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Direct Analysis of Traditional Chinese Medicines by Mass Spectrometry

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Abstract

Analysis of traditional Chinese medicines (TCMs) plays important roles in quality control of TCMs and understanding their pharmacological effects. Mass spectrometry (MS) is a technique of choice for analysis of TCMs due to its superiority in speed, sensitivity and specificity. However, conventional MS analysis of TCMs typically requires extensive sample pretreatment and chromatographic separation, which could be time-consuming and laborious, prior to the analysis. The expanding usage of TCMs worldwide nowadays demands development of rapid, cost-effective and reliable methods for analysis of TCMs. In recent years, new sample preparation and ionization techniques have been developed to enable direct analysis of TCMs by MS, significantly reducing the analysis time and cost. In this review, various MS-based techniques, mainly including ambient ionization-MS and MALDI-MS based techniques, applied for direct analysis of TCMs are summarized and their applicability and future prospects are discussed.

1. Introduction

Traditional Chinese medicines (TCMs) have been using for thousands of years for curing diseases and promoting human health in China. Due to their high efficacy and less adverse effects on human body, public recognition and interests on TCMs treatment have been significantly increased in western countries in the past decades. It is expected that the global herbal medicines and supplements marketplace would reach about US$93 billion by 2015 [1]. Along with the rapid expansion of the global needs on TCMs, the safety and quality of TCMs have become major concerns for health authorities and public [2-4]. However, different from chemical drugs, the chemical compositions of TCMs are highly complex in nature. Each TCM herb may contain hundreds of chemical constituents and every constituent may have its own therapeutic effects on biological systems [5]. In fact, in a typical TCM treatment, mixture of herbal medicines might be used in one formulation, which poses a great challenge to TCM analysis in terms of separation and detection. Furthermore, it is well-known that the chemical constituents of TCMs could vary due to numerous factors such as species, growing conditions, harvest season and processing procedures [6, 7]. Apart from determining the chemical composition of TCMs, screening of pesticides / herbicides residues, adulterated compounds and contaminants in TCM is also an important task. Overall, quality assessment and control of TCMs are therefore exclusively important to safeguard the TCM safety and efficacy for medical use, yet are highly challenging missions [8, 9].

Analytical techniques are indispensable for TCM quality control and better understanding of TCMs on scientific basis. Mass spectrometry (MS) is a fast, sensitive and specific technique that allows reliable detection of molecules from measurement of the *mass–to-charge ratio* (*m/z*) of

their ions. It is a label-free technique that is suitable for analysis of diverse analytes. The sensitivity and specificity of detection can be further improved by various tandem mass spectrometric scanning modes, e.g., product ion scan, selected ion monitoring, selected reaction monitoring, precursor ion scan, and neutral loss scan, with related MS instruments, i.e., triple-quadrupole MS, and accurate *m/z* measurement with high resolution instruments, e.g., time-of-flight (TOF), Orbitrap and Fourier transform ion cyclotron resonance MS [10-12]. The availability of chemical compound databases further facilitates compound identification in TCM studies [13]. So far, many bioactive TCM constituents such as flavonoids, ginosensides, saponins, alkaloids, monoterpene glycosides and steroids can be successfully detected using different MS techniques [14-16].

Despite the desired features of MS, TCM analysis has been remaining a challenging task, mainly due to the fact that the detection sensitivity and specificity of MS could be greatly affected by sample matrix. Ion suppression effect from the matrix hinders the sensitive detection of targeted analytes [17, 18]. Moreover, complicated mass spectra could be resulted due to the presence of matrix interference peaks which make it difficult for data interpretation. To reduce the sample complexity and matrix interference for sensitive and specific MS detection, extensive sample extraction, sample pretreatment and chromatographic separation, which could be time-consuming and laborious, are usually needed [19-21]. With the fast growing demand for TCM analysis, it is imperative to develop rapid, cost-effective, and high-throughput analytical techniques. In recent years, development of methods for direct analysis of complex samples with no or only little sample preparation has been being an important area in MS. For examples, various ambient ionization techniques, in which ionization takes place under atmospheric

pressure and no or only little sample preparation is involved [22, 23], and a wide range of MALDI-MS based methods have been developed to facilitate analysis of complex samples. Many of these methods and techniques have been successfully applied in direct analysis of TCMs without major sample preparation, facilitating TCM analysis by reducing the time and cost required for analysis. In this review, various MS-based methods, mainly including ambient ionization-MS based and MALDI-MS based methods, applied for rapid and directly analysis of TCMs are summarized and their advantageous and disadvantageous features and prospects are discussed. In addition, the applications of MS in imaging study, which allows determination of spatial distribution chemicals in plants and thus detailed knowledge in their properties, are also summarized and discussed.

2. Traditional methods for TCM analysis

Traditional methods for authentication of TCMs mainly include physical and molecular level analysis. In physical analysis, morphological and microscopic features of TCM tissues are examined [24]. Physical characteristics of TCMs such as color, size, smell and texture are common parameters for herb identification. To a more in-depth extent, microscopic examination could be performed to determine the structural, internal and cellular tissue features. Molecular-level analysis mainly includes analysis of genetic materials, e.g., DNA [25], and chemical and biological molecules, e.g, small organic molecules, peptides and proteins, with modern analytical techniques, such as MS. This review mainly focuses on the MS-based methods in TCM analysis, which are the most commonly applied and reliable methods in TCM standardization and authentication nowadays.

3. Traditional MS-based methods for TCM analysis

Liquid Chromatography/Mass Spectrometry (LC/MS) with electrospray ionization (ESI) or atmospheric pressure chemical ionization and Gas Chromatography/Mass Spectrometry (GC/MS) with electron ionization or chemical ionization are traditional MS-based techniques applied in TCM analysis. Analysis of TCMs with these techniques usually involves sample extraction, sample pretreatment for reducing impurity contents, and chromatographic separation for reducing sample complexity and matrix interference upon detection. Practically, after sample extraction with desired organic solvents, sample extracts could be first subjected to solid phase extraction or liquid-liquid extraction for sample clean-up [26, 27], then further separated by gas chromatography or liquid chromatography before MS detection. Comparatively, LC/MS is the mainstream in TCM analysis as approximately 80% of the TCM constituents are non-volatile and thermally labile compounds which cannot be analyzed using GC/MS without derivatization [28]. The uses of LC/MS and GC/MS in various TCM related applications, e.g., fingerprinting analysis, marker identification, screening of pesticide residues, and analysis of essential oils, etc, have been successfully demonstrated in a wide range of studies [29-33].

The LC/MS and GC/MS techniques have demonstrated their success and important roles in TCM researches in the past decades. However, due to the increasing popularity of TCM usage nowadays, development of more rapid and high-throughput analytical techniques for TCM analysis is of great importance. In parallel with the further development of sample pretreatment and chromatographic techniques, development of MS-based methods that allow rapid and direct analysis of TCMs is highly beneficial to the field of TCM analysis.

4. MS methods for direct analysis of TCMs

4.1. Matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS)-based methods

Matrix-assisted laser/desorption ionization (MALDI) is a soft ionization technique in which analyte ions can be generated without causing significant fragmentation [34]. More importantly, MALDI-MS has desirable features that it generates simple mass spectra with mainly singly charged ions and has high tolerance to impurity, allowing direct analysis of crude mixtures, e.g., extracts of TCMs, without sample pretreatment and chromatographic separation [34, 35]. The MALDI ionization mechanism has been extensively studied in the past decades and thoroughly reported in several reviews [36-38]. In general, analytes and UV absorbing matrices (e.g., α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid) are first mixed in solution phase and applied on a MALDI target plate. Crystallization of sample-matrix mixture will occur when the solvent is evaporated. Upon laser irradiation on the sample-matrix crystals, analytes are desorbed/ionized for MS detection.

MALDI-MS has been widely applied in direct analysis of extracts of TCMs or solid herbal materials, e.g, slices or grinded powders, and the related studies are well summarized in a literature review [34]. For example, MALDI-MS was applied in rapid differentiation of *Panax ginseng* and *Panax quinquefolius*, two of the most widely used TCMs with similar physical properties but with different therapeutic effects [35]. By direct analysis of the extracts of *Panax ginseng* and *Panax quinquefolius* from brief extraction with ACN/H_2O 50/50 (v/v), the two herbs could be well differentiated by their characteristic ginsenosides, small molecule patterns or the intensity ratios of some of these components (Fig. 1). This study also demonstrated the quantitative determination of adulteration of red ginseng or *Panax quinquefolius.* with *Panax*

ginseng based on the relative abundance of characteristic ginsenosides and small molecules. In the same study, attempts were made in direct analysis of solid herbal powders and raw pieces of *Panax ginseng* and *Panax quinquefolius* by MALDI-MS. By loading small amount (i.e. 0.1 mg) of solid herbal powder or adhering a small piece of herbal sample onto the MALDI target plate and adding 1 μL of CHCA matrix onto the sample, ginsenoside profiles similar to the traditional solution-based method could be observed, indicating that the solid sample preparation methods could be rapid and reliable methods in analysis of ginseng herbs.

In an earlier study, MALDI-MS was applied in direct analysis of *Aconitum Carmichaeli* Debx. (Fuzi in Chinese), a TCM widely used to treat cadianeuria, neuralgia, rheumatalgia, and inflammation [39]. It is well-known that unprocessed Fuzi is highly toxic in nature [39]. The toxicity of Fuzi was believed to be brought out by aconitine-type alkaloids including aconitine, mesaconitine and hypaconitine, which can be converted into less toxic benzoylaconine, benzoylmesaconitine and benzoylhypaconitine, respectively, through heating, boiling and steaming processes [39]. In this study, the Fuzi herbs were cut into slices with thickness of 10 – 20 μm and adhered onto a MALDI target plate. Matrix solution was deposited onto the slice surface for analysis, and various alkaloids could be well detected. In a semi-quantitative manner, the results of this study showed that the relative intensities of the toxic alkaloids for unprocessed Fuzi were much higher than those of processed Fuzi, while the corresponding less toxic forms were more abundant in processed Fuzi. These data demonstrated the use of MALDI-MS analysis for rapid and reliable assessment of safety of TCM herbs. Another study combined MALDI-MS and LC/MS in comprehensive alkaloids profiling of Fuzi from different suppliers [40]. The results showed a significant difference in chemical composition of Fuzi from different

sources, revealing extensive quality assurance (QA)/quality control (QC) procedures are required to ensure the safety and efficacy of this TCM.

Furthermore, Ng *et al*. reported an alternative approach in direct analysis of plant tissues by MALDI-MS [41]. In this approach, plant tissues were cut into slices and MALDI matrix was applied by aerospraying the matrix solution onto the slices. This aerospraying method was reported to allow the formation of more homogeneous crystal layers thus improved spot-to-spot reproducibility. By this sample preparation method, different alkaloids could be directly detected from *Sinomenium acutum*, a well-known TCM for treatment of inflammatory and rheumatic diseases. Chemical components in different regions of the stem tissues, e.g., cortex, xylem, rim, and pith, were semi-quantitatively determined and the chemical distribution characteristics allowed unambiguous differentiation of the herbs from different growing areas.

MALDI-MS was also employed for direct analysis of crude extract of Chinese gall (Wubeizi), a common TCM used as astringent, haemostatic, antiphlogistic, and antiseptic agents for treating various diseases [42]. By extracting the grinded herbal power with 80% methanol for 5 minutes and directly mixing the crude extract with matrix on the MALDI target plate, different gallotannins, bioactive hydrolysable tannins known to exhibit antioxidant properties, were well identified by a quadrupole ion trap-TOF MS. This study demonstrated the applicability of MALDI-MS in rapid screening and evaluation of hydrolysable tannins in medicinal herbs.

Although various successful applications have been demonstrated, MALDI-MS analysis of TCMs could be challenged by the problem of matrix interference at low *m/z* range as most active

compounds in TCMs are relatively small molecules [34]. Alternative matrices, e.g., graphene or graphene oxide [43, 44], have been developed to reduce matrix interference effect and enhance detection of TCM compounds. Apart from matrix interference, samples are ionized under vacuum in MALDI, making sampling of raw herbal materials less convenient. These limitations could be solved by ambient ionization-MS techniques, which do not require any matrix for ionization and allow sampling and ionization in an open ambient environment. The applications of various ambient ionization-MS techniques in TCMs analysis are discussed in the following sections.

4.2 Ambient ionization-MS techniques

Ambient ionization-MS techniques have general features that sampling and ionization are performed in an opened ambient environment under atmospheric pressure and no or only little sample preparation is involved [45-51]. The invention of desorption electrospray ionization (DESI) in 2004 by Cooks' research group [52] was an important event in the development of ambient ionization-MS. Afterward, a wide range of ambient ionization techniques have been developed. The details of different ambient ionization techniques and their applications have been well summarized in several reviews [45-51]. Ambient ionization techniques allow direct analysis of complex samples with no or little sample preparation, which could facilitate TCM analysis by reducing the time and cost required for sample preparation and analysis. Various ambient ionization techniques applied in TCM analysis are summarized as follows.

4.2.1 Desorption Electrospray Ionization (DESI)

DESI allows *in situ* desorption and ionization of analytes on sample surfaces in ambient environment [52]. With pneumatic assistance, charged solvent droplets are generated from the electrospray emitter and hit on the sample surface (Fig. 2a). The analytes on the sample surface are dissolved, desorbed and ionized, as proposed by Cooks *et al* [53, 54].

Applications of DESI-MS in direct analysis of herbal materials were demonstrated in recent years. DESI-MS was applied in direct analysis of *Salvia divinorum*, a common herbal medicine producing hallucinogenic effect [55]. In this study, *Salvia divinorum* leaves were simply fixed on microscope glass slides by double-sided tapes for direct DESI-MS analysis. No additional pretreatment was needed prior to analysis. Major constituents of *Salvia divinorum*, including salvinorin A, B, C, D/E, and divinatorin B, were identified and semi-quantified effectively. The same study also demonstrated that combining DESI-MS with thin layer chromatography (TLC), i.e., direct analysis of sample spots separated on TLC plate with DESI-MS, could allow improved detection of salvinorins in *Salvia divinorum.*

In addition, DESI-MS was employed in direct analysis of *Stevia* leaves, one natural material believed to be an alternative of sucrose [56]. By directly analyzing a small leaf fragment, diterpene glycosides, major chemical components of *Stevia* leave, could be identified and semi-quantified (Fig. 2b). This study showed that untreated leaves and leaves extracted with hexane for removal of naturally-occurred oil layer showed similar mass spectral results, revealing that the naturally-occurred oil layer did not significantly hinder the desorption/ionization of chemical components on tissue surface of *Stevia* leaves.

DESI-MS was also used for rapid and direct detection of an anti-tumor drug component, camptothecin, and its derivative, 9-methoxycamptothecin, in *Nothapodytes nimmoniana*, a medicinal herb exhibiting anti-tumor activity [57]. Various regions of the plant, including leaves, stems and barks, were subjected to analysis. The results showed that the anti-tumor chemical components were more abundant in barks than in leaves and stems. This study demonstrated the use of DESI in rapid screening of bioactive components in different parts of medicinal plants for efficacy evaluation.

Although a number of successful cases reported, DESI-MS analysis of herbal tissues, i.e., leaves, could be challenging due to the presence of wax, cuticle and epidermal cell wall on plant material surface [58, 59]. These materials make the ionizing solvent difficult to penetrate to the tissue for detection of exogenous or endogenous chemicals. Li *et al*. reported that direct DESI-MS analysis was not possible for raw barley leaf, possibly due to the presence of cuticular wax layer which shielded the underlying chemicals [59]. Although major components, hydroxynitrile glucosides, could be detected after washing the leaves with chloroform, unstable and irreproducible signals were obtained. Recently, an indirect DESI technique was developed to solve this technical problem. In this technique, the sample leaves were imprinted onto a porous surface, such as PTFE, instead of direct sampling for DESI-MS analysis [58].

4.2.2 Direct Analysis in Real Time (DART)

DART is the first plasma-based ambient ionization technique developed by Cody *et al*. in 2005 (Fig. 3a) [60]. In DART, molecules on the sample surface are ionized by reacting with a hot

plasma of excited atoms and ions generated by applying electrical potential to a gas, usually nitrogen or helium [60].

DART-MS analysis has been performed on various solid, liquid and gas samples and widely applied to analyze TCMs for rapid identification and quality control purposes. Wang *et al*. developed a rapid and reliable DART-MS approach in rapid analysis of a series of well-known TCMs, e.g., *Coptidis Rhizoma (Coptis chinensis Franch.), Scutellariae Radix (Scutellaria baicalensis Georgi)*, ginseng *(Panax ginseng C.A. Mey.*), etc [61]. With a short ultrasonic extraction with 50% (v/v) methanol / water followed by direct DART-MS analysis, major constituents of those TCMs such as alkaloids, flavonoids and ginsenosides were readily detected and identified for rapid differentiation of these herb species (Fig. 3b). This study showed that optimization of temperature of ionizing gas was critical for generation of high quality spectra. In addition, as demonstrated by analysis of ginosenosides, derivatization for enhancing the volatility and proton affinity was necessary for detection of molecules having relatively low volatility and proton affinity.

DART-MS was also used for quality control of *Radix Salviae Miltiorrhizae (*Danshen in Chinese) injections, a widely used TCM herbal injection [62]. A total of forty-seven samples of herbal injections from different suppliers were subjected to direct DART-MS analysis for detection of representative chemical components. With the aid of principle component analysis (PCA), various chemical components, including fructose, glucose, sucrose, protocatechuic aldehyde and salvianolic acid A, were identified as potential chemical markers for quality control purpose.

Furthermore, DART-MS was utilized in analysis of *Radix Aconiti Preparata*, a commonly used traditional herbal medicine worldwide [63]. Unprocessed *Radix Aconiti*. is highly toxic in nature and misuse of unprocessed or improperly processed *Radix Aconiti*. can be life-threatening [63]. Similar to Fuzi, the toxicities of unprocessed *Radix Aconiti*. are associated with aconitine-type alkaloids including aconitine, mesaconitine and hypaconitine. These toxic alkaloids can be converted into less toxic monoester diterpenoid aconitines and lipoalkaloids through heating, boiling and steaming processes. In the study, the methanolic extracts of processed and unprocessed *Radix Aconiti*. were directly analyzed and their mass spectral profiles were compared. The detected ions for raw and processed *Radix Aconiti*. samples were found to have no large difference, yet the relative abundances of some ion peaks, especially those of the toxic alkaloid components, were different. For example, the relative abundance of toxic alkaloid hypaconitine was significantly reduced in the spectra of qualified processed *Radix Aconiti*. By statistical analysis (i.e., principal component analysis), some potential chemical markers, mainly monoester diterpenoid aconitines and diester diterpenoid aconitines, were identified for the differentiation of processed and unprocessed *Radix Aconiti*..

4.2.3 Electrospray Ionization with Solid Substrates

The techniques of electrospray ionization on solid substrates (solid-substrate ESI) facilitate MS analysis by avoiding the clogging problem in conventional capillary-based ESI and allowing more convenient sampling [64, 65]. So far, solid-substrate ESI with various materials, e.g., metals, paper, wood, fibers and biological tissues, have been developed [64, 65]. Particularly, some solid-substrate ESI techniques have been demonstrated to permit direct analysis of complex samples, leading to its increasingly important role in various biological and chemical

fields, including TCM analysis, nowadays. A wide range of solid-substrate ESI techniques have been demonstrated to be applicable in rapid identification of TCMs for QA and QC purposes.

Paper spray

Paper spray, a technique making use of typical chromatography paper as solid substrate for ESI, was developed by Wang *et al.* in 2010 [66]. In this technique, a piece of chromatography paper was first cut into a small triangle and samples in solution or solid form were loaded onto the paper [66, 67]. By adding extraction solvent and applying of a high voltage, the loaded sample is extracted, transferred to the tip end, and ionized for MS detection. This technique was shown to be able to directly analyze raw complex samples, e.g., herbal materials, foods, and biological fluids, because of the separation capability of paper. For example, this technique was applied in chemical fingerprinting analysis of Bansha herbal tea (BHT) [68]. By directly applying only 2 μL of BHT sample onto the triangular chromatography paper and 10 μL of extraction solvent, a wide range of active ingredients, such as amino acids, organic acids and trierpenoide saponins, were identified. Assisted by PCA analysis, samples from different manufacturers as well as qualified and expired products could be well differentiated.

Wooden-tip ESI

The technique of wooden tip-ESI, which was developed in 2011, makes use of disposable wooden tips (wooden toothpicks) for sampling [69]. By loading sample, which could be in form of solution, semi-solid or solid, to the sharp tip end and applying of a high voltage to the wooden tip, spray ionization could be induced for MS detection (Fig. 4a). This technique was

demonstrated to allow direct analysis of raw complex samples, e.g., biological fluids [70], with no or only little sample preparation.

Wooden tips are hard, slim and very convenient for sampling of samples in various forms. Wooden tip-ESI was demonstrated to be well suitable for rapid analysis of pharmaceutical samples in various forms, including tablets, capsules, granules, dry suspensions, suspensions, drops, and oral liquids [71]. Chemical fingerprints provided by direct analysis of herbal products using wooden-tip ESI had allowed quality assessment and origin tracing of herbal products. Wooden-tip ESI was also applied in rapid and direct analysis of powders of *Fritillariae Cirrhosae Bulbus* for species differentiation [72]. In this study, a wooden tip was first pre-wetted with solvent (MeOH/H_2O 1/1 (v/v)) and then scraped with the herbal powders until a layer of powder was adhered on the tip surface. Afterward, extraction and spraying solvent (MeOH/H_2O 50/50, 0.1% formic acid (v/v)) was added onto the sample layer for extraction and ionization of analytes. A wide range of chemical components, e.g., amino acid, sugars, and alkaloids, were well detected by this simple sample preparation method. With the aid of multivariate statistical analysis, six species of *Fritillariae Cirrhosae Bulbus* were successfully differentiated and *Fritillariae Pallidiflorae* Bulbus, a common adulterant of *Fritillariae Cirrhosae* Bulbus, was also unambiguously identified.

Another study by Yang and Deng applied wooden tip-ESI in rapid QA/QC of Shuang-Huang-Lian (SHL) oral liquid, a well-known TCM herbal preparation [73]. A particular merit of this study is the use of an internal standard, which allows correction of various experimental factors, to semi-quantitatively determine the chemical contents, including organic acids, flavonoids,

phenylethanoid glycosides, etc, of the SHL oral liquid products. By combining with multivariate statistical analysis, SHL oral liquid products from different manufacturers as well as long expired, short expired, and qualified SHL oral liquid products could be well differentiated (Fig 4b-g), indicating the applicability of this technique in rapid QA and QC of herbal medicine preparations.

ESI with Aluminium Foil

Apart from cellulose-based materials such as paper and wooden tips, metals were also applied as solid substrates for ESI-MS [64]. Recently, household aluminium foil was employed as the solid substrate for ESI [21]. Similar to other solid-substrate ESI methods, samples in solution, semi-solid or solid forms are applied onto the surface of aluminium foil and ionized upon application of a high voltage and, if necessary, extraction solvent. As a solid substrate for ESI, aluminium foil has advantages that it is impermeable, rigid and can be readily folded and cut into desired configuration for holding sample solution in bulk [21]. These features not only allow acquisition of more durable signals, but also permit solid samples, e.g., herbal powders, to completely soak in the extraction solvent for more effective sample extraction. ESI with aluminium foil was applied in direct analysis of grinded powder of *Panax quinquefolius* and Fuzi [21]. By loading only small amount, i.e., 5 mg, of herbal powder onto a folded aluminium foil and addition of methanol as extraction solvent, various active components, such as ginsenosides and alkaloids, could be well detected. Due to the desirable features of rapid and high efficiency of on-target extraction, this technique will be dedicated in analysis of more TCM systems later on.

4.2.4 Pipette-tip ESI

This technique combines pipette tips with syringe and syringe pump for direct analysis of powder samples with only little sample preparation (Fig. 5a) [19]. A small piece of cotton swab is placed inside a typical pipette tip at a position near the opening of the tip. A small amount of solid powder sample is loaded into the pipette tip and retained inside the tip by the cotton swab. A stainless steel needle of a glass syringe was then inserted into the pipette tip for delivery of extraction and spraying solvent. When the solvent is delivered from the syringe with a syringe pump and a high voltage is applied to the stainless steel needle of the syringe, analytes could be extracted by the flowing solvent charged with a high voltage and ionized when reaching the tip end for MS detection. This technique was applied to analyze various TCMs and quality mass spectra with stable and durable signals could be obtained. For example, *Panax ginseng*, *Panax quinquefolius* and *Panax notoginseng,* three closely related ginseng herbs, could be rapidly differentiated based on the ginsenoside patterns detected (Fig. 5b-d) [19]. In addition, *F. Schisandrae Chinensis (FSC)* and *F. Schisandrae Sphenantherae (FSS),* two commonly used TCMs having different quality and efficacy, were well differentiated by their lignan patterns [19]. The durable, stable and reproducible signals offered by the technique enable it to have good capability in quantitative analysis, which was demonstrated by quantitative determination of caffeine content in tea samples in the study [19].

Pipette-tip ESI was further advanced by another version of experiment setup, in which a typical pipette tip was replaced by a pipette tip with a C18 sorbent material [20]. The C18 sorbent material could act as a medium for rapid purification and enrichment of analytes in raw sample solutions. This technique was demonstrated to allow rapid and highly effective purification of protein samples with interfering agents, e.g., salts and detergents, and rapid quantification of

drugs and metabolites in raw urine with high analytical performance. It is expected that this technique could be readily extended to rapid qualitative and quantitative analysis of raw extracts of TCMs, which might contain high salt contents and/or could be easily contaminated during processing and handling.

4.2.5 Direct ionization (DI)

DI is a rapid and simple technique for direct analysis of plant and animal tissues [74]. In this technique, a small piece of tissue is fixed ~ 0.5 – 1 cm away from the mass spectrometer inlet with a clip or needle (Fig. 6a). By applying a high voltage to the tissue sample and adding extraction/spraying solvent if necessary, spray ionization could be directly induced from the bulky tissue sample. This technique was applied to directly analyze *Coptis chinensis* Franch (*CCF*) and various known alkaloids of this herb could be predominately detected [74]. In the same study, DI was also used for differentiation of *F. Schisandrae Chinensis (FSC)* and *F. Schisandrae Sphenantherae (FSS)*. Quality spectra with specific lignan patterns could be obtained for the two herbs, allowing unambiguous differentiation of the two species (Fig. 6b & c) [74].

In coherent with the development of DI, similar techniques, termed as tissue spray [75] and leaf spray [76], were also developed for direct analysis of plant tissues. Tissue spray was successfully applied in rapid differentiation of wild-type and cultivated *Panax quinquefolius,* which are significantly different in price and efficacy, based on the chemical components detected, e.g., ginsenosides, amino acids and oligosaccharides [75]. Leaf spray was successfully employed in various natural product analysis, including direct analysis of steviol glycosides from *Stevia*

leaves [77], rapid identification of molecular changes upon ageing of plants (*Ocimum sanctum* Linn) [78], detection of allergenic urushoils directly from poisonous ivy leaves [79], determination of pesticides in peel and pulp of different fruits and vegetables [80], and differentiation of Chinese and Japanese star anises based on their differences in anisatin content [81]. Another similar technique, named as internal extractive electrospray ionization, was also developed for analysis of chemical contents inside bulk samples [82]. In this technique, solvents are supplied into the bulk sample for extraction of the phytochemicals embedded in the herbal sample, followed by direct ionization MS analysis. Stable signals could be obtained and variation of chemicals at different extraction times could be observed with this technique.

More recently, an alternative prototype of DI, field-induced DI, was developed [83]. In this technique, the high voltage for ionization is applied to the mass spectrometer inlet and the sample for analysis is maintained at electric ground (Fig. 7a). This setup avoids connection of the sample to high voltage and significantly facilitates the sampling and analysis, particularly for *in vivo* study. The results showed that variation in alkaloids in a living plant, *Catharanthus roseus*, upon heat stimulation could be readily monitored in real-time using this technique (Fig. 7b). This technique was also used for real-time monitoring of chemical response of living animals upon stimulation in the study. For example, chemical compositions of fresh venom secreted by living toad upon stimulation were detected by this technique. Dried toad venom, i.e., Changsu, is a well-known TCM.

In addition, in order to further speed up and facilitate TCM analysis, a high-throughput field-induced DI was developed and applied for rapid screening of raw herbal materials [84]. In a

high-throughput field-induced DI setup, a two-dimensional (2D) moving stage equipped with a sample plate, controlled by the stepper motor controller, was used to place sharpened raw herbal materials or sample-preloaded wooden tips with an equal distance of ~5 mm. The results showed that major active ingredients of 4 raw herbal materials, i.e, *CCF, Sophora flavescens, Radix Scutellariae, and Glycyrrhiza uralensis Fisch* were detected consecutively at a time of less than 30 s (with a sampling rate of 6 s/sample) in positive or negative ion mode. For example, three major active ingredients of *Coptis chinensis Franch*, coptisine, berberine and palmatine were detected in positive ion mode while malic acid and quinic acid were detected in negative ion mode. Protonated signals of sophocarpine, matrine, oxysophocarpine, and oxymatrine were observed in the positive ion mass spectrum of *Sophora flavescens* while deprotonated Z-4,2',4'-trihydroxychalcone, sophoraflavanone, norkurarinone, kurarinone, kushenol L, and kushenol I were detected in the negative ion mass spectrum. Furthermore, this high-throughput technique was also used for the quality assessment of Yin-Huang (YH) granules. A total of 25 YH granules samples produced by 5 different manufacturers were analyzed and their MS fingerprints were obtained. Orthogonal partial-least squares to latent structures discriminate analysis (OPLS-DA) was performed to the obtained MS fingerprints for quality assessment and origin tracing of YH granules. Discrete clusters peaks were found from different sample groups in the score plot and loading plot of OPLC-DA, showing that this high-throughput technique was a reliable tool for quality control of TCMs.

4.3 Imaging studies of TCMs by MS

An important contribution of MS in plant research is due to its capability in performing imaging study, which allows determination of spatial distribution of chemical components in tissues thus

acquisition of detailed knowledge on biological properties of plants. Three major MS techniques applied in imaging study are MALDI, DESI, and secondary ion mass spectrometry (SIMS). The principle, pros and cons, and applications of these techniques in imaging study are well summarized in several comprehensive literature reviews [85-87]. SIMS, although it is the firstly introduced imaging technique and offers very high spatial resolution, i.e., 100 nm, was rarely applied in plant imaging mainly because of its relatively harsh ionization conditions and poor sensitivity in analyzing large molecules [85-87]. Among the three techniques, MALDI is the most widely used because high spatial resolution down to 20 μm could be readily achieved by commercially available instruments [85]. The uses of MALDI imaging in study of various plant materials, including leaf, stem, seed, and fruit, were widely demonstrated previously [85]. However, a major challenging in MALDI imaging is the matrix interference at low *m/z* range as most plant metabolites are small molecules [85]. This problem could be alleviated by the use of infrared LDI, which does not require addition of matrix for sample desorption/ionization [85, 88]. Infrared LDI imaging offers an added advantage that it can make use of physiological water as matrix, thus allows imaging of samples in their natural state [85, 88]. However, the spatial resolution achieved by infrared LDI is typically lower than UV-MALDI [85].

Apart from MALDI, various ambient ionization techniques have been also applied for imaging studies, which are summarized in a recent review paper [89]. DESI has been widely utilized for imaging of plants in recent years. Although the spatial resolution achieved by DESI imaging is around 200 μm, which is around ten folds less than MALDI imaging, no addition of matrix, relatively simple experimental setup, and only little sample preparation are involved in DESI, allowing imaging study to be carried out conveniently without matrix interference [85, 89]. A

number of recent studies successfully applying DESI for imaging study plants were reported [90-92]. Prospectively, as DESI operates under opened ambient environment, fine tuning of various instrumental conditions could be more readily performed in order to further enhance the applicability of this technique. The uses of other ambient ionization techniques, e.g., easy ambient sonic spray ionization [93], laser ablation electrospray ionization [94], probe electrospray ionization [95], infrared laser ablation metastable-induced chemical ionization [96], low-temperature plasma probe [97, 98], etc, in imaging studies were also attempted previously. The applicability of more ambient ionization techniques in imaging study is worth to be further explored.

5. Conclusions and prospects

In line with the global expansion in the demand of TCMs, development of rapid, cost-effective, and reliable analytical techniques for TCM analysis is an important research topic. This review summarizes various MS-based techniques, mainly ambient ionization-MS and MALDI-MS, applied in rapid and direct analysis of TCMs. For ambient ionization-MS, it is expected that more applications and further development would be seen in near future because of the increasing recognition of these techniques and availability of these techniques in commercial instruments. Also, there are more than 30 ambient ionization techniques reported so far, therefore more explorations on the applicability of different techniques in TCM analysis are expected. For MALDI-MS, development of new sample preparation method for facilitating sampling is of wide interest and highly beneficial to TCM analysis. For example, an oil-assisted solid sample preparation method has been developed to facilitate direct analysis of solid samples [99]. This method may be further extended to direct analysis of solid herbal samples.

Although various successful applications are stated in this review, direct analysis of complex mixtures like TCMs by MS-based techniques could face various challenges. For example, no or only little separation of analytes is involved during analysis in many cases, therefore peak overlapping could be a potential problem. Coupling various direct MS-based techniques with ion mobility mass spectrometers, which allow separation of molecules based on size and shape thus add another dimension of separation, could significantly enhance the detection specificity in direct analysis of complex samples such as TCMs. In addition, it is a general phenomenon that the number of chemical components observed in a mass spectrum obtained by direct MS analysis is far less than the number actually exists in the herb samples. More investigation should be performed to understand the detailed ionization mechanism of various ionization techniques in order to obtain insight into the strategies for increasing chemical coverage. In addition, more efforts are worth to put on investigating the capability of various techniques in absolute quantitative analysis, which is still less readily achieved by ambient ionization-MS techniques.

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7. References

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Figure captions

Figure 1. MALDI-MS spectra acquired for *Panax ginseng* (a) and *Panax quinquefolius* (Canada) (b) respectively. (c) A chart showing the intensity ratio of *m/z* 1147 to *m/z* 1117 (I_{1147}/I_{1117}) in the MALDI-MS spectra obtained for *Panax ginseng* and *Panax quinquefolius*. (Reproduced from ref. 35 with permission)

Figure 2. (a) Schematic diagram of DESI setup. (Reproduced from ref. 52 with permission) (b) DESI mass spectra for a *Stevia* leaf fragment acquired in negative (upper) and positive (lower) ion mode. (Reproduced from ref. 56 with permission)

Figure 3. (a) Schematic diagram of DART setup. (Reproduced from ref. 48 with permission) (b) DART-MS spectrum for *Scutellariae Radix* and MS/MS spectra for baicalein and wogonin, two major chemical components of *Scutellariae Radix*. (Reproduced from ref. 61 with permission)

Figure 4. (a) Schematic diagram of wooden tip-ESI setup. (Reproduced from ref. 69 with permission) (b) – (e) Wooden tip-ESI-MS spectra obtained for qualified SHL oral liquids from four different manufactures. (Reproduced from ref. 73 with permission) (f) & (g) Wooden tip-ESI-MS spectra obtained for short expired (f) and long expired (g) SHL oral liquids. (Reproduced from ref. 73 with permission)

Figure 5. (a) Schematic diagram of the setup of pipette tip ESI. (b) – (d) Mass spectra obtained for direct analysis of (b) *Panax quinquefolius,* (c) *Panax ginseng,* and (d) *Panax notoginseng* by pipette tip ESI. (Reproduced from ref. 19 with permission)

Figure 6. (a) Experimental setup for DI-MS analysis of a tissue sample. (b) & (c) DI-MS spectra obtained for *FSS* & *FSC*, respectively. (Reproduced from ref. 74 with permission)

Figure 7. (a) Experimental setup of field-induced DI. (b) Mass spectra obtained by analysis of a living *Catharanthus. rosues* leaf before (upper) and after (lower) heat stimulation. (Reproduced from ref. 83 with permission)

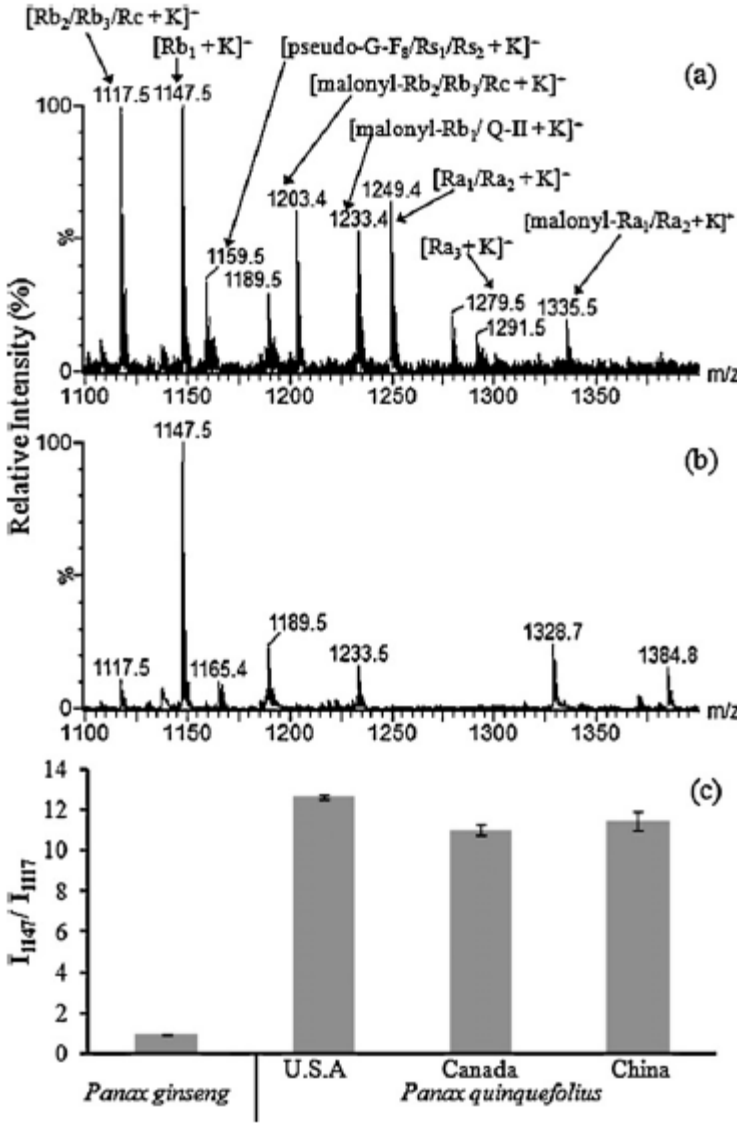
[Rb2/Rb3/Rc + K]-
[Rb1 + K]-
[pseudo-G-F8/Rs1/Rs2 + K]-
[malonyl-Rb2/Rb3/Rc + K]-
[malonyl-Rb1/ Q-II + K]-
[Ra1/Ra2 + K]-
[malonyl-Ra1/Ra2+K]+
[Ra3+K]-
(a)
(b)
(c)
Relative Intensity (%)
m/z
I1147/ I1117
Panax ginseng
U.S.A
Canada
China
Panax quinquefolius

Figure 1

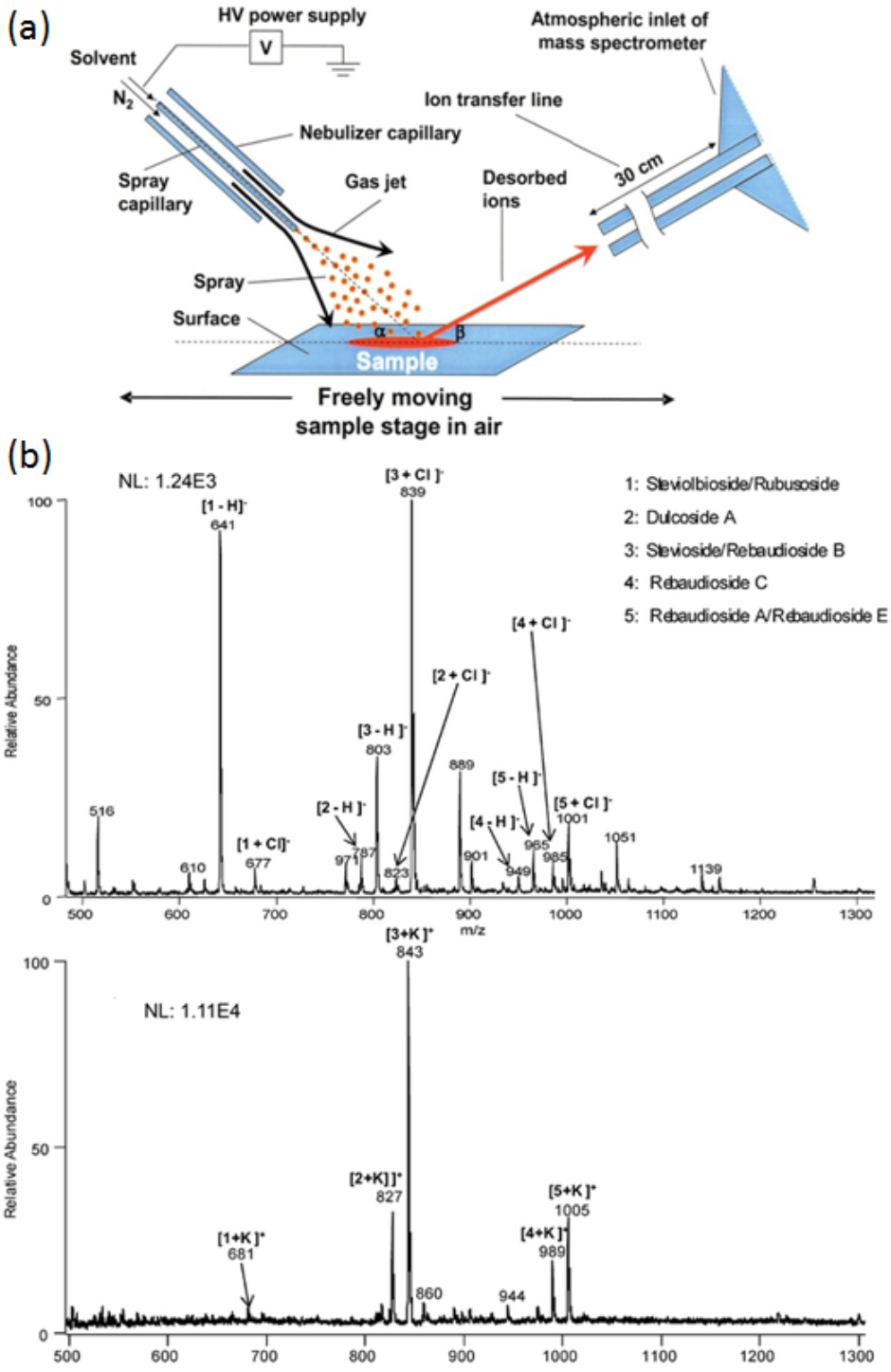
(a)
HV power supply
V
Solvent
N2
Nebulizer capillary
Spray capillary
Gas jet
Spray
Surface
α
β
Sample
Atmospheric inlet of mass spectrometer
Ion transfer line
30 cm
Desorbed ions
Freely moving sample stage in air
(b)
NL: 1.24E3
1: Steviolbioside/Rubusoside
2: Dulcoside A
3: Stevioside/Rebaudioside B
4: Rebaudioside C
5: Rebaudioside A/Rebaudioside E
[1 - H]-
641
[3 + Cl]-
839
[4 + Cl]-
[2 + Cl]-
[3 - H]-
803
889
[5 - H]-
[2 - H]-
[4 - H]-
[5 + Cl]-
1001
516
[1 + Cl]-
677
610
971
787
823
901
949
965
985
1051
1139
Relative Abundance
m/z
[3+K]+
843
NL: 1.11E4
[2+K]]+
827
[5+K]+
1005
[1+K]+
681
[4+K]+
989
860
944

Figure 2

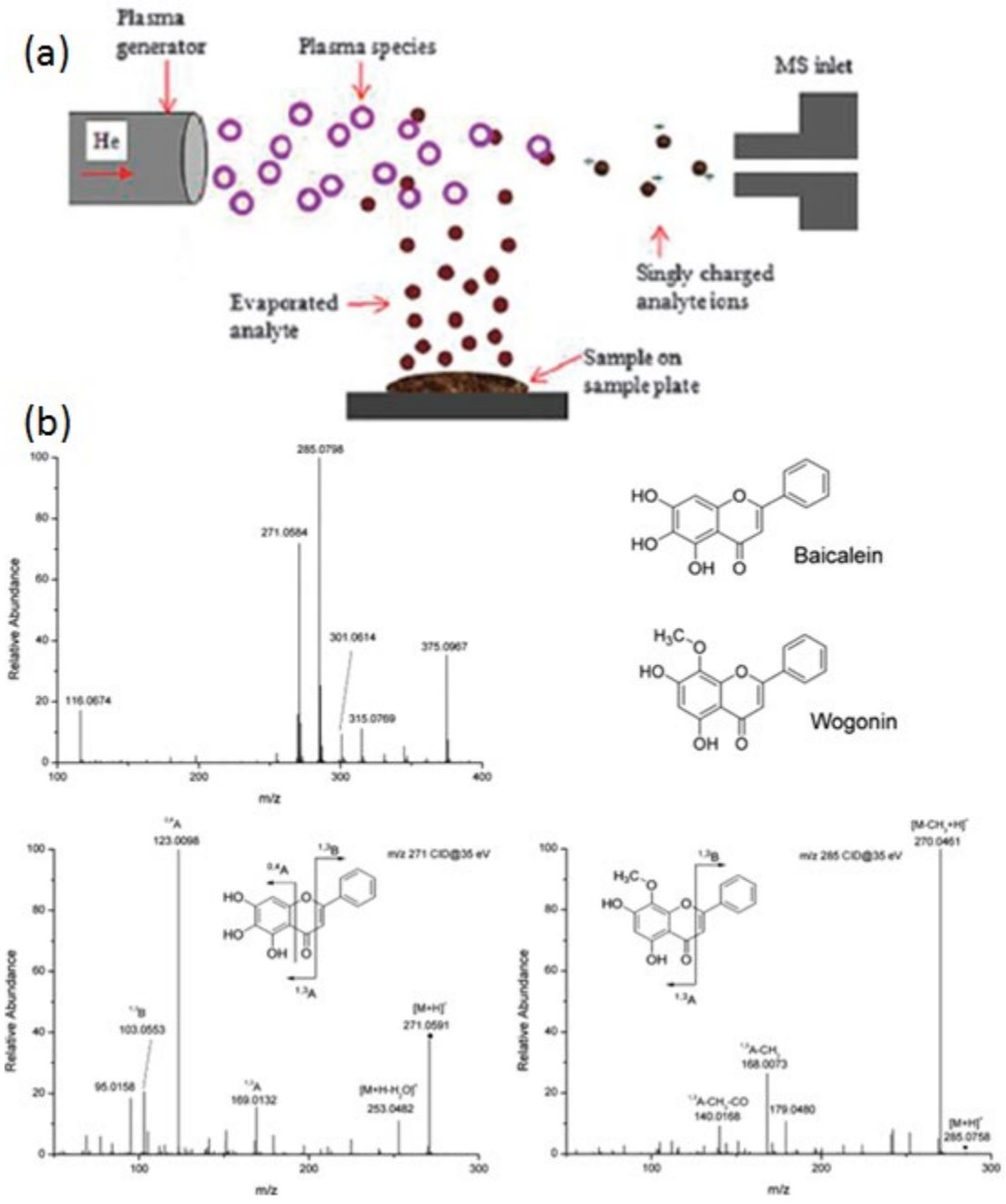
(a)
Plasma generator
Plasma species
MS inlet
He
Singly charged analyte ions
Evaporated analyte
Sample on sample plate
(b)
Relative Abundance
m/z
285.0798
271.0684
301.0614
375.0967
116.0674
315.0769
Baicalein
Wogonin
m/z 271 CID@35 eV
123.0088
103.0553
95.0158
169.0132
253.0482
271.0591
m/z 285 CID@35 eV
270.0461
168.0073
140.0168
179.0480
285.0758

Figure 3

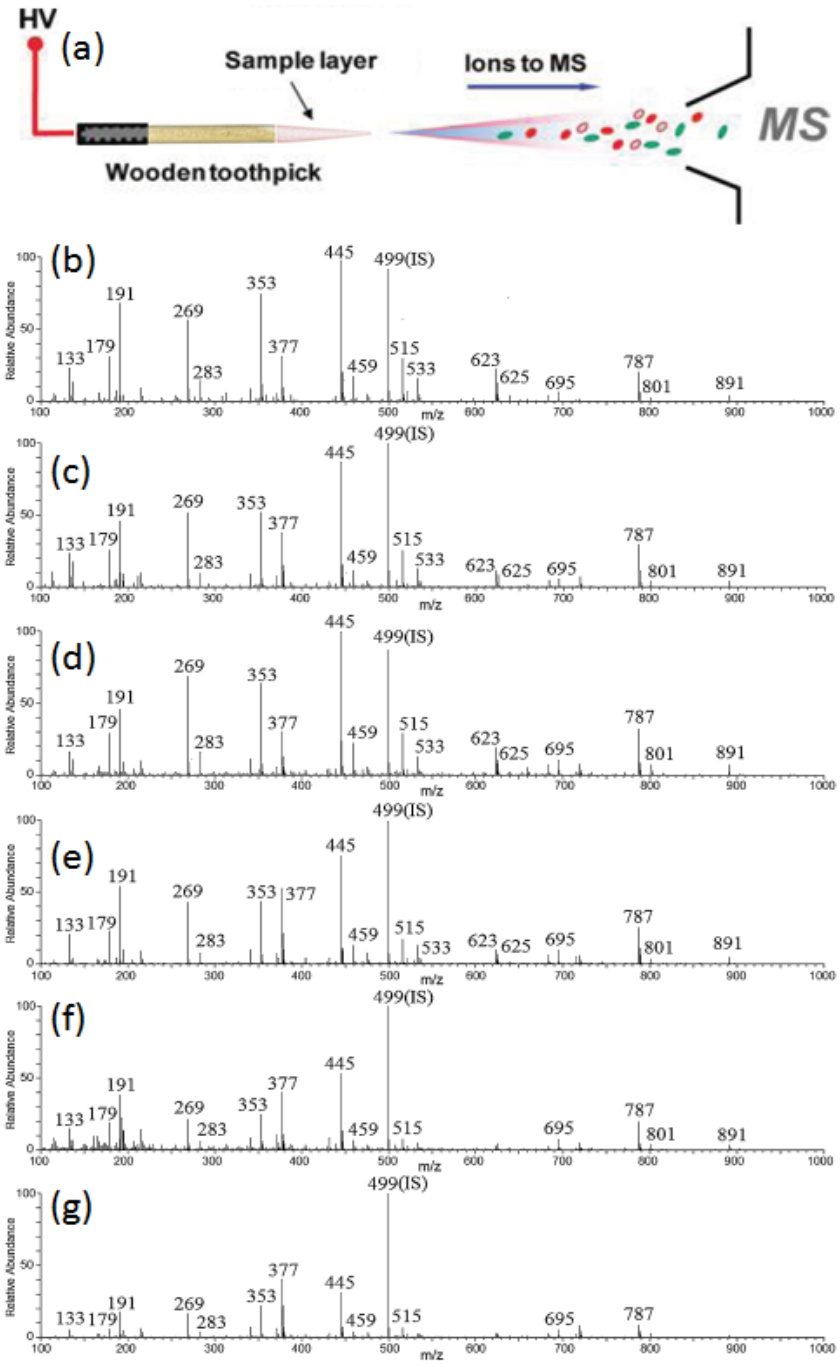
HV
(a)
Sample layer
Ions to MS
MS
Wooden toothpick
(b)
(c)
(d)
(e)
(f)
(g)
Relative Abundance
m/z
499(IS)
445
353
191
269
377
133
179
283
459
515
533
623
625
695
787
801
891

Figure 4

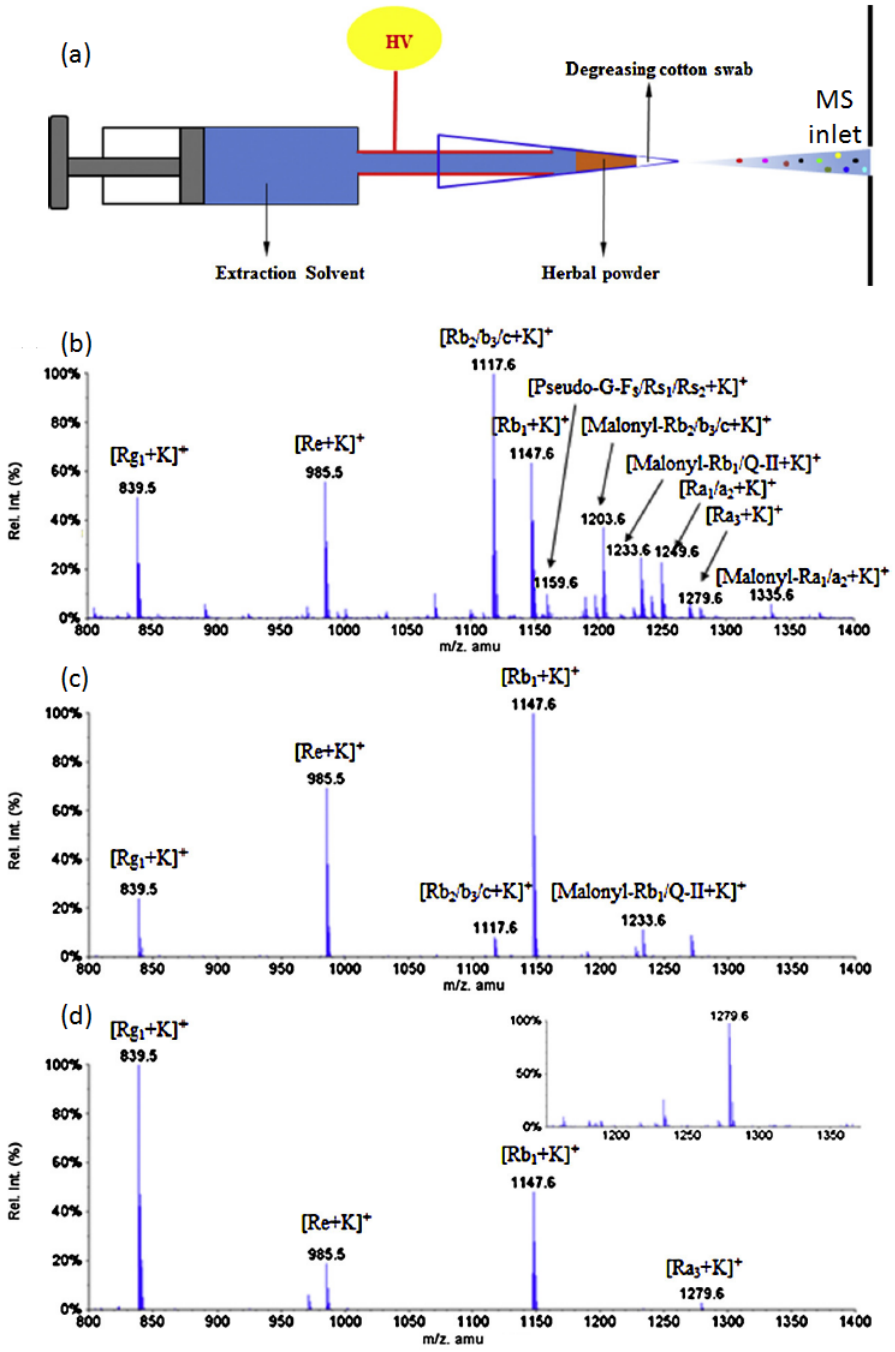
(a)
HV
Degreasing cotton swab
MS
inlet
Extraction Solvent
Herbal powder
(b)
[Rb2/b3/c+K]+
1117.6
[Pseudo-G-F3/Rs1/Rs2+K]+
[Rb1+K]+
1147.6
[Malonyl-Rb2/b3/c+K]+
[Malonyl-Rb1/Q-II+K]+
[Ra1/a2+K]+
[Ra3+K]+
[Rg1+K]+
839.5
[Re+K]+
985.5
1203.6
1233.6
1249.6
1159.6
1279.6
1335.6
[Malonyl-Ra1/a2+K]+
Rel. Int. (%)
m/z. amu
(c)
[Rb1+K]+
1147.6
[Re+K]+
985.5
[Rg1+K]+
839.5
[Rb2/b3/c+K]+
1117.6
[Malonyl-Rb1/Q-II+K]+
1233.6
Rel. Int. (%)
m/z. amu
(d)
[Rg1+K]+
839.5
1279.6
[Rb1+K]+
1147.6
[Re+K]+
985.5
[Ra3+K]+
1279.6
Rel. Int. (%)
m/z. amu

Figure 5

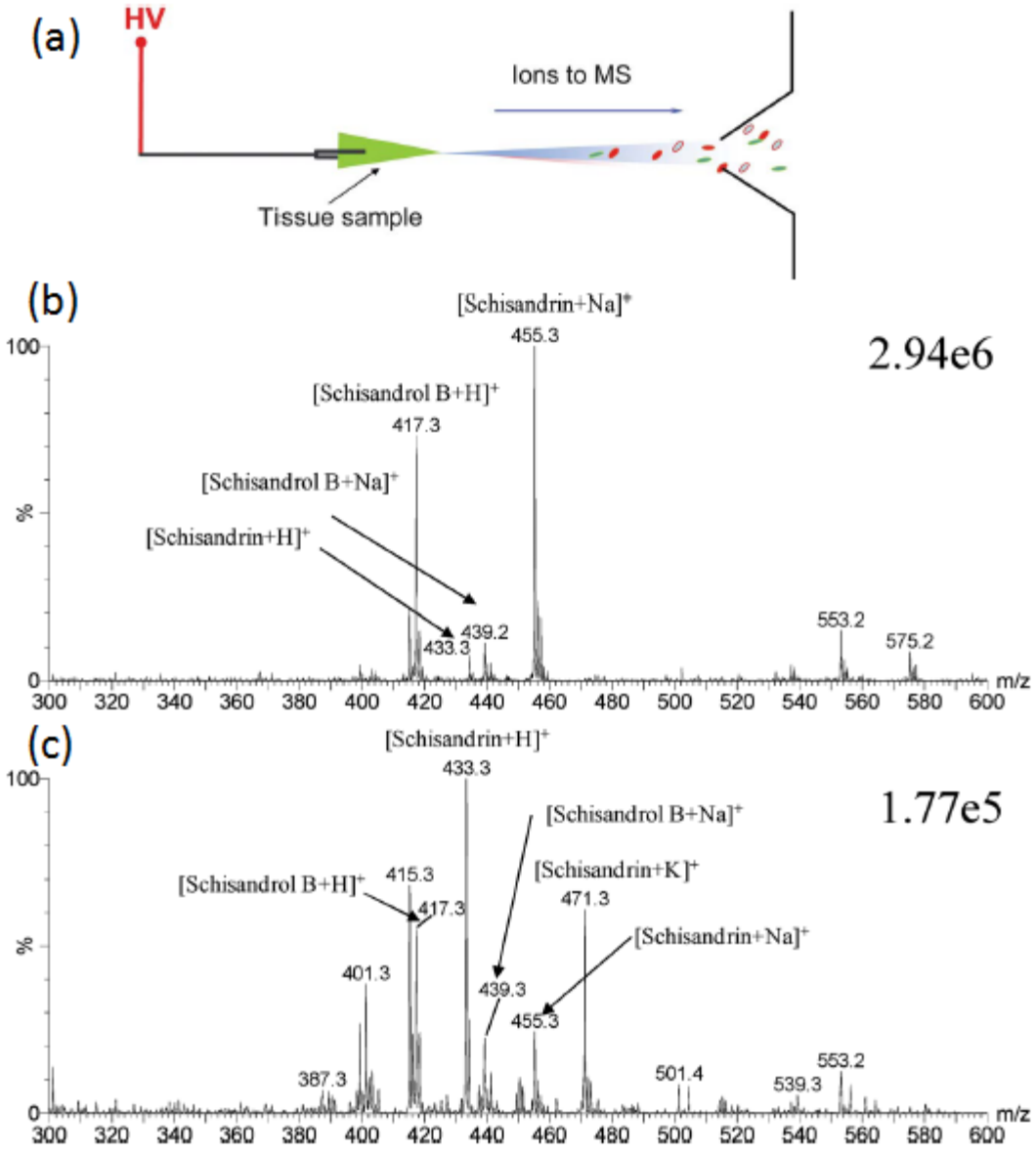
(a)
HV
Ions to MS
Tissue sample
(b)
[Schisandrin+Na]*
455.3
2.94e6
[Schisandrol B+H]+
417.3
[Schisandrol B+Na]+
[Schisandrin+H]+
439.2
433.3
553.2
575.2
100
%
0
300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600
m/z
(c)
[Schisandrin+H]+
433.3
1.77e5
[Schisandrol B+Na]+
[Schisandrin+K]+
471.3
[Schisandrol B+H]+
415.3
417.3
[Schisandrin+Na]+
401.3
439.3
455.3
387.3
501.4
539.3
553.2
100
%
0
300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600
m/z

Figure 6

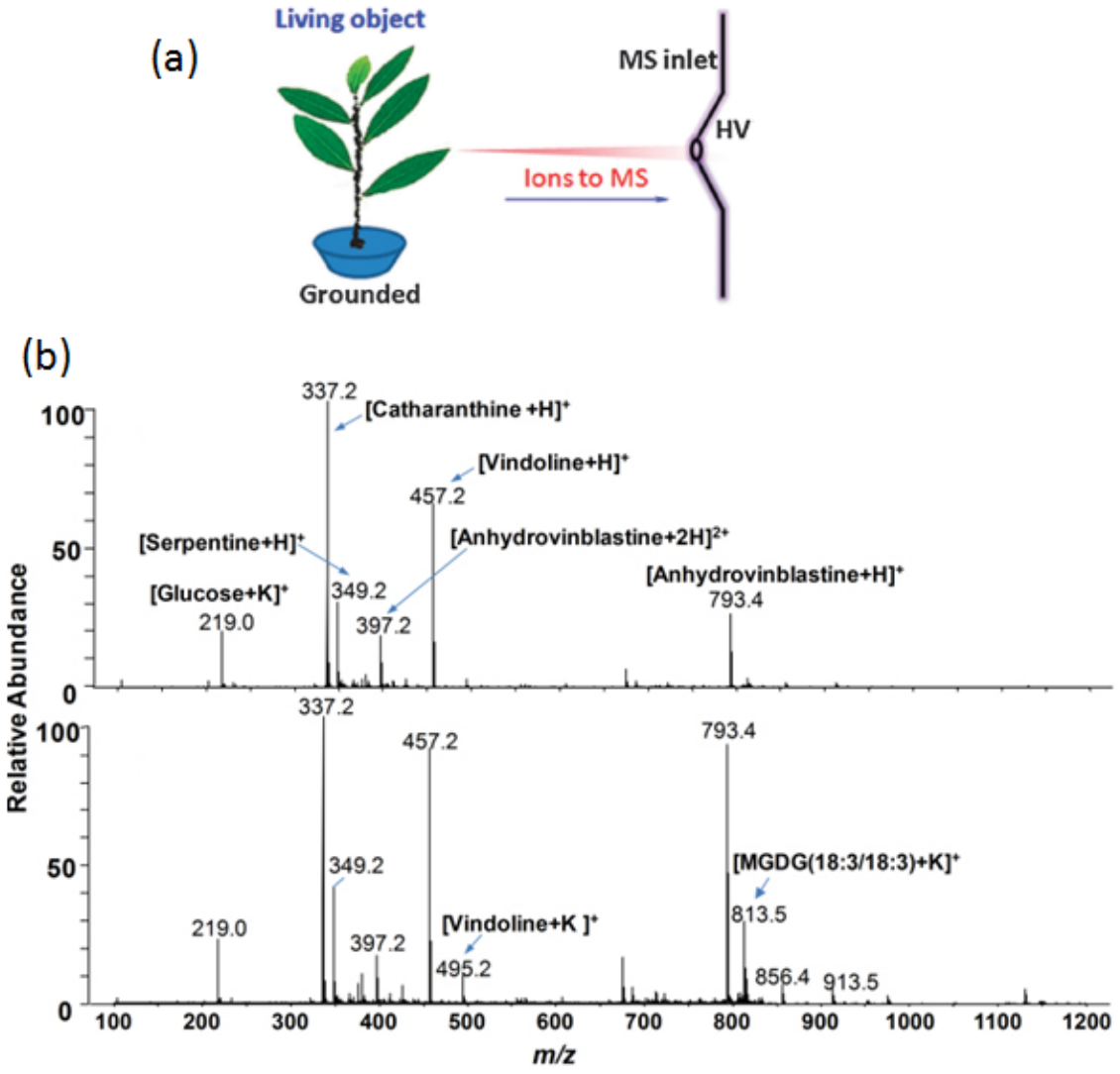
(a)
Living object
MS inlet
HV
Ions to MS
Grounded
(b)
Relative Abundance
m/z
337.2
[Catharanthine +H]+
[Vindoline+H]+
457.2
[Serpentine+H]+
[Anhydrovinblastine+2H]2+
[Anhydrovinblastine+H]+
793.4
[Glucose+K]+
219.0
349.2
397.2
[MGDG(18:3/18:3)+K]+
813.5
[Vindoline+K]+
495.2
856.4
913.5

Figure 7