Abstract: The inherent complexity of the human biological matrix and its importance in modern medical diagnosis and medical research promote the development of modern analytical technology. Solid-phase microextraction technology has been widely used in the treatment and analysis of different complex biological matrices due to its smaller sample size, simpler sample preparation and setting, and lower consumption of harmful chemicals. This review provides updated information on headspace solid-phase microextraction combined with gas chromatography technique applications, focusing on the analysis of volatile and semivolatile compounds in human biological matrices. The application of headspace solid-phase microextraction combined with gas chromatography techniques in human biological matrix analysis is mainly summarized into three aspects, namely, to discover biomarkers, to investigate volatile metabolomics, and to explore the effect of the external environment on volatile metabolomics of the human biological matrix. In addition, the frequently used statistical analytical methods are summarized, and the application prospect of solid-phase microextraction in the analysis of human biological matrices is proposed.

Keywords: headspace solid-phase microextraction, gas chromatography, human biological matrices, volatile organic compounds, statistical analysis methods

1 Introduction

Many important fields related to human health, such as disease diagnosis and medical research, rely on the analysis of one or several chemical and biochemical substances in biological matrix samples [1]. The analysis of chemical and biochemical substances usually involves compound structure identification or structure elucidation and then quantification to measure the actual concentration level in the sample. These human biological samples are usually complex matrices, such as urine, blood, saliva, breast, and hair. Volatile organic compounds (VOCs) can reflect the human metabolic level, hormone changes, microbial activities, and the presence of environmental pollutants [2]. These compounds and their subtle changes during the disease are clearly reflected in body fluids and exhaled gases [3]. In recent years, the application of VOCs in diagnosis has aroused people’s interest, which provides a faster and cheaper method than the existing diagnosis. An increasing number of studies are concerning assessing health by analyzing VOCs in biological matrices [4].

Sample pretreatment is an important step in the analysis of complex biological matrix samples. There are two main reasons. First, human biological matrices are usually composed of various ions, proteins, lipids, and carbohydrates, which cannot be directly injected into the analytical instrument. Second, highly abundant interfering compounds interfere with the identification and quantification of microanalyses. The purpose of the sample pretreatment is to eliminate the interference of compounds in the matrix samples to the analysis to meet the requirements of matrix effect, accuracy, repeatability, and lower limit of sensitivity. The traditional methods for preparing human biological samples mainly include two categories, namely, solid-phase extraction (SPE) and liquid–liquid extraction (LLE), which are widely used [5]. However, these methods have some disadvantages to varying degrees, such as being time consuming and
laborious, requiring large quantities of samples and solvents, affecting trace analysis due to solvent residues, and increasing environmental pollution [6,7]. In addition, due to the complexity of human biological matrices, complex pretreatment is usually required before analysis. A long sample pretreatment time may not meet the rapid detection requirements of many analytes. Therefore, rapid and sensitive detection is highly required in the direct analysis of raw samples.

To overcome the limitations of traditional sample preparation methods, many attempts have been made. The modern trend of green chemical analysis has led to the development of microscale extraction methods, which aim to minimize the consumption of organic solvents, sample throughput, and recovery of the analyte. Therefore, in the past few years, microextraction has become the frontier of analytical chemistry [8]. Solid-phase microextraction (SPME) is a new sample pretreatment method. The analyte is extracted directly and concentrated on the solid phase of the SPME fiber. Compared with LLE and SPE, this method saves time, labor, and solvent and has high detection sensitivity [9]. Headspace solid-phase microextraction (HS-SPME) has become the preferred method for the analysis of volatiles mixed in complex human biological matrices. An illustration of the analysis process for HS-SPME is presented in Scheme 1. Moreover, this method has been widely used in food analysis [10], environmental analysis [11], biomedical studies [12], and judicial expertise [13]. As expected, SPME is usually combined with powerful separation and optical technologies to allow enhanced recovery, selectivity, and sensitivity of analytes. Volatile and semi-volatile compounds are usually detected by gas chromatography (GC) or gas chromatography–mass spectrometry (GC-MS) [14]. Weak volatile and thermally unstable compounds were detected by liquid chromatography or high-performance liquid chromatography mass spectrometry [15]. This technique can also be directly combined with capillary electrophoresis [16], GC plasma coupled mass spectrometry, and other detection methods [17]. Therefore, SPME is widely considered and applied by scientific researchers and analytical practitioners.

SPME mainly includes two steps: adsorption (extraction) process and desorption process. The whole process is the dynamic balance between three phases. In general, the substances dissolved in the analytical sample diffuse into the headspace (HS) with increasing sample temperature and stirring, and the compounds are then distributed between the HS and fiber or droplet solvent [18]. The adsorption methods mainly include HS-SPME and direct solid-phase microextraction (DI-SPME). After the analyte enters the coating of the SPME fiber from the sample, the adsorption process is completed, and then the SPME fiber is directly inserted into the chromatographic sample inlet for desorption and separation of the adsorbate. HS-SPME places the fiber in the evaporation phase above the sample, which can avoid the interference of substances with high molecular weight or nonvolatile substances in the sample matrix. This technique is suitable for the analysis of volatile and semi-volatile substances [19]. HS-SPME-GC has become a valuable technique for the separation of volatile analytes, which simplifies the analysis by eliminating interference and matrix effects. In addition, when the sample matrix is solid and nonuniform, such as hair, tissue, and insects, the HS extraction procedure can be used. In contrast, in the DI-SPME process, the SPME fiber is directly inserted into the liquid sample or exposed to the gas sample, and the analyte is directly transferred from the sample to the SPME fiber, which is suitable for the analysis of clean samples and gaseous samples [20]. Therefore, HS-SPME has attracted increasing attention from medical researchers and human biological matrix analytical technicians.

This article summarizes the application of HS-SPME in a human biological matrix from three aspects, namely, to discover biomarkers, to investigate volatile metabolomics, and to explore the effect of the external environment on the volatile metabolomics of the human biological matrix. In addition, the commonly used data analysis methods are summarized, and the application prospect of SPME in the analysis of human biological matrices is proposed. The keyword searches were conducted in the Web of Science database.

2 Application of HS-SPME-GC for the analysis of human biological matrices

The chemical components in the human biological matrix usually have different physical and chemical properties.

![Scheme 1: Illustration of the analysis process for HS-SPME-GC.](image)
These chemicals can provide a large amount of information about people’s health. Research on the chemical composition of these samples has always been a hot spot in the scientific community, because these samples contain much information that can indicate the relevant information of an organism’s toxicological exposure [21], habit preference [22], drug fate [23], and metabolic cycle [24]. Volatile compounds with biological significance are key substances because they can reflect the characteristics of the biological state. HS-SPME-GC provides convenience for the detection of VOCs in complex biological matrices. In general, HS-SPME has been applied to human biological matrices with the aim of discovering new biomarkers, investigating volatile metabolites, and exploring the important ingredients or the effect of the external environment on volatile metabolites of the human biological matrix.

2.1 To discover biomarkers

VOCs are used as biomarkers in a variety of disease and environmental exposure studies. For example, the chromatographic patterns of VOCs extracted from normal cells and cancer cells are very different, indicating that these compounds may be used as new volatile biomarkers. These biomarkers may become a new diagnostic method.

Among them, the analysis of urinary VOC profiling has attracted the most attention in recent years because it is a noninvasive and convenient method. It has been successfully used in the detection of a variety of diseases and can provide unique biomarkers [25]. Currently, a large number of trials mainly focus on urinary metabolomic biomarkers (such as VOCs) of many diseases. Liu et al. found that urinary VOCs could be used as biomarkers for the minimal change-type nephric syndrome. These VOCs included trans-2,2-dimethyl-4-decene, pyrrole, carboxamic acid, monoammonium salt, 3,3-dimethyl-1-butene, diisopropylamine, and 4-heptanone [26]. SPME coupled with GC-MS was used to analyze the VOCs. Drabinska et al. quantitatively analyzed 15 biomarkers in urine to distinguish healthy children from children with celiac disease. They found that the most effective fiber for the analysis of the selected biomarkers was carboxen/polydimethylsiloxane fiber in the HS mode [27]. Naccarato et al. provided a reliable and rapid method for the determination of one of the most important cancer biomarkers for early diagnosis and treatment. The protocol was based on SPME-GC-MS/MS analysis, before which propyl chloroformate is directly used in the derivatization step in raw urine [28]. Other selected reports using the HS-SPME-GC to discover biomarkers in human biological matrices are presented in Table 1.

In addition, blood is the most informative part of the human physiological state, because it is in constant contact with the whole body and in balance with organs and tissues [33]. Therefore, some volatiles exist not only in the blood but also in other liquids. Therefore, the search for biomarkers of some diseases in the VOCs of other human body fluid matrices has also attracted extensive attention. These fluid matrices include saliva [34], plasma [35], skin [38], and hair [39].

2.2 To investigate volatile metabolomics

Metabolomics, in general, is the study of metabolites or metabolite fingerprints produced in metabolic processes. Metabolic profiling outlines a person’s cellular and physiological state. Therefore, it is widely recommended to diagnose the health state in the clinical environment [40]. A plethora of studies have documented that the extraction method of SPME is considered important in volatile metabolomics. Studies have shown that the odor substances emitted from human secretions and respiration mainly contain VOCs that reflect the physiological conditions of animals and humans. In the case of illness, VOCs change considerably. Therefore, the analysis of VOCs in human biological matrices can provide information on metabolic changes caused by disease. The study of volatile metabolites using HS-SPME-GC in body secretions of diseased individuals has attracted increasing attention. Metabonomics describes a person’s health and disease status. This method is becoming an attractive tool for the development of precision medicine.

Urine is considered to be a potential source of volatile metabolomics to reveal diseases. Compared with other biological matrices, urinary VOCs have been studied more. Approximately 230 VOCs have been detected and identified in human urine [26]. These VOCs include ketones, hydrocarbons, aldehydes, terpenes, heterocyclic compounds, and sulfur-containing compounds. VOCs in urine contain potential substances that can be used as biomarkers of health status. Bannaga et al. found that urinary VOCs detected by applying the SPME technique can distinguish between control and liver cancer, prostate cancer, and bladder cancer [41]. The multivariate optimization method first reported by Semren et al., including reduction factor and Doehlert matrix design, was used to
Table 1: Selected reports using the HS-SPME to discover biomarker in human biological matrices

| Matrix          | SPME Fiber          | Analytical method | Major statistical analysis method | Disease diagnosis                                                                 |
|-----------------|---------------------|-------------------|-----------------------------------|-----------------------------------------------------------------------------------|
| Urine           | 75 µm CAR/PDMS GC   | PCA, PLS-DA       | PCA, PLS-DA; ROC curves           | Minimal change type nephrotic syndrome [26], Celiac disease [27], Clear cell renal carcinoma [28], Cystic disease [29] |
| Urine           | 75 µm CAR/PDMS GC   | PLS-DA            | PCA, PLS-DA; ROC curves           | Minimal change type nephrotic syndrome [26], Celiac disease [27], Clear cell renal carcinoma [28], Cystic disease [29] |
| Urine           | 50/30 µm DVB/CAR/PDMS GC-MS | PCA, PLS-DA     | PCA, PLS-DA; ROC curves           | Minimal change type nephrotic syndrome [26], Celiac disease [27], Clear cell renal carcinoma [28], Cystic disease [29] |
| Urine; serum    | 65 µm PDMS/DVB GC   | PCA, PLS-DA       | PCA, PLS-DA; ROC curves           | Minimal change type nephrotic syndrome [26], Celiac disease [27], Clear cell renal carcinoma [28], Cystic disease [29] |
| Plasma          | 50/30 µm CAR/PDMS GC-MS | PLS-DA           | PCA, PLS-DA; ROC curves           | Minimal change type nephrotic syndrome [26], Celiac disease [27], Clear cell renal carcinoma [28], Cystic disease [29] |
| Blood           | 75 µm CAR/PDMS GC   | PLS-DA            | PCA, PLS-DA; ROC curves           | Minimal change type nephrotic syndrome [26], Celiac disease [27], Clear cell renal carcinoma [28], Cystic disease [29] |

Note: PDMS/DVB, polydimethylsiloxane/divinylbenzene; VOCs, volatile organic compounds; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane; PCA, principal component analysis; PLS-DA, partial least squares discrimination analysis; ROC curves, receiver operating characteristic curves.

has attracted increasing attention in recent years, as VOCs can indicate the state of the internal and external environment, which people are exposed to. The characteristics of volatile compounds in human biological matrices are summarized in Table 1.

Long-term exposure to some chemicals may have an impact on human health. These substances may come from food additives, organic combustion (such as fuel, wood, and tobacco), and water disinfection byproducts via ozonation. Therefore, the detection of these substances in the human matrix is very important. VOCs in different human matrices have important biological significance. Their subtle changes can reflect the state of organisms. Therefore, changes in VOCs can be detected to evaluate the impact of the external environment on human health. These include VOCs associated with environmental pollution, such as VOCs in saliva, which may change as a result of human diet, tobacco, drugs, etc. The HS-SPME-GC-MS technique has been used to study VOCs in saliva samples [42]. Cozzolino et al. used HS-SPME-GC-MS to evaluate the characteristics of urinary volatile metabolites in samples of normal weight and overweight/obese children [43].

Although there are relatively few studies on VOCs in other human biological matrices compared with urine, they have also attracted some attention. Longo’s team analyzed the composition of VOCs in human semen in detail by the HS-SPME-GC-MS method and evaluated the different frequencies of specific compounds in men with normal sperm count, oligospermia, and asthenospermia [44]. Campanella et al. proposed a simple analytical method based on HS-SPME-GC-MS for the analysis of VOCs in salivary glands without any derivatization steps [45]. Cavaco et al. explored the potential components of saliva samples as for the noninvasive diagnosis of breast cancer using HS-SPME-GC-MS followed by multivariate statistical analysis [46]. Other selected reports using the HS-SPME-GC to investigate volatile metabolomics in human biological matrices are presented in Table 2.

### 2.3 To explore the important components or the effect of the external environment on volatile metabolites of the human biological matrix

Long-term exposure to some chemicals may have an impact on human health. These substances may come from food additives, organic combustion (such as fuel, wood, and tobacco), and water disinfection byproducts via ozonation. Therefore, the detection of these substances in the human matrix is very important. VOCs in different human matrices have important biological significance. Their subtle changes can reflect the state of organisms. Therefore, changes in VOCs can be detected to evaluate the impact on humans caused by external environmental factors (air pollution, chemical exposure, workplace environment, diet, tobacco, drugs, etc.) to which people are exposed.

As a tool to describe the characteristics of environmental pollution exposure, human biological monitoring has attracted increasing attention [51]. Silva et al. used HS-SPME-GC-high-resolution mass spectrometry to quantitatively analyze 19 aldehydes in human serum, which easily diffuse through the cell membrane and covalently bind with cell macromolecules, destroy function, and...
cause mutation [54]. In addition, Liu et al. pointed out that 3-vinylpyridine (a unique component in tobacco) is a promising biomarker and can be used in tobacco exposure and toxicology research. Therefore, they determined the characteristics of 3-vinylpyridine content in the urine of active and passive smokers by HS-SPME-GC-MS [55]. Sankarganesh et al. used the optimized SPME conditions to explore the significant effect of different storage conditions (refrigerator (4°C), freezing (20°C), or deep freezing (80°C)) on the change in urinary metabolite concentration with time. The results show that storing human urine at 80°C for 6 months, and no more than two freeze–thaw cycles can be considered suitable for metabolomics research [40]. Silva et al. developed and validated a new, high-throughput method for the detection and quantitative analysis of seven terpenoids in human serum using HS-SPME GC-MS/MS. A high-throughput method to quantify terpene levels is useful for population-wide studies of terpene exposure and its potential health effects [56]. Zanella et al. compared the effects of chemically and biologically induced inflammation on the production of volatile metabolites in lung epithelial cells by SPME/GC × GC time-of-flight mass [57]. Tian et al. established an HS-SPME-GC-MS method for the analysis of acetid acid, propionic acid, and butyric acid in feces and serum. The results showed that the intestinal microbial metabolites propionic acid and butyric acid in diarrhea–predominant irritable bowel syndrome patients increased in serum, but not in feces [58]. Blackshaw et al. used HS-SPME-GC-MS to explore the effects of thermal pasteurization, freeze–drying, and gamma radiation on donor human milk. The results show that freeze–drying can be considered to prolong the shelf life of donor human milk at room temperature [59].

3 Frequently used statistical analysis methods

The biological matrix has inherent complexity, and its VOCs are rich and numerous. Therefore, it is of great significance to study the relationship between the changes in VOCs and diseases. Currently, statistical analysis methods are commonly used in the analysis of VOCs in the human biological matrix: principal component analysis (PCA), cluster analysis (CA), partial least squares discrimination analysis (PLS-DA), and receiver operating characteristic (ROC) curves.

PCA is a mathematical method commonly used in the statistical analysis of VOCs, which is a method to reduce dimensions or convert multiple indicators into a few comprehensive indicators. The purpose of PCA is to simplify data and reveal the relationship between VOCs [60]. Therefore, PCA can eliminate the correlation between evaluation indicators and reduce the workload of indicator selection. At the same time, when there are many rating indicators, a few comprehensive indicators can be used to replace the original indicators for analysis while retaining most of the information [61]. Deev et al. used the PCA method to study the possible clustering of urine samples in the process of analyzing the VOCs of urine samples by SPME and GC-MS to develop a noninvasive prostate cancer screening method [62]. Gao and Lee applied the PCA method to analyze the relationship between minimally changed type nephrotic syndrome and healthy subjects when exploring urinary VOCs as biomarkers of minimally changed nephrotic syndrome and obtained a two-dimensional PCA score map with 215 parameters, which has a good separation tendency [25].

Table 2: Selected reports using the HS-SPME to investigate volatile metabolomics in human biological matrices

| Matrix    | SPME fiber | Analytical method | Major statistical analysis method | Ref. |
|-----------|------------|-------------------|-----------------------------------|------|
| Urine     | 75 µm CAR/PDMS | GC-MS             | Response surface plots           | [42] |
| Urine     | 50/30 µm DVB/CAR/PDMS | GC-MS           | PCA; PLS-DA; ROC                 | [43] |
| Saliva    | 85 µm CAR/PDMS | GC-MS             | Correlation plot                  | [45] |
| Saliva    | 75 µm CAR/PDMS | GC-MS             | PCA; PLS-DA; ROC curve; HCA      | [46] |
| Blood     | 65 µm DVB/CAR/PDMS | 1D-GC/Orbitrap-MS; 2D-GC/TOF-MS | PCA                               | [47] |
| Blood     | 100 µm PDMS | GC-MS             | —                                 | [48] |
| Urine     | 85 µm CAR/PDMS | GC-MS             | —                                 | [49] |
| Feces     | 85 µm CAR/PDMS | GC-MS             | PCA                               | [50] |
| Urine     | 85 µm CAR/PDMS | GC-MS             | LDA; ROC                          | [52] |
| Feces     | 75 µm CAR/PDMS | GC-MS             | —                                 | [53] |

Note: PDMS/DVB, polydimethylsiloxane/divinylbenzene; CAR/PDMS, carboxen/polydimethylsiloxane; VOCs, volatile organic compounds; DVB/CAR/PDMS, divinylbenzene/carboxen/polydimethylsiloxane; PCA, principal component analysis; CA, cluster analysis; PLS-DA, partial least squares discrimination analysis; ROC curves, receiver operating characteristic curves; LDA, linear discriminant analysis.
Monedeiro et al. used HS-SPME-GC-MS technology to evaluate the VOC distribution in the saliva of halitosis and submandibular abscesses, and PCA can show the clusters formed according to the evaluation conditions [63].

Different from PCA, PLS-DA is a supervised statistical method of discriminant analysis. In this method, PLS-DA was used to establish the relationship model between metabolite expression and sample category to predict the sample category. The main advantage of PLS-DA is that it allows visual evaluation of group separation [64]. Mesquita et al. found that several metabolites can be used as potential biomarkers of non-Hodgkin lymphoma patients and can describe the metabolism of patients before and after each chemotherapy cycle by using PLS-DA, when they explored the optimization of urine VOC analysis and biomarkers in patients with non-Hodgkin lymphoma before and after chemotherapy [65]. Lima et al. also analyzed volatile metabolites in urine through the PLS-DA model and identified a biomarker to improve the diagnosis of prostate cancer [30].

CA refers to the analysis process of grouping the collection of physical or abstract objects into multiple classes composed of similar objects. The goal of CA is to collect data for classification on the basis of similarity [66]. The results of CA can be presented as a tree diagram or dendrogram, providing a very useful image for processing or the overall relationship between varieties [67]. In a study of VOCs as potential tumor markers in patients with head and neck squamous cell carcinoma, Opitz and Herbarth applied CA to Cluster 81 analyzed volatile metabolites and 135 cases of the whole study population [68]. Pinto et al. used urine metabolomics to identify volatile biomarkers for bladder cancer detection and staging. CA was used to analyze urinary metabolite differences between nonmuscle invasive tumors and muscle invasive tumors [69].

The ROC curve is a coordinate diagram composed of false alarm probability as the horizontal axis and hit probability as the vertical axis, and the curve is drawn by the different results obtained by the subjects under specific stimulation conditions due to different judgment criteria [70]. ROC curves are useful for organizing classifiers and visualizing their performance. In recent years, ROC graphs have been used in medical decision-making, machine learning, and data mining research in abundance [71]. For example, Silva et al. used the ROC curves to verify which metabolites have the highest sensitivity/specificity for the diagnosis of potential BC [31]. Hua et al. evaluated the diagnostic values of the potential biomarkers by the ROC curves [36].

4 Conclusion

The inherent complexity and importance of biological matrices promote the development of modern analytical technology [72]. SPME technology has attracted increasing attention because of its high efficiency, rapidity, simplicity, and lack of solvent. Such a simple and practical sample pretreatment technology for the analysis of complex biological matrices has become the general development trend [73]. This article summarizes the application of HS-SPME in a human biological matrix from three aspects, namely, to discover biomarkers, to investigate volatile metabolomics, and to explore the effect of the external environment on the volatile metabolomics of the human biological matrix. Inevitably, SPME also needs to be improved and further developed. With the deepening of basic theoretical research, the continuous improvement of extraction devices, the continuous development of new coatings with more stability and selectivity, and the development of SPME combined with other technologies, it can be predicted that SPME technology will have a broader prospects in the application of qualitative and quantitative detection of trace components in complex matrices.

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