Trends in Analytical Chemistry 72 (2015) 169–180

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

journal homepage: www.elsevier.com/locate/trac

Quadrupole time-of-flight mass spectrometry as a powerful tool for demystifying traditional Chinese medicine

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ARTICLE INFO

Keywords:
Active ingredient
Intervention effect
Metabolomics
Quadrupole time-of-flight mass spectrometry
Quality control
Screening
Toxicity
Traditional Chinese medicine

ABSTRACT

Traditional Chinese medicine (TCM) has been effective in prevention and treatment of chronic diseases in a synergic manner with mild healing effects and lower side effects than Western medicines. Researchers have made great efforts to explore the real theory of TCM for many years with different strategies. With the rapid advances in instrumentation in chromatography and mass spectrometry, more scientific outcomes have been achieved for disclosing the mysteries of TCM. In this article, we review the achievements of quadrupole time-of-flight mass spectrometry in TCM analysis, including profiling active ingredients and their metabolites, screening harmful components, and applying cutting-edge metabolomics strategies.

Contents

1. Introduction

Traditional Chinese Medicine (TCM) has been used in clinical and healthcare practice for more than 2000 years in China [1]. More overseas countries and regions have taken active action to accept TCM as an alternative to Western medicine. Medicinal plants, animals, and minerals are major starting materials to make TCM preparations, in which medicinal plants are the most dominant. TCM is becoming more popular because it is widely available and relatively inexpensive, and has fewer adverse effects [2]. More importantly, TCM mainly focuses on the aim of promoting health and improving quality of life, with therapeutic strategies for treatment of specific diseases or symptoms in a holistic fashion based on two fundamental theories (i.e., Yin-Yang and Five Elements) [3]. There are usually four kinds of drugs in a Chinese formula, including the principal drug, the ministerial drug, the adjunctive drug, and the messenger drug [4]. They coordinate with each other and enhance the effect of the formula. However, there has been a long time for people to reveal the mysteries of TCM, to recognize and to understand fully how and why TCM works, and what its strengths and

Abbreviations: AA, Aristolochic acid; CE, Capillary electrophoresis; DDA, Data-dependent acquisition; DIA, Data-independent acquisition; GC, Gas chromatography; PCDL, Personal compound database and library; Q-TOF-MS, Quadrupole time-of-flight mass spectrometry; SFC, Supercritical-fluid chromatography; TCM, Traditional Chinese medicine; UHPLC, Ultrahigh-performance liquid chromatography.

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http://dx.doi.org/10.1016/j.trac.2015.04.021
0165-9936/

weaknesses are. The fast development of mass spectrometry (MS) and its coupling with chromatography, especially high-resolution instrumentation, significantly enhanced the ability to dissect TCM and there has been a rapid increase in interest in TCM research.

Quadrupole (Q)-Orbitrap and Q-time-of-flight (TOF) are two dominant high-resolution MS platforms employed in analysis of both small and large molecules. They can provide accurate monoisotopic mass measurement and high-resolution MS/MS spectra for target confirmation and unknown identification. Q-Orbitrap can achieve multiplexing selected-ion monitoring (SIM) for target compounds with higher sensitivity than Q-TOF, thanks to ion accumulation in the C-Trap. It is superior to Q-TOF in terms of mass-resolving power, especially for chemical entities with low *m/z*. It was utilized to investigate the intervention effect of TCM Yi Tang Kang on the metabolic syndrome of spleen deficiency using a proteomics strategy [5]. It was also employed in profiling *Rhizoma coptidis* [6] and *Paeoniae Radix Alba-Atractylodis Macrocephalae Rhizoma*, a pair of herbal extracts [7].

However, with the increase in spectral acquisition rate, the mass resolution of Orbitrap decreases dramatically, so its performance is compromised when it is coupled to ultrahigh-performance liquid chromatography (UHPLC) for fast separation. With Orbitrap, chromatography plays an important role to reduce the mass shift due to peak coalescence [8]. Q-TOF can provide a much faster spectral acquisition rate without a drop in mass resolution.

For TCM-related analysis, Q-TOF-MS combined with powerful separation techniques is more popular and reasonable. Separation process can greatly reduce sample complexity and the matrix effect, and discriminate structural isomers. LC and its derivative strategy, heart-cutting and comprehensive two-dimensional LC [9], and supercritical-fluid chromatography (SFC) [10] are reported to be the dominant approaches used for separation.

Commercially-available Q-TOF-MS incorporating the ion-mobility technique is also effective in discriminating isomers in complex samples [11,12].

TCM is a complex collective of many organic and inorganic chemical entities. For organic compounds, their empirical formula is a combination of several elements and each combination corresponds to an exact monoisotopic mass. To distinguish formulas of different combinations but with very close exact mass, especially in the case of mass difference less than 2 ppm, isotope-pattern information, including monoisotopic mass, isotope-abundance ratio, and isotope spacing, is usually taken into consideration as an orthogonal filter [13].

The Orbitrap-based mass analyzer is reported to have an inherent drawback, resulting in poor fidelity of the isotope pattern [14,15]. Spectral error increases with mass-resolving power [16].

The TOF-based analyzer is capable of generating a consistent isotope pattern within a reasonable ion-abundance range.

Narrow dynamic range is another inherent limitation of Q-Orbitrap. With the transfer from TDC to ADC dual gain signal processor used on Q-TOF-MS, an in-spectrum dynamic range with five orders of magnitude is achievable for analysis of very complex samples, such as TCM. Q-TOF-MS, as the most sophisticated, promising accurate-mass instrumentation, with strong qualitative and quantitative capabilities, is therefore widely employed in TCM analysis [17].

From molecular-formula generation to structure elucidation for characterization of unknowns, additional important qualitative information (e.g., MS/MS spectrum, collision cross-section, and chromatographic retention time or electrophoretic migration time) is necessarily utilized with the help of dedicated home-made or public databases and libraries [18], and software {e.g., Molecular Structure Correlator [19], MassFragment [20], Mass Frontier [21]}. With the development of a new-generation Q-TOF-MS with sensitivity and robustness comparable to triple-quadrupole (QqQ)-MS, quantitative analysis based on the Q-TOF-MS platform becomes more reasonable and flexible. Q-TOF-MS is extensively adapted from relative quantitation in various omics study to absolute quantitation in routine applications. Its quantitation is based on the response of either parent ions or product ions, depending on real situation.

Most Q-TOF-MS platforms are capable of acquiring data in three different modes:

1) MS full scan, no filter in quadrupole, no fragmentation in collision cell;
2) data-dependent acquisition (DDA), only specific parent ions passing quadrupole filter and entering into collision cell for fragmentation; and,
3) data-independent acquisition (DIA), all ions in a specified and wide *m/z* range passing through a quadrupole filter and entering into a collision cell for fragmentation; DIA is relatively new technique developed most recently to improve analytical throughput significantly.

In this article, we provide a comprehensive review on recent advances in TCM-related analysis by Q-TOF-MS-based strategies.

2. Active constituent identification and their metabolite profiling

Significant value of TCM lies in its various pharmacological activities {e.g., antitumor [22,23], reduction of blood lipids [24,25], and protection of the liver [26]}. The real sources of activity are functional components in TCM. Q-TOF-MS has been well used as a powerful tool for characterizing active constituents in TCM formulae (e.g., injections and decoctions) or extracts of herbal plants.

For analysis of TCM formulae, various classes of components were investigated by profiling [27]. In most cases, the purpose of research is to evaluate TCM quality that can be affected by geographical origin, growing condition, harvesting time, and processing. For crude-extract analysis, research mainly focused on one or two functional groups {e.g., saponins [28–31], flavonoids [32], steroidal alkaloids [33], and other antioxidants [34,35]}.

Fig. 1 shows a general strategy based on Q-TOF-MS for identification of the flavonoids in *Spatholobus suberectus* samples. Fragmentation pathways of reference standards and known components were well investigated and diagnostic product-ion-based strategies were employed to screen and identify similar compounds of the same group efficiently. Chromatographic retention behavior and information from previous literature were also involved in the discrimination of structural isomers, especially positional isomers.

Besides this strategy, identification of effective components of TCM in biological fluids or tissues after oral or intravenous administration has become an way to clarify absorbed components [36].

To answer another question as to which components are metabolized, the whole metabolism of TCM has been well investigated. Identification of metabolites of single active component *in vitro* [37] and *in vivo* [38,39] followed strategies similar to those for Western drug. However, TCM is a complicated mixture of many bioactive components, which make it more challenging than a single compound.

Another big challenge lies in the study of *in vivo* metabolites of TCM at an oral clinical dosage. Cheng et al. proposed a three-step strategy to characterize *in vivo* metabolites of licorice at a normal clinical dosage systematically [40]. They combined metabolites of a single reference compound and those after high-dose oral administration to generate a list of target metabolites for a normal clinical dosage study. *In vivo* metabolism studies are usually performed using rat after oral administration of certain TCM. Metabolites in rat biological fluids (e.g., plasma, serum, urine, and bile) are identified.