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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 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].

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Despite the desired features of MS, TCM analysis has remained 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 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 (DHB)) 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 *P. ginseng* and *P. 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 *P. quinquefolius.* with *P. 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 *P. ginseng* and *P. 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,