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REVIEW PAPER

# Liquid chromatography coupled with time-of-flight and ion trap mass spectrometry for qualitative analysis of herbal medicines

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High-performance liquid chromatography (HPLC);
Time-of-flight mass spectrometry (TOF-MS);
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Herbal medicine (HM)

**Abstract** With the expansion of herbal medicine (HM) market, the issue on how to apply up-to-date analytical tools on qualitative analysis of HMs to assure their quality, safety and efficacy has been arousing great attention. Due to its inherent characteristics of accurate mass measurements and multiple stages analysis, the integrated strategy of liquid chromatography (LC) coupled with time-of-flight mass spectrometry (TOF-MS) and ion trap mass spectrometry (IT-MS) is well-suited to be performed as qualitative analysis tool in this field. The purpose of this review is to provide an overview on the potential of this integrated strategy, including the review of general features of LC-IT-MS and LC-TOF-MS, the advantages of their combination, the common procedures for structure elucidation, the potential of LC-hybrid-IT-TOF/MS and also the summary and discussion of the applications of the integrated strategy for HM qualitative analysis (2006–2011). The advantages and future developments of LC coupled with IT and TOF-MS are highlighted.



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## 1. Introduction

Herbal medicines (HMs), also called botanical medicine or phytomedicine, refers to using a plant's seeds, berries, roots, leaves, bark or flowers with minimal or no industrial processing that have been used for medicinal purposes. It is becoming more mainstream as improvements in analysis and quality control along with advances in clinical research show the value of herbal medicine in the treating and preventing disease [1]. According to the World Health Organization, about 80% of world's population relies on HMs for some aspect of their primary healthcare, and the worldwide annual market for HM products approaches US$ 60 billion [2].

With the expansion of HMs' market, the issue on how to apply up-to-date scientific technologies on HMs to assure their quality, safety and efficacy has been arousing great attention in a broad range of fields [3]. Most herbal medicines and their derivative products were often prepared from crude plant extracts, which comprise a complex mixture of different phytochemical constituents (plant secondary metabolites). The chemical features of these constituents differ considerably among different species [4]. Therefore, the qualitative analysis of HMs was described as "complex system research", which is really a challenging task for scientists.

In recent decade, liquid chromatography coupled with mass spectrometry (LC–MS) has become the most selective technique for rapid screening and characterization of known and unknown constituents from the extracts of HMs. Interfaces including atmospheric pressure chemical ionization (APCI) and electrospray ionization (ESI) have been successfully used in LC–MS configuration, which are well-suited for HM analysis. There are three common single mass analyzers: quadrupole (Q), ion trap (IT) and time-of-flight (TOF). With purposeful combination, triple quadrupole (TQ), Q-IT, IT-TOF, Q-TOF and TOF–TOF, as well as 3D traps including Fourier transform ion cyclotron resonance (FTICR) and Orbitrap, were commercially available and have already been wildly used for HM analysis.

It must be pointed out that TOF-MS is a powerful tool, which is capable of 10,000 or more resolving power expressed in terms of full peak width at one-half maximum (FWHM). TOF-MS has a high acquisition speed and provides accurate mass measurement (possibility to yield mass accuracy $<2$ ppm with an adequate calibration range) as well as full scan spectral sensitivity. Accurate mass measurement gives the elemental composition of parent and fragment ions, used for the identification of unknown species and a greater differentiation of isobaric species (two different compounds with the same nominal mass but different elemental composition, also with different exact masses) [3,5]. Correspondingly, ion trap analyzers are especially suitable for multiple fragmentation steps ($MS^n$).

In linear ion traps, ions are isolated and accumulated due to a special arrangement of hyperbolic and ring shaped electrodes as well as oscillating electric fields. Then the ions can be fragmented in a similar way as described above by collision-induced decomposition (CID). This process can be repeated in a sequential manner, so that valuable structural information is obtained [6], which can be used for the differentiation of isomers (two different compounds with the same exact mass and elemental composition).

In this sense, the combination of LC/TOF-MS accurate mass measurements to generate empirical formulae and LC/IT-$MS^n$ providing additional fragmentation data for structure confirmation represents a powerful methodology for the analysis of complex systems. Currently, this strategy has been successfully developed and applied in the analysis of environmental contaminants [7,8], HMs [9–12], metabolites [13–18] and many other fields.

This review intends to summarize the advantages of the combination of TOF-MS and IT-$MS^n$, hyphenated to LC for qualitative analysis of complex constituents in HM products and metabolites of HM-treated biological samples. Two main aspects are involved in this review: (1) a brief introduction of general features of TOF-MS and IT-MS, including the recent advances in the application of hybrid IT-TOF/MS and (2) discussion of the integrated strategy applied to HM qualitative analysis, including the summary of relevant reports from 2006 to the present.

## 2. General features and advantages of the combination of LC-TOF-MS and LC-IT-MS

### *2.1. TOF-MS*

#### *2.1.1. Accurate mass measurements*

One of the main attributes of TOF instrument is its accurate mass measurement, which gives the elemental composition of parent