiode array detector; ICP-AES: inductively coupled plasma-atomic emission spectrometry.

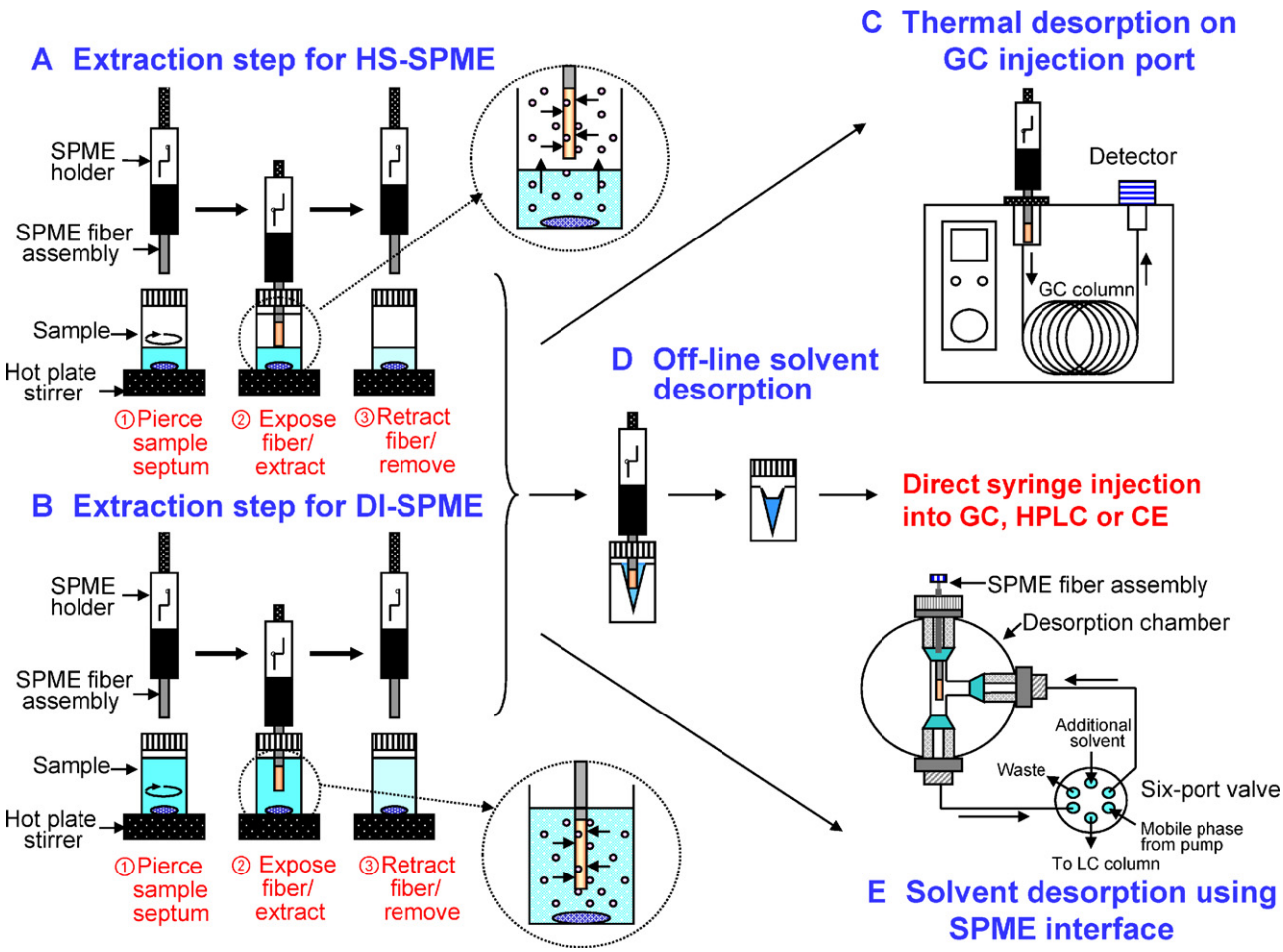

Fig. 2. Schematic diagram of fiber SPME coupled to GC or HPLC.

In addition to commercially available fibers, several types of new coating fibers have been recently developed, including polypyrrole (PPY) [62,106–108,114–116,120,127,128,132], immunosorbent [103,104], restricted access materials (RAM) [149], sol–gel porous silica [111,112,150], molecularly imprinted polymers (MIPs) [113], ion liquid [131], pencil lead [133] and various new biocompatible coating phases [62] suitable for bioanalysis. Some of these devices permit application of *in vivo* SPME (described in the next section) to a variety of analytic assays, including those for pharmacokinetics, bioaccumulation and metabolomics, with good temporal and spatial resolution. PPY coatings are intrinsic conducting polymers, which are positively charged and can more efficiently extract polar compounds, aromatic compounds and anionic species. Their enhanced extraction efficiencies are likely due to the numerous types of interactions between these multifunctional (i.e. acid–base, π–π and dipole interactions, ion-exchange, hydrogen bonding) coatings and the analytes. PPY coating fibers are usually prepared on the surface of metal (Pt, Au, or stainless steel) wires by electrochemical polymerization. For selective and sensitive immunoaffinity SPME, antibodies to a drug are covalently immobilized on the surface of a fused-silica fiber previously modified with 3-aminopropyltriethoxysilane and glutaraldehyde. MIPs are cross-linked synthetic polymers synthesized by copolymerization of a monomer with a cross-linker in the presence of a template molecule, and are used to coat SPME fibers. For direct extraction from blood, the principle of RAM was adopted for SPME. Commercial 35-μm LiChrospher RP-18 alkyl-diol-silica (ADS)–RAM particles is glued to a cleaned stainless steel wire. In contrast, sol–gel coatings, which deposit organic structures onto inorganic polymeric structures, have been introduced to overcome some of the problems of commercial fibers, such as solvent instability and swelling, low operating temperature and stripping of coating. In the sol–gel coating technique, hydroxyl-terminated siloxane polymers or mixed polymers with polyethylene or polypropylene glycols are bonded to Si-OH groups at the fused-silica surface. The sol–gel technology can be utilized effectively to selectively coat SPME fibers. Ionic and molecular recognition in these materials can be achieved by controlling the pore size and morphology of the silicate host structure, by introducing specific functional groups such as crown ethers, calixarenes, β-cyclodextrin and polyethylene glycol (PEG) into the dense framework, or by utilizing molecular imprinting or templating strategies. A remarkable feature of these fibers is their high thermal stability, when compared with conventional pure polymeric PDMS films. These fibers can be heated up to 320 °C without degradation of their performance or significant bleeding, because the sol–gel coating is chemically bonded to the silica core. The details of the properties of the new SPME fiber coatings have also been reviewed [34,62,70,73,74,76,79,80,93–96].

2.3. *In vivo* SPME sampling system

In vivo research involves the study of dynamic chemical processes continuously occurring in living systems. It is generally more suited for monitoring overall effects than *in vitro* research, because a more complex biological system gives a better indication of what will happen in a real body. However, the development of techniques appropriate for *in vivo* analysis poses significant dif-

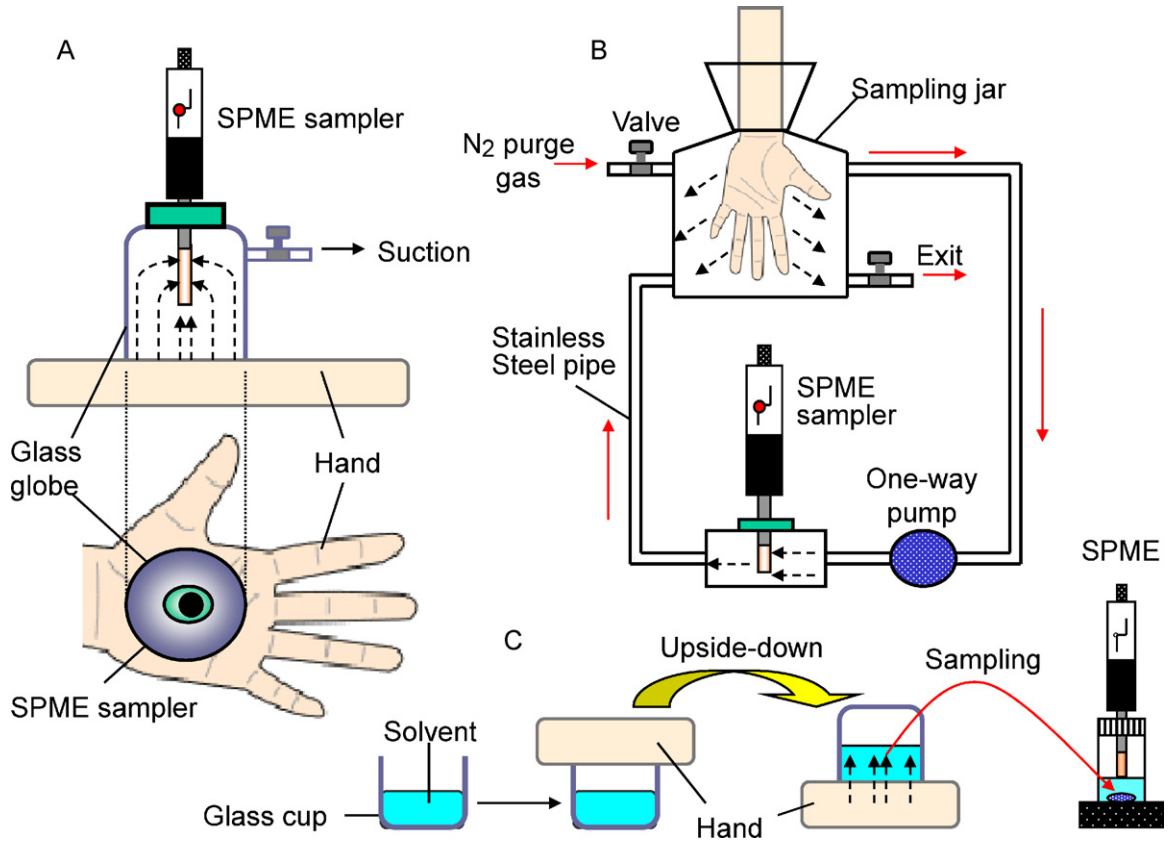

Fig. 3. Systems for sampling volatile compounds emitted from the skin. (A) Direct SPME in sealed glass globes, (B) direct SPME in flow sampling chambers and (C) liquid sampling in glass cups.

ficulties, due to the low and unceasingly changing concentrations of target analytes in complex biological matrices. An ideal *in vivo* sampling technique should be portable, solvent-free and integrate the sampling, sample preparation and analysis steps. The small dimensions of SPME devices, their ability to directly sample on-site and their solvent-free features enable convenient *in vivo* sampling. One *in vivo* SPME sampling system has focused on biogenic VOCs emitted from human skin or breathe that make up human odors. More recently, SPME techniques have been successfully applied to *in vivo* pharmacokinetic analysis of the concentrations of drugs intravenously injected into living animals [106–112].

Configurations of *in vivo* SPME sampling systems are illustrated in Figs. 3 and 4. *In vivo* SPME in the HS-mode can be used to study volatile emanations such as breath exhalations and skin emissions in combination with GC or GC–MS using commercially available devices. In general, human breath is collected into Tedlar bag and the analytes are extracted by direct exposure of SPME fiber into the bag. For skin emissions, three approaches of SPME sampling include direct SPME in sealed glass globes (Fig. 3A), direct SPME in flow sampling chambers (Fig. 3B) and liquid sampling in glass cups (Fig. 3C). In the first and second approaches, SPME fiber can be placed directly over the skin emissions. The third approach can be used to further increase the extraction of more polar and non-volatile compounds. The most complex *in vivo* sampling procedures include insertion of the SPME fiber directly into the system under study, whether it is the vein of an animal to sample flowing blood (Fig. 4A) or a tissue, such as muscle. In the case of polar nonvolatile analytes, only direct extraction is feasible. The fiber coating is either carefully rubbed against the sample or inserted with a special *in vivo* device (Fig. 4A). The design of direct exposure devices must incorporate a mechanically strong, flexible and unbreakable fiber core. A modified device with 1–2 cm long coatings housed inside a hypodermic needle can be particularly useful for this purpose, and small device dimensions minimize tissue damage. In contrast, the sampling of small rodents requires an interface due to the small size of blood vessels, which prevents direct insertion of SPME probes (Fig. 4B) [107]. Y-shaped interfaces, designed to allow the recirculation of blood to the animal, are prone to clotting problems, such that adequate blood flow through the interface could not be maintained for prolonged periods of time. An alternative approach, in which only one tube is connected to a catheter, was found to work much better, and blood flow in this design is provided by manual push/pull with a syringe.

In vivo SPME sampling relies on direct immersion of SPME coatings into a living system in order to directly extract the analyte onto the coating without having to remove a representative sample of biofluid or tissue from the living system. Therefore, this technique eliminates the need to draw blood during pharmacokinetic studies and allows the direct study of various biochemical processes *in vivo*. For example, it is possible to determine the degree of protein-binding of drugs in blood samples, since SPME usually extracts only free drug. Hence, protein binding can easily be determined if the equilibrium between bound and free drug is not disturbed by the SPME procedure. However, direct *in vivo* sampling of flowing blood is much more demanding than conventional sampling. SPME devices must be biocompatible and sterilizable, preferably by autoclaving, to prevent adverse and/or toxic reactions in the living system, such as clotting, or immunological rejection, and to prevent adhesion of biological molecules, such as proteins. Furthermore, SPME coatings with high distribution constants for the analytes of interest are preferable to improve analytical sensitivity. The creation of nonfouling surfaces is one of the major prerequisites for microdevices for biomedical applications. Because none of the commercially available fibers is suitable for direct *in vivo*

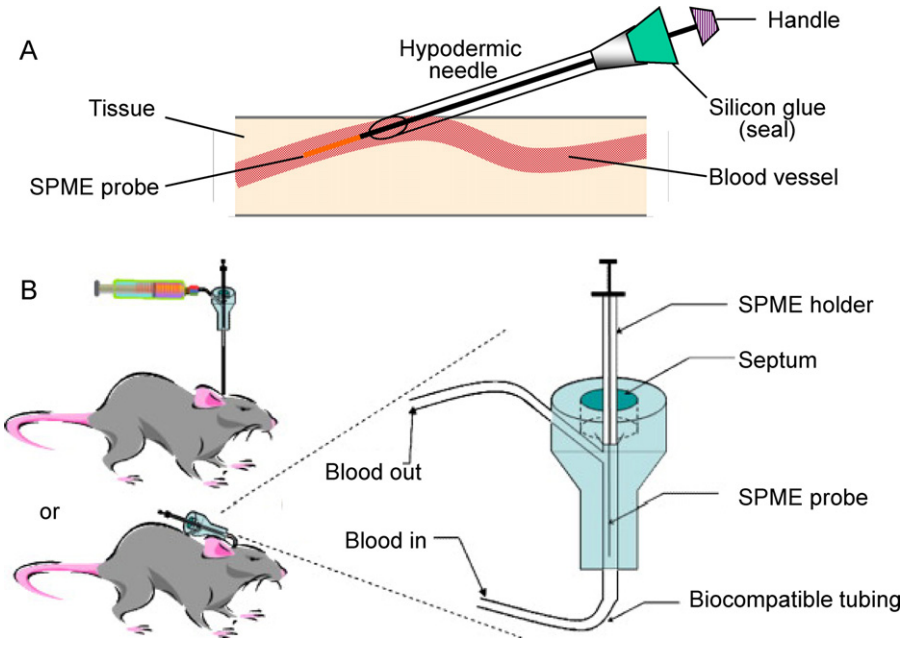

Fig. 4. Direct *in vivo* SPME systems for sampling from flowing blood vessels. (A) Direct insertion of SPME device into blood vessel and (B) placement of SPME device and interface connection to the carotid artery. Reproduced from [107] with permission.

extraction, custom-designed coatings based on PPY, PEG and RAM, deposited as a thin layer on medical grade stainless steel wires, have been utilized for *in vivo* studies in living animals due to their biocompatibility [106–112]. Methodologies similar to those utilized in experimental animals to monitor free and total circulating drug concentrations may also be utilized in human subjects, using biocompatible materials and the SPME devices of dimensions appropriate for human veins, although more tests are required to ensure safety. The details of *in vivo* SPME sampling have also been reviewed [60–63].

2.4. Automated fiber SPME system

Automation of an analytical method provides a number of advantages, including reduced analysis time both for routine analysis and method development, faster sample throughput and greater reproducibility. The fiber SPME method has been automated using commercially available Combi-PAL autosampler (CTC Analytics, Zwingen, Switzerland and other suppliers). This autosampler can be easily programmed to perform various sample preparation steps, such as dilution, agitation and extraction. In the automated system, samples are agitated by fiber vibration or sample tray rotation in contrast to manual fiber SPME, in which magnetic stirring is the most common technique. The Combi-PAL (Fig. 5A) has various additional capabilities, including full temperature control of individual samples, stirring, fiber conditioning and baking out of the fiber outside the injection port. Recently, the automation of fiber SPME procedures has been enhanced by the introduction of the new generation TriPlus autosampler (Thermo Fisher Scientific, Milan, Italy), which utilizes a rocking agitation procedure rather than the orbital procedure used by the Combi-PAL. The TriPlus autosampler also allows the injection of sample into two separate GC systems, thus increasing productivity and high-throughput sample analysis. Furthermore, the Concept 96 robotic system (Fig. 5B), the first fully automated, commercially available sample preparation station from Professional Analytical Systems (PAS) Technology (Magdala, Germany), can be utilized for automated high-throughput analysis with a multi-fiber SPME configuration (described in the next section). This autosampler can be interfaced directly with commercial HPLC equipment and is fully software-controlled. The Concept 96 system has been utilized in drug–protein binding studies [105]. The details of automated SPME sampling systems have also been reviewed [40,60,62,63,68].

2.5. Multiple well-plate fiber SPME system

In off-line desorption, samples are desorbed into a small amount of appropriate solvent contained in a small HPLC vial (Fig. 2D), allowing for simultaneous processing of multiple samples. The use of multiple SPME fibers in conjunction with multi-well plates can be easily automated for parallel extractions and desorptions. This multiplexing approach has been found to significantly increase the throughput of peptide analysis by SPME–MALDI (matrix-assisted laser desorption ionization) [216]. The SPME device can simultaneous extract samples from 16 wells on a multi-well plate, and can be directly coupled to a modified atmospheric pressure MALDI source. Alternatively, a device capable of holding 96 SPME fibers has been developed as an extension of the automation of SPME–HPLC using 96-well plate technology [68]. A suitable brush-like array of SPME fibers can be utilized for robotic automation of the extraction, agitation and liquid desorption steps involved in SPME. The Concept 96 autosampler described above section (Fig. 5B) can perform all steps of multi-fiber SPME with parallel extractions, thus providing very accurate timing control of SPME steps and precise, reproducible positioning of all the fibers in the wells. This automated multi-fiber SPME can improve assay precision, by reducting human intervention, and improve the reproducibility of extraction/desorption times. The multiple SPME technique allows for high-throughput screening suitable for drug discovery or *in vivo* monitoring purposes. The details of SPME automation have been reviewed [3,40,62,63,68].

3. In-tube SPME technique

3.1. Capillary microextraction techniques

Microextraction techniques using capillary columns as extraction devices are of value for miniaturization, automation,

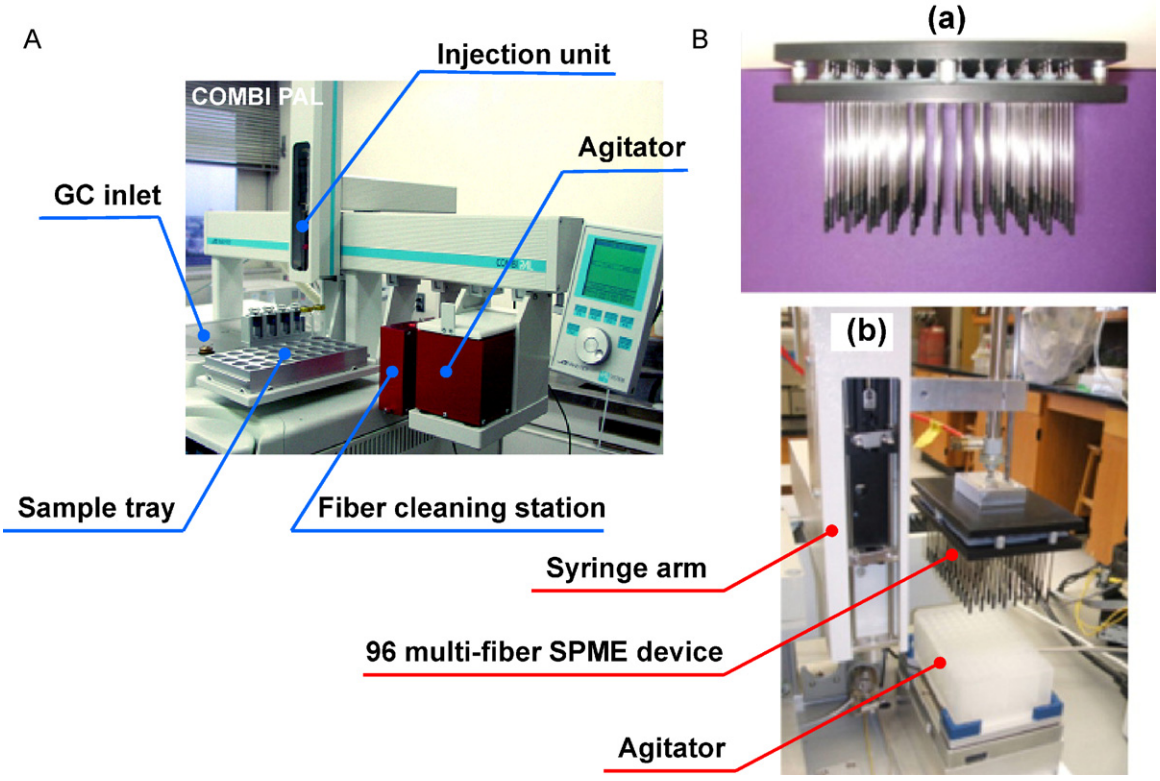

Fig. 5. Automated fiber SPME systems. (A) Combi PAL autosampler, (B) Concept 96 autosampler. (a) 96 multi-fiber SPME device consisting of stainless-steel fibers coated with self-adhesive carbon-tape 12 mm wide; (b) robot arm that inserts the multi-fiber SPME device into the 96-well plate. Reproduced from [190] with permission.

high-throughput performance, on-line coupling with analytical instruments and to avoid solvent consumption. In-tube SPME was primarily developed to provide an automation option for fiber SPME-HPLC. This technique can overcome some problems related to the use of conventional fiber SPME, such as fragility, low sorption capacity, and bleeding of thick-film coatings of fiber. In open-tubular capillary in-tube SPME by flow through and draw/eject systems, an aqueous sample is passed through a capillary column and the analytes absorbed or adsorbed on the capillary coating are analyzed by solvent desorption coupled off-line or on-line with a GC, HPLC or CE system. Unlike fiber SPME, where a sorbent coating on the outer surface of a small-diameter solid rod serves as the extraction medium, in-tube SPME typically uses a short piece of fused-silica capillary with an appropriate stationary phase coating on its inner surface for extraction (Fig. 1B-a). In contrast, fiber-packed, sorbent-packed and rod-type monolith capillaries have been developed to improve extraction efficiency and specificity (Fig. 1B). Fiber-packed capillary microextraction, called "fiber-in-tube SPE", is a modified method using capillary tubes (Fig. 1B-b) packed with fibrous rigid-rod heterocyclic polymers, In contrast, sorbent-packed (Fig. 1B-c) and rod-type monolith (Fig. 1B-d) capillary microextraction use pieces of micro-LC capillary columns packed with extracting phase. In these techniques, analytes are absorbed or adsorbed at the outer surface of the packed sorbent.

Open-tubular and packed capillary in-tube SPME techniques can be used to analyze particularly clean water samples, and the analytes can be highly enriched by passing the sample through the capillary. The main advantage of the in-tube SPME technique is that it enables automation of the SPME–HPLC process, allowing extraction, desorption and injection to be performed continuously using a standard autosampler. In addition, its limits of detection are lower than those of fiber SPME–HPLC systems, due to higher capacity of capillary. Automated sample handling procedures not only shorten the total analysis time, but are more accurate and precise than manual techniques. Furthermore, in-tube SPME enables on-line hyphenation with chromatography at low operating costs and with no environmental pollution. Another important advantage is that it can be used with GC commercial columns, thus increasing the number of stationary phases and allowing a wider range of applications. To choice of an appropriate capillary coating, the columns that have a bonded phase and can be rinsed with solvent are good candidates for in-tube SPME. Although these stationary phases are unsuitable for the extraction of polar compounds, extraction may be improved by derivatization of these compounds. In-tube derivatization techniques improve detectability by increasing selectivity and sensitivity, and enhance the separation of analytes with poor chromatographic behavior. The main disadvantage of this technique is its requirement for very clean samples, because the capillary can be easily blocked. It is therefore necessary to prevent plugging of the capillary column and flow lines by filtering the sample solution before extraction. Although the extraction yields are generally low which is consistent with other microextraction techniques, these compounds may be extracted reproducibly using an autosampler, and all of the extracts may be introduced into the LC column after in-tube SPME, usually resulting in good precision and sensitivity. The details of on-line in-tube SPME techniques for sample preparation have been described in several well-documented reviews [26,29,34,36,37,39,52,57,59,68,70].

3.2. Open tubular capillary in-tube SPME

In-tube SPME can be utilized to directly extract target analytes in aqueous matrices by column switching techniques, and the analytes in the stationary phase of a capillary can be desorbed by introducing a stream of mobile phase or a static desorption solvent for analytes more strongly adsorbed to the capillary coating. In-tube SPME operating systems can be categorized as flow through extraction systems, in which solutions are passed continuously in one

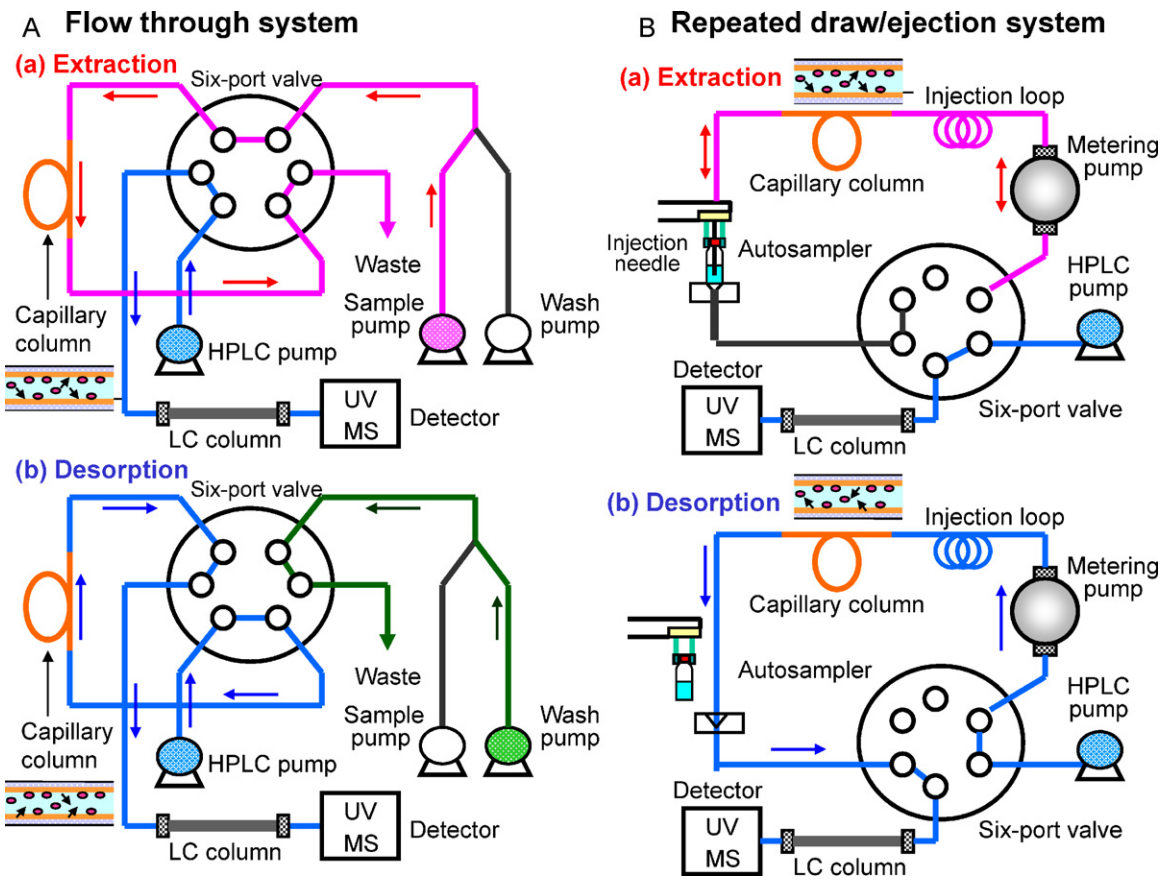

Fig. 6. Schematic diagram of automated in-tube SPME. (A) Flow through system, (B) draw/eject system.

direction through an extraction capillary column; or as draw/eject extraction systems, in which the sample solution is repeatedly aspirated into and dispensed from an extraction capillary column. Fig. 6 illustrates schematic diagrams of on-line in-tube SPME systems. In-tube SPME depends on the distribution coefficient of each analyte as well as fiber SPME, making it important to increase the distribution factor of each in the stationary phase to optimize the rapidity and efficiency of extraction. The selectivity and efficiency of extraction depend on the extraction rate, the sample volume, the pH of the sample, the type of stationary phase, and the internal diameter, length, and film thickness of the capillary column [36]. Therefore, these extraction parameters must be optimized for each type of analyte or sample.

A flow through extraction system is shown in Fig. 6A. The complete analytical system consists of an automatic six-port valve, two pumps (a sample pump and a wash pump) and an LC system. The capillary column is installed in the switching 6-port valve. The enrichment procedure is divided into 4 steps: conditioning, extraction, washing and desorption. The extraction capillary column is rinsed and conditioned with water. During extraction (Fig. 6A-a), the 6-port valve is switched to the load position, and the aqueous sample is pumped through the column. The capillary column is then washed with water to remove any remaining matrix and inorganic residues from the capillary. For desorption (Fig. 6A-b), the 6-port valve is switched to the inject position, and the LC mobile phase is passed through the column (dynamic desorption). During this step, the flow rate of the LC pump is reduced to minimize the back pressure of the analytical column on the capillary. The desorbed analytes are transferred to the analytical column for separation and detection. In a similar system, using double column switching valves [201], the extraction and analysis segments were independent, enabling the rapid, simultaneous performance of several runs, thus shortening analysis time. These systems, however, may cause some systematic problems, including contamination of the switching valve with sample solution due to direct fixation of the capillary column on the 6-port valve. This may result in inaccurate quantitative information and overestimation of analyte. The in-tube SPME method using flow through extraction systems has been applied to the analysis of various biological samples [193–201,204–207,212–215].

In the draw/eject extraction system (Fig. 6B), an extraction capillary column is placed between the injection loop and the injection needle of the HPLC autosampler. An injection loop is installed to prevent sample contamination of the metering pump and switching valve. Building in UV, diode arrays or fluorescence detectors between the HPLC and the MS can enhance multidimensional and simultaneous detection, improving analyte identification. As shown in Fig. 6B-a, a computer controls the injection syringe, which repeatedly draws and ejects sample from the vial, with the analytes partitioning from the sample matrix into the stationary phase until equilibrium is almost reached. After switching the 6-port valve, the extracted analytes can be directly desorbed from the capillary coating by mobile phase flow (dynamic desorption) or by an aspirated desorption solvent (static desorption) (Fig. 6B-b). The computer controls the drawing and ejection of sample solution, switching of the valves, the control of peripheral equipment such as the HPLC and MS, and analytical data processing, thus reducing labor and enhancing precision. In addition, a large number of samples can be automatically processed by the autosampler without carryover, because the injection needle and capillary column

are washed in methanol and the mobile phase before the sample is extracted. The in-tube SPME method using draw/eject extraction systems have been applied to the analysis of various biological samples [191,192,202,203,208–210].

3.3. New capillary coating devices

During in-tube SPME, the amount of analyte extracted into the stationary phase of the capillary column depends on the polarity and film thickness of the capillary coating. Several commercial capillary columns are currently available, depending on analyte characteristics [36]. For example, a low polarity column with a methyl silicon liquid phase selectively retains hydrophobic compounds, whereas a high polarity column with a polyethylene glycol liquid phase selectively retains hydrophilic compounds. The use of thinly coated capillaries often results in low stationary-phase loading, reducing sample capacity and extraction sensitivity, while minimizing the time to reach extraction equilibrium. Although sample capacity and extraction sensitivity may be increased by increasing the film thickness of the stationary phase, it is extremely difficult to reliably immobilize thicker coatings using conventional approaches, and conventionally prepared GC coatings do not bind chemically to fused-silica capillary inner surfaces. This lack of chemical bonds is mainly responsible for low solvent stability, preventing effective hyphenation of in-tube SPME techniques that employ organic or organo-aqueous mobile phases. Although capillary columns with chemically bonded or cross-linked liquid phases are very stable in water and organic solvents, they readily deteriorate in the presence of strong inorganic acids or alkalis. However, capillary columns are generally stable for the mobile phase usually used in HPLC. Among the commercially available capillary columns, the silica modified columns have been found more suitable for the analysis of nonpolar compounds [36]. Due to its large surface area and enhancing mass-transfer kinetics, the adsorptive coated capillary Supel-Q-PLOT has been found to be more efficient for the analysis of steroid compounds [208,209]. Since these PLOT columns have a larger adsorption surface area and a thicker film layer than liquid-phase type columns, they could extract higher amounts of analytes. For example, a CP-Pora PLOT amine could extract more nicotine because of its affinity to relatively polar compounds [210].

In contrast, several unique phases and technical solutions have been developed to improve extraction efficiency and selectivity. Extraction phases better suited to the extraction of relatively polar compounds from aqueous samples have been found to enhance the sensitivity and overall utility of this method. These include the preparation of a series of electrochemical coatings based on polypyrrole by an oxidative polymerization method [203]. Due likely to the numerous types of interactions between analytes and these multifunctional (i.e. π–π, polar, hydrogen bonding and ionic interactions) coatings, PPY coated capillaries have higher extraction efficiencies than commercial GC capillaries. Moreover, the extraction efficiency and selectivity of electrochemical polymer-coated capillaries can be manipulated by regulating the thickness of the coating (i.e. the number of electrochemical polymer cycles). In contrast, sol–gel 3-mercaptopropyl-trimethoxysilane modified silica-coated capillaries have been developed for on-line automated in-tube SPME and have been applied to the analysis of inorganic metals in hair samples by ICP-AES [215]. The details of the properties of new capillary coatings have been reviewed [70,73,74,76,79,80,93–96].

3.4. Packed capillary in-tube SPME

Unique capillary microextraction techniques developed to increase extraction efficiencies are “wire-in-tube SPME”, which uses modified capillary columns with inserted stainless steel wires; and “fiber-in-tube SPE”, which uses capillary tubes packed with fibrous rigid-rod heterocyclic polymers (Fig. 1B-b) [33,67,71,92]. The internal capacity of wire-in-tube SPME can be significantly reduced by inserting a narrow stainless steel wire into the extraction capillary while maintaining the surface area of the polymeric coating material. For fiber-in-tube SPE, several hundred fine filaments of polymeric materials packed longitudinally into a short polyether ether ketone (PEEK) tube serve as the extraction medium. This technique not only can reduce the internal void volume of the extraction tube, but allows the fine polymer filaments to be employed as the extraction medium. Because the filaments are arranged parallel to the outer tubing, narrow coaxial channels can form inside the tube. Therefore, the pressure drop during extraction and desorption is lower in a fiber-in-tube SPE device than in a conventional particle-packed SPE cartridge. Furthermore, the effective interaction of the sample solution with a number of fine fibrous extraction tubes suggests that it can be further miniaturized as a microscale sample preconcentration device, as this technology is convenient for coupling of miniaturized samples to micro or nano-scale separation technologies such as micro-LC and CE. These fiber-packed microextraction techniques have been applied to the analysis of tricyclic antidepressant drugs [33,67]. The fiber-in-tube SPE technique in combination with CE resulted in a 3–76-fold increase in preconcentration of these drugs, depending on whether analytes interacted significantly with the sorbent fiber in the lumen of the capillary tube.

An approach using a small section of capillary packed with microsphere beads is similar to SPE. Although this technique is easy to implement in existing autosampler systems, sorbent-packed capillaries can easily break under high pressure. In analyzing liquid samples by direct immersion, the main disadvantage of this technique is that even very tiny particles can block the capillaries, making it necessary to use very clean samples. Phases better suited to the extraction of relatively polar compounds from aqueous samples have been developed to enhance the sensitivity and overall utility of capillary microextraction methods. For example, an MIP consisting of cross-linked synthetic polymers produced by copolymerizing a monomer with a cross-linker in the presence of a template molecule has been used as an in-tube SPME adsorbent, and a capillary packed with MIP particles in a PEEK tube has been used for the selective analysis of β-blockers in biological fluids [29,74]. Moreover, a highly biocompatible SPME capillary packed with ADS particles was developed as a RAM, preventing fouling of the capillary by protein adsorption while simultaneously trapping the analytes in the hydrophobic porous interior [29]. This approach required a simpler apparatus than used for existing RAM column switching procedures, as well as overcoming the need for ultrafiltration or other deproteinization steps prior to handling biological samples, thus further minimizing sample preparation requirements. The ability to pre-concentrate resulted in low-ng/mL detection limits. Furthermore, a simple SPME device has been fabricated for use in on-line immunoaffinity capillaries [202]. Immunoaffinity-SPME columns, which combine the inherent selectivity of antibodies and the advantages of SPME, are prepared by immobilization of an antibody in an in-tube SPME, using a sensitive, selective, and reproducible method. Important aspects of the optimization of in-tube SPME conditions and the evaluation of the capacity of immunoaffinity capillaries have been described [202]. The details of these new packed capillaries have been reviewed [70,73,74,76,80,93,96].

3.5. Monolithic capillary in-tube SPME

A recent trend in in-tube SPME is the use of polymeric monolithic capillaries, composed of a single piece of organic polymer or silica with a unique flow-through double-pore

structure. Monolithic capillaries can be easily synthesized in situ, initiated thermally or by radiation, using appropriate mixtures of monomers, cross-linkers and proper porogenic solvents [94]. Synthesis generally results in monolithic structures with different functional groups that are biocompatible and pH-stable. A C18-bonded monolithic silica column, prepared by in situ hydrolysis and polycondensation of alkoxysilane, showed preconcentration efficiencies higher than those obtained by conventional in-tube SPME. Various monolithic capillaries have been developed based on poly(N-isopropylacrylamide-co-ethylene dimethacrylate) [193], poly(methacrylic acid-ethylene glycol dimethacrylate) [195–197,199,204–208] and poly(glycidyl methacrylate-co-ethylene dimethacrylate) [198]. The hydrophobic polymeric bone structure and the acidic pendant groups of poly(methacrylic acid-ethylene glycol dimethacrylate) make it superior for extracting basic analytes from aqueous matrices. Various monolithic capillary columns have been used as in-tube SPME devices in combination with LC or CE to assay drug concentrations in biological samples [193–201,204–208,213]. The biocompatibility of these monolithic structures allows the direct analysis of drugs of abuse in urine samples with no manipulations other than dilution and/or centrifugation, simplifying the entire determination procedure [205]. These newly developed monolithic capillaries showed excellent reusability and high stability under extreme pH conditions during in-tube SPME, because of their unique features, including a low pressure drop allowing a high flow-rate to achieve high throughput, and total porosity higher than that of a particle column.

More recently, a new molecularly imprinted monolith was developed for capillary in-tube SPME of 8-hydroxy-2′-deoxyguanosine, a biomarker of *in vivo* oxidative DNA damage [212]. An alternative approach consists of an in-line coupled SPME-capillary zone electrophoresis (CZE) using a continuous bed monolithic RAM capillary insert [201]. Hyperlinked robust biocompatible SPME devices could be interfaced with a CZE system, resulting in a fully automated analysis for sample preconcentration, desorption, separation and quantification of analytes. The RAM based SPME approach was able to simultaneously separate proteins from a biological sample, while directly extracting the active components from a natural drug. The details of these monolithic capillaries have been reviewed [70,73,94].

4. Recent applications of SPME techniques in biomedical analysis

4.1. General aspects in biomedical application of SPME techniques

The main advantages of SPME are its simplicity, rapidity, solvent elimination, high sensitivity, small sample volume, relatively low cost and simple automation. A number of SPME methods have been developed to assay compounds in biological samples, including urine, serum, plasma, whole blood, saliva and hair. The number of publications reporting pharmaceutical and biomedical applications of SPME has increased exponentially, with over 150 reports appearing during the last five years. Their selected applications in pharmacotherapeutic, forensic, clinical diagnostic, and environmental and occupational exposure studies are summarized in Tables 1 and 2. Applications before 2004 have been summarized previously [37,39,52,61]. In this section, we summarize these applications and discuss the characteristics of the approaches employed.

Although various biological specimens, particularly blood (plasma, serum) and urine, have been analyzed for the simultaneous screening and quantification of various analytes, blood samples generally require deproteinization to prevent increased matrix viscosity and protein binding prior to sample pretreatment and chromatography or electrophoresis. Urine may be used for the comprehensive screening and identification of unknown compounds and their metabolites, because of their relatively high concentrations in urine. Although urine collection is easy, it is necessary to prevent the contamination and degradation of analytes during storage. In most analyses, urine samples are diluted with a suitable buffer and filtered through a syringe filter to suppress matrix effects and to prevent contamination of the fiber. Analyses of saliva, sweat and hair have been recently considered as useful adjuncts to conventional drug testing. In particular, analysis of hair is frequently used for the long-term monitoring of drug and alcohol users, and has proved reliable for the retrospective detection of chronic drugs of abuse in clinical and forensic toxicology. However, the interpretation of results may be unclear, particularly with regard to external contamination, cosmetic treatment and ethnic bias. Although the use of milk, tissue and breath samples are unusual in clinical applications, biomarker analysis in breath is utilized in disease diagnosis and for biomonitoring of environmental and occupational exposure to hazardous substances.

Most analytes in biological samples are extracted with 100 μm PDMS, 85 μm PA, a porous polymer DVB or mixed phase PDMS/DVB and CAR/DVB fibers, by HS techniques for volatile analytes or by DI and derivatization techniques for less volatile analytes, followed by analysis in combination with GC–MS. These methods have low limits of detection and excellent quantitation. However, the application of SPME to low volatility drugs and metabolites in plasma may be limited to compounds with high therapeutic concentrations, because of the relatively low partition coefficients between polar analytes and the SPME fibers. The situation can be improved by using LC–MS–MS, allowing the analysis of compounds at concentrations approaching 1 ng/mL in plasma [57,133]. This process, however, may be hindered by various matrix effects, such as fouling and the disturbance of uptake kinetics of target analytes directly extracted from complex biological samples. Interfering compounds or suspended particles can be adsorbed by fiber coatings during the DI–SPME technique. These problems may be minimized by using RAM or biocompatible extraction phases, and/or by using a hollow membrane to form a concentric sheath around each coated SPME fiber [37,102,133]. *In vivo* SPME sampling methods based on blood flow [107–111] are faster than current methods based on blood drawing, can minimize the errors associated with sample preparation and can limit the exposure of personnel to hazardous biological samples. SPME devices based on hypophilic PPY or PEG are used for direct extraction of drugs from the flowing blood of animals, with sampling times of 2 min for external calibration and 30 s for standard on fiber approaches, which are great improvements compared with conventional approaches [107–111].

The in-tube SPME method can be applied to polar and non-polar compounds in liquid samples using a commercially available GC capillary column, and can be easily coupled with various analytical methods such as HPLC and LC–MS [36,37,39,52,57,59]. On-line in-tube SPME systems have been applied to many pharmaceutical and biomedical analyses (Table 2). Extraction efficiency and selectivity may be improved by developing capillaries coated or packed with new materials, such as PPY, ADS, MIP and polymeric monolith [29,36,193–207,213]. For example, a biocompatible in-tube SPME method using a PEEK tube packed with ADS particles has been used for the direct extraction of drugs from serum samples [29]. This approach required a more simplified apparatus than existing RAM column switching procedures, as well as overcoming the need for ultrafiltration or another deproteinization step prior to handling biological samples, thus further minimizing sample preparation requirements. Recently, a new in-tube SPME method using a monolithic capillary was developed for biomedical analysis [36,193–199].

4.2. Applications in pharmacotherapeutic analysis

An overview of recent applications of fiber and in-tube SPME techniques in biological and pharmaceutical analysis is shown in Tables 1 and 2, respectively. Most of these methods have been used to analyze therapeutic drugs in biofluids, such as urine, plasma and serum. These methods show high chromatographic selectivity, linearity, precision, and sensitivity, in line with international criteria for validating application procedures, such as TDM, bioavailability, and pharmacokinetics. Their applications demonstrate the versatility of this method and its potential use in analyzing other drugs in clinical, metabolic, toxicological and pharmaceutical studies.

A number of fiber SPME methods have been developed to extract drugs from various biological samples. For example, an HS–SPME/GC–NPD method using a PDMS fiber was developed for the determination of anesthetics and analgesics in human urine and applied to patients who had undergone coronary bypass surgery operations [97]. The proposed protocol may be an attractive alternative for assaying clinically acute intoxication and medico-legal cases. In addition to commercial fibers, unique extraction devices were recently developed. For example, an SPME fiber, consisting of a piece of copper wire coated with a polypropylene hollow-fiber membrane (HFM) was used to assay valproic acid in human serum [102]. Due to the high porosity of the HFM, polar molecules were efficiently trapped in the porous structure of the durable metallic-based fiber. PPY coated onto stainless steel is a useful SPME sorbent, and has been used to effectively extract various drugs [117–119,123]. A DI–SPME/LC–MS method using a PPY fiber has been used for the determination of five adrenolytic drugs in human plasma [117], and a novel MIP-coated fiber with propranolol as a template has been used in the selective analysis of β-blockers in urine and plasma samples [116]. As shown in Fig. 7, the sensitivities of propranolol and pindolol were greatly enhanced with the MIP-coated fiber, and matrix interference was markedly reduced.

A new approach consists of using biocompatible SPME fibers coated with immunosorbent for direct sampling in biological matrices [103,104]. A new ADS–RAM fiber was prepared by immobilizing ADS particles on a cleaned silica fiber with Locktite 349 adhesive. This fiber can simultaneously fractionate the protein component from a biological sample by size-exclusion while directly measuring several drugs. Other newly developed biocompatible coatings include hydrophilic PPY [107,108,130–132] and immobilized silica (octadecyl, polar embedded and cyano) particles [108–112,133] on a metal fiber core, and these new SPME fibers have been evaluated for *in vivo* SPME applications to drug analysis. For example, these fibers were used in *in vivo* pharmacokinetic determinations of diazepam in rats [107–110]. As shown in Fig. 4B, the catheters were plugged and exteriorized at the nape of the neck. The lower tube of the interface was connected to the carotid artery catheter, and the upper tube of the interface was either recirculated to the carotid artery catheter or connected to a syringe. After administration of diazepam by bolus injection into the jugular carotid artery catheter, the SPME device was placed through the septum into the interface and exposed to flowing blood, and the drug and its metabolites were analyzed by LC–MS–MS. In contrast, an automated SPME method coupled to LC–MS–MS using a 96-multiwell plate format, and consisting of an SPME multifiber device, two orbital shakers, and a three-arm robotic system, was used for high-throughput analysis of diazepam, lorazepam, nordiazepam, and oxazepam in human whole blood [105]. This method allowed the automated preparation of 96 samples in 100 min.

For in-tube SPME, commercial GC capillaries have been used to assay for neuroleptic drugs [191] and nontricyclic antidepressants [192] in plasma. These systems were on-line and fully automated, with no evaporation or reconstitution required. New capillaries, consisting of antibody-immobilized [202] and PPY-coated [203] fused silica, have been used for in-tube SPME analysis of the selective serotonin inhibitor fluoxetine and its metabolite in serum and plasma samples. These extraction efficiency of these capillaries was better than that of commercial capillaries, allowing successful analysis of the enantiomers of these compounds in patient plasma samples (Fig. 8). In contrast, several on-line monolithic capillary in-tube SPME methods have been developed to measure therapeutic drugs in biological fluid samples [193–200]. Most of these methods use poly(methacrylic acid-ethylene glycol dimethacrylate) monolithic capillaries, which contain hydrophobic main chains and acidic pendant groups, which are highly efficient for extracting basic analytes from an aqueous matrix. Monolithic RAM capillaries have been used to simultaneously separate proteins from biological samples, while directly extracting the active components of caffeine, paracetamol and acetylsalicylic acid from the drug NeoCitramonum [201].

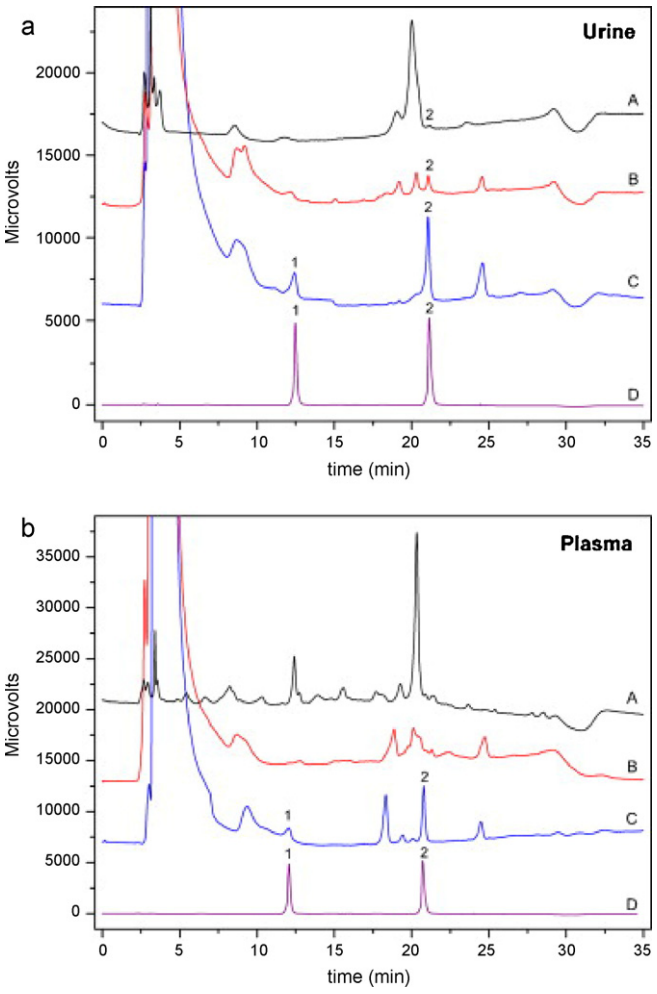

Fig. 7. HPLC chromatograms of propranolol and pindolol obtained from a 2000 ng/mL standard mixture, and from 100 ng/mL spiked (a) urine and (b) plasma samples. (A) Spiked sample by LLE, (B) spiked sample by SPME with non-MIP fiber, (C) spiked sample by SPME with MIP fiber, and (D) standard mixture (10 μL direct injection). Peaks: 1, pindolol; 2, propranolol. Reproduced from [116] with permission.

4.3. Applications in forensic analysis

Drugs of abuse, illicit drugs and incidental/accidental intoxication by drugs and poisons are often analyzed in clinical and forensic toxicology. An overview of recent applications of fiber and in-tube SPME techniques in forensic and toxicological analyses is shown

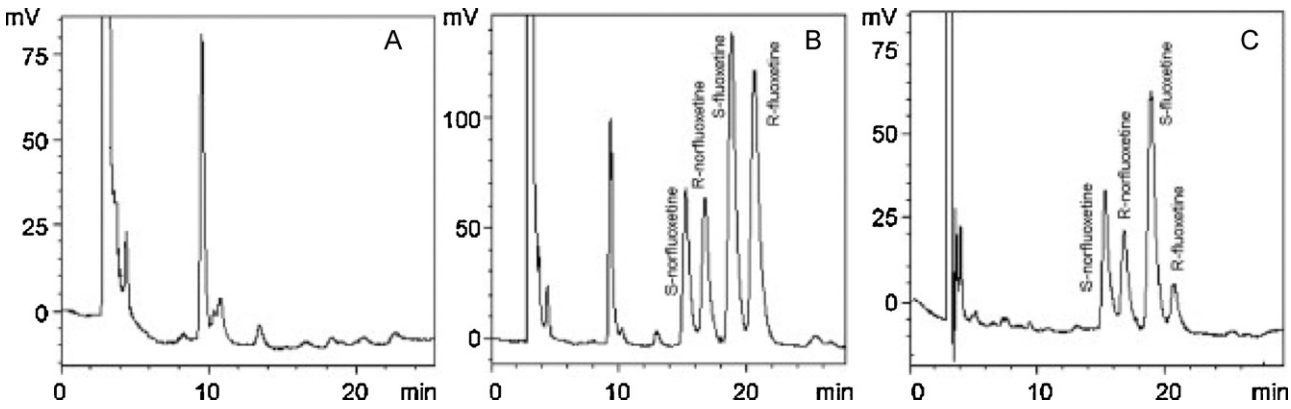

Fig. 8. PPY-coated in-tube SPME/HPLC chromatograms of (A) Blank plasma sample, (B) blank plasma sample spiked with 300 ng/mL fluoxetine and norfluoxetine, and (C) plasma sample from an elderly depressed patient receiving a therapeutic dosage of fluoxetine. Reproduced from [203] with permission.

in Tables 1 and 2, respectively. Most of these methods have been used to analyze forensic drugs in urine, serum and hair. These methods show high chromatographic selectivity, linearity, precision, and sensitivity, in line with international criteria for validating applications in forensic toxicology.

Various illicit drugs, recreational drugs [138,140], cocaine [141–143] and cannabinoids [148–150], can be extracted from hair, urine and plasma samples by HS– or DI–SPME techniques using PDMS fibers and subsequently analyzed by GC–MS. The analysis of drugs in hair samples has become particularly popular in recent years, with possible applications in forensic and clinical toxicology for the retrospective detection of chronic drugs of abuse. A HS–SPME/GC–MS–MS method was recently developed for the determination of Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) in human hair samples [150]. As shown in Fig. 9, these compounds were directly analyzed by GC–ion trap MS–MS after alkaline digestion of 10 mg hair and HS-SPME using 100 μm PDMS. This method was simple, selective and sensitive, and detected higher concentrations of THC, CBD and CBN (0.013–0.300 ng/mg hair) in the hair of a cannabis user than in a non-user (Fig. 10). A new SPME method using an ionic liquid (IL)-based fused silica fiber has been used to assay for amphetamine and methamphetamine in human urine [135]. Although the extraction efficiency of IL fiber is lower than that of 100 μm PDMS, this method results in simple, fast and sensitive sample preparation because of its ability to select a wider number of both cations and anions, and has advantageous physical and chemical properties, including hydrophobicity, viscosity, thermal stability, selectivity and vapor pressure. Furthermore, a pencil lead fiber with a custom-designed unique extraction phase was found very useful for SPME sampling of trace amounts of methamphetamine from human saliva [137].

Recent applications in in-tube SPME include the use of monolithic capillaries for the analysis of illicit drugs in urine and plasma samples [204–207]. In addition, an automated on-line in-tube SPME/LC–MS method was developed to assay seven anabolic steroids in human urine [208]. The steroids were extracted by 20 draw/eject cycles of sample size 40 μL using a Supel-Q PLOT capillary column, and desorbed readily from the capillary column by flow of the mobile phase without carryover. As shown in Fig. 11, the steroids were separated within 14 min by HPLC on a Chromolith RP-18e column, and the urine samples from healthy volunteers were analyzed successfully without interference peaks. This method was sensitive (LOD, 9–182 pg/mL) and useful for anti-doping tests.

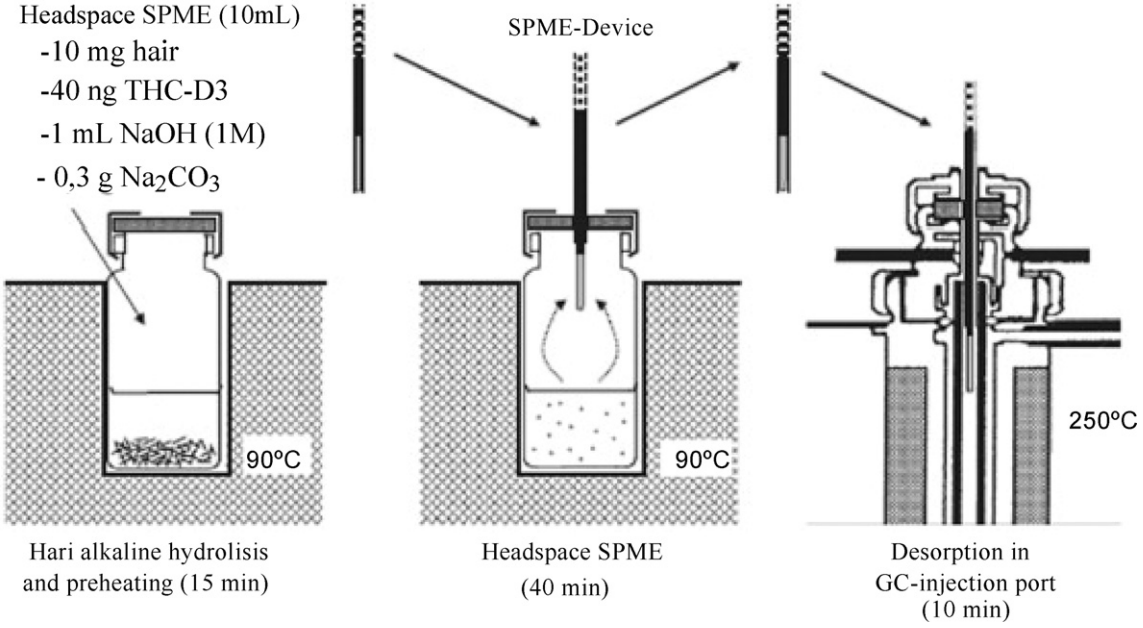

Fig. 9. Steps of HS-SPME in hair analysis. Reproduced from [150] with permission.

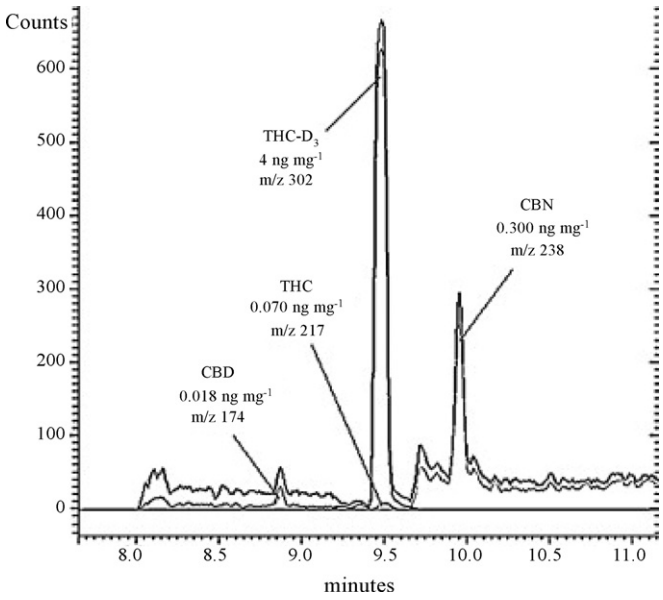

Fig. 10. Chromatogram obtained from the hair sample of a cannabis drug user (22 years old) by HS-SPME/GC–MS–MS. Reproduced from [150] with permission.

4.4. Applications in clinical diagnostic analysis

Endogenous substances such as neurotransmitters, hormones and various bioactive compounds are useful as diagnostic or prognostic biomarkers in disorders or healthcare, and are often analyzed during population screening, disease diagnosis, and biomonitoring. An overview of recent applications of fiber and in-tube SPME techniques in clinical diagnostic analyses is shown in Tables 1 and 2, respectively. Most of these methods have been used to analyze blood, urine, saliva, skin and breath. For example, biogases synthesized during metabolism and emanations from human skin or breath are human odors (body odor and halitosis, respectively). Hundreds of substances are present in human odors, with the actions of skin glands and excreted organic compounds resulting in individual human odors. Any changes in metabolism can alter human emanations. Therefore, the characteristics of human odors are actually informative biomarkers and have been successfully used to identify criminals and to diagnose diseases.

Volatile compounds in biological samples are important markers of disease. For example, since the concentration of acetone in breath is higher in diabetic patients than in controls, an analysis of breath acetone may be diagnostic of diabetes. Although it is very difficult to accurately measure the concentration of acetone in human breath, due to its volatility and activity, these drawbacks are minimized by an HS–SPME method with on-fiber derivatization (OFD) and GC–MS [37,57,62]. An analysis of volatile aldehydes in breath samples by HS–SPME-OFD/GC–MS found that exhaled pentanal, hexanal, octanal and nonanal concentrations were significantly higher in lung cancer patients than in smokers and healthy controls, suggesting that exhaled aldehydes reflect aspects of oxidative stress and tumor-specific composition and metabolism [158]. Several SPME methods have been developed to assay volatile compounds in breath, blood and cancer cell lines as biomarkers of lung and liver cancers [161,169–173], and we recently developed a new SPME sampling technique to measure volatile odor mercaptans exhaled from breath, palm skin and saliva. As shown in Fig. 12, dimethylsulfide and dimethyltrisulfide, which may be biomarkers for breast cancer, were detected in breath exhalations and saliva and in skin emissions, respectively. In these assays of

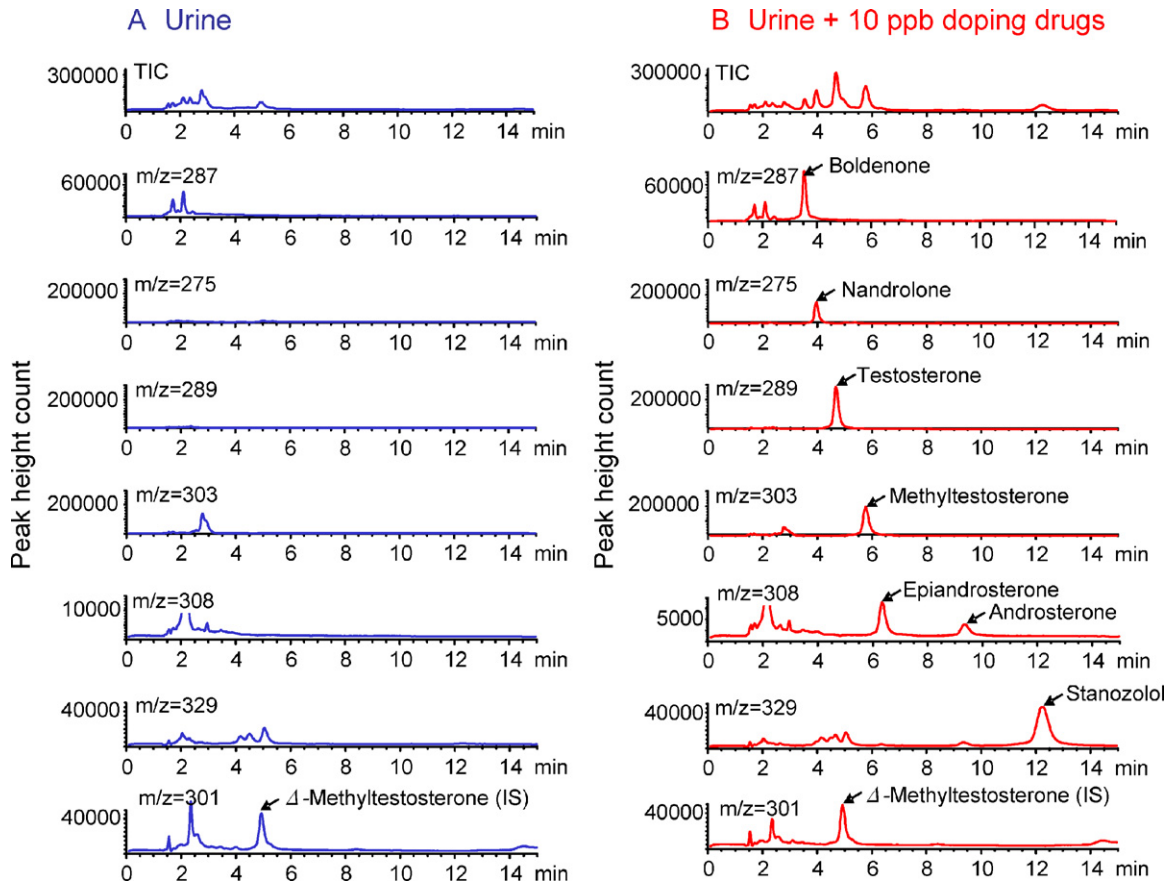

Fig. 11. LC–MS chromatograms of steroids obtained from urine samples. (A) Blank urine; (B) spiked urine (10 ng/mL each steroid). Reproduced from [208] with permission.

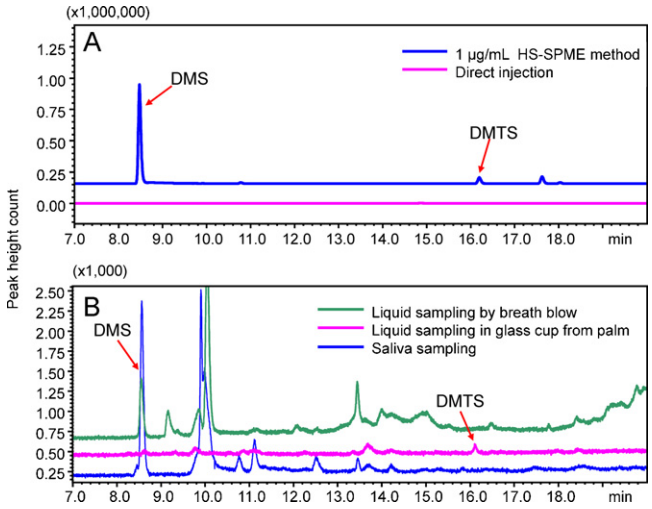

Fig. 12. GC–MS chromatograms obtained from (A) a standard mixture of dimethylsulfide (DMS) and dimethyltrisulfide (DMTS) and (B) breath (collected in water by breath blow), skin gas (collected in glass cup containing ethanol from palm) and saliva samples.

volatile compounds, PDMS/DVB fibers were usually used for fiber SPME. In contrast, a method based on DI–SPME was developed using a sol–gel derived fiber and post-derivatization on the fiber coupled with GC–MS for the analysis of fatty acids in the sputum of patients with pulmonary tuberculosis [154]. This method was fast and sensitive, and useful for the detection of *Mycobacterium tuberculosis* in sputum.

In-tube SPME methods have also been adapted for clinical diagnostic analyses. For example, we have used a Supel-Q PLOT capillary coated with porous DVB polymer as an in-tube SPME device for the analysis of the stress biomarker cortisol in saliva [205]. We also developed an automated on-line in-tube SPME coupled with LC–MS for the determination of nicotine, cotinine and related alkaloids in urine and saliva [206]. These compounds were effectively extracted on a CP-pora PLOT capillary (concentrated 20–46-fold in comparison with direct injection), with detection limits of 15–40 pg/mL. This method was useful for monitoring tobacco smoking, for estimating the uptake of nicotine and tobacco-related toxicants, for understanding the pharmacologic effects of nicotine and nicotine addiction, and for optimizing nicotine dependency treatment. Moreover, an in-tube SPME/HPLC-UV method was recently developed for the determination of urinary 8-OHdG, a biomarker of oxidative DNA damage [208]. In this method, MIP monolithic capillary prepared with guanosine as a template was used for selective extraction, and applied to urine samples from healthy volunteers, cooking plant workers, and cancer patients.

4.5. Applications in environmental and occupational health analysis

Environmental and occupational exposure to chemicals may induce various diseases in individuals and populations and may lead to major public health problems. Therefore, monitoring exposure based on biomarkers allows the evaluation of individual and group hazards and the early detection of exposure to hazardous chemicals, thus significantly reducing their effects on health. Several markers have been proposed to estimate the internal dose of respective chemical agents in the body. For example, environmental pollutants such as heavy metals, pesticides, herbicides, VOCs and PAHs can be analyzed in biological samples during biomonitoring and the investigation of environmental and occupational exposure to these hazardous substances (Tables 1 and 2). Most of these methods have been used for analyses of blood and urine, and sometimes saliva, hair and breath.

VOCs target the central nervous system and are easily absorbed by humans through the lungs and, in some cases, through the skin. Moreover, VOCs have great clinical interest as biomarkers of diseases such as lung cancer, liver disease, myocardial infarction and diabetes. The determination of VOCs in exhaled breath has been used as a biomarker of exposure to environmental and occupational chemicals since it is noninvasive and involves a simpler matrix than other biological samples such as urine and blood. However, the concentrations of VOCs in breath are very low, and pre-concentration is necessary prior to analysis. SPME has been found to have great potential in analyzing VOCs in breath and has been utilized in the analysis of nanomolar concentrations of chemical substances in human breath. For routine use, however, it is important to establish relationships between exposure (dose) and concentration in breath samples. Using HS–SPME/GC–MS with PDMS/DVB fiber, urinary concentrations of the unmetabolized VOCs, toluene, ethylbenzene and xylene, have been assayed as markers of low level occupational exposure in workplaces (paint and footwear factories) [177]. In addition, a simple HS–SPME/GC–FID method was developed to measure urinary 2,5-hexanedione (2,5-HD), the most important metabolite of n-hexane and methyl ethyl ketone, as a marker of exposuere to n-hexane [181]. Moreover, fiber and in-tube SPME techniques have been widely used to analyze various contaminants, such as pesticides [183–185], inorganic compounds [186–189,214,215] and mycotoxin [190], in biological samples. These methods are useful for biomonitoring in toxicology and environmental chemistry. Using SPME/LC–MS–MS coupled with a Concept 96 autosampler system, an automated method was recently developed to measure ochratoxin A (mycotoxin as food contaminant) in human urine samples [190]. This method achieved high throughput by simultaneous preparation of up to 96 samples using a carbon-tape coated multi-fiber SPME device and multi-well plates, allowing analysis of >1500 samples/day.

5. Conclusion and future perspective

Since most analytical instruments cannot directly handle complex matrices such as biological samples, sample preparation is usually necessary to extract, concentrate and isolate the analytes of interest from these complex matrices. However, no universal sample preparation technique is suitable for all types of samples, and sample preparation is dependent on the nature of the analytes, the matrix, and the final separation method. Therefore, selecting and optimizing an appropriate sample preparation scheme for various types of analytes and sample matrix is a key factor in the final success of any analysis, and the choice of an appropriate procedure greatly influences the reliability and accuracy of a given analysis. Furthermore, practical factors such as simplicity of operation, efficiency and time required for pre-treatment, reproducibility, and costs of the method must be considered. Recent trends in sample preparation include miniaturization, automation, high-throughput performance, on-line coupling with analytical instruments, use of less solvent, and maximizing cost effectiveness, speed and safety (especially when using potentially infectious samples of biological material). Minimizing the number of sample preparation steps is not only effective in reducing sources of error but is associated with higher accuracy and precision. Introducing automated techniques into sample preparation is also highly effective in saving time and in improving reproducibility compared with manual methods but involves some costs.

The SPME techniques described in this review are very effective sample preparation tools for qualitative and quantitative analyses of biological and pharmaceutical samples, and can be easily cou-

pled with various analytical methods such as GC–MS and LC–MS. By combining extraction and concentration, all of the analyte extracted can be directly and automatically introduced into the analytical system, resulting in high sensitivity and reproducibility. Therefore, convenient fiber SPME and in-tube SPME are becoming attractive alternatives to SPE and LLE, and have been applied widely to pharmaceutical, forensic and clinical analyses. However, their uses in combination with CE are still rather limited. Moreover, the choice of selectivity is lower in SPME than in SPE since only few stationary phases are available for the former. Thus, despite being a very promising tool, SPME requires further development to solve its inherent drawbacks. New trends in SPME are focused on several development of new sorbents and formats, on-line automation and coupling to state-of-the-art instruments.

The extraction efficiency and selectivity of SPME may be further improved by the development of new extraction devices with new coating materials, and its range of applications may increase after further combination with different analytical instruments. With the development of more sensitive and selective phases, it may be possible to further miniaturize these techniques. Additional customized coatings may become available in the future, including chirally active phases, ion exchangers, ionic liquids, monolithic polymers, carbon nanomaterials, sol–gel porous silicas, immunoaffinity sorbents and MIPs. Furthermore, biocompatible RAM and monolithic sorbents may allow the direct analysis of biological samples with no other manipulation except for dilution and/or centrifugation, simplifying the entire procedure. Monolithic capillaries are especially suitable for in-tube SPME media because of their unique features, including low pressure drop allowing a higher flow-rate to achieve high throughput, and total porosity greater than that of particle-packed capillaries. The development of new coatings has significantly extended the range of analytes amenable to direct extraction by SPME, thus successfully addressing the limitations of this technology. Furthermore, the development of new sorbent supports based on various column dimensions and chemical properties and compatible with HPLC–UV and LC–MS methods is the next step in on-line extraction research. Silicon rods or silica tubes [217] and carbon nanocones/disks [80] may be very promising materials, in addition to the coating materials described in this review, such that more research is expected in this field.

Minimizing the time and manipulations that can be required to process samples is not only effective in reducing sources of error but is associated with high throughput analysis. Future research will also focus on the development of multi-dimensional analytical systems employing on-line SPME techniques. There is also increasing interest in automating sample preparation, thus speeding up these procedures and improving their precision and cost-effectiveness. The key attractive features of automated sample preparation techniques include miniaturization, high throughput, reproducibility, and traceability. Over the last decade, new concepts have been developed, allowing the on-line coupling of sample preparation devices to separation and detection systems especially designed for automation. Automated SPME–GC is now a firmly established technique with the original fiber-type SPME device and, to a lesser extent, with in-tube approaches. Future developments in this area are likely to focus on the automation of more complicated procedures that use devices such as the dual-arm sampler. Importantly, off-line desorption strategies allow the use of multiple SPME devices in parallel extractions, in situations where sequential extraction is not practical, followd by analysis with a single probe. Automation of in-tip SPME can be achieved by commercially available systems using 96-well extraction plates and a robot [37,62,63]. The availability of fully automated multi-well SPME permits large numbers of samples to be prepared in parallel, and will allow the high-throughput screening suitable for drug discovery or *in vivo* monitoring purposes, particularly for applications in pharmacokinetics, pharmacodynamics, metabolomics and toxicology. The construction of a suitable brush-like array of SPME fibers enhances the robotic automation of the extraction, agitation and liquid desorption steps involved in the SPME technique. Currently, the main limitations of automated multi-fiber SPME are the lack of commercially available coatings suitable for use with the system and the unsuitability of the system for the analysis of volatile compounds. Although SPME–HPLC automation has been made possible by the use of in-tube based extraction devices, applications to multiple samples are difficult. The challenge is to develop a sampler that can successfully automate the fiber approach or another SPME configuration with HPLC. Automated coupling with other analytical instrumentation, such as CE and spectroscopic techniques, is likely to receive more attention in the future.

Other unique approaches using a capillary tube are reactor functions such as in-tube derivatization and biomimetic microreactors. Recently, an open-tubular trypsin reactor was developed for on-line digestion without prior protein denaturation, followed by micro-LC separation and photodiode array detection [218]. The miniaturized enzyme-reactor can be produced easily and reproducibly, and seems promising for coupling to other analytical techniques such as CE and surface plasmon resonance, because there is no back pressure. Such capillary microextraction techniques are useful for on-line peptide mapping and inhibitor screening, and novel applications in proteomics, metabolomics (related to environmental, nutritional and disease cases) and biomarker discovery are expected in the future. Furthermore, the development of multifunctional SPME coatings with both selective extraction and biomimetic transformation effects may be useful for toxicological evaluation of compounds, and may be applied to drug discovery and biomonitoring of pollutants.

In summary, SPME has proven very useful for sample preparation in biomedical analysis. Recent advances in SPME technology and methodology open up new possibilities in biomedical analysis, especially in combination with powerful mass spectrometry. In future, the range of potential applications of SPME will be further extended by better integration of sampling/sample preparation and instrumental analysis. Finally, we hope that this review will serve as a guide to choosing the most effective sample preparation technique for each biomedical analysis.

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