HHS Public Access

Author manuscript

Trends Analyt Chem. Author manuscript; available in PMC 2016 December 01.

Published in final edited form as:

Trends Analyt Chem. 2016 June ; 80: 57–65. doi:10.1016/j.trac.2016.02.017.

Solid-phase microextraction technology for *in vitro* and *in vivo* metabolite analysis

Qihui Zhang[a,*], **Liandi Zhou**[b], **Hua Chen**[a], **Chong-Zhi Wang**[c], **Zhining Xia**[a,*], and **Chun-Su Yuan**[c]

[a]Department of Pharmacy, School of Chemistry and Chemical Engineering, Chongqing University, Chongqing 400044, China

[b]Department of Immunology, Basic Medical College, Chongqing Medical University, Chongqing 400016, China

[c]Tang Center for Herbal Medicine Research and Department of Anesthesia & Critical Care, University of Chicago, Chicago, IL 60637, U.S.A.

Abstract

Analysis of endogenous metabolites in biological samples may lead to the identification of biomarkers in metabolomics studies. To achieve accurate sample analysis, a combined method of continuous quick sampling and extraction is required for online compound detection. Solid-phase microextraction (SPME) integrates sampling, extraction and concentration into a single solvent-free step for chemical analysis. SPME has a number of advantages, including simplicity, high sensitivity and a relatively non-invasive nature. In this article, we reviewed SPME technology in *in vitro* and *in vivo* analyses of metabolites after the ingestion of herbal medicines, foods and pharmaceutical agents. The metabolites of microorganisms in dietary supplements and in the gastrointestinal tract will also be examined. As a promising technology in biomedical and pharmaceutical research, SPME and its future applications will depend on advances in analytical technologies and material science.

Keywords

Solid-phase microextraction; endogenous metabolite; metabolomics; pharmaceuticals; medical diagnosis; microorganisms

1. Introduction

Metabolomics systematically investigates the biochemical processes that produce endogenous metabolites in a given organism. Detecting metabolites in biological samples is critical for interpreting health status, medical diagnostics, disease conditions and treatment outcomes [1,2]. For example, cancer is characterized by the abnormal growth of malignant cells beyond their natural boundaries. A successful search for cancer-related biomarkers

*Correspondence to: Qihui Zhang, Ph.D., Associate Professor, Zhining Xia, Ph.D., Professor, School of Chemistry and Chemical Engineering, Chongqing University, Shapingba Zhengjie 174, Shapingba District, Chongqing 400044, China, Tel: 86-23-6510-2531, qhzhang@cqu.edu.cn (Qihui Zhang)., tcm_anal_cqu@163.com (Zhining Xia). .

would contribute to the early diagnosis of malignancy [2]. Early stage melanoma can be detected by volatile organic compounds (VOCs) in the skin [3].

The key technical difficulty to achieving online accurate sample analysis is the establishment of a continuous combined sampling and extraction method [1,4,5]. SPME has a number of advantages: (1) the small sampling needle is relatively nonintrusive and is cost-effective, (2) the coated material on the needle can be diverse, allowing for a wide range of applications, and (3) the two-step integration method for sampling and sample preparation is convenient and time-efficient. SPME also reduces potential extraction or operational errors. Thus, the use of this stable apparatus can achieve identical analytical results with minimal injury to laboratory animals [6-9].

In this article, we will describe the SPME methodology for the analysis of metabolites after the ingestion of herbal medicines, foods and other nutrients, and pharmaceuticals. Then the application of SPME in pharmaceutical research and medical diagnosis will be reviewed in detail. The metabolites of microorganisms in dietary supplements and in the gastrointestinal tract will also be examined (Fig. 1).

2. Solid-phase microextraction (SPME)

2.1 Conventional sample extractions and their limitations

The first extraction technique was liquid-liquid extraction (LLE), a time-consuming, labor-intensive and multi-stage operation [4]. During the concentration of solution, LLE can introduce errors or lose its specificity for analyzing VOCs. To overcome these limitations, solid-phase extraction (SPE) was introduced [10]. SPE, however, also needs a sample concentration step, in which VOCs could be lost. Other common problems of SPE include material aggregation, percolation, and channeling formation [10,11]. To overcome the limitations inherent in LLE and SPE, SPME was introduced by Arthur and Pawliszyn in 1989 [12].

2.2 Advantages of SPME and its potential development

SPME integrates sampling, extraction, concentration and sample introduction into a single, solvent-free step [13,14]. SPME saves preparation time by producing a concentrated extract, applicable for direct MS analysis with high accuracy and low operational and disposal cost [4,15,16]. The advantages of SPME have become well-recognized, and many research articles, book chapters and books on the technology have been published [1,17].

A key driving force of the development of SPME is the progress in the coating material technology [18]. Fig. 2 is a schematic diagram of the *in vitro* and *in vivo* SPME needle apparatuses. The commonly used needle-coating chemicals are polydimethylsiloxane (PDMS), carboxen (CAR), hydrophilic lipophilic balanced (HLB), divinylbenzene (DVB), octadecylsilyl derivatized silica column packing material (C18), carbowax (CW), templated resin (TPR), polyacrylonitrile (PAN), benzenesulfonic acid (BSA) [1,4,15]. Coating chemicals with different thicknesses and polarities are now commercially available [4,16]. For example, SPME coating fibers can be characterized with a DNA aptamer for selective enrichment of a low abundance protein from diluted human plasma, and with antibody-

linked immunoaffinity sorbents for diagnosing methicillin-resistant *Staphylococcus aureus* [19,20].

Fig. 3 is a flow chart of the analytical process using SPME as sample preparation technology. The coating is cleaned firstly to remove contaminants, which prevents a high background in the chromatogram. Subsequently, absorption, desorption optimization, a washing-out process and derivatization are performed [4,21]. The sampling processes can be affected by several factors, such as sample location, selection and time [1,4,22]. Data collection and statistical analysis are continuously implemented until a conclusion is obtained [1,4]. During the analysis process, SPME can be matched with GC or LC chromatography methods and MS or UV detection methods [21,23,24]. For instance, the detection of VOCs in the blood stream using SPME-GC/MS provides accurate results, and metabolic biomarkers could be found to screen colorectal cancer patients [25]. The sensitive, selective and reproducible in-tube SPME-LC/UV can also be used to analyze lidocaine and its metabolites in human plasma for anesthesiology research [26]. SPME can be considered a new sample preparation method for global metabolomics studies from living biological samples, supported by newly developed analytical techniques [27].

3. SPME applications for metabolite analysis

3.1 *In vitro* analysis

To establish a reliable SPME method, many *in vitro* studies were first conducted using test samples from botanicals, food, pharmaceuticals, microorganisms, and environmental pollutants. After stable *in vitro* sample collections and extractions were achieved, SPME was subsequently applied to *in vivo* experiments (Fig. 4).

3.1.1 Plant extracts—Botanical extracts from the liverwort *Scapania aspera* and citrus essential oil vapor from *Peucedanum cervaria* are good candidates for SPME *in vitro* exploration [28-30]. These extracts can be detected by SPME coupled with GC/MS. Extracts of *Hypericum perforatum*, sunflower oil, *Dracaena draco* leaf, and *Ficus carica* were analyzed for their VOCs by head space-SPME (HS-SPME) coupled with GC-ion trap (IT)-MS. The data showed that these extracts are promising antioxidant agents. When the antioxidant effects of these botanicals were evaluated by several chemical assays *in vitro*, the results showed that DPPH, nitric oxide and superoxide radicals were inhibited in a concentration-dependent manner [31-33].

Other researchers have discovered the existence of anti-malarial VOC components in the *Plinia corrocampanensis* leaf [34]. The hypoglycemic and hypolipidemic effects of *Pelargonium graveolens* were studied for their potential application in obesity, diabetes, and metabolic syndrome [35]. The essential oil was detected by HS-SPME-GC/MS.

The composition of volatiles in micropropagated and field-grown botanicals from the islands of Tuscany were identified using HS-SPME with GC/MS. These botanicals have the same aromatic flavor and produce massive materials [36]. In a separate study, designed to detect the activation of purified compounds, data were obtained from *Plectranthus ornatus.* The researchers focused on the effects of different concentrations of 2,4-dichlorophenoxyacetic

acid and 1-naphthaleneacetic acid on the induction of callus and the production of VOCs. The VOCs were detected by HS-SPME followed with GC/MS [37]. *Artemisia umbelliformis*, a protected species, was also studied with SPME, and only a very small amount of the fresh botanical was needed [38].

The influence of growth regulators on biomass production and the volatile profile of the botanicals of *Thymus vulgaris* were also studied *in vitro* with SPME-GC [39]. In another study, five different banana cultivars were distinguished by HS-SPME combined with one-dimensional GC/MS [40].

3.1.2 Diet samples—Eucalyptus essential oil as a natural food preservative was studied using SPME-GC/MS. This essential oil was used for fruit juice preservation against food-spoiling yeast [41]. The effects of kefir culture entrapped in casein and in whey protein as starter cultures for the production of feta-type cheese were also evaluated by SPME-GC/MS. The researchers reported that the VOCs of the different cheese types depended on the nature of the starter culture. When they used kefir culture as the starter, the products showed a soft, fine taste with improved quality [42].

The antioxidant properties of a Chinese gingko wine were accessed using SPME-GC/MS. The results indicated that the total phenol content of gingko wine was 456 mg/L gallic acid equivalents. The antioxidant capacity was higher than that of typical Chinese liquors [43].

Olive oils from two geographic areas were identified and differentiated. The VOCs of the monovarietal virgin olive oils were detected by HS-SPME. The results indicated that the volatile formation was affected by both genetic factors and agronomic conditions [44]. The VOCs in berries were considered at three developmental stages of *Vitis vinifera*, and were exposed (or not) to UV-B both *in vitro* and in field experiments. Of the VOCs that were detected by HS-SPME-GC/MS, 10 VOCs were found at all developmental stages and were found to affect wine flavor [45]. HS-SPME-GC/MS was also used to characterize three coffee monoterpene synthases to help improve the quality control of various types of coffee [46].

3.1.3 Biomedical research—To advance our understanding of the uptake, transportation, and transformation dynamics of compounds for pharmaceutical research, SPME was used for compound measurement [47]. Transport of chlorpromazine was investigated in a Caco-2 cell permeability assay. SPME detected free chlorpromazine concentration, and a precise evaluation was made of the Caco-2 cell model [48]. Another study introduced SPME to determine the free concentration of chlorpromazine in aqueous samples containing albumin [49]. Binding studies of carbamazepine were conducted *in vitro* with SPME and LC/UV [50].

For an *in vitro* metabolism study, a high-throughput bioanalytical method was applied using 96-blade thin-film SPME and LC/MS/MS for the study of selected compounds and their main metabolites [51]. A special SPME with a new type of β-cyclodextrin-modified nanocellulose as a sorbent material showed a wide linear fluorimetric response against danofloxacin. This special SPME's recognition has been proven to be highly selective and

efficient against this metabolite and other fluoroquinolones [52]. In a stereo-selective study of fungal biotransformation, SPME determined whether fungi could biotransform risperidone into its active metabolite [53]. Previous sample collection and complicated processing after biotransformation may be associated with errors, and these errors can be avoided by applying SPME technology, which is advantageous due to its simplicity.

3.1.4 Naturally occurring microorganisms—The applications and dynamics of lactic acid bacteria for the four-season production of Vastedda-like cheese were investigated. SPME with GC/MS detected the VOCs of the experimental cheeses, obtained with raw milk and inoculated with single and multiple combinations of lactococci [54].

Preculturing of *Lactobacillus rhamnosus* and *Bifidobacterium animalis* sub-sp. lactis BB12 under sublethal stress conditions for survival and metabolite formation was investigated in set-yoghurt. 35 volatile and 43 non-volatile polar metabolites were identified by SPME-GC/MS and proton nuclear magnetic spectroscopy (SPME-H^1NMR). These data contribute to the possibility of placing stress-adapted probiotics in a food-carrier environment [55]. The effects of probiotics and prebiotics on the metabolic profile of human microbiota have been analyzed with sampling from human feces by SPME-GC/MS [56].

The identification of VOCs produced by *Cladosporium cladosporioides*, which could accelerate botanical growth, was detected by SPME-GC/MS. Promotion of fungal VOC-mediated botanical growth requires in-depth study for application to large scale crops, particularly those grown under greenhouse conditions [57]. The VOCs in solid-state and submerge-cultured *Antrodia camphorate* were also accessed by HS-SPME-GC/MS [58]. The starters of yoghurt are critical to the quality of product. Thus, the discovery of different proteolytic strains of *Streptococcus thermophilus* in the production of set-yoghurt should allow for better control of the quality of yoghurt starters [59].

3.1.5 Environmental pollutants—Indoor molds produce microbial VOCs (MVOCs). MVOCs have been detected with automated HS-SPME and GC/MS analysis. MVOCs produced by malt extract agar, plasterboard, and wallpaper have been compared [60]. In soil, the bioavailability of estrogen-like endocrine-disrupting compounds contributed to the assessment of risk to the environment using thin film geometry SPME (TF-SPME) [61]. With respect to water pollution, petroleum-related substances from refineries and refinery effluents in water were studied with biologically based SPME methods [62]. The technique also can be used to screen environmental petrochemical contamination in seafood [63].

3.1.6 Medical diagnosis from biological samples—TF-SPME coupled to LC/MS/MS analysis was developed to measure bile acids in fluid samples from the bronchoalveolar lavage. Thus, metabolites may provide additional information about the occurrence and severity of gastric reflux/aspiration in lung patients, which will lead to a more accurate diagnosis [64]. Cocaethylene (CE) is a metabolite formed during alcohol and cocaine co-consumption. CE in hair is a biomarker indicating chronic alcohol consumption among individuals who have consumed cocaine. HS-SPME coupled with GC/MS analysis is able to reveal CE as biomarker. Specificity for chronic excessive alcohol consumption was high among cocaine users and other drug addicts [65].

Another study used SPME to collect headspace vapors from methicillin-sensitive *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) grown *in vitro* in liquid growth medium. The collected molecules were separated and identified by GC/MS. The data distinguished the two strains and provided the foundation for a biomarker library to identify specific bacterial infections [66]. Penicillin-binding protein 2a also can be detected by antibody-linked immuno-affinity SPME sorbents. The established immuno-affinity platform is expected to provide insights into the development of a specific, sensitive, accurate and practical assay for diagnosing MRSA [20].

HS-SPME greatly facilitated the identification of VOCs in human feces for diagnosis and health implications [67]. After investigating the effect of certain organophosphorus pesticides on breast cancer risk, other researchers concluded that endocrine-disrupting chemicals alter normal functioning. *In vitro* experiments were conducted utilizing HS-SPME combined with HPLC. The results revealed that chlorpyrifos binds to one class of sites on sex hormones [68].

Results of SPME technology from *in vitro* samples are shown in Table 1. Only after accurate SPME analysis is achieved with *in vitro* samples can the new technique be applied to *in vivo* samples.

3.2 *In vivo* analysis

3.2.1 Botanicals—The health benefits of herbs and edible plants are remarkable, and botanical materials have been used as preventative measures or as treatments for disease for thousands of years. Thus, the composition of botanical matrixes from living plants has gained more attention in recent years. With the rapid development of technology, botanicals and their metabolites can be determined using SPME coupled with other advanced analytical methods.

Orchids are one example of botanicals that can be analyzed using SPME coupled methods. When SPME was applied to Italian grown orchids, HS-SPME-GC/MS proved a suitable technique for distinguishing the volatile fingerprint of different orchid species [69]. One study tested whether SPME could uncover the fingerprints of volatile and semi-volatile metabolites in complex samples. With SPME-comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry (GC/GC/TOF-MS), 399 metabolites of apples were identified [70]. These results confirm that metabolites can be characterized in botanical samples *in vivo*.

3.2.2 Food science—SPME is compatible with methods for analyzing food samples. For rapid food analysis and cleanliness monitoring, the volatile metabolites in farmed and wild European sea bass have been accurately distinguished by SPME followed by GC/MS. These data increased our knowledge of the quality of raw and processed sea bass. For example, the off-flavor components in live fish were detected by SPME, and the detection limit was as low as 0.12ng/g for geosmin and 0.21 ng/g for 2-methylisoborneol, which are far below human sensory thresholds [71,72]. Similarly, the uptake and elimination of organic pesticides in fish muscle were traced by SPME plus GC/MS [73].

One *in vivo* experiment showed that SPME was reliable for detecting changes in the volatile fingerprint of *Achillea collina*, changes separately induced by an infestation of aphids, mechanical damage, or jasmonic acid. Differences were clear between control, infested, damaged and jasmonic-treated plants [74]. The responses of plants to external stimuli are traceable and of value in food science. In another example, the biosynthesis of sesquiterpenes in the grape berry exocarp of *Vitis vinifera* was evidence for transport of farnesyl diphosphate precursors from plastids to the cytosol. The method used was HS-SPME-GC/MS [75]. This SPME has also been applied in the deep processing of grapes. Notable changes in some aroma compounds of Moscatel sparking-wine were detected in the production process. Data showed the 75 compounds were co-eluted [76]. Even extremely small differences in the components could be detected.

3.2.3 Pharmaceutical research and development—Most pharmaceuticals are synthetic or semi-synthetic compounds. The absorption, distribution, metabolism and excretion of pharmaceutical compounds have been studied with SPME. The technology has also contributed to drug discovery. The anti-microbial potential of extracts of the liverwort *Scapania aspera* was investigated, and the chemical composition of the extracts was determined by SPME-GC/MS. The experimental results suggested that *S. aspera* contains natural anti-microbial agents [28].

The diffusion-based calibration interface model of SPME has been proposed for the analysis of the pharmacokinetics of selected drugs. For *in vivo* SPME sampling, this model has several advantages over other kinetic calibration models: (1) it does not require the addition of a standard into the sample matrix, (2) it eliminates the need to pre-load a standard onto the SPME extraction phase, and (3) the calibration constant can be calculated [77]. The *in vivo* detection of drugs and their metabolites can contribute to pharmaceutical research. A SPME probe inserted directly into the peripheral vein of a living animal can monitor and quantify the concentration of drugs and their metabolites [1].

A space-resolved SPME technique was used to study the tissue-specific bio-concentration of pharmaceutical agents in live fish, which is critical for monitoring antibiotic abuse. SPME needles were segmented and coated with novel fibers to detect specific pharmaceutical residues in fish dorsal epaxial muscles and adipose tissues with repeat *in vivo* sampling of tissues. Precision was acceptable [78,79]. As material science and analytical technology further develop, the accuracy of this technique will be further improved.

3.2.4 Microorganisms in the body—Metabolomics studies the anabolic and catabolic pathways of bacteria and fungi and the dynamics of their metabolism. Metabolites with low molecular weights can be qualitatively and quantitatively analyzed. VOCs fingerprinting of *Listeria monocytogenes* was recognized by SPME-GC/MS and E-nose in pure culture medium. Analysis of the VOCs fingerprint of microorganisms has the potential to become routine in microbiology studies [80].

Aliphatic amides were applied to detect *Helicobacter pylori* using SPME coupled with GC/MS. Propionic and butyric acid were the biomarkers for *H. pylori* after incubation with

the corresponding amides. SPME also detected the acids and verified their hepatic stability. The sensitivity of detection of both acids was in amounts as low as 0.8 μg [81].

Human gastrointestinal microbiota have become the subject of extensive research in recent years. Fecal microbiota excreted by 30 healthy volunteers after treatment with Khorasan wheat was detected by SPME-GC/MS. The data verified the anti-inflammatory effect and counteraction of oxidative stress by Khorasan-based cereal foods [82].

3.2.5 Analysis of environmental pollutants—Environmental pollution is becoming a serious problem in the world, so effective sampling methods may strengthen environmental governance. *In vivo* sampling of organic contaminants in fish with SPME improved the sensitivity and extraction kinetics of the determination of trace pharmaceutical pollutants in fish tissue. A novel thin film micro-extraction configuration based on C18 thin film was introduced [83]. Another study developed and improved SPME for the sampling of pharmaceuticals in fish tissue. SPME with a PDMS extraction phase was a robust tool and was simpler than the traditional device. The new device is a platform for rapid sampling of carbamazepine, diazepam, and nordiazepam in fish muscle with acceptable precision [83].

The quantitative evaluation of (+)-Δ^3-carene metabolites from the living larvae of *Spodoptera litura* was possible with HS-SPME. The method was sensitive enough to quantitate the (+)-Δ^3-carene metabolites released from the *Spodoptera* larvae [84]. The medication lindane, widely used to treat agricultural pests, was evaluated for its toxicity, persistence and tendency to bioaccumulate in terrestrial and aquatic ecosystems. In the study, lindane was extracted by SPME and identified by GC/MS. Its metabolite, 1,3,4,5,6-pentachloro-cyclohexene, contributed to the *in situ* bioremediation of this pollutant [85]. In the common reed, *in vivo* sampling was performed on a site highly polluted with methyl tertiary butyl ether. SPME fiber was directly introduced into the aerenchyma of the botanical stem. This method seems feasible for the screening of VOCs in wetlands [86].

3.2.6 Disease diagnosis—Supported by many studies, SPME is a reliable technology for early diagnosis. Rapid breath analysis was performed by SPME in cystic fibrosis patients, and the analytical data helped the diagnosis of the disease in the near future [87]. TF-SPME combines sampling and sample preparation into a single step. *In vivo* sampling using TF-SPME coupled with GC and LC for 5 minutes revealed a wide range of analytes with different physical and chemical properties [7]. HS-SPME sampling for VOCs in humans significantly reduced background signal intensity, and resulted in reproducible analysis. The method can be used to detect the biomarkers of garlic intake and alcohol ingestion [88].

During surgery, metabolites can be collected directly with SPME for biochemical analysis and for biomarker identification [89]. SPME coatings functionalized with a DNA aptamer can selectively enrich aspecific proteins from diluted human plasma. This SPME was successfully applied to detect thrombin in human plasma [19]. When SPME was used to screen for VOCs, the results suggested a possible cause of death from a rare case of captan ingestion [90]. With convenient sample preparation [91], SPME could be a valuable tool for the early diagnosis of cardiovascular, oncologic and neurodegenerative illnesses. Selected reports that used SPME for *in vivo* sample analysis are shown in Table 2.

4. Conclusion and outlook

Sample collection is critical to the determination of various compounds and their metabolites in different matrices. In the last few years, there has been a notable increase in the application of SPME. This new technology is sensitive, selective, simple, reproducible and relatively noninvasive. Thus, SPME is a well-established technique for the study of the chemical composition of botanicals, food and pharmaceuticals, all of which could be affected by microorganisms and the environmental factors. SPME is particularly useful for medical diagnosis because its tissue damage is minimal. The application of SPME to the diagnosis of human diseases, however, is still in its early stage. In the future, the application of SPME in metabolite analysis will depend on advances in specific analytical technologies and material science. It is anticipated that the sensitivity and accuracy of the detection and analysis of non-volatile compounds from minute samples will be increased. SPME has a promising future in biomedical and pharmaceutical research.

Acknowledgement

This work was supported by the Fundamental and Frontier Research Fund of Chongqing under Grant cstc2014jcyjA10108; Fundamental Research Funds for the Central Universities under Grant CQDXWL-2014-Z007; Special Fund for Basic Scientific Research of Central Colleges, Chongqing University under Grant No. 201310611045 and Fundamental Research Funds for the Central Universities under Grant CQDXWL-2012-031; Tang Foundations. We thank Sally Kozlik for editing the manuscript.

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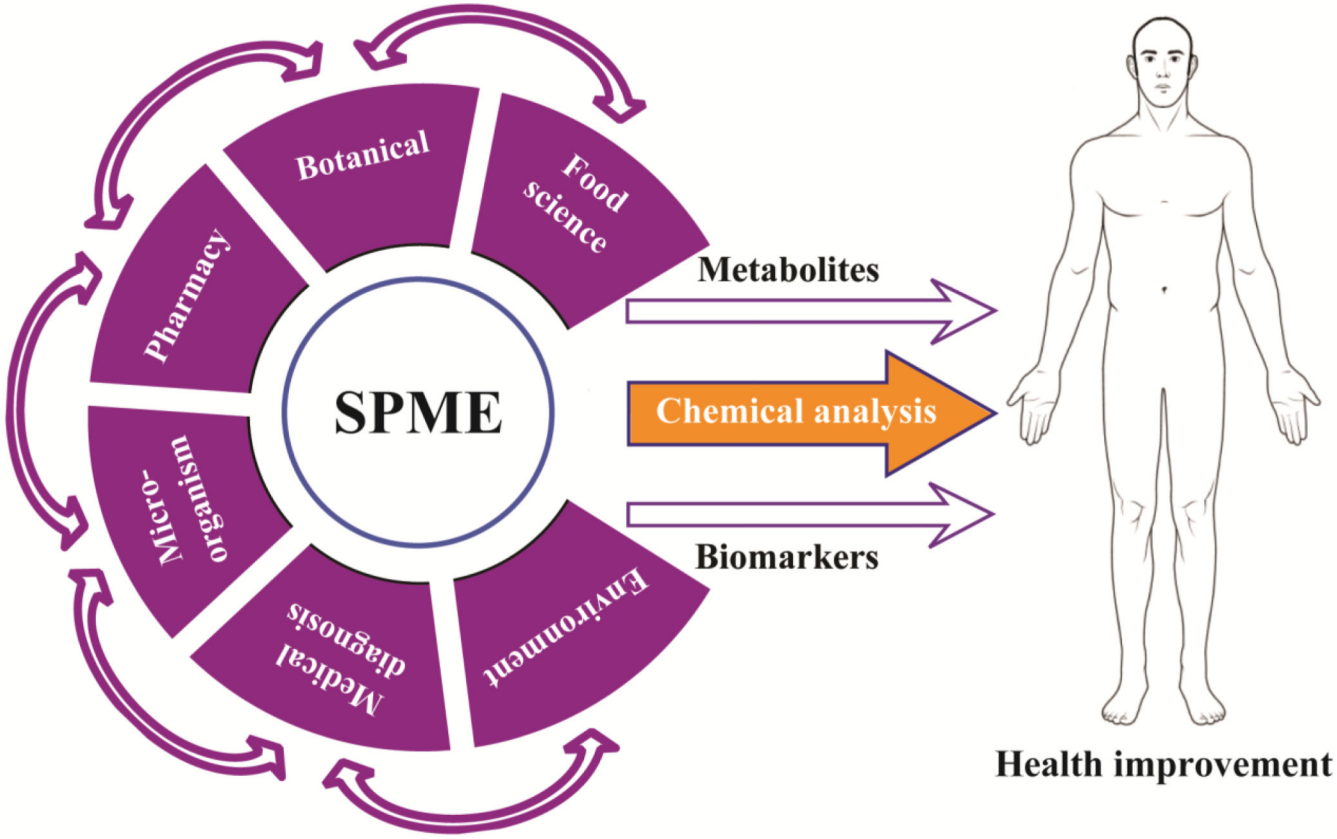

Fig. 1.

Application of solid-phase microextraction (SPME) technology. This technique has been used in food science research, botanical component analysis, and pharmaceutical studies. In addition, the SPME has also been reported in research related to microorganisms, environmental factors, and medical diagnosis. The small endogenous molecules detected using SPME investigations are critical for metabolomic analysis and biomarker identification to improve human health.

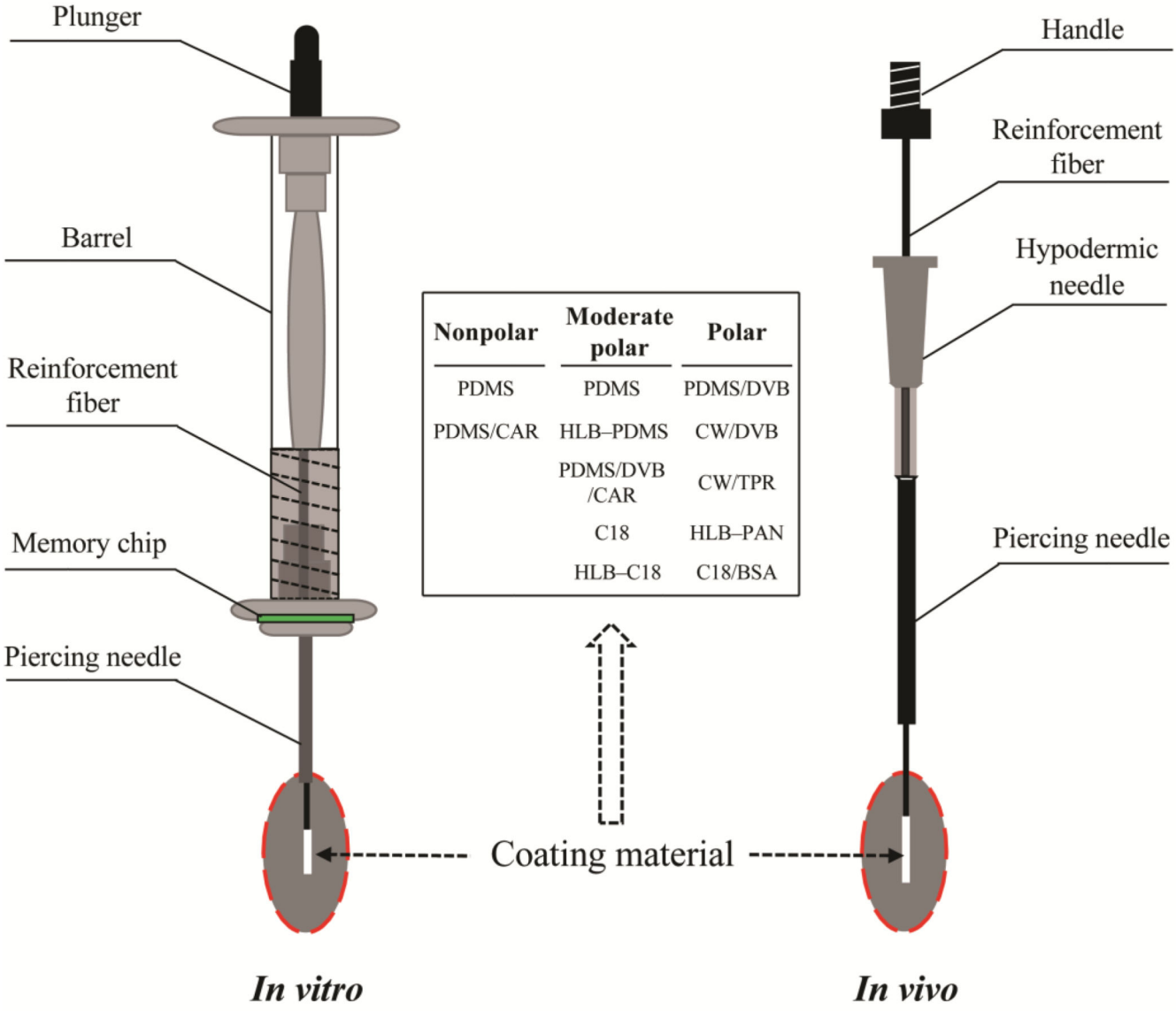

Fig. 2.

Schematic diagram of the *in vitro* and *in vivo* SPME needle apparatuses for metabolite analysis. The tips of the needles can be covered by various coating materials. PDMS, polydimethylsiloxane; CAR, carboxen; HLB, hydrophilic lipophilic balanced; DVB, divinylbenzene; C18, octadecylsilyl derivatized silica column packing material; CW, carbowax; TPR, templated resin; PAN, polyacrylonitrile; BSA, benzenesulfonic acid.

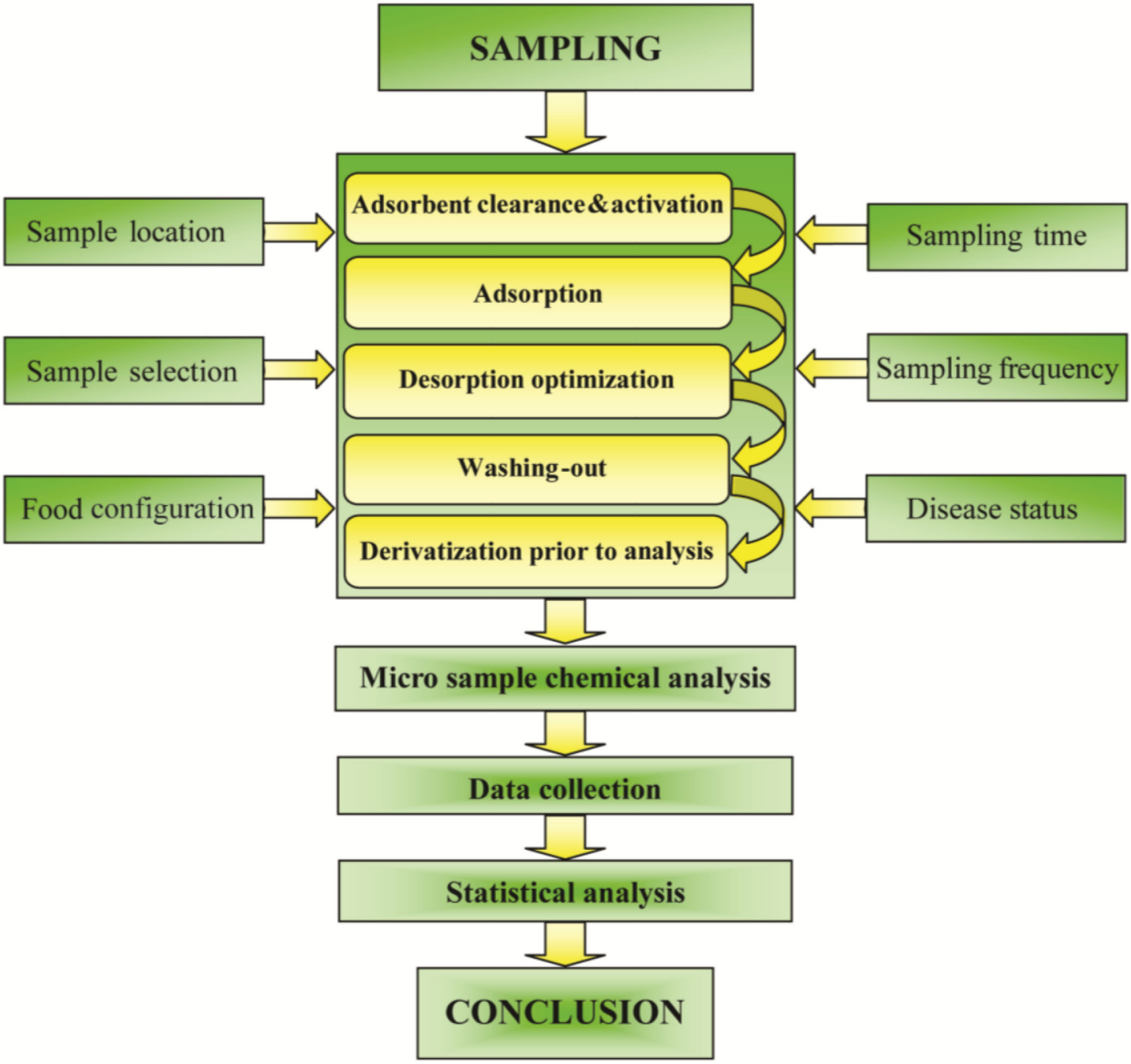

Fig. 3.
Flow chart of the analytical process using SPME as sample preparation technology. The sampling is influenced by six factors, which are listed on the right and left.

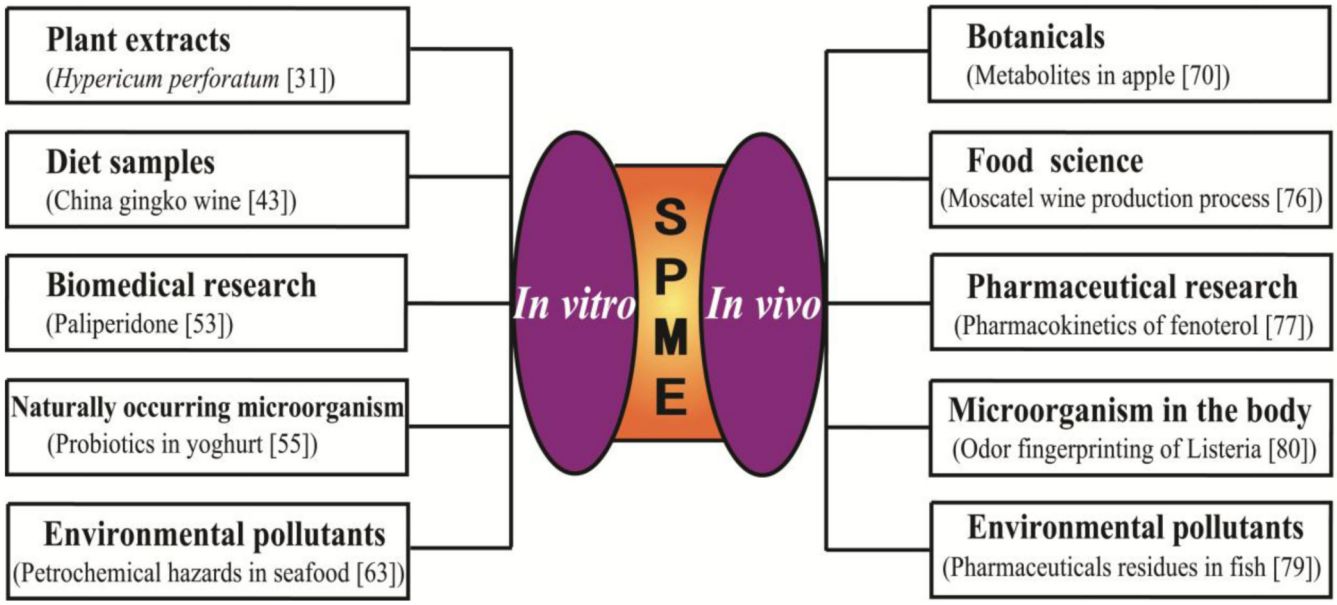

Fig. 4.

Different investigations using SPME technology. A representative study in each category is selected.

Table 1

Selected reports using the SPME technology in *in vitro* sample analysis

	Study subject	Fiber coating	Thickness (μm)	Sample (g), part	Condition (°C/min)	Analytical method	Ref.
Plant Extract							
Pinene, sabinene	*Peucedanum cervaria*	PDMS	30	2.0, fruit	RT/30	GC/MS	[30]
Aldehydes	*Hypericum perforatum*	PDMS; PDMS/CAR	75; 100	Extract	40/20	GC/ECD	[31]
VOCs, carotenoid	*Dracaena draco*	PDMS/DVB	65	0.1, leaf	45/20	GC/MS	[32]
Sesquiterpene, monoterpenes	*Ficuscarica*	PDMS/DVB	65	Fruit	40/60	GC/MS	[33]
VOCs	*Plinia cerrocampanensis*	DVB/CAR/PDMS	30; 50	0.5, leaf	49/14	GC/MS	[34]
VOCs	*Pelargonium graveolens*	PDMS/DVB	65	0.1/0.2, leaf	RT/2	GC/MS	[35]
Terpinyl acetate, monoterpenes	*Plectranthus* spp.	PDMS	100	1.0, callus	60/20	GC/MS	[37]
Terpinene, cymene, thymol	*Thymus vulgaris*	PDMS/DVB	65	0.2, leaf	60/15	GC/MS	[39]
Volatile ethyl esters	Dwarf Cavendish banana	PDMS/DVB	65	0.5, fruit	50/60	GC/MS	[40]
Diet Sample							
Cineole, limonene, pinene, terpinene	*Eucalyptus*	PDMS/DVB	65	5.0, essential oil	RT/10	GC/MS	[41]
Ester, alcohol, acid, hydrocarbon	Gingko wine	PDMS /CAR	75	5.0 ml, wine	50/45	GC/MS	[43]
Monoterpene, aldehyde, alcohol	*Vitis vinifera* grapevine	DVB/CAR/PDMS	30; 50	5.0, grape berry	40/40	GC/MS	[45]
Metabolite	Fecal microbiota	PDMS/CAR	85	3.0, stool/urine	45/40	GC/MS	[82]
Biomedical Research							
Chlorpromazine	Aqueous containing albumin	PA	30	0.2, solution	RT/420	LC/UV	[49]
Carbamazepine	Albumin or mouse plasma	C18		1.8 ml, plasma	RT/50	LC/NMR	[50]
Risperidone/9-OH risperidone	*Cunninghamella* fungal	C18	45	2.0 ml, supernatant	RT/30	LC/MS	[53]
Naturally-occurring Microorganisms							
Acid, alcohol, aldehyde, ester	Vastedda-like cheese	DBV/CAR/PDMS		10.0, cheese	60/30	GC/MS	[54]
Metabolites	Stool sample	CAR/PDMS	85	3.0, feces	45/40	GC/MS	[56]
Pinene, caryophyllene	Tobacco	PDMS/DVB	65	6.0 ml, culture	25/30	GC/MS	[57]
VOCs: alcohol, heterocycle, etc.	Yoghurt	Carboxen/PDMS	75	3.0 ml, culture	RT/10	GC/NMR	[59]
Environmental Pollutants							
MVOCs: methylpropanol, etc.	Indoor mold	DVB/CAR/PDMS	30; 50	Fresh culture	35/30	GC/MS	[60]
Alkylbenzene, tetralin, naphthalene	Seafood	PDMS; PDMS/DVB	65; 100	20.0, seafood	65/3	GC/MS	[63]
Sample for Medical Diagnosis							

	Study subject	Fiber coating	Thickness (μm)	Sample (g), part	Condition (°C/min)	Analytical method	Ref.
Penicillin binding protein 2a	PBP2a extraction solution	Immuno-SPME		25.0, extraction	RT/720	LC/MS	[20]
Biomarker library	*Staphylococcus aureus*	CW/DVB; CAR/PDMS	70;75	5.0 ml, culture	RT/10-480	GC/MS	[66]
Chlorpyrfos, sex hormone	CPS and hormone solution	PA-HF	400	Solution	42/30	LC/UV	[68]

Abbreviations: Apt-PANCMA-aptamer functionalized poly (acrylonitiile-co-maleic acid); CAR-Carboxen; HLB-hydrophilic lipophilic balanced particles; MVOCs-microbial volatile organic compounds; PA-polyacrylate; PDMS-polydimethylsiloxane; RT-room tempreture; VOCs-volatile organic compounds.

Table 2

Selected reports using the SPME technology in *in vivo* sample analysis

	Study subject	Fiber coating	Thickness (μm)	Sample (g), part	Condition (°C/min)	Analytical method	Ref.
Botanical							
Hydrocarbon, aldehyde, furan, etc.	*Ophrys sphegodes*	DVB/CAR/PDMS	50; 30	Flowering plant	280/180	GC/MS	[69]
Food Science							
Geosmin, 2-methyl isoborneol	Rainbow trout	PDMS	65	4.0, tissue	RT/30	GC/MS	[72]
Pesticide Residue	Tilapias, Pomfrets	PDMS	44; 165	Epaxial muscle	RT/10,20	GC/LC/MS	[73]
Precursor of sesquiterpene	*Vitis vinifera*	PA	85	1×10^{-4} , precursor	60/10	GC/MS	[75]
Monoterpene, ester, alcohol	Moscatel sparkling wine	PDMS/DVB	65	2.0 ml, wine	40/10	GC/GC/MS	[76]
Pharmacy Research & Development							
Methoxy fenoterol/fenoterol	Rat	C18/Cyanopropyl		Organ	RT /4	LC/MS	[77]
Drug residues	Rainbow trout	PDMS	330	Organ	RT /10	LC/MS	[78]
Mefenamic acid/fluoxetine	Fish	PS@PDA-GA	100	Organ	RT /10	LC/MS	[79]
Microorganisms in the Body							
Propionic / butyric acid	*H. pylori* reference strain	CW/DVB		25.0 ml, fresh culture	37/5	GC/MS	[81]
Analysis of Environmental Pollutants							
Fluoxetine, venlafaxine, sertraline, etc.	Rainbow trout, Fathead minnow	C18	45	Epaxial muscle	RT/1440	LC/MS	[83]
(+)-Δ^3-carene metabolite	*Spodoptera litura*	DVB/CAR/PDMS	50; 30	0.5, larvae	25/30	GC/MS	[84]
Pentachloro cyclohexene	*Hymeniacidon perlevis*	PDMS	100	15.0, seawater	50/30	GC/MS	[85]
Methyl *tert*-butyl ether	*Phragmites australis*	CAR/PDMS	85	Plant sampling	RT/120	GC/MS	[86]
Disease Diagnosis							
Endogenous steroid	Human saliva	PDMS/HLB/C18	65	1.0 ml, saliva	RT/5	GC/MS	[7]
Thrombin	Human plasma	Apt-PANCMA		2.0 ml, plasma	RT/60	LC/MS	[19]
VOCs	Human exhaled breath	CAR/PDMS	75	100.0 ml, EB	RT/10	GC/MS	[87]
DMSO, AMS, allyl-mercaptan	Skin	PDMS	254	Skin	40/60	GC/MS	[88]
Methylprednisolone	Male Yorkshire pig	C18/BA	45	Organ	RT/20,30	LC/MS	[89]
Captan and its metabolites	Human viscera content	PDMS	100	1.0 ml, blood/GI	40/20	GC/MS	[90]

Abbreviations: Apt-PANCMA-aptamer functionalized poly (acrylonitiile-co-maleic acid); BA-benzenesulfonic acid; CAR-Carboxen; EB-exhaled breath; HLB-hydrophilic lipophilic balanced particles; MVOCs-microbial volatile organic compounds; PA-polyacrylate; PDMS-polydimethylsiloxane; RT-room tempreture; VOCs-volatile organic compounds.