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Advances of MALDI-TOF MS in the Analysis of Traditional Chinese Medicines

Minghua Lu and Zongwei Cai

Abstract Traditional Chinese medicines (TCMs) are attracting more and more attention because of their long historical clinical experience and reliable therapeutic efficacy for preventing and/or treating various human diseases. Many techniques and methods were developed for the analysis of TCMs to support new drug discovery and quality control. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a soft ionization mass spectrometric technique, has been widely used in the analysis of a wide variety of large molecular compounds including proteins, peptides, and polymers since it was introduced in the late 1980s. In the present chapter, advances of MALDI-TOF MS in TCMs analysis have been reviewed. The review covers MALDI-TOF MS applications in the identification of new bioactive ingredients, analysis of alkaloids, determination of small molecular compounds with new matrices, proteomics analysis associated with TCMs, direct analysis of plant tissue, and other applications in TCMs.

Keywords Chinese herbs · MALDI-TOF MS · Traditional Chinese medicines (TCMs)

Contents

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Abbreviations

2-DE	Two-dimensional gel electrophoresis
α-CHCA	α-Cyano-4-hydroxycinnamic acid
DHB	2,5-Dihydroxybenzoic acid
ESI	Electrospray ionization
HPLC	High performance liquid chromatography
IEC	Ion exchange chromatography
MALDI	Matrix-assisted laser desorption/ionization
MS	Mass spectrometry
TCMs	Traditional Chinese medicines
TLC	Thin-layer chromatography
TOF	Time-of-flight

1 Introduction

Traditional Chinese medicines (TCMs) have been widely used in China for the prevention and/or treatment of human diseases for thousands of years. Due to their long historical clinical experience and reliable therapeutic efficacy, TCMs applications have also been extended to other countries, including Korea, Japan, India, and even European [1] and North American countries [2]. Chinese people have accumulated rich clinical experimental and human clinical data with a long history of the development of theory and clinical practice. However, compared to modern drugs that contain one or two active compounds with known concentration, TCMs are complex mixtures which usually contain hundreds of chemically different constituents. Moreover, the content of the active ingredients in TCMs may be influenced by breed, region of growth, season of harvest, and processing procedures. So far, there have been 12,806 medical resources discovered in China, including 11,145 medicinal plants, 1,581 medicinal animals, and 80 medicinal minerals [3]. In the Pharmacopoeia of the People's Republic of China (2010 edition), a total of 2,136 Chinese medicine products have been compiled [4].

In the process of "modernization" and "globalization" of TCMs, quality control, identification of active ingredients, and study of the toxicology of TCMs, many methods were developed for the analysis of TCMs. Thin-layer chromatography (TLC) is a simple, low-cost, versatile, and specific method for the identification of

herbal medicines [5]. Due to its high resolution, selectivity, and sensitivity, gas chromatography-mass spectrometry (GC-MS) is the most commonly used technique for analysis of liposoluble constituents, especially volatile/semi-volatile compounds and their metabolites in TCMs [6]. Liquid chromatography-mass spectrometry (LC-MS) is another promising technique for the quantitative analysis of bioactive compounds and their metabolites of herbal extract and medicines in biological fluids because the technique can be directly applied for analysis of nonvolatile and low thermal stable compounds without derivatization [7]. Capillary electrophoresis (CE) and capillary electrophoresis-mass spectrometry (CE-MS), with the advantages of high resolution, minimal sample and solvents consumption, short analysis time, and high separation efficiency, are very effective tools for analyzing TCMs and related compounds [8, 9]. Multi-dimensional chromatographic separation systems have emerged with obvious advantages (e.g., strong separation ability, high resolution, and high peak capacity) in analysis of complex samples [10]. In addition to chromatographic and hyphenated techniques, spectroscopic methods, including Fourier transform infrared spectroscopy (FT-IR), near-infrared spectroscopy (NIR), and nuclear magnetic resonance (NMR) spectroscopy, have also been widely used in the quality analysis of TCMs [11].

Matrix-assisted laser desorption/ionization (MALDI), a powerful soft ionization technique proposed in the late 1980s [12, 13], has become a preeminent technique for the analysis of complex samples. Applications of MALDI coupled with time-of-flight mass spectrometry (TOF MS) have been extended to various fields, especially in identification of large molecular compounds, such as peptides/proteins [14–16], lipids [17, 18], polymers [19, 20], oligonucleotides [21], and oligosaccharides [22]. Compared to other mass analyzers such as Fourier transform ion cyclotron (FT-ICR) MS, ion trap MS, quadrupole-based MS, and magnetic-sector MS, TOF MS is the most frequently used detector in MALDI because it lends itself naturally to ion production by a short laser pulse [23, 24]. Therefore the configuration of MALDI-TOF MS is characterized by a large mass range, high sensitivity for ion detection, high tolerance to salts and buffers, and simple and fast analysis [25]. In recent years, MALDI-TOF MS was also applied to analyze small molecular compounds (MW $<$ 1,000) followed by the introduction of various new matrices [26, 27].

This chapter reviews the advances of MALDI-TOF MS in TCMs analysis in the last decade. MALDI-TOF MS as a very useful and powerful tool in the identification of new bioactive ingredients in TCMs is discussed in Sect. 2.1. Applications of MALDI-TOF MS to the analysis of alkaloids that are the large type of bioactive compounds existing in most TCMs are reviewed in Sect. 2.2. Following this, the determination of small molecular compounds in TCMs by MALDI-TOF MS with new matrices is discussed in Sect. 2.3. Proteomic analysis associated with TCMs and the direct analysis of plant tissue by MALDI-TOF MS are reviewed in Sects. 2.4 and 2.5, respectively. In the last section (Sect. 2.6), other applications of MALDI-TOF MS in TCMs are summarized.

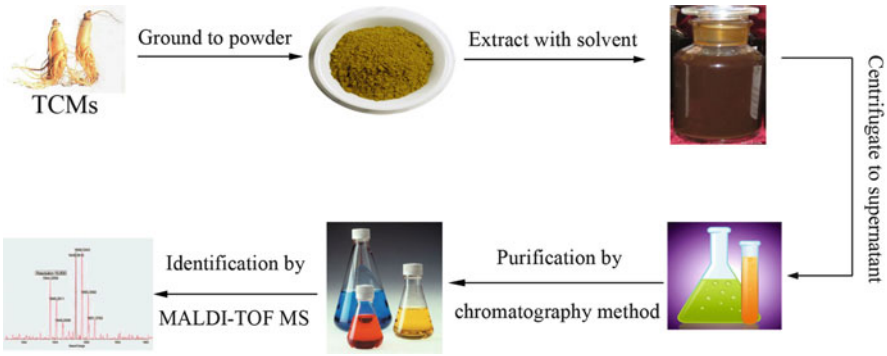


Fig. 1 Scheme for identification of new bioactive ingredients from TCMs by MALDI-TOF MS

2 Methods

2.1 Identification of New Bioactive Ingredients in TCMs

MALDI-TOF MS is a very useful and powerful tool that can be used to identify rapidly new bioactive ingredients in TCMs. Prior to the MALDI-TOF MS analysis, sample preparation procedures, including extraction, purification, and isolation, are usually required. To extract bioactive ingredients effectively from TCMs, herbal samples were usually cut into pieces and ground to powder, and then extracted with suitable extraction solvent. Supernatant obtained by centrifugation was then subjected to purification with chromatography methods, including gel filtration chromatography or size exclusion chromatography, gel electrophoresis, ion exchange chromatography (IEC), and RP-HPLC. The purified bioactive ingredients, such as peptides, proteins, anticoagulants, antifungals, and lectins, were identified by MALDI-TOF MS. A brief scheme for identification of bioactive ingredients in TCMs by MALDI-TOF MS is illustrated in Fig. 1.

Liu and coworkers [28] purified and identified three novel peptides with antioxidant properties from *Cornu Bubali* (water buffalo horn, WBH), a TCM that was used for dispelling heat, counteracting toxins, and relieving convulsions. In this research paper, consecutive chromatographic methods including gel filtration chromatography, IEC, and HPLC were applied to purify and isolate three novel antioxidant peptides from aqueous extract of WBH. The purified peptides were identified by MALDI-TOF/TOF MS in positive ion delayed extraction reflector mode with α-cyano-4-hydroxycinnamic acid (CHCA) as the matrix. The amino acid sequences of the peptides WBH-1, WBH-2, and WBH-3 were identified as hexapeptide Gln-Tyr-Asp-Gln-Gly-Val (708 Da), nonapeptide Tyr-Glu-Asp-Cys-Thr-Asp-Cys-Gly-Asn (1,018 Da), and dodecapeptide Ala-Ala-Asp-Asn-Ala-Asn-Glu-Leu-Phe-Pro-Pro-Asn (1,271 Da), respectively.

Two novel antifungal peptides named EAFP1 and EAFP2 were purified and identified by Huang and colleagues [29] in the bark of *Eucommia ulmoides* Oliv (Du-Zhong in Chinese). EAFPs were obtained from the isolation and purification of bark of *E. ulmoides* Oliv using chromatography methods. The molecular weight and sequence analysis were performed by MALDI-TOF MS combined with CPY time-dependent and concentration-dependent digestion. For MALD-TOF MS analysis, the matrix was prepared by dissolving CHCA in 50% ACN solution containing 0.1% TFA to a saturated solution. Two microliters (about 1–5 pmol/μL) of peptide dissolved in a 0.1% TFA solution in water was mixed with 20 μL of prepared matrix solution. Prior to the MALDI-TOF MS analysis, 1 μL of this mixture solution was deposited on the target and dried. The molecular weight of EAFP1 and EAFP2 were determined as 4,201.4 Da and 4,518.9 Da, respectively. A conclusion that both peptides contain ten cysteines forming five pairs of disulfides was obtained.

Cyclotides are macrocyclic peptides isolated from plants. The compounds typically contain 28–37 amino acids and are characterized by their head-to-tail cyclized peptide backbone and the interlocking arrangement of three disulfide bonds [30]. They were initially discovered based on the potent insecticidal activity, in addition to a range of other biological activities including anti-HIV, antimicrobial, and cytotoxic activities [31]. Wang and co-workers [32] isolated and identified five new and three known cyclotides that were shown to have anti-HIV activity from *Viola yedoensis*, an important Chinese herb from the Violaceae family that has been reported to contain potential anti-HIV agents. Before MALDI-TOF MS analysis, isolation and purification of cyclotides from *V. yedoensis* were performed. The desalted samples were mixed in a 1:1 ratio (v/v) with matrix consisting of a saturated solution of CHCA in 50% ACN (0.5% formic acid) and confirmed by MALDI-TOF MS.

Zhong et al. [33] developed a method for rapid isolation and purification of an anticoagulant from *Whitmania pigra*, a common traditional Chinese anticoagulant medicine in China. A new anticoagulant was isolated and purified from *W. pigra*. This novel component was named whitmanin and its molecular weight was determined as 8,608 Da by MALDI-TOF MS with sinapinic acid (SA) as matrix. *Rosa chinensis* (Yuejihua in Chinese) is a well-known ornamental plant, and its flowers are commonly used in TCM for treating catamenia disorder, trauma, and blood disorders. A total of 36 known and unknown phenolic antioxidants were simultaneously determined in methanolic crude extracts of dried *R. chinensis* flowers by LC coupled with single-quadrupole electrospray ionization MS (ESI-MS) and MALDI-quadrupole ion trap (QIT)-TOF MS [34]. MALDI-QIT-TOF MS was applied not only to confirm 31 of all 36 known and unknown phenolics isolated and identified by LC-ESI-MS, but also to identify tentatively two ellagitannins (rugosins B and C). Additionally, MALDI-QIT-TOF MS analysis only took a few minutes per run whereas LC-ESI-MS analysis took >100 min per run.

Clematis Montana lectin, a novel mannose-binding lectin with antiviral and apoptosis-inducing activities, was isolated from *Clematis montana* Buch.-Han stem (*Ranunculaceae*)[35]. *C. Montana* lectin was a homodimer of 11,968.9-Da subunits as determined by gel filtration and MALDI-TOF MS. The peptide mass

fingerprinting of *C. Montana* lectin was identified by MALDI-Q-TOF MS/MS, which indicated that *C. Montana* lectin may be a novel plant lectin. Yan and coworkers [36] isolated a novel homodimeric lectin (AMML) with antifungal activity from a Chinese herb, that is, the root of Astragalus mongholicus, by using a combination of ammonium sulfate fraction chromatography and IEC. The molecular mass of intact AMML was determined to be 66,396 Da by MALDI-TOF MS and 61.8 kDa by gel filtration, respectively. Experimental results also demonstrated that AMML was a dimeric protein composed of two identical subunits each with a molecular mass of 29.6 kDa.

Copper-zinc superoxide dismutase (Cu, Zn SOD) was extracted, purified, and characterized by Haddad and Yuan [37] from Radix lethospermi seed (RLS), a kind of TCM. Extraction and purification were carried out by consecutive chromatographic methods including ammonium sulfate fractionation, IEC, column chromatography, and a second step of IEC before MALDI-TOF MS analysis. The molecular weight of RLS Cu, Zn SOD was determined by following several methods. On gel filtration, a single peak was obtained with a molecular weight of 32 kDa for a whole enzyme. The rough size of the subunit determined by SDS-PAGE was 16 kDa. The exact mass of one subunit of SOD was determined as 15.166 kDa by MALDI-TOF MS with CHCA as matrix.

2.2 Analysis of Alkaloids in TCMs

Alkaloids, a group of compounds that mostly contain basic nitrogen atoms, are widely distributed in various living organisms including plants. Alkaloid-containing plants have been used by humans since ancient times for therapeutic and recreational purposes, especially TCM herbs. Therein, more than 2,000 alkaloids were identified since morphine, the first individual alkaloid, was isolated from the opium poppy plant by Friedrich Sertürner in 1804. Many alkaloids were discovered to have medicinal properties. TCMs containing tropane alkaloids have been used to treat asthma, chronic bronchitis, pain, and flu symptoms. However, not all TCM herbs can be directly used because they may contain toxics alkaloids, such as aconitum alkaloids in Fuzi. Thus, proper processing of the herb is usually required to reduce the amount of toxic alkaloids prior to use [38]. Many qualitative and quantitative methods have been developed for the purposes of quality control, forensic medicine, and therapeutic drug monitoring of TCMs [39–43]. MALDI-TOF MS, a relatively new analytical technique, with characteristics of easy sample preparation, high throughput, and strong identification ability, was also applied to the analysis of alkaloids in TCMs [44–50].

A method for direct analysis of alkaloid profiling in Chinese herbs tissue by using MALDI-TOF MS was established in our laboratory, which will be discussed in detail in Sect. 2.5 [45, 46]. Feng and Lu [47] developed a method for analyzing low molecular weight compounds and applied to the determination of carcinogenic

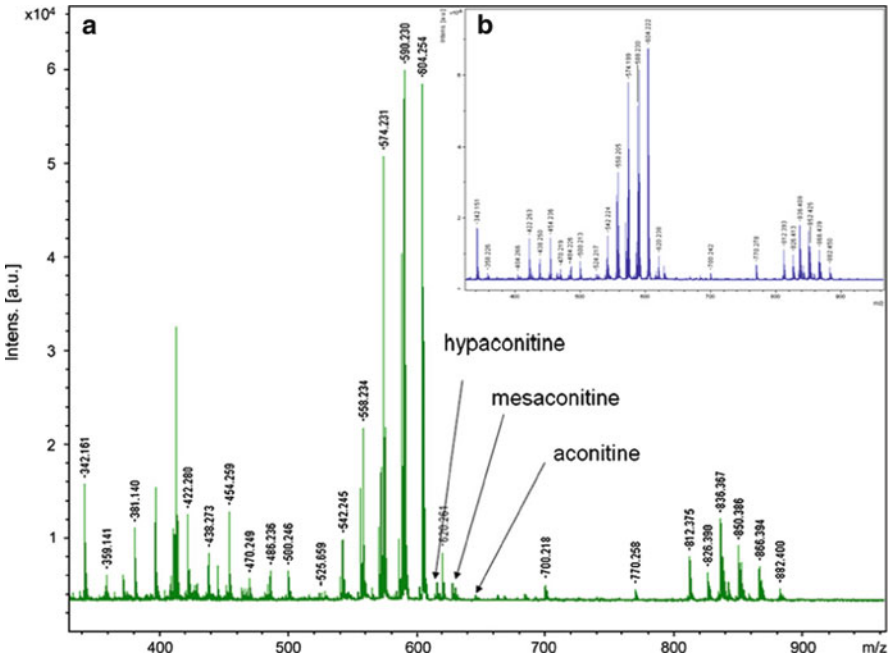


Fig. 2 (**a**) Typical MALDI-TOF MS spectrum of Aconitum alkaloids from *A. carmichaeli* root. (**b**) Typical MALDI-TOF MS spectrum of Aconitum alkaloids from direct tissue analysis. Copyright with permission from Elsevier Science B.V [49]

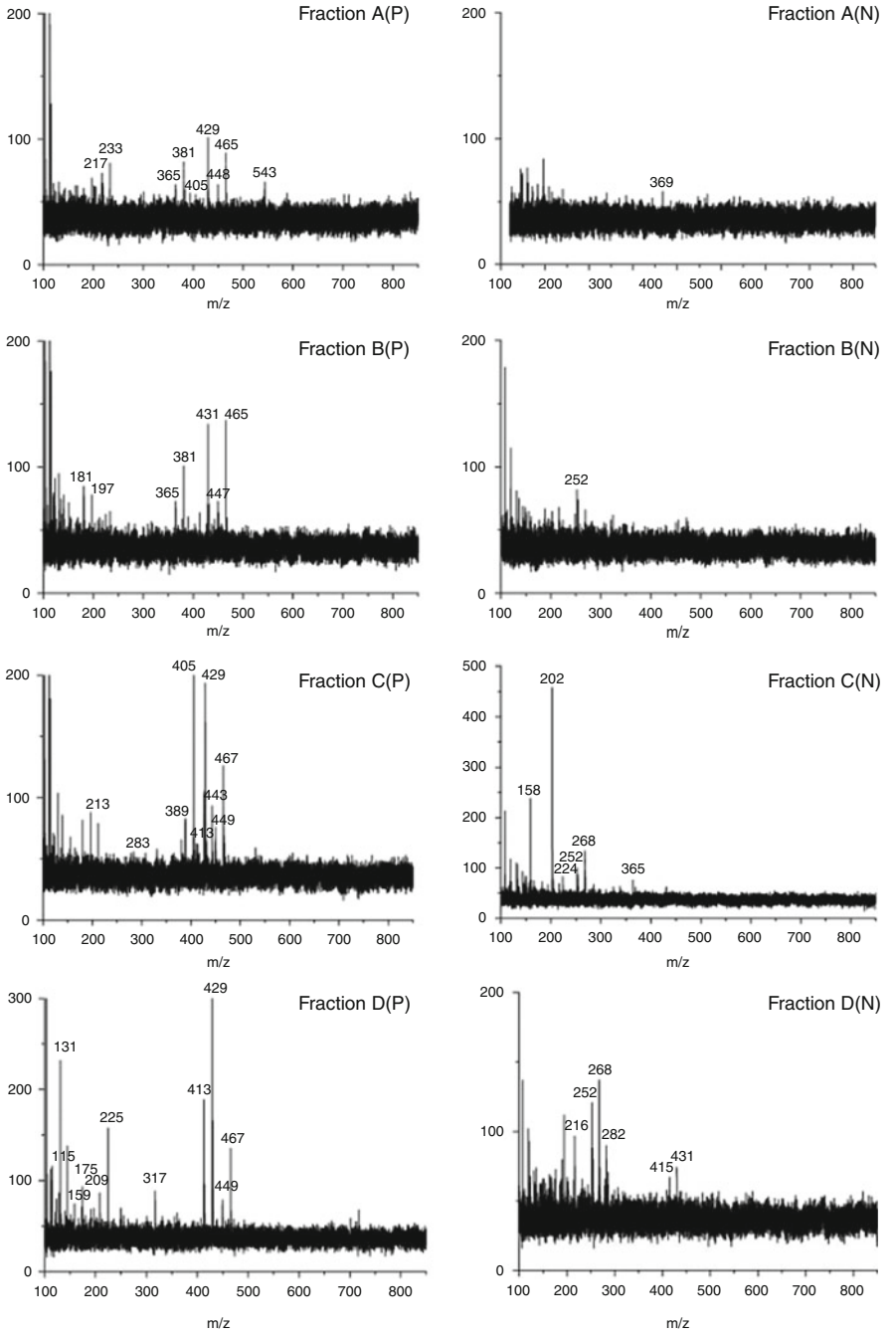
areca alkaloids by MALDI-TOF MS with a new matrix, which will be reviewed in the next section.

TLC combined with MALDI-TOF MS is a powerful technique for fast and high throughput analysis of compounds in complex samples. However, this technique usually suffers from dilution of the TLC bands resulting in decreased sensitivity and masking of signals in the low-mass region because of addition of matrix. A matrix-free TLC and laser desorption/ionization MS method was developed for separation and identification of medicinal alkaloids, berberine and palmatine from *Berberis barandana*, by Shariatgorji and coworkers [48]. Wang and coworkers [49] developed a high throughput and robust qualitative MALDI-TOF MS method for profiling alkaloids in Fuzi, the processed lateral roots of the TCM *Aconitum carmichaeli* Debx (*A. carmichaeli*). Under optimized conditions, a typical MALDI-TOF MS profiling spectrum of a Fuzi extract using DHB as matrix was obtained and is shown in Fig. 2. A solid sample analysis method was also investigated by applying the matrix solution onto the powdered samples directly on the sample plate. The ratio of sample mass to DHB matrix volume was proved to have a significant impact on the mass spectrum. When the ratio was 10 μg to 0.5 μL, similar mass spectra to that with the conventional solvent extraction method was achieved (Fig. 2a, b). Furthermore, the semi-quantitative potential of MALDI-TOF MS was studied and compared by using LC-MS as reference.

A MALDI-TOF MS method for rapid and direct profiling of alkaloids in medical herbs was developed by Lu and colleagues [50]. The dry herbs were ground to powder and passed through a stainless steel sieve, mixed with DHB matrix solution to form a homogeneous suspension, and directly subjected to MALDI-TOF MS analysis. DHB was considered as optimized matrix for analysis of alkaloids in TCM herbs by MALDI-TOF MS, which agreed with previous work published by Wang and coworkers [49].

2.3 *Determination of Small Molecular Weight Compounds in TCMs with New Matrices*

Since it was first introduced in the late 1980s [12, 13], MALDI-TOF MS has been successfully applied to the analysis of various types of large molecules such as peptides, proteins, and polymers. However, using conventional MALDI-TOF MS for the analysis of small molecular weight compounds is difficult since it suffers from strong interference in the low-mass range from the matrices. To overcome the drawbacks of MALDI-TOF MS in analysis of small molecular compounds (<1,000 Da), many methods and matrices, including various matrix-free desorption/ionization MS [26, 51, 52], various carbon nano-materials [27, 53, 54], as well as other inorganic [55, 56] and organic [57, 58] materials, were introduced in recent years. A method based on oxidized carbon nanotubes as matrix for analysis of a TCM *Psoralea corylifolia* by MALDI-TOF MS was developed by Chen and his coworkers [59]. By using oxidized carbon nanotubes as matrix, all 11 fractions were analyzed by MALDI-TOF MS in both negative and positive ionization modes. A large number of individual species of small molecular weight compounds were detected in the fractions with high detection accuracy and sensitivity by MALDI-TOF MS (Fig. 3). A total of more than 188 components were isolated and identified from the extract of *P. corylifolia* by integration of IEC fractionation with RPLC-APCI MS and MALDI-TOF MS. Among these compounds, a total of 92 dominant molecular ion peaks could only be detected by MALDI-TOF MS. Therefore, MALDI-TOF MS with matrix of oxidized carbon nanotubes is a complementary technique with LC-APCI MS for detection and analysis of small molecules in a complex sample. Chen and colleagues [60] also established a hyphenated method (including IEC, RPLC-APCI-MS, and MALDI-TOF MS) for isolation and identification of components in a TCM of Honeysuckle. For MALDI-TOF MS analysis, oxidized carbon nanotubes were used as matrix. A total of 262 components were detected from the extract of Honeysuckle by UV detector, APCI-MS, or MALDI-TOF MS. Among these compounds, 145 components only could be detected by MALDI-TOF MS alone. Compared with LC-UV and LC-APCI-MS, MALDI-TOF MS using oxidized carbon nanotubes as matrix exhibited a very powerful ability in the identification of low-mass compounds in complex samples, such as TCMs and biological samples. Using



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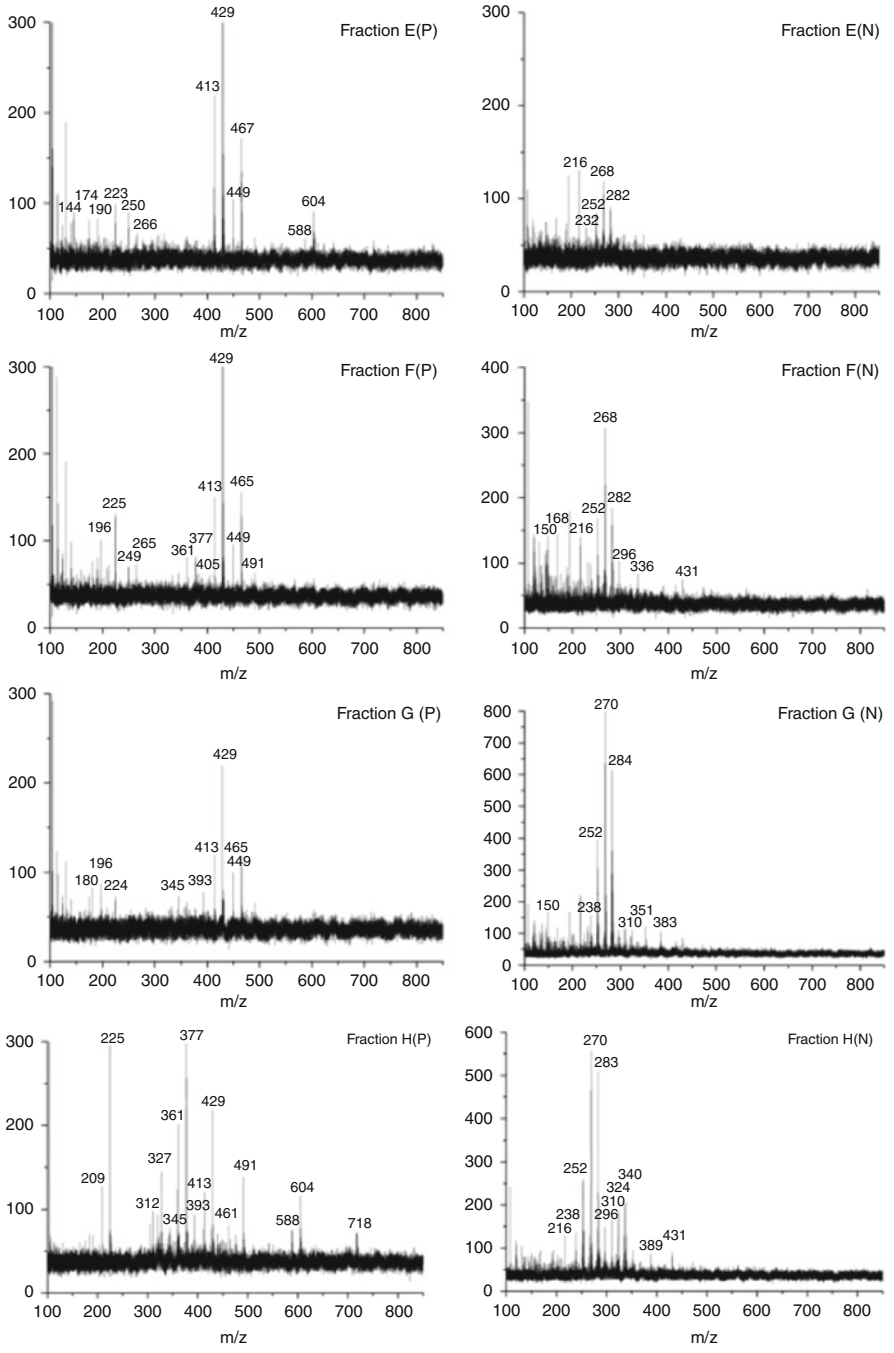

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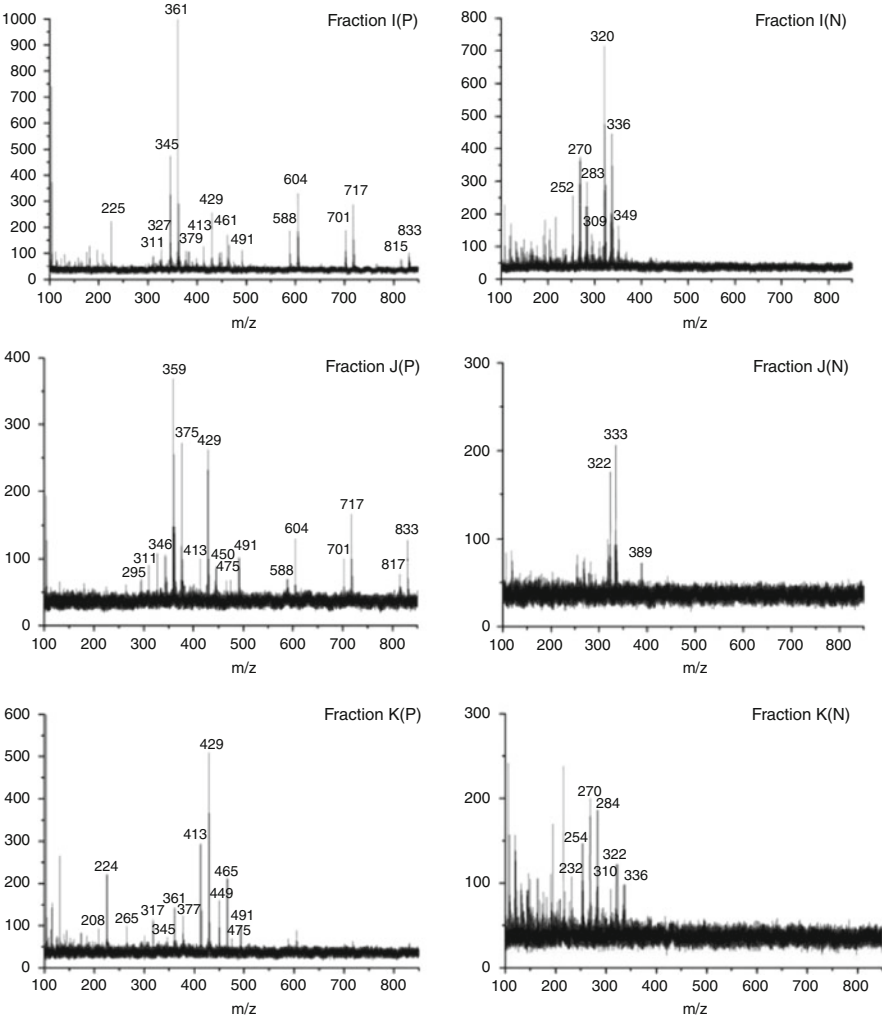

Fig. 3 MALDI-TOF MS spectra of the fractions A to K obtained from SCX column separation of *Psoralea corylifolia* extract. The mass spectra of all fractions were acquired with laser power adjusted to slightly above the threshold energy for all of the components with oxidized carbon nanotubes as matrix and detected in positive ion mode (P) and negative ion mode (N). Copyright with permission from Elsevier Science B.V [59]

oxidized carbon nanotubes as matrix, Pan et al. [53] developed a method for quantitative determination of the concentrations of jatrorrhizine (8.65 mg/mL) and palmatine (10.4 mg/mL) in an extract of *Coptis chinensis* Franch. Hu and his coworkers [61] established a MALDI-TOF MS using carbon nanotubes for the analysis of low-mass compounds in environmental samples. In this article, two arsenic speciations ($HAsO_2$ and H_3AsO_4) in the extracts of TCMs were directly

detected without any further separation and purification by MALDI-TOF MS with carbon nanotubes as matrix.

Feng and Lu [47] developed a MALDI-TOF MS method with a new matrix "7-mercapto-4-methylcoumarin" for analyzing low molecular mass compounds and it was applied to the determination of carcinogenic areca alkaloids. Furthermore, the new matrix was also used for determination of the signals of arecoline and arecaidine in the MALDI imaging experiment. The established method was succeeded to trace analysis of arecoline in human plasma at sub-micromolar level.

2.4 Proteomic Analysis Associated with TCMs

TCMs are great sources in which to discover new bioactive compounds with anticancer, antitumor, antifungal, and/or other biological activities [62–64]. To elucidate their working mechanism(s) in the treatment of various diseases, a comparative proteomics analysis was usually carried out by 2-DE and MALDI-TOF MS. Comparisons were made by gel images between TCMs treated samples and untreated controls. Differentially expressed proteins (usually greater than twofold difference) between treated samples and untreated controls that were excised from one or more gels were identified by MALDI-TOF MS.

Some TCMs, such as saponins, have great value as potent cancer prevention and chemotherapeutic agents. However, the active mechanisms of these compounds were not clear. Wang and colleagues [65] used a proteomic method to examine the cytotoxic effect of dioscin, a glucoside saponin, on human myeloblast leukemia HL-60 cells. Thirty-nine differentially expressed proteins after dioscin treatment for 24 h were identified by separation of the microsomal fraction and subsequently analyzed by MALDI-TOF MS. Ge and co-workers [66] applied proteomics to analyze the arsenic trioxide (ATO)-induced protein alterations in multiple myeloma (MM) cell line U266 and then investigated the molecular pathways responsible for the anticancer actions of ATO. It is noted that 84 differentially expressed proteins were excised from 2-DE and identified by MALDI-TOF/TOF MS followed by database interpretation in this study. Among them, 76 proteins including 29 up-regulated and 47 down-regulated proteins were identified successfully.

Tubeimoside-1 (TBMS1) is a triterpenoid saponin extracted from *Bolbostemma paniculatum* (Maxim.) Franquet (Cucurbitaceae), a Chinese herb with anticancer potential called "Tu Bei Mu". Xu et al. [67] studied the cytotoxic effects of TBMS1 on HeLa cells by a comparative proteomics approach to delineate the possible molecular basis of TBMS1-induced cancer cell death. 2-DE and MALDI-TOF MS/MS were applied to identify altered proteins related to energy metabolism and protein synthesis in this research work. To elucidate the anti-tumor mechanism of Rhizoma Paridis total saponin (RPTS) from the herb Rhizoma Paridis, a proteomic analysis for studying the change of proteins between the control and RPTS treated cells was carried out using MALDI-TOF MS by Cheng et al. [68]. The digests obtained were spotted onto the anchorchips with 1 μL of analyte in duplicate and

0.05 μL of 2 mg/mL CHCA in 0.1% TFA/33% ACN which contained 2 mM ammonium phosphate. The experiments were performed on a TOF Biflex IV mass spectrometer (Bruker Daltonics) in positive ion reflectron mode. The data were searched against NCBInr database by MASCOT search engine. More than 50 proteins showed a significant change between control (0.01% DMSO) and RPTS (IC50 approximately 10 μg/mL) treated cells after 48 h. Twelve proteins were identified by MALDI-TOF MS using peptide fingerprinting from 15 protein spots (density difference greater than twofold between the control and RPTS-treated groups).

Sanqi, also called tianqi, the root of *Panax notoginseng*, is an important medicinal substance in TCM with blood-stanching and blood-quickening actions. Notoginsengnosides (NG) isolated from Sanqi could inhibit ADP-induced platelet aggregation of rat washed platelets. To identify the possible target proteins of NG in platelets, 2-DE-based comparative proteomics was performed and proteins altered in expressional level after NG treatment were identified with MALDI-TOF MS/MS by Yao and coworkers [69]. The proteins were digested with trypsin and the peptides were analyzed using an ABI 4700 Proteomics Analyzer with delayed ion extraction. The MS data obtained were investigated using the MASCOT search engine against the NCBI protein sequence database with a score of more than 50.

To clarify the mechanism regarding the concomitant use of berberine (BBR) and fluconazole (FLC) could provide a synergistic action against FLC-resistant *Candida albicans* (C. albicans) clinical strains in vitro, a comparative proteomic study was performed in untreated control cells and cells treated with FLC and/or BBR in two clinical strains of *C. albicans* resistant to FLC by Xu et al. [70]. The peptides obtained were spotted onto a MALDI target and overlaid with 0.8 μL of matrix solution (CHCA in 0.1% TFA and 50% ACN). The samples were analyzed on an Applied Biosystems TOF-TOF Proteomics Analyzer in positive reflection mode. The MS and MS/MS spectra were searched using the MASCOT engine to identify the proteins. A total of 16 differentially expressed proteins, most of which were related to energy metabolisms (e.g., Gap1, Adh1, and Aco1) were identified by 2-DE and MALDI-TOF MS in this research work.

2.5 *Direct Analysis of Plant Tissue*

In recent years, direct tissue analysis has gained more and more attention because it does not require complicated and tedious extraction and purification procedures prior to MS analysis. Although new techniques have been introduced recently, including desorption electrospray ionization MS (DESI MS) [71] and surface-enhanced laser desorption ionization TOF MS (SELDI TOF MS) [72], most reports on direct analysis were focused on MALDI-TOF MS [14, 17, 22, 73–76]. Up to now there are two types of experimental methods using MALDI-TOF MS for direct tissue analysis, namely profiling and imaging. According to the type of experiment being performed, there are also two different methods of matrix deposition techniques used: spotting and coating. The profiling experiments focus on comparison of different regions in tissue by analyzing several spots (typically

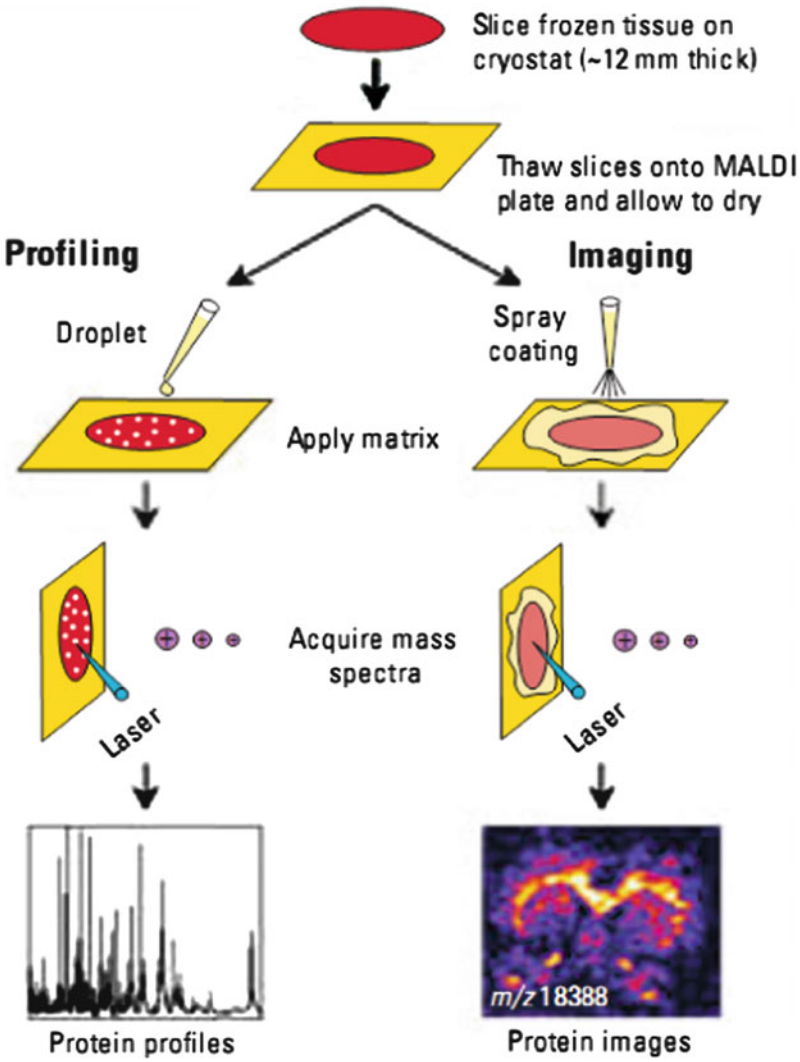

Fig. 4 Procedures involved in profiling and imaging MS of mammalian tissue samples. Copyright with permission from American Chemical Society [77]

ten spots) per tissue section from large numbers of samples. Thus, for profiling experiments, the matrix is deposited directly onto specific regions of interest in the tissue section. For imaging experiments, to obtain high-resolution two-dimensional images, the experiments are usually run on one or two prototypical samples. Therefore, the matrix must be homogeneously distributed over the entire tissue section and the individual mass spectra are automatically acquired in a raster pattern across the entire tissue section. A general scheme for profiling and imaging sample preparation is shown in Fig. 4 [77].

The application of MALDI-TOF MS to direct profiling and imaging has made great progress in tissue analysis. Most of the reports about direct profiling and imaging tissue were focused on animal tissue for proteins and peptides analysis. Only a few research papers for direct profiling and imaging plant tissues of TCMs were reported, probably because most of the compounds in TCMs have a molecular weight below 1,000 Da. In this mass range it is difficult to identify analytes by using traditional MALDI-TOF MS because the signals may be interfered with by matrix background ions. Direct analysis of plant tissue by MALDI-TOF MS has become more and more applicable with the development of many new matrices for analysis of small molecular compounds. A method for the direct determination of alkaloid profiling in plant tissue by MALDI-TOF MS was developed in our laboratory [45]. Four commonly used Chinese medicinal herbs including Aconitum Carmichaeli Debx. (Fuzi in Chinese), Processed Fuzi, *Coptis chinensis* Franch. (Huanglian in Chinese), and Corydalis yanhusuo W.T.Wang (Yanhusuo in Chinese) were studied for herb differentiation and explanation of the significant differences in their toxicities. Briefly, plant tissues were cut into slices with a thickness of 10–20 μm and then these slices were adhered to MALDI target plate. The matrix was deposited onto the tissue surface directly before the sample plant was placed in a vacuum desiccator to improve crystal homogeneity prior to the MALDI-TOF MS analysis. Among all commonly used matrices, CHCA and DHB were found to be effective for analyzing the low-molecular-weight compounds in complex samples [78, 79]. In optimized conditions, alkaloid profiles in Fuzi tissue were investigated by MALDI-TOF MS with CHCA as matrix and the profiling spectrum is illustrated in Fig. 5a. To study the differentiation of Fuzi and Processed Fuzi as well as their toxicity, the direct analysis of Processed Fuzi under the same experimental conditions was performed and the spectrum is shown in Fig. 5b. The usability of direct analysis on plant tissue for detecting the alkaloid profiles indicated that MALDI-TOF MS could be applicable for the discovery of new compounds. In addition, a rapid and straightforward method for direct alkaloid profiling in crude and processed Strychnos nux-vomica seeds by MALDI-TOF MS was also used in our laboratory [46]. Alkaloid profiles in tissues from different parts (endosperm and epidermis) of crude Semen Strychni and the tissues from different heating processes (sand and oil) were analyzed and differentiated by direct MALDI-TOF MS (data not shown here). The spectrum of alkaloid profiles obtained from MALDI-TOF MS could provide much valuable information for the differentiation of crude and processed Strychnos nux-vomica seeds and for explanation of the significantly different toxicity.

A method for direct spatial profiling of phytochemicals and secondary metabolites in integrated herbal tissue by MALDI-TOF MS was developed by Ng and coworkers [80]. Experiment demonstrated that among all the different matrices used, including CHCA, SA, and DHB, CHCA could form relatively uniform layers of crystals on the stem tissue and assist the desorption/ionization of the alkaloids effectively upon N_2 laser ablation. For example, the abundance of alkaloid ions desorbed with CHCA matrix is ten times that of SA and DHB matrices. The established method was applied to determine the spatial distributions

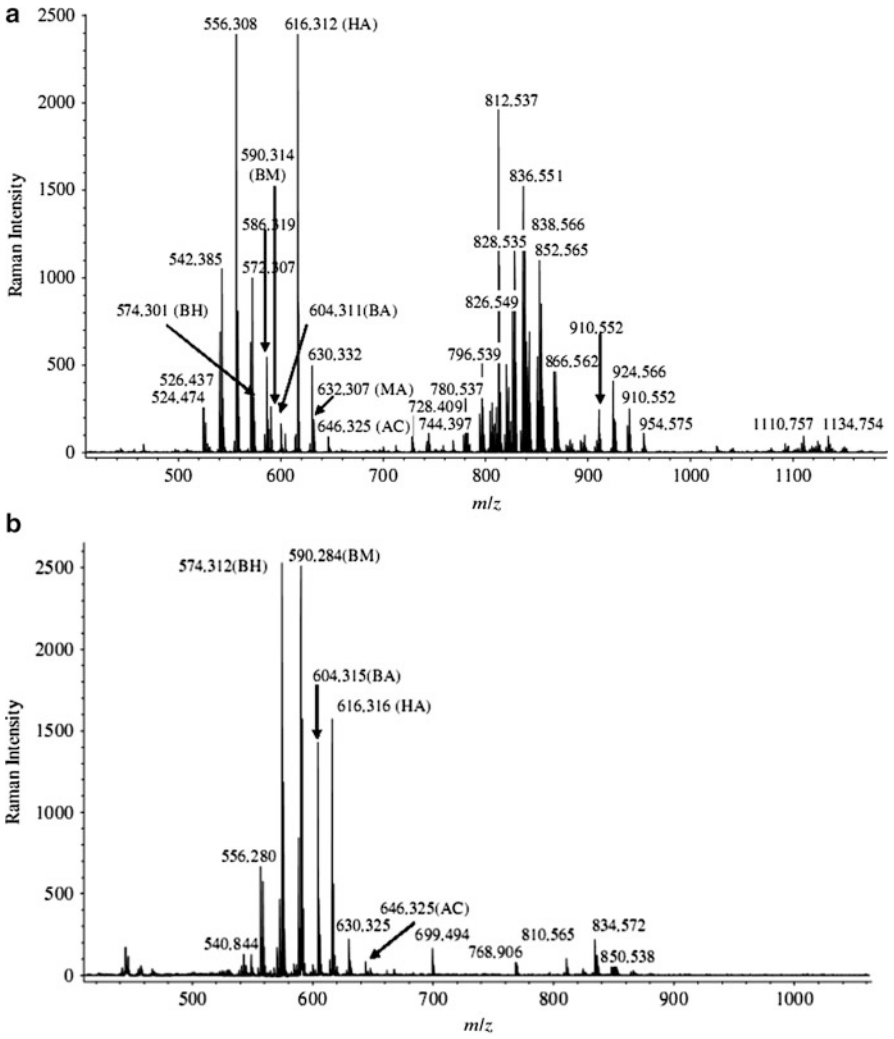

Fig. 5 MALDI-TOF MS spectra of alkaloids from the direct analysis of Fuzi root tissue (**a**) and Processed Fuzi (**b**) deposited with CHCA in 50:50 acetonitrile/0.1% TFA as matrix, about 20 μL/cm^2 under 22% laser energy. Copyright with permission from John Wiley and Sons Ltd [45]

of the metabolites and measure semiquantitatively their relative abundances in different tissue regions. A mass spectrum for direct desorption/ionization of secondary metabolites (alkaloids) from the cortex region of CHCA-coated stem tissue of Sinomenium acutum by MALDI-TOF MS is illustrated in Fig. 6. Compared with other techniques for analysis of Chinese herbs, this method showed some distinguished merits including simple, fast, clear, low sample consumption without any extraction process.

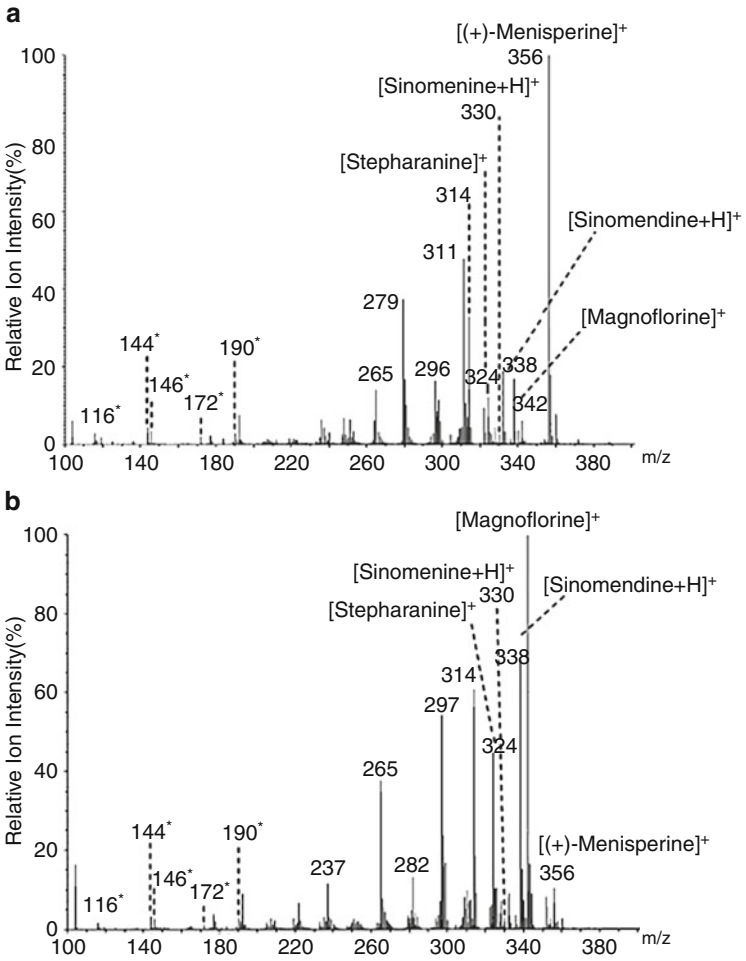

Fig. 6 Direct desorption/ionization of secondary metabolites (alkaloids) from the cortex region of CHCA-coated stem tissue of Sinomenium acutum. Samples were collected from Shaanxi (**a**) and Anhui (**b**) provinces, China. The solvent composition of CHCA solution (10 mg/mL) used for the matrix deposition was 80 vol.% acetone and 20 vol.% H_2O. (Asterisks denote ion peaks from CHCA matrix). Copyright with permission from The American Chemical Society [80]

2.6 Other Applications of MALDI-TOF MS in TCMs

Chinese gall (Wubeizi), a conventional TCM that contains high levels of gallotannins, has been mainly used as astringent, haemostatic, antiphlogistic, and antiseptic agents. Zhu and co-workers [81] demonstrated that MALDI-QIT-TOF MS

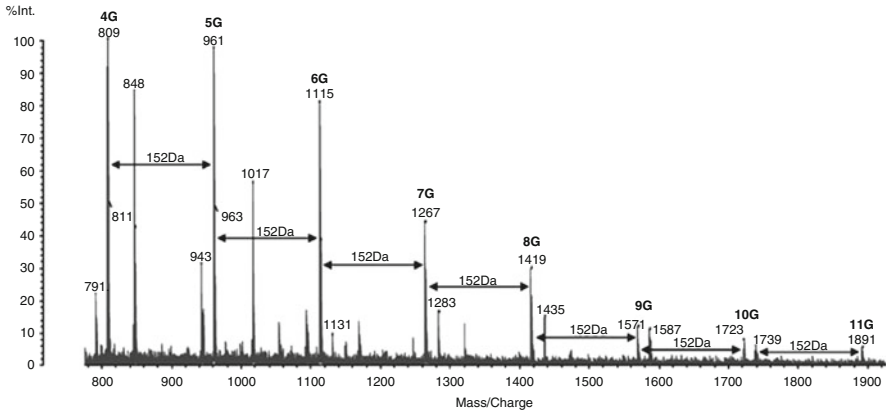

Fig. 7 MALDI-QIT-TOF MS positive ion spectrum of the gallotannins with DP of 4G to 11G in the crude extract of Chinese galls. Copyright with permission from John Wiley and Sons Ltd [81]

was a powerful and efficient technique for rapid direct analysis of the gallotannins in the crude extract of Chinese gall without any troublesome sample pretreatments. Several matrices such as DHB, 2',4',6'-trihydroxyacetophenone (THAP), and CHCA were initially examined for the measurement of the crude extract from Chinese galls by MALDI-QIT-TOF MS. Experimental results demonstrated that the use of THAP provided excellent peak intensity. A series of gallotannins with 4–11 galloyl units were identified in Chinese galls by MALDI-QIT-TOF MS. Figure 7 shows a typical MALDI-QIT-TOF positive ion spectrum of the gallotannins in the crude extract of Chinese galls by using THAP as matrix.

Glycyrrhizin (GC) is the main sweet tasting compound from liquorice root, which has protein kinase inhibitory, antiulcer, and antiviral activities. It is known that the hapten number in an antigen conjugate is important for immunization against small molecular compounds. Shan and coworkers [82] reported an analysis of hapten-carrier protein conjugates by MALDI-TOF MS that can directly describe the suitability of hapten number for immunization. By using BAS with molecular weight 66,433 Da as conjugate, a broad peak coinciding with the conjugate of GC and BSA appeared at *m*/*z* 70021 in the MALDI-TOF MS spectrum. Therefore the calculated value of the GC component (MW 823) is 3,588, indicating that at least four molecules of GC conjugated with the BAS.

Lin wa pi, the dried skin of the Heilongjiang brown frog, *Rana amurensis*, is commonly used as an ingredient of many medicines, as a general tonic, and as a topical antimicrobial/wound dressing. By using RP-HPLC and MALDI-TOF MS, Zhou and colleagues [83] identified components of the peptidome and transcriptome of the cutaneous granular gland, the source of skin-derived bioactive peptides in *Lin wa pi*. The molecular masses of polypeptides in fractionation of skin extract were detected by using MALDI-TOF MS in positive detection mode with CHCA as the matrix (data not shown).

3 Conclusions and Perspectives

MALDI-TOF MS, as a useful and powerful tool, has been successfully applied to solve a wide range of problems from TCMs analysis, such as rapid identification of new bioactive compounds, proteomics analysis to elucidate activity mechanism of TCMs, and direct tissue analysis with easy sample preparation. Compared with other techniques for analysis of TCMs, MALDI-TOF MS possesses some prominent advantages, including fast analysis, high sensitivity, low sample consumption, relative high tolerance towards salts and buffers, possibility to store sample on the target plate, and the ability of direct tissue analysis. Despite the higher salt and buffer tolerance of MALDI-TOF MS compared to ESI, the technique has limitations for qualitative and quantitative analysis of low molecular weight compounds (<1,000 Da). Poor reproducibility (sample to sample and shot to shot) is the main problem that hampers the application of the MALDI technique to the analysis of small molecular weight compounds. The presence of "hot spots" that comes from the co-crystallization process is the main cause of the poor reproducibility of MALDI-TOF MS. Various approaches have been developed to improve reproducibility, which can be divided into two main groups, namely the enhancement of homogeneous matrix crystallization and the averaging out of variations in instrument response [84]. Another challenge for analysis of low molecular weight compounds by MALDI-TOF MS is the interferences from matrix in low-mass range. To suppress or eliminate the interferences from matrix, various matrix-free methods and new matrices that are suitable for analysis of small molecular compounds were introduced, including desorption on porous silicon [51], sol-gel derived film [85], carbon nanostructure [54], and so on.

Direct analysis of plant tissue is another promising direction for analyzing TCMs by MALDI-TOF MS. The compositions of TCMs are very complicated and they usually contain hundreds of chemical constituents including flavonoids, alkaloids, phenols, and so on. The inherent advantages of MALDI-TOF MS, such as high throughput, easy sample preparation, and rapid analysis ability, make this technique most suitable for the direct analysis of complex samples. Although little work on the direct analysis of TCM tissue has been reported so far, it is believed that direct profiling or imaging of plant tissue by MALDI-TOF MS will be more and more applicable in future TCMs analysis.

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