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Review

Applications of liquid-phase microextraction techniques in natural product analysis: A review

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ABSTRACT

Over the last years, liquid-phase microextraction (LPME) as a simple, rapid, practical and effective sample-preparation technique, coupled with various instrumental analytical methods, has been increasingly and widely used to research and determine trace or ultra-micro-levels of both inorganic and organic analytes from different matrix-complex samples. In this review, different kinds of LPMEs such as single drop liquid-phase microextraction, dispersive liquid–liquid microextraction, and hollow fibre liquid-phase microextraction are summarized and recent applications of LPMEs in trace compounds in *vivo* and in *vitro* from different natural product matrice analysis such as tea, vegetables, seeds, herbs, and galenical are also discussed. Finally, future developments and applications of LPMEs in complex sample analysis are prospected.

Contents

1. Introduction

Natural product analysis constitutes one of the most important fields of the science at present. The analysis of organic and inorganic compounds in natural products has gained considerable interest in recent years [1–3]. This interest is particularly focused on herbal medicines (HMs), including herbs, herbal preparations, herbal materials and finished herbal products, which contain active ingredients of natural products, other natural product materials or combinations [4]. HMs, such as traditional Chinese medicines (TCMs), have performed crucial functions in clinical therapy for numerous diseases and have provided valuable and easily obtainable healthcare resource in many oriental countries for thousands of years [5]. During the last few decades, the use of TCMs has expanded globally, both as primary health care of the poor in developing countries and as the predominant medical treatment in the national health care system [6]. With the widespread use of traditional medicine, safety and effectivity, as well as quality control of HMs and traditional preparation procedure, have become important concerns for both health authorities and the public. However, little is known about the chemical compositions, pharmacokinetics, pharmacodynamics and metabolomics of TCMs to date. In addition, data about identification, efficacy and safety of TCMs are far from sufficiently meeting the criteria to support their use worldwide. This state is mainly due to the following considerations: (1) TCMs are complex matrix, multi-component

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integration and collaborative systems; (2) TCMs contain low active ingredient content; and (3) pretreatment technology or method is not adequate or acceptable for TCMs research and evaluation.

Target compound analysis in complex samples generally includes two steps of sample pretreatment (extraction, separation, purification and concentration) and target compound determination (identification, qualitative and quantitative analysis). Given the evolution of computer, information, and instrument technologies, analysis methods and technologies have been greatly promoted and developed. However, sample pretreatment technologies or methodology have yet to adapt instrument development and meet target analyte requirements in identification, qualitative and quantitative analysis. Therefore, increased efforts are currently being focused on sample pretreatment improvement. In this sense, current trends are moving towards (1) reducing the number of steps required for the procedure, (2) reducing or totally eliminating solvents required for extraction, (3) extending the adaptability to field sampling, and (4) automation [7]. Such enhancement poses a challenging task, especially in the field of natural product analysis, in which the sample is a high complex, multi-component and has low levels of active ingredients systems. Therefore, it is essential to establish a sample pretreatment technology that can retain the effective ingredient at the extreme, as well as to separate and remove ineffective ingredient and impurities. Sample preparation is the basic and crucial step in the success for any analytical method.

Sample preparation is the basic and most crucial step in the success of any analytical method. A successful sample pretreatment method typically has three major objectives: (1) sample matrix simplification and/or replacement, (2) analyte enhancement or concentration, and (3) sample clean-up [7]. Over the last years, several new miniaturized solvent-based extraction procedures, which are known as liquid-phase microextraction (LPME) techniques, have been introduced and applied with success [8–14]. LPME emerged from liquid–liquid extraction, which is probably the most widely used sample extraction and separation procedure despite its clear disadvantages such as high consumption of time and strong toxicity of solvent, as well as its tedious application [15]. LPME normally takes place between several microliters of water-immiscible solvent extraction phase or acceptor phase (AP) and an aqueous sample phase or donor phase (DP), which contains the target analytes of interest. LPME can be classified into three main categories [7,16]: single drop liquid-phase microextraction (SD–LPME) [5,17,18], dispersive liquid–liquid microextraction (DLLME) [19–22] and hollow fibre liquid–phase microextraction (HF–LPME) [23–26]. Fig. 1 shows a schematic diagram of several LPME modes. Several variations have also recently been introduced in each mode, which clearly demonstrate the methodology's versatility. The LPME technique combines sampling, extraction, separation and concentration in one step, meanwhile a relatively high enrichment factor (EF) of analyte is obtained since its low AP volume (V_a) and high DP volume (V_d) (that is $\beta = \frac{V_d}{V_a}$, phase ratio to be the higher), and the AP is easily introduced into a chromatographic or electrophoretic system.

LPME performs a primary function in the extraction of inorganic and organic compounds from water samples, such as inorganic substance (fluorine [27], vanadium [28], arsenic, stibium, bismuth, plumbum, stannum and mercury [29], iodate [30], and arsenic (III) and arsenic (V) [31]); as well as organic substance (polycyclic aromatic hydrocarbon (PAH) [32,33], organochlorine pesticides [34–36], chloroacetanilide herbicide [37], sulphur compounds [38]) and so on. However, the matrix complexity, as well as the varied, low level contents of components in natural samples is a considerable drawback that makes the application in this area difficult. Even so, multiple developments and applications highly focused on the TCMs analysis field have been proposed, which represent the start of the expansion of LPME in TCMs or complex samples analysis. Therefore, this review will primarily focus on the different applications of LPME techniques, such as SD–LPME, D–LLME, HF–LPME and so on, coupled with various instrumental analytical methods, such as high performance liquid chromatography (HPLC), gas chromatography (GC), liquid chromatography/mass spectrometry (LC/MS), ultra-high performance liquid chromatography/mass spectrometry (UHPLC/MS), gas chromatography/mass spectrometry (GC/MS), capillary electrophoresis (CE), ultraviolet–visible spectrophotometry (UV) and atomic absorption spectrophotometry (AAS), in the field of natural product analyses during the past few years.

The advances of LPME technique, in *vivo* and in *vitro*, for the analysis of organic and inorganic compounds from different natural product types, such as tea, vegetables, seeds, herbs and galenical, which will be summarized and discussed below, clearly demonstrate the potential of the LPME technique as a powerful sample preparation tool in complex sample analysis. To the best of our knowledge, the present paper is the first review article dealing with the specific application of LPME techniques in natural product analysis.

2. Applications

2.1. Single drop liquid-phase microextraction

One of the modes of the LPME that was first reported by Liu and Dasgupta [39] is termed SD-LPME, in which the extraction medium is in the form of a single drop (Fig. 1A and B). The technique is based on the distribution of analytes between a microdrop of extraction solvent (usually few microliters) at the tip of a microsyringe needle and an aqueous sample phase containing the analytes. After extraction, the microdrop is retracted back into the microsyringe and injected into a chromatographic or electrophoretic system for further analysis. Jeannot and Cantwell [40,41], as well as He and Lee [11], performed the technique in combination with chromatographic analysis based on a previous work of Liu and Dasgupta [39,42], in which a single drop was used as the analyte collector. SD-LPME can be classified into two-phase [11,40,43] and three-phase mode [7,44]. In the two-phase mode, such as direct immersion SD-LPME (DI-SD-LPME) (Fig. 1A) and continuous flow SD-LPME (CF-SD-LPME), analytes are extracted from the sample solution (DP) into the organic solvent (AP) as a microdrop suspended from a microsyringe needle. Considering that suspended particles or impurities in sample solution may disturb the microdrop or even make that drop highly unstable and easily fall off, the two-phase mode is more suitable for simple matrix sample. In the three-phase mode, such as headspace SD-LPME (HS-SD-LPME) [45] (Fig. 1B) and drop-to-drop SD-LPME (DD-SD-LPME) [46], analytes are firstly extracted from the DP into the organic solvent or the headspace, and then back-extracted into the single drop aqueous AP. Compared with the two-phase mode, the three-phase mode is more suitable for the analysis of volatile components in complex samples. In the process, the microdrop remains relatively stable and nearly not influenced by the stirring, impurities or sample matrix interference.

To reduce evaporation risk during the extraction period and obtain the desired results, several important factors of extraction solvent, such as relatively high boiling point or relatively low vapour pressure, density, high viscosity, suitable chromatographic behaviour and high extraction efficiency (EE) or EF for the target analytes, must be considered [47]. Based on these factors, the most common extraction solvents used are toluene [48], hexane, octane [49], dodecane [50] and xylene [5,45]. Except that, certain ionic liquids (ILs) [51], such as 1-butyl-3-methylimidazollium hexafluorophosphate ([BMIM][PF_6]) [14], 1-hexyl-3-methylimidazolium