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A review of modern sample-preparation techniques for the extraction and analysis of medicinal plants

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Abstract Sample preparation is the crucial first step in the analysis of herbs. In recent years there has been increasing interest worldwide in the use of alternative/herbal medicine for the prevention and treatment of various illnesses. Currently, however, quality-related problems (lack of consistency, safety, and efficacy) seem to be overshadowing the potential genuine health benefits of various herbal products. Thus, the development of "modern" sample-preparation techniques with significant advantages over conventional methods for the extraction and analysis of medicinal plants is likely to play an important role in the overall effort of ensuring and providing high-quality herbal products to consumers worldwide. In this article, recent developments and applications of modern sample-preparation techniques for the extraction, clean-up, and concentration of analytes from medicinal plants or herbal materials are reviewed. These modern techniques include solid-phase microextraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave-assisted extraction, solid-phase extraction, and surfactant-mediated extraction.

Keywords Sample preparation · Extraction · Quality control · Medicinal plants · Herbs

Introduction

Plants are naturally gifted at the synthesis of medicinal compounds. The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value [1, 2]. A classic example is aspirin, which was initially discovered as salicylic acid in willow bark and leaves [1]; another noted example is taxol, recently proven to be effective against breast and ovarian cancers, which was initially discovered in bark of yew trees [2].

The use of medicinal plants (herbs) has a long history throughout the world and herbal preparations, including herbal extracts, can be found in the pharmacopoeias of numerous countries [3]. In recent years there have been a renaissance of interest in natural or herbal remedies worldwide, partly because of the realization that modern medicine is not capable of providing a "cure-all" solution against human diseases and that the presence of unwanted side-effects is almost unavoidable. Unlike modern drugs that invariably comprise a single active species, herb extracts and/or prescriptions contain multiple active constituents. Interestingly, natural compounds contained in these "herbal cocktails" can act in a synergistic manner within the human body, and can provide unique therapeutic properties with minimal or no undesirable side-effects [4].

A key factor in the widespread acceptance of natural or alternative therapies by the international community involves the "modernization" of herbal medicine. In other words, the standardization and quality control of herbal materials by use of modern science and technology is critical. At present, however, quality-related problems (lack of consistency, safety, and efficacy) seem to be overshadowing the potential genuine health benefits of various herbal products, and a major cause of these problems seems to be related to the lack of simple and reliable analytical techniques and methodologies for the chemical analysis of herbal materials [5, 6].

Sample preparation is the crucial first step in the analysis of herbs, because it is necessary to extract the desired chemical components from the herbal materials for further separation and characterization. Thus, the development of "modern" sample-preparation techniques with significant advantages over conventional methods (e.g. reduction in organic solvent consumption and in sample degradation, elimination of additional sample clean-up and concentration steps before chromatographic analysis, improvement in extraction efficiency, selectivity, and/or kinetics, ease of automation, etc.) for the extraction and

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analysis of medicinal plants is likely to play an important role in the overall effort of ensuring and providing high-quality herbal products to consumers worldwide.

In this article recent developments and applications of modern sample-preparation techniques for the extraction, clean-up, and concentration of analytes from medicinal plants or herbal materials are reviewed. These modern techniques include solid-phase microextraction, supercritical-fluid extraction, pressurized-liquid extraction, microwave- assisted extraction, solid-phase extraction, and surfactant-mediated extraction. Emphasis is placed on brief description of the unique capabilities and advantages and disadvantages of each modern sample-preparation techniques and of how these techniques were exploited to improve the extraction and analysis of a variety of medicinal plants. More detailed description of the basic principles of these modern sample-preparation techniques for the extraction of solid materials in general is available in number of excellent review articles recently appeared in the literature [7, 8, 9, 10].

Modern techniques for sample preparation in the analysis of medicinal plants

Headspace analysis

The medicinal properties of plants can be related in part to the presence of volatile constituents (e.g. essential oils) in the plant matrix, and gas chromatography (GC) is frequently used for determination of the volatile composition of plant materials. Because the sample to be injected should be free from non-volatile components, a fractionation step is necessary before GC analysis. The disadvantages of commonly used sample-preparation techniques such as distillation and liquid solvent extraction are that they usually require large amounts of organic solvents and manpower; these methods also tend to be destructive in nature (i.e. significant artifact formation can occur owing to sample decomposition at high temperatures) [11].

Headspace (HS) sampling is well suited for the fractionation of volatile compounds from complex solid matrices such as plant materials. Sanz and co-workers [12] have shown that reproducible and rapid identification of volatile compounds in aromatic plants can be achieved when static HS sampling is coupled with GC–mass spectrometry (MS), with the advantages of eliminating the extraction or fractionation step, reducing artifact formation, and providing on-line capacity. More recently the same research group has extended the capabilities of automated HS sampling in the GC–MS analysis of volatile compounds from *Origanum vulgare* [13].

Another example of herb analysis by automatic HS–GC is recent work by Stuppner and Ganzera [14] on determination of the safrole content from different *Asarum* species from China and Europe. These *Asarum* species contain up to 5.5% essential oils with methyleugenol as the major constituent and several minor components, e.g. safrole, which is known to have mutagenic and carcinogenic effects. The HS–GC results for safrole were found to be in good agreement with those obtained by "classical" GC analysis, i.e., using an organic solvent (dichloromethane) for extraction.

A novel and effective approach, known as solid-phase microextraction (SPME), was recently developed by Pawliszyn and co-workers [15] as a solvent-free sampling technique. In this approach, the analytes from the sample are adsorbed directly onto an adsorbent coated fused-silica fiber (which fits inside a syringe needle) and then either thermally desorbed directly into a GC injection port or high-performance liquid chromatography (HPLC) injection valve. By placing the fiber in head space at equilibrium with a sample (i.e. the technique of HS-SPME), a rapid and inexpensive method for isolation of volatile organic analytes for subsequent GC–MS analysis has been successfully demonstrated for the quality control of herbal medicine and other formulations containing herb extracts, such as terpenoids, peppermint, rosemary, sage, and thyme [16]. In addition to endogenous active components in medicinal plants, the usefulness of SPME as a sampling tool has also been recently demonstrated by Hwang and Lee [17] for the GC–MS analysis of toxic contaminants, i.e., organochlorine pesticide residues, present in Chinese herbal materials.

In a recent paper Pawliszyn and co-workers [18] reported successful demonstration of the feasibility and unique advantages of SPME for the characterization and quantification of the biogenic volatile organic compounds (e.g. isoprene and terpenoid compounds) emitted by living plants (leaves of *Eucalyptus citriodora*). By use of coated SPME fibers they were able to identify 33 compounds emitted by the living plant and, using diffusion-based SPME quantitation, it was possible to quantify sub-parts-per-billion amounts of isoprene after a very short extraction time (15 s).

The effect of the fiber coating in HS-SPME–GC analysis of aromatic and medicinal plants has been investigated in depth by Bicchi and co-workers [19]. Interestingly, it was found that fibers consisting of two components, a liquid polymeric coating for the less polar analytes and a solid polymeric coating for the more polar analytes, were more effective for HS-SPME analysis of the volatile fraction of the four aromatic and medicinal plants investigated (rosemary, sage, thyme, and valerian). More recently, replacement of the fiber needle with a stir-bar, a new sampling technique known as stir-bar sorptive extraction (SBSE), has been described by Bicchi and co-workers [20] for the headspace sampling of the same four aromatic/medicinal plants. Because of the larger amounts of trapping material (e.g. polydimethylsiloxane) coated on the stir-bar, the concentration capability is significantly greater than that of SPME.

Supercritical- and subcritical-fluid extraction

Supercritical fluid extraction (SFE) has been used for many years for the extraction of volatile components, e.g.

essential oils and aroma compounds from plant materials, on an industrial scale [21]. Recently, the application of this technique on an analytical scale has started to attract wide interest for sample preparation before chromatographic analysis [22]. The potential advantages include the ability to perform rapid (often less than 30 min) extractions, to reduce the use of hazardous solvents (i.e. carbon dioxide is commonly used as the extraction solvent), and to couple the extraction step with gas, liquid, or supercritical-fluid chromatography.

An important advantage of applying SFE to the extraction of active compounds from medicinal plants is that degradation as a result of lengthy exposure to elevated temperatures and atmospheric oxygen are avoided. For example:

1. Smith and Burford [23] showed that the active compound of feverfew, i.e. the sesquiterpene lactone parthenolide (well known to be unstable and to degrade during storage), can be efficiently and selectively extracted from dried feverfew by SFE without thermal degradation;
2. Bartley and Foley [24] demonstrated that although volatile compounds (essential oils) in ginger were expected to be strongly influenced by heat treatment, possibly as a result of hydrolysis, oxidation, and rearrangement, a very low concentration of gingerol degradation products were, however, found after SFE of Australian-grown ginger, which attested to the mild nature of the extraction procedure; and
3. in the determination of the enantiomeric purity of atropine (a tropane alkaloid with significant medical interest found in plants of the Solanaceae family), Mateus et al. [25] found that SFE induced less racemization than classical liquid–solid extraction procedures.

In SFE several experimental conditions can be adjusted to optimize the recovery, kinetics, and selectivity of the extraction. For example:

1. during optimization of SFE conditions for extraction of active ingredients from *Curcuma zedoaria*, Ma and co-workers [26] found that the density of the CO_2 and the fluid volume passing through the plant matrix are the most important factors affecting extraction efficiency, whereas increasing the temperature has little effect;
2. when using SFE to extract indirubin (the active ingredient in the herbs *Strobilanthes cusia*, *Isatis tinctoria*, and *Polygonum tinctorium*) Li et al. [27] found that faster extraction kinetics were achieved by employing factorial design to optimize experimental variables such as temperature, pressure, modifier concentration, static extracting time, and CO_2 dynamic extracting volume; and
3. it is interesting to note from another study by Hawthorne and co-workers [28] that the rate of extraction of volatile active components (essential oils) from plant matrices by SFE seemed to be governed by analyte–matrix interaction rather than the bulk solubility of the analyte in pure CO_2, because the extraction rates were found to increase greatly due to an addition of organic modifiers.

In another fundamental study Fahmy and co-workers [29] clearly demonstrated that swelling of the plant matrix, as a result of interaction between modifiers and matrix, was an important factor in enhancing extraction recovery and kinetics in SFE. Also, by use of different conditions of pressure and temperature, Reglero and co-workers [30] found that SFE conditions can be fined tuned for selective extraction of an antioxidant fraction with almost no residual aroma from rosemary plants. It has also been shown, by operating under sub-critical temperature and pressure conditions, that CO_2 fluid can be used as solvent for the selective extraction of essential oils [31] and diterpene glycosides [32] from plants of medicinal interest. More recent examples of the application of SFE for the optimum extraction of analytes from medicinal plants under different extraction conditions are listed in Table 1.

The on-line coupling of SFE with supercritical-fluid chromatography (SFC) has recently been shown to afford enhanced speed and sensitivity, as a result of the ability to use this technique to perform consecutive extraction, concentration, and separation of the constituents of herbal materials. For example, using an amino column for trapping and separation, Suto and co-workers [44] demonstrated that the on-line SFE–SFC analysis of the active compounds (magnolol and honokiol) in Magnoloae cortex can be completed within 5 min. Also, by using silica gel as the trapping and separation column and sodium sulfosuccinate as the counter-ion for ion-pair formation, the same research group recently showed that the on-line coupling of ion-pair SFE–SFC enabled the rapid analysis (within 10 min) of berberine and palmatine (positively charged active species) in Phellodendri cortex [45].

Another interesting recent development is the on-line coupling of SFE to a uterotonic bioassay by Sewram and co-workers [46]. In South Africa, the use of traditional medicine is popular and the ingestion of plant extracts during pregnancy to provide health supplements or to induce labor is common. In the on-line SFE uterotonic bioassay system [46] SFE extracts from four local medicinal plants were transferred directly to a uterus muscle chamber to identify the active fractions (i.e. the fractions capable of inducing muscle contraction can be determined rapidly, safely, and sensitively). This novel on-line SFE bioassay method could also be adapted for screening plants with other therapeutic properties, e.g. those used for treatment of diabetes mellitus and hypertension. Another novel approach involving the off-line coupling of SFE to GC in such a way that the glass liner of a programmed temperature vaporizer is placed after the separation vessels of the SFE extraction module has been recently demonstrated by Blanch et al. [47] to be effective for the sensitive and selective analysis of complex plant matrices.

Table 1 Recent applications of SFE in medicinal plant analysis

Analyte	Medicinal plant	Extraction conditions/remarks	References
Taxanes (Taxol)	English yew tree (*Taxus baccata*)	Methanol-modified CO_2; 50 °C; 400 atm; 100 min; extraction efficiency comparable with that of liquid solvent extraction	[33]
Volatile compounds	Frankincense, myrrh and *Evodia rutaecarpa* (traditional Chinese medicine)	CO_2 only: 20 MPa; 50 °C; 40 min; good extraction efficiency for high molecular-weight and oxygenated components	[34]
Diosgenin (a steroid intermediate)	Tubers of *Dioscorea nipponica*	CO_2; range of temperature and pressure studied: 3100 psig; 44 °C; 70 min gave the highest recovery	[35]
Taxol and baccatin III	Needles of *Taxus cuspidata*	Ethanol-modified CO_2; 40 °C; 300 bar; using a continuous flow-through cell for extraction	[36]
Essential oils	Feverfew, tansy, and German chamomile	CO_2: 250 bar and 450 °C; GC chromatograms can be used to discriminate between the different plant materials	[37]
Oxindole alkaloids	*Uncaria tormentosa* (one of the most important botanicals in the rain forest of Peru)	CO_2 only and methanol-modified CO_2; 250 atm; 60 °C; 30 to 60 min	[38]
Kava lactones	*Piper methysticum* Forst	CO_2 alone and ethanol-modified CO_2; 250 to 450 atm; 60 °C; 60 min; to maximize extraction efficiency, use CO_2 alone for another 4 h and 3 min	[39]
Volatile compounds	Roots from different species of *Echinacea* (herbal extracts marketed widely in Europe)	CO_2: 15 MPa; 60 °C; 30 min; GC patterns can be used for rapid identification and to verify the authenticity of different species	[40]
Tanshinone II A	Root and rhizome of *Salvia miltiorrhiza* bunge (popular traditional Chinese medicines)	Methanol–CO_2 gave the highest recovery; 350 kg cm^{-2}; 60 °C; 30 min	[41]
Flavanones and xanthones	Root bark of osage orange tree (*Maclura ponifera*)	CO_2 alone and methanol-modified CO_2; 40.5 MPa; 40 to 100 °C; 45 min; addition of methanol to CO_2 essential for achieving high yields	[42]
Flavonoids	Roots of *Scutellaria baicalensis* (traditional Chinese medicine)	A range of conditions were studied; optimum conditions: CO_2 + methanol + H_2O (20:2.1:0.9), 50 °C and 200 bar	[43]

Microwave-assisted extraction

In contrast with conventional liquid–solid extraction methods (e.g. Soxhlet extraction) in which a relatively long extraction time (typically 3–48 h) is required, the use of microwave energy for solution heating (the technique known as microwave-assisted extraction, MAE) results in significant reduction of the extraction time (usually less than 30 min), because the microwaves heat the solvent or solvent mixture directly, thus accelerating the speed of heating. Besides having the advantage of high extraction speed, MAE also enables a significant reduction in the consumption of organic solvent (typically less than 40 mL, compared with the 100–500 mL required in Soxhlet extraction) [48].

Among the conditions commonly studied for optimization of MAE process, the effects of solvent composition, solvent volume, extraction temperature, and matrix characteristics seem to be most important for plant materials. For example, Chen and Spiro [49] recently performed a kinetic study of the effects of these conditions on the MAE extraction of plant materials with medicinal significance, i.e. leaves from rosemary and peppermint. Their results indicated that for a sample matrix such as a plant, which usually contains water as a component with dielectric loss (a measure of the efficiency of converting microwave energy into heat), the use of pure, microwave-transparent solvents such as hexane could result in the rapid extraction of essential oil components. This is probably because of the direct interaction of microwaves with the free water molecules present in the glands and vascular systems, which results in the subsequent rupture of the plant tissue and the release of the essential oil into the organic solvent (hexane). More effective microwave heating of this particular system (hexane+leaves) could therefore be achieved by increasing the weight of leaves relative to the volume of hexane. For a system involving use of an organic solvent which absorbs microwaves more strongly (ethanol+leaves), more effective heating of sample mixture could, on the other hand, be achieved by increasing the microwave power output, because in this system the ethanol (rather than water molecules in the leaves) absorbs the bulk of the microwave energy.

In traditional medicine the preparation of “herbal drinks” for oral intake usually involves cooking the herbal materials with water for 30–60 min. In modern herbal medicines the herbs are cooked with water or ethanol and then processed into tablets, pills, liquid, or injection solutions. The use of conventional extraction methods, e.g. ultrasonic processing and heating under reflux, to leach the active compounds from herbs is very time consuming. To resolve this problem, Gou et al. [50] recently demonstrated that with the aid of MAE the extraction of puerarin from *Radix puerariae* took 1 min only when water was

used as the extraction solvent, because water not only absorbed the microwaves efficiently but also readily dissolved the polar active constituents (iso-flavone compounds) of this important Chinese herb.

For the analysis of Chinese herbs Liu and co-workers [51, 52, 53] have developed new MAE methodologies for extraction of glycyrrhizic acid from licorice root, which has been used in traditional medicines for the treatment of stomach ulcers for over 2000 years [51, 52]. The root contains approximately 1–10% (w/w) of glycosides including two glucuronic acid units and glycyrrhetinic acid and avloids. Their results indicated that the use of water and a mixture of water and ethanol were suitable for rapid and efficient extraction of glycyrrhizic acid. The recovery could be enhanced by addition of appropriate amounts of ammonia or salt to form more water soluble species, e.g. glycyrrhizic ammoniate and the potassium salt of glycyrrhizic acid. New MAE methodologies were also developed for the optimum extraction of tanshinones from the dried roots of *Salvia miltiorrhiza*; this was complete in 2 min only [53]. Other recent applications of MAE to the extraction of medicinal plants include the leaching of:

- alpha-hederin and hedera-saponin from *Hedera helix* leaves [54];
- saponin and sapoganin from *Paris polyphylla* [55];
- sallidroside and tyrosol from *Rhodioda sachalinensis* [56];
- essential oils from the leaves of *Lippoa sidoides* [57];
- Lupin alkaloid (sparteine) from seeds [58]; and
- Taxanes from Taxus biomass [59].

The effectiveness of using microwave and aqueous mineral acids for the digestion of solid materials in metal analysis is well known. In herbal medicine the significance of measuring the concentration of metals is related to their clinical efficacy, safety, and toxicity. For example, raw and processed herbs frequently contain heavy metals that exceed the safety regulation limits set by many countries. Clinical studies, on the other hand, show that some heavy metals, despite their reported toxicity, actual play an important role in the therapeutic effects of the herbs containing these metals [60, 61, 62]. It is, therefore, of critical importance to develop reliable sample treatment and analytical techniques for metal analysis and speciation in herbal products [5, 6].

In a recent study Wang and co-workers [63] compared the effectiveness of three different digestion methods (two conventional wet acid digestion and one microwave acid digestion) for the determination of metals in traditional Chinese medicine (TCM). Although all three methods led to comparable results using inductively-coupled plasma (ICP)–MS for analysis, microwave acid digestion was concluded to be the method of choice because of its higher speed and lower reagent consumption.

Similarly Ong and co-workers [64] have recently reported a simple and rapid method for determination of mercury in TCM. Two different types of vessel for closed-vessel microwave were compared; the results indicated that, in addition to improved speed, closed-vessel microwave digestion also minimizes loss of volatile analytes such as mercury. To enable higher sample throughput and precision, lower washout time and memory effect, and smaller sample volume capability, the same research group recently coupled closed-vessel microwave digestion and flow injection methods with ICP–MS for the determination of arsenic and lead [65, 66].

Pressurized liquid extraction

For rapid and efficient extraction of analytes from solid matrices such as plant materials, extraction temperature is an important experimental factor, because elevated temperatures could lead to significant improvements in the capacity of extraction solvents to dissolve the analytes, in the rates of mass transport, and in the effectiveness of sample wetting and matrix penetration, all of which lead to overall improvement in the extraction and desorption of analytes from the surface and active sites of solid sample matrices. To achieve all these advantages, however, elevated pressure is needed to maintain the extraction solvents as liquids at high temperatures (usually above their boiling points); this can be accomplished by use of a modern extraction and sampling technique known as pressurized-liquid extraction (PLE) or, more commonly, by its trade name (accelerated solvent extraction) [67].

PLE emerged in the mid-nineteen-nineties, but it is surprising to find it has rarely been applied to the extraction/analysis of plant materials. Benthin et al. [68] were among the first to conduct a comprehensive study on the feasibility/usefulness of applying PLE in medicinal herb analysis. In their study PLE extracts from a selection of representative herbs were compared with extracts obtained according to pharmacopoeia monographs; their results indicated that PLE is often superior to other extraction methods currently used in crude herb analysis in terms of recovery, extraction time, and solvent consumption (i.e. for all the herbs studied, a significant saving in time and solvents was realized and extraction recoveries of the analytes were equivalent or higher). Similarly, Ong and co-workers [69] recently found that PLE is superior to conventional extraction methods (ultrasonic and Soxhlet extraction) for the extraction of berberine and aristolochic acids in medicinal plants.

In addition to extraction temperature, the choice of extraction solvent is another important factor in PLE. Most PLE applications reported in the literature employed the organic solvents commonly used in conventional techniques, e.g. methanol, in which many organic compounds are very soluble. A recent application of PLE reported by Kawamura and co-workers [70] for the extraction of an active compound with significant medicinal interest, paclitaxel (commonly known as taxol, which has anticancer activity), from the bark of *Taxus cuspidata* indicated, however, that use of water alone as the extraction solvent is a viable alternative. An interesting result from this study was that although in conventional extraction methods the taxol content of the water extract was very low,

this was dramatically improved by use of the elevated temperature and pressure conditions of PLE. Sumni and co-workers [71] compared conventional and modern extraction methods for the rapid and efficient extraction of medicinal iridoid glycosides from a plant matrix (*Veronica longifoloa* leaves) and reported that use of hot water as the extraction solvent under atmospheric or higher pressure conditions was the most efficient.

Ultrasonic extraction

Although the use of ultrasonic energy to aid the extraction of medicinal compounds from plant materials can be found in the literature as early as the nineteen-fifties, mechanistic aspects of the usefulness of ultrasonically assisted extraction are worth noting. Fundamentally, the effects of ultrasound on the cell walls of plants can be described as follows [72]:

1. Some plant cells occur in the form of glands (external or internal) filled with essential oil. A characteristic of external glands is that their skin is very thin and can be easily destroyed by sonication, thus facilitating release of essential oil contents into the extraction solvent; and
2. Ultrasound can also facilitate the swelling and hydration of plant materials to cause enlargement of the pores of the cell wall. Better swelling will improve the rate of mass transfer and, occasionally, break the cell walls, thus resulting in increased extraction efficiency and/or reduced extraction time.

As a novel approach to extraction and sample preparation for medicinal herbs, Huie and co-workers [73] recently employed ultrasound to assist the surfactant-mediated extraction of ginsenosides from American ginseng (a very popular herb consumed worldwide). In this approach the surfactant-mediated extraction process can be divided into two parts:

1. solubilization of active ingredients from the solid herbal material into the extraction solvent (aqueous surfactant solution); and
2. the cloud-point phase separation of the aqueous surfactant solution containing the active ingredients into a bulk aqueous phase and a smaller volume surfactant-rich phase (analyte concentration).

In ultrasonically assisted extraction the use of aqueous surfactant solution containing 10% Triton X-100 as the extraction solvent was found to result in faster extraction kinetics and higher recovery compared to methanol and water.

Solid-phase extraction

A common drawback of classical and modern extraction methods in sample preparation for complex matrices is that additional clean-up procedures are often required before gas or liquid chromatographic analysis. For medicinal plants the use of sampling techniques such as Soxhlet extraction, MAE, or PLE often results in non-selective co-extraction of relatively large amounts of undesirable components (e.g. lipids, sterols, chlorophylls, etc.), which can severely affect the separation and detection performance of subsequent GC or HPLC analysis [74].

Solid-phase extraction (SPE) is a simple preparation technique based on the principles used in liquid chromatography, in which the solubility and functional group interactions of sample, solvent, and adsorbent are optimized to effect sample fractionation and/or concentration. A wide range of chemically modified adsorbent materials (silica gel or synthetic resins) enable precise group separation on the basis of different types of physicochemical interaction, i.e. reversed-phase (C_2, C_8, C_{18}), cation- and anion-exchange, etc. It should, in particular, be noted that SPE is well suited to the treatment of sample matrices with high water content, e.g. extracts of herbal materials [75].

Most traditional medicines, e.g. TCM, involve use of boiling water to extract the herbal materials for preparation of the medicinal prescriptions. For example, the leaves of a tea plant known as Theaw folium, which contain caffeine as a major constituent, are commonly used as a Chinese herb [76]. Without pretreatment HPLC analysis of aqueous extracts of this tea plant was rather difficult, because of the presence of many highly polar complex constituents. Ku and co-workers [76] recently resolved these difficulties by combining SPE (C_{18} reversed-phase adsorbent) and HPLC for the sensitive and reproducible determination of caffeine in six different TCM prescriptions (complex herb mixtures) that contained Theae folium as one of the herbs.

Similarly, Hurlbut and co-workers [77] from the US Food and Drug Administration (FDA) demonstrated the importance and effectiveness of using SPE and HPLC for the determination of ephedrine alkaloids extracted from plants of the genus *Ephedra*. Large amounts of these extracts are imported into the US annually for sale to consumers as dietary supplements that promote weight loss, body building, and energy increase. Ephedrine alkaloids can, however, affect the cardiovascular and nervous system and are known to cause illnesses and injuries to consumers. Using a propylsulfonic acid SPE column (cation-exchange clean-up) the FDA developed a simple and reliable method for HPLC analysis of ephedrine alkaloids in herbal products. Lino and co-workers [78] have recently shown that, in addition to biologically active compounds, SPE with C_{18} or Florisil adsorbents was also very useful for extraction and clean-up of herbal materials before analysis of the organochlorine pesticide residues present in medicinal plants.

In addition to conventional SPE columns, new polymeric adsorbents have recently been developed for the improved retention of polar organic compounds, which is a major limitation of the C_{18} adsorbent. Klejdus et al. [79] recently compared classical adsorbents and new polymeric adsorbents (e.g. Waters Oasis HLB extraction cartridges, which contain a unique copolymeric adsorbent

designed to have a hydrophilic–lipophilic balance) for determination of isoflavones in plants; these compounds are known to be important in cancer prevention and to have other health benefits. The results indicated that the use of polymeric adsorbents for sample pretreatment enabled higher recoveries, higher reproducibility, and lower consumption of plant materials for the HPLC analysis of these isoflavones. As an alternative to a single SPE column, the use of combined or mixed-mode SPE columns was shown to be effective for the purification and isolation of active components from medicinal plants. For example, Glowniak et al. [80] demonstrated the usefulness of combining C_{18} and quaternary amine adsorbents for the fractionation of free phenolic acids (naturally occurring compounds with a broad spectrum of pharmaceutical activity) in *Echinacea* species. Stobiecki et al. [81] combined C_{18} and an adsorbent containing benzene sulfonic groups as an effective sample-preparation method for profiling quinolizidine alkaloids and phenolic compounds in *Lupinus albus*.

Concluding remarks

Among the modern sampling techniques described in this review, SFE seems to offer unique advantages in the extraction of medicinal plants – high selectivity, minimum degradation of thermally labile analytes, and elimination of the use of hazardous organic solvents (e.g. use of pure CO_2 as the extractant). The main drawbacks of SFE, on the other hand, such as difficulties in extracting polar compounds and high susceptibility to matrix effects, are problematic in the extraction of herbal materials. As plant matrices are highly complex, factors such as the water content and particle size of the matrix and strong analyte–matrix interactions, etc., can severely limit the capacity of SFE to effect high extraction efficiency and rapid kinetics, especially for polar analytes.

The basic principles of MAE and PLE are very similar to those of classic extraction techniques, i.e. use of liquid solvents is still needed; however, partly because these newer technologies are automated and the solvents are under "superheated" conditions (the effect of microwaves in MAE or elevated temperature and/or pressure in PLE), they are more user-friendly, much quicker, and require significantly less organic solvent. Compared with SFE, MAE and PLE are more easily optimized and should be preferred when less selectivity is acceptable, because, for example, the use of "hot" liquid solvents in these techniques can partly overcome strong analyte–matrix interactions, even for more polar compounds. For some medicinal plants, however, thermal degradation of the analytes can be a problem under typical MAE and PLE conditions and co-extraction of large amounts of matrix materials, e.g. lipids and chlorophylls, would require extensive additional sample clean-up and concentration before chromatographic analysis.

In the coming years, it seems that the use of PLE with subcritical water as the extractant for the extraction and analysis of analytes from medicinal plants might have interesting potential [70, 71], because water is inexpensive, non-toxic, and environmentally friendly. Also, by simply increasing the temperature at constant pressure, the relative permittivity of water can be reduced, so that analytes with a wide range of polarity can be extracted. The use of SPME, especially head-space SPME, also shows good promise as a convenient and effective analytical tool for the sampling of volatile compounds, e.g. essential oils from medicinal herbs, from plant materials before GC analysis [15, 17]. Although SPE and ultrasonic extraction have been around for a relatively long time, it is likely these methods will remain popular and effective tools for the extraction, clean-up, and/or concentration of analytes from a variety of herbal materials.

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