Journal of Pharmaceutical Analysis 2011;1(4):235–245

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

www.elsevier.com/locate/jpa
www.sciencedirect.com

REVIEW PAPER

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

Xiao-Fei Chen[a,b], Hai-Tang Wu[a,b], Guang-Guo Tan[a,b], Zhen-Yu Zhu[a,b], Yi-Feng Chai[a,b,*]

[a]*Department of Pharmaceutical Analysis, School of Pharmacy, Second Military Medical University, Shanghai 200433, China*
[b]*Shanghai Key Laboratory for Pharmaceutical Metabolite Research, Shanghai 200433, China*

Received 1 August 2011; accepted 7 September 2011
Available online 21 October 2011

KEYWORDS

High-performance liquid chromatography (HPLC);
Time-of-flight mass spectrometry (TOF-MS);
Ion trap mass spectrometry (IT-MS);
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.

*Corresponding author at: Department of Pharmaceutical Analysis, School of Pharmacy, Second Military Medical University, Shanghai 200433, China.
E-mail address: yfchai@smmu.edu.cn (Y.-F. Chai).

Peer review under responsibility of Xi'an Jiaotong University.
doi:10.1016/j.jpha.2011.09.008

Contents

1. Introduction . . . 236
2. General features and advantages of the combination of LC-TOF-MS and LC-IT-MS. . . . 236
2.1. TOF-MS. . . . 236
2.1.1. Accurate mass measurements . . . 236
2.1.2. Fragmentation . . . 237
2.2. IT-MS . . . 237
2.3. Procedures of the combination of LC-TOF-MS and LC-IT-MS for structure elucidation . . . 237
2.4. LC-hybrid-IT-TOF/MS. . . . 237
3. Application of HPLC-IT/TOF-MS for the qualitative analysis of medicinal plants . . . 237
3.1. Study of chemical constituents. . . . 237
3.1.1. Identification of components . . . 237
3.1.2. Differentiation of isomers . . . 241
3.2. Study of metabolites. . . . 242
4. Conclusion . . . 242
References . . . 243

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

and fragment ions and can be used for the identification of unknown compounds and the differentiation of isobaric compounds. The measurement of accurate masses within 5 ppm is widely accepted for the verification of the elemental compositions [5]. To achieve such accurate mass measurement, TOF instruments require frequent tuning and calibration of the spectrometer.

In compatible with TOF-MS analyzer, an 'in-house library' (containing the chemical information of components from relative HMs) is always constructed for screening constituents from HMs. For non-target compounds recorded in the in-house library, the exact elemental compositions are generally deduced from several steps. First, low fragmentor voltages are used and the software of TOF-MS will list the possible molecular composition according to accurate mass measurements and different criteria such as double bond equivalent (DBE) index, 'show isotopic' function and so on. Second, the most probable molecular formulae (corresponding to the criteria) are selected and searched against exhaustive in-house chemical library. At last, the library hits by each chemical formula are recorded [19]. This strategy can make screening non-target components from HMs an easy task on the basis of accurate mass measurements by TOF-MS.

2.1.2. Fragmentation

Fragmentor voltage is crucial for providing characteristic fragment ions in the MS spectra resulted from CID in-source, which corresponds to the MS^n fragmentation. Generally, the fragmentor voltage needs to be adjusted since various types of components in HMs produce characteristic fragment ions at different fragmentor voltages [19]. Thus, with dynamic adjustment of fragmentor voltage, TOF-MS can provide valuable structural information by producing various characteristic fragment ions together with their elemental compositions [3]. However, the parent ion of the fragment ions may not be easier to confirm, particularly when analyzing complex matrix samples. This situation may complicate the identification and confirmation process and affect the deduction of fragmentation pathways for the analytes of interest. Fortunately, IT-MS can solve this problem easily by its own features, which will be introduced in detail in the following section.

2.2. IT-MS

A different concept is followed in ion trap analyzer, which is especially suitable for multiple fragmentation steps (MS^n) rather than quantitative studies. 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 CID in TOF-MS analyzer. This process can be repeated up to 12 stages as needed [6]. Thus, the structures of target components could be tentatively elucidated by applying the MS^n process, which solve the problem that TOF-MS cannot lock the target parent and fragment ions. This feature has made IT-MS to be a powerful tool for the differentiation of isomers, and even the structure elucidation of unknown compounds. However, IT-MS cannot provide information with high resolution and may not ensure good precursor ion selectivity. Apparently, the combination of TOF-MS and IT-MS has complementary advantages, which can overcome their respective defects during the analysis procedures.

2.3. Procedures of the combination of LC-TOF-MS and LC-IT-MS for structure elucidation

According to large amounts of literatures, we come to the multiple relative procedures for the identification and confirmation of non-target compounds: (1) accurate mass data within 5 ppm, provide the authentic molecular formula with well-matching isotope profile and DBE; (2) under certain conditions, the fragment ions due to CID in-source fragmentation appear in TOF-MS spectrum assisted structure elucidation; (3) utilize IT-MS to conduct multiple stages analysis and confirm the target fragment ions, including the differentiation of isomers. Finally, reference standard (if available) can be used to validate the conclusion. By applying these procedures, the unambiguous identification of multiple components and the differentiation of isobaric species and isomers will be feasible.

2.4. LC-hybrid-IT-TOF/MS

As one of the latest LC/MS instrumentation designs, hybrid ion trap/time-of-flight mass spectrometry coupled with high-performance liquid chromatography (HPLC-hybrid-IT-TOF-MS) provides higher sensitivity and accuracy than both TOF and IT-MS. In particular, multiple scans of natural products in MS^n modes and accurate mass measurements can be performed simultaneously through data-dependent acquisition [20]. This tandem mass technique has raised the qualitative analysis of HMs to a new height both in analytic speed and accuracy, which integrate the advantages of both IT and TOF. Recently, it has been applied to HMs analysis and also been confirmed to be a very powerful tool on the global identifications of both target and non-target components [21].

3. Application of HPLC-IT/TOF-MS for the qualitative analysis of medicinal plants

3.1. Study of chemical constituents

3.1.1. Identification of components

The LC coupled with IT-MS and TOF-MS strategy has now been widely used in many fields, especially in qualitative analysis of HMs. In spite of its enormous analytical potential, not many articles have been published as yet on the analysis of HMs mainly due to the still high costs of the technique. It should be noticed that, the use of LC-hybrid-IT-TOF/MS on HM analysis has increased quickly in the last two years (see Tables 1 and 2), which will dominate the field of HM qualitative analysis in the near future.

Qualitative analysis of HMs involves the confirmation of target components and elucidation of non-target components and the identification of unknown compounds. Table 1 summarizes the different studies performed regarding the qualitative analysis of chemical constituents and biotransformation products in HMs or HM-treated biological matrices using the integrated strategy. The benefit of using a TOF analyzer that allows it to perform full-scan acquisitions with superior sensitivity and high mass accuracy, makes the qualitative analysis of chemical constituents in HMs easier, quicker and more accurate. This is due to the fact that monitoring a specific mass of an analyte does not need to be predefined before data acquisition and this fact allows us to detect the

Table 1 Combination of TOF-MS and IT-MS for qualitative analysis of HM samples.

Analytes	Matrix	LC/stationary phase	Analyzers	Year	Ref.
4 N-alkylamides	*Spilanthes acmella*	HPLC/C_{18}	IT-TOF/MS^n	2011	[25]
21 compounds including amino acids, phenolic acids, flavonoid glycoside, terpene glycoside and phthalide	*Xuebijing injection*	HPLC/C_{18}	DAD-TOF-MS; IT-MS^n	2011	[26]
Over 39 compounds	Fructus *corni*	UFLC/C_{18}	IT-TOF/MS^n	2011	[27]
18 phenolic constituents, including moscatilin and gigantol	*Dendrobium*	HPLC/C_{18}	IT-TOF/MS^n	2010	[11]
Rutaecarpine and its 2 derivatives	Standard compound		IT-TOF/MS^n	2010	[28]
46 compounds including 3 phenolic compounds and 1 triterpenoid in licorice extract; 25 compounds in rat plasma	Licorice and rat plasma	HPLC/C_{18}	DAD; TOF-MS;QIT-MS^n	2010	[12]
62 flavone and isoflavone glycoconjugate	Root and leaf tissues of *Lupinus reflexus*	HPLC/C_{18}	IT-MS^n; Q-TOF-MS	2010	[29]
5 lignans components	*Schisandra* lignans extract and rat plasma	HPLC/C_{18}	IT-TOF/MS^n	2010	[21]
Salvinorin A	*Salvia divinorum*	TLC/SiO_2	DESI-TOF-MS; IT-MS^n	2010	[30]
Phenolic compounds	Olive leaf	HPLC/C_{18}	TOF-MS; IT-MS^n	2010	[31]
Flavonoids, phenolic acids, ellagitanins, hexahydroxydiphenoyl and derivatives, etc.	*Cistus ladanifer shrub* aqueous extract	HPLC/C_{18}	DAD; TOF-MS; IT-MS^n	2010	[32]
Gallotannins	Chinese galls	–	MALDI-QIT-TOF/MS^n	2009	[33]
30 ginsenosides and 20 lignans	*Shengmai injection*	HPLC/C_{18}	IT-TOF/MS^n	2009	[24]
28 cynandione A derivatives	Radix *Cynanchum wilfordii* and *C. auriculatum*	HPLC/C_{18}	IT-MS^n; QTOF-MS	2009	[34]
23 furocoumarins	Radix *Angelica dahurica*	HPLC/C_{18}	DAD; QIT-MS^n; TOF-MS	2009	[35]
Proanthocyanidin	Blueberry leaves	HPLC/C_{18}	IT-TOF/MS^n	2009	[36]
25 dibenzocyclooctadiene lignans, including seven groups of lignan isomers	*Schisandra Chinensis*	HPLC/C_{18}	UV; IT-MS^n; TOF-MS	2009	[37]
Phenolic compounds including verbascoside and its derivatives, diglucuronide derivatives of apigenin and luteolin, and eukovoside. gardoside, verbasoside, cistanoside F, theveside, campneoside I, chrysoeriol-7-diglucuronide, forsythoside A and acacetin-7-diglucuronide	Lemon verbena extract	HPLC/C_{18}	DAD-TOF-MS; IT-MS^n	2009	[38]
Pregnane glycosides	*Cynanchum auriculatum*	HPLC/C_{18}	IT-TOF/MS^n	2009	[39]
21 lignans in Schisandra chinensis; 11 lignans in rat plasma	*Schisandra chinensis* and rat plasma	HPLC/C_{18}	DAD; TOF-MS; QIT-MS^n	2009	[40]
10 constituents including resveratroloside, polydatin, emodin-8-O-glucoside, resveratrol, torachryson-8-O-glucoside, emodin-1-O-glucoside, torachryson-8-O-(6′-acetyl)glucoside, physcion-8-O-glucoside, physcion-8-O-(6′-acetyl)glucoside and emodin	*Polygonum cuspidatum* Sieb. et Zucc	HPLC/C_{18}	QIT-TOF/MS^n	2009	[41]
25 anthocyanins	Blueberry	HPLC/C_{18}	UV; IT-TOF/MS^n	2009	[42]
C-20-nonoxygenated ent-kauranes and two subtypes of C-20-oxygenated ent-kauranes	Standard compounds	–	IT-MS^n; QTOF-MS	2008	[43]
Major components from Astragali Radix	43 Radix *Astragali* samples	HPLC/C_{18}	IT-TOF/MS^n	2008	[44]
Anthocyanins and chlorogenic acid	Dried calyces of *Hibiscus sabdariffa* L	CE	IT-MS^n; TOF-MS	2008	[45]
87 compounds including non-target components	*Mai-Luo-Ning injection*	HPLC/C_{18}	IT-TOF/MS^n	2008	[46]
Terpene lactones	Standard compounds	–	IT-MS^n; QTOF-MS	2008	[47]

Table 1 (*continued*)

Analytes	Matrix	LC/stationary phase	Analyzers	Year	Ref.
8 C-21 steroidal glycosides	*Hoodia gordonii*	HPLC/C_{18}	IT-MS^n;TOF-MS	2008	[48]
7 tropane alkaloid including tropine, belladonnine, norhyoscyamine, apoatropine, hyoscyamine, 6beta-hydroxyhyoscyamine, and scopolamine	*Atropa belladonna* L	CE	IT-MS^n;TOF-MS	2008	[49]
40 phenolic and diterpenoid constituents	Radix *Salvia miltiorrhiza*	HPLC/C_{18}	DAD; QIT-MS^n;TOF-MS	2007	[23]
Dammarane-type triterpenoid saponins	*Bacopa monnieri*	–	ESI-IT-MS^n, AP-MALDI-IT-MS^n, MALDI- IT-TOF/MS^n	2007	[50]
Sesamin and gmelinol	Standard compounds	–	IT-TOF/MS^n	2007	[51]
7 flavonolignans including Silychristins A and B , silydianin , silybins A and B, and isosilybins A and B	*Silybum marianum*	Semi-microHPLC/C_{18}	IT-TOF/MS^n	2007	[52]
Resveratrol glycosides including resveratrol diglucoside (M1), trans- and cis-resveratrol acetylhexosides	Transgenic *Arabidopsis*	HPLC/C_{18}	UV; QTOF-MS; IT-MS^n	2007	[53]
Podophyllotoxin and its 4′-demethyl-4beta-substituted derivatives	Standard compounds	–	IT-TOF/MS^n	2007	[54]
Puerarin	Standard compounds	–	IT-TOF/MS^n	2007	[55]
13 steroid saponins	Rhizomes of *Dioscorea panthaica*	–	QTOF-MS; IT-MS^n	2006	[56]
Differentiation of 3 pairs of aconite alkaloid isomers	*Aconitum nagarum* var. *lasiandrum*	–	QTOF-MS;IT-MS^n	2006	[57]
14 betacyanins	*Amaranthus tricolor, Gomphrena globosa,* and *Hylocereus polyrhizus*	–	MALDI-QIT-TOF/MS^n	2006	[58]

presence of an unlimited number of chemical constituents in a certain HM without reanalysis, which is not easily achieved by the SIM or MRM mode of quadrupole mass analyzers (Q, TQ) [22]. On the other hand, besides the screening of fragment ions procedure performed by CID in-source fragmentation of TOF-MS, the IT analyzer can deal with the MS/MS and MS^n processes with higher selectivity and stability of target ions than TOF-MS. This is because the (Q)-IT analyzer consisting of a ring electrode and two endcap electrodes with hyperbolic surfaces and IT is operated in a ''mass-selective stability'' mode of operation. In this mode, analogous to the operation of a quadrupole mass filter, rf and dc voltages applied to the ring electrode are ramped to allow stability, hence storage, of a single (increasing) value of m/z in the IT analyzer [6].

An illustrative example has been reported by our research group [23], in which Radix *Salvia miltiorrhizae* (Dan-shen) was analyzed by using the integrated strategy of HPLC coupled with DAD, TOF-MS and QIT-MS. In order to elucidate the components of *S. miltiorrhizae*, the first step was to propose the fragmentation pathways of the reference compounds. We tried two approaches: one was based on the accurate masses of the parent ions and fragment ions produced by dynamic adjustment of the fragmentor voltage in TOF-MS; the other was based on MS^n analysis ($n=2$–6) of IT-MS for the confirmation of parent and fragment ions. Take salvianolic acid B as an example (see Fig. 1), from the TOF-MS spectrum (Fig. 1(a)) we can see that the accurate mass of salvianolic acid B can be obtained under low fragmentor voltage. Afterwards, a series of fragment ions were characterized under high fragmentor voltage, which were less than the fragment information acquired by Q-IT-MS^n (see Fig. 1(b)). In addition, Q-IT-MS can ''trap'' the target parent ion with high selectivity, so Q-IT-MS was performed as structure elucidation tool while TOF-MS as component identification tool, combined with DAD for more reliable components recognition. Fig. 2 shows the DAD, TOF-MS and IT-MS spectra. By applying this strategy, 40 constituents including phenolic and diterpenoid constituents were identified within 30 min based on their positive and negative ion ESI mass spectra and liquid chromatographic information.

Another research reported by Zheng et al. [24] must be highlighted, which presents a modified and universally applicable diagnostic fragment-ion-based extension strategy (DFIBES) to efficiently process the information acquired by LC-(ESI)-hybrid-IT-TOF/MS, facilitating the structural determination of serial components contained in traditional Chinese medicine prescription (TCMP). The key advantage of DFIBES is that it facilitates the rapid classification of the complicated peaks into well-known chemical families, which significantly simplifies the complicated procedures of structural characterization due to the specific advantages of hybrid-IT-TOF/MS analyzer. *Shengmai* injection, composed of *Panax ginseng*, Radix *ophiopogonis* and *Schisandra chinensis*, was taken as a TCMP example to conduct and validate the proposed DFIBES. Diagnostic fragment ions (DFIs) for each chemical family contained in *Shengmai* injection were firstly determined or proposed from the separated analysis of 15 authentic standards and the extract of S. *chinensis*. The ESI–MS^n fragmentation patterns of ginsenosides and lignans were then systematically studied for developing the 'structure extension'

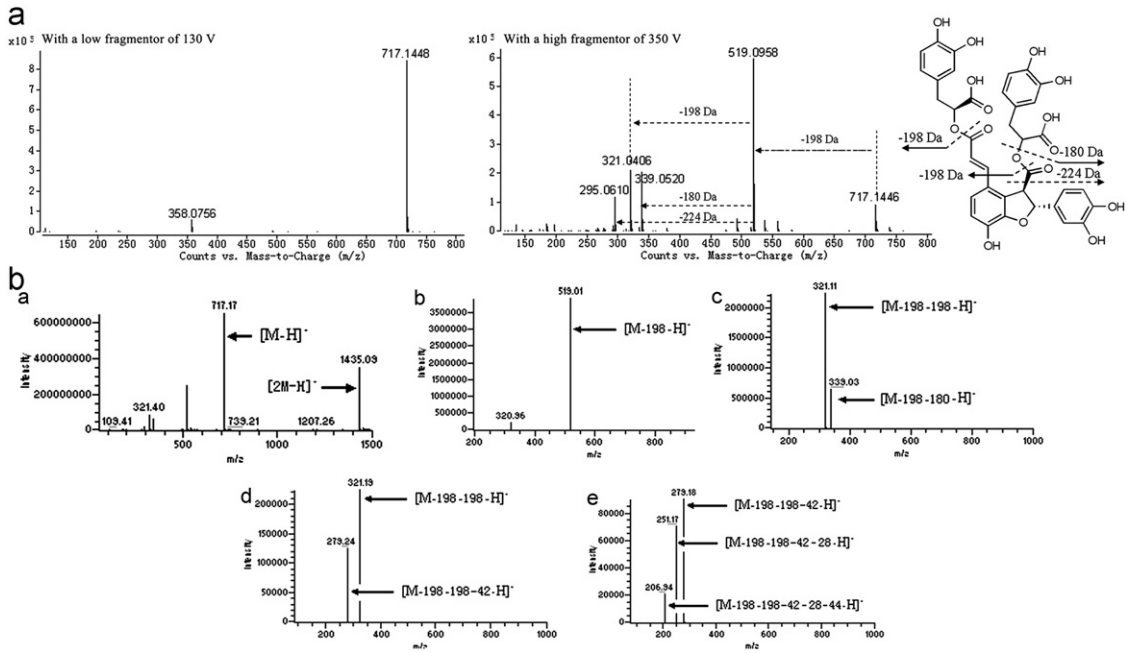

Figure 1 TOF-MS spectra (a) and IT-MS$^{1-5}$ spectra (b) of salvianolic acid B.

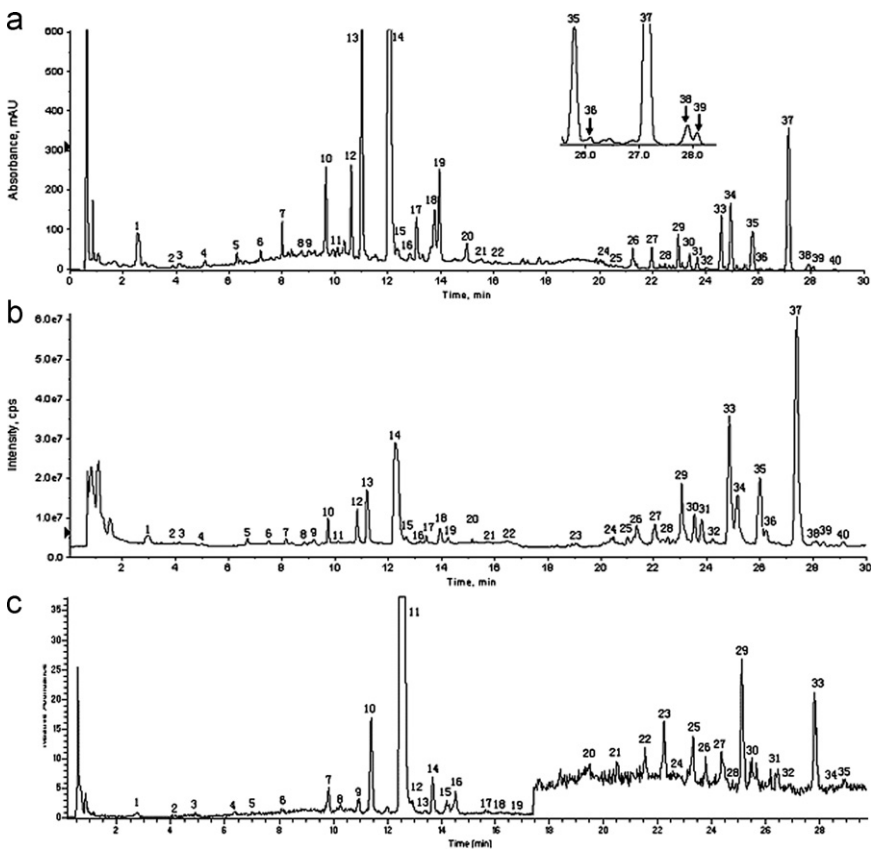

Figure 2 HPLC–UV chromatogram monitored at 280 nm (a), TIC profile from HPLC/ESI-TOF-MS (b), and TIC profile from HPLC/ESI–MS^n (c) of phenolics and diterpenoids in Radix *Salvia miltiorrhizae*.

approach. Upon LC-IT-TOF/MS analysis and DFIBES, more than 30 ginsenosides and 20 lignans have been rapidly detected and identified from *Shengmai* injection, supporting that the DFIBES achieved by LC-hybrid-IT-TOF/MS is a powerful strategy and would be applicable for the components identification from TCMP and other complicated mixtures.

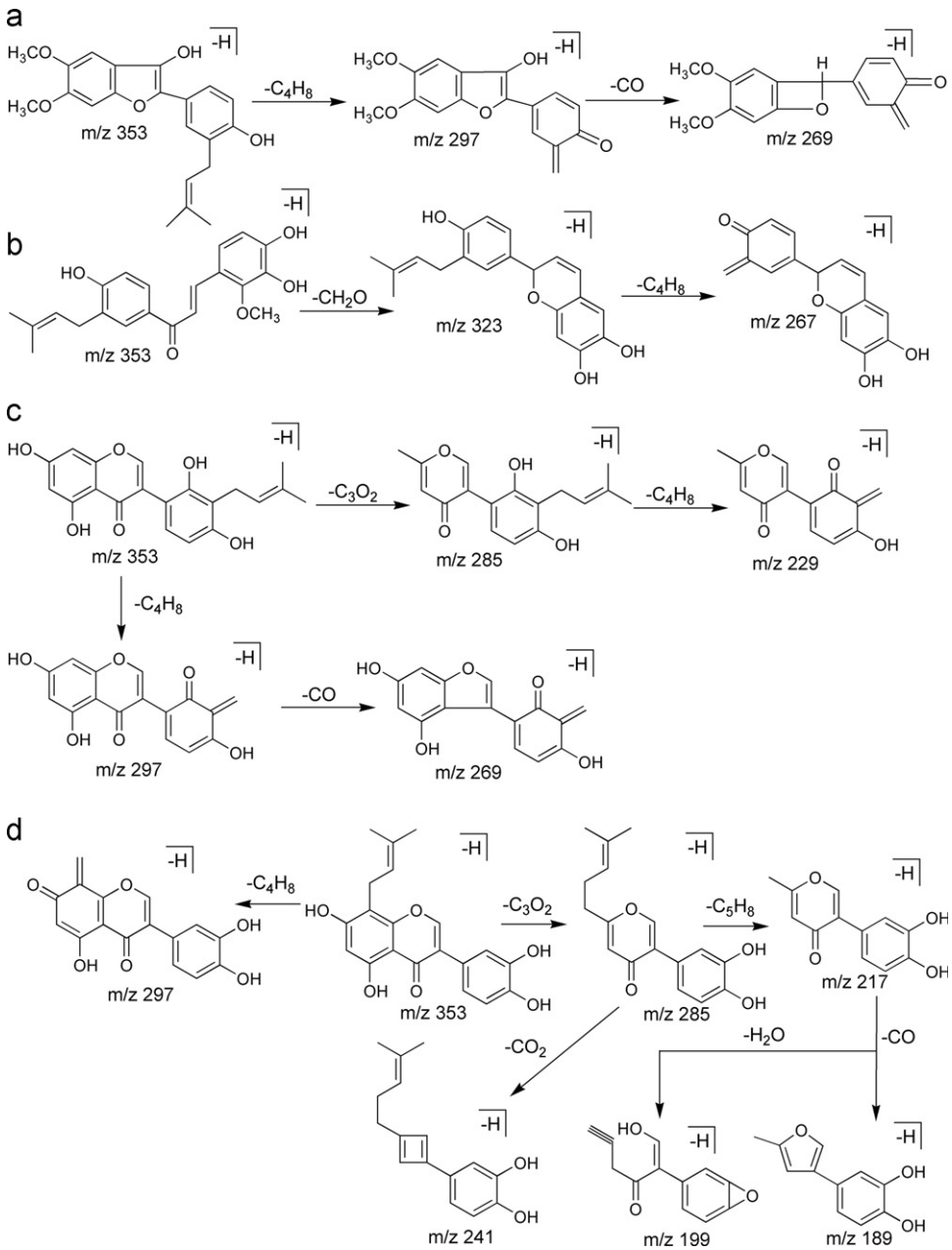

Figure 3 The proposed fragmentation pathways of licobenzofuran (a), licochalcone D (b), licoisoflavone (c) and gancaonin L (d).

3.1.2. Differentiation of isomers

Isomers are the groups of compounds with the same exact masses and elemental compositions. In most cases, these compounds are similar in structure and polarity and also have similar cleavage pathways, which is difficult for analysts to deal with. Fortunately, the strategy that TOF-MS combined with IT-MS is a very powerful tool for the differentiation of isomers due to the accurate mass and multiple stages ability.

One example of our previous report [12] showed that there are four compounds (a, b, c and d, see Fig. 3) identified from licorice with the same $[M-H]^-$ ion at m/z 353. Firstly, by using HPLC-TOF-MS, four compounds could be assigned two group of isomers. Compound (a) and (b) [group 1] were assigned as licobenzofuran or licochalcone D with the accurate mass of $[M+H]^+$ ions at m/z 355.1542, while compound (c) and (d) [group 2] as licoisoflavone or gancaonin L with the accurate mass of $[M+H]^+$ ions at m/z 355.1179 by database-matching. Then we applied HPLC-QIT-MS^n to differentiate the compounds from each group. The QIT-MS spectra of group 1 showed that the product ion $[M-56-28-H]^-$ at m/z 269 of compound (a) was triggered by initial loss of $-C_4H_8$, followed by loss of CO, as proposed in Fig. 3(a); and the product ion $[M-30-56-H]^-$ at m/z 267 of compound (b) was produced by initial lose of $-CH_2O$, followed by the elimination of $-C_4H_8$, as depicted in Fig. 3(b). Thus, the compounds (a) and (b) were tentatively characterized as licobenzofuran and licochalcone D, respectively. As to group 2, the product ion $[M-68-H]^-$ at m/z 285 and $[M-56-H]^-$ at m/z 297 were for both compounds triggered by initial loss of $-C_3O_2$ at m/z 68 and of $-C_4H_8$, respectively. When the product ion at m/z

Table 2 Combination of TOF-MS and IT-MS for analysis of metabolites in HMs and HM-treated biological samples.

Analytes	Metabolite	Matrix	LC/stationary phase	Analyzers	Year	Ref.
(+)-Praeruptorin B (dPB) and (+)-praeruptorin E (dPE) from *Peucedani* Radix	Human: dPB:B1–B9; dPE:E1–E13 rat: dPB:B1–B8; dPE:E1–E13	Human and rat liver microsomes	HPLC/C_{18}	IT-MS^n; TOF-MS	2011	[13]
Traditional Chinese medicine tongxinluo	1-Methyladenosine, indoxyl sulfate, hippuric acid, riboflavin, coproporphyrin, and p-cresol glucuronide	Endothelial dysfunction rats urinary	UFLC/C_{18}	IT-TOF/MS^n	2011	[59]
5 Schisandra lignans from *Schisandra* lignans extract	44 metabolites	Rat urine	HPLC/C_{18}	IT-TOF/MS^n	2010	[60]
Salvianolic acid A from *Salvia miltiorrhiza*	5 metabolites including SalA-monoglucuronide, monomethyl-SalA-monoglucuronide, monomethyl-SalA, dimethyl-SalA and dimethyl-SalA-monoglucuronide,	Rat plasma	HPLC/C_{18}	IT-MS^n; TOF-MS	2009	[61]
Tectorigenin	7 phase II metabolities	Rat bile	HPLC/C_{18}	IT-MS^n; TOF-MS	2008	[62]
Ginkgolide B	3 metabolites in urine;1 hydroxyl metabolite in liver	Rat urine and liver	HPLC/C_{18}	IT-MS^n; TOF-MS	2008	[63]
Interaction between tanshinone IIA and warfarin	Warfarin metabolite	Rat blood and urine, human serum albumin	HPLC/C_{18}	IT-MS^n; TOF-MS	2008	[64]

285 was further selected for MS^3 analysis, the two compounds displayed different MS^3 spectra. Compound (c) exhibited the product ion $[M-68-56-H]^-$ at m/z 229 by sequential loss of $-C_4H_8$, while compound (d) yielded the product ion $[M-68-68-H]^-$ at m/z 217 by sequential loss of $-C_5H_8$. The product ion at m/z 217 of compound (c) fragmented into two ions at m/z 199 and 189 in the MS^4 spectra corresponding to the losses of H_2O and CO, respectively. On the basis of the above deduced fragmentation behaviors, compound (c) and (d) were tentatively identified as licoisoflavone and gancaonin L, respectively. The possible fragment pathways are proposed in Figs. 3(c) and (d). From the discussion above we can draw the conclusion that the combination of TOF-MS and IT-MS has the complementary advantages, which is very suitable for the differentiation of isomers in complex HM samples.

3.2. *Study of metabolites*

The metabolites in HM-treated biological matrices are always non-target or unknown constituents of extremely low concentration, which require high sensitivity, selectivity and strong identification ability of the analyzers. As discussed above, the combination of TOF-MS and IT-MS or hybrid-IT-TOF/MS technique both have all these abilities and are really suitable for the study of metabolites from HMs or HM-treated biological matrices. Table 2 summarizes the recent applications of this strategy on the study of metabolites. From the summarized data we can conclude that the applications are still limited possibly due to lacking of the global strategy for the analysis of complicated metabolites. However, with the development of mass spectrometer technique and data acquiring and processing strategy, the advantages of this integrated strategy for analysis of metabolites will be revealed, and the rapid, accurate analysis of the subtle metabolites in HMs and HM-treated biological samples will be achieved.

4. Conclusion

The manuscript reviews the general features and the recent applications of LC coupled with IT and TOF-MS for qualitative analysis of HMs. Based on the currently available literatures, the advantages of this approach were described and discussed. It gives complementary advantages and becomes one of the most important methods in the field of qualitative research of HMs or HM-treated biological samples.

The inherent characteristics of TOF-MS in accurate mass measurements and high resolution make this analyzer attractive in the qualitative analysis of chemical constituents in herbal samples. While the parent ion of the fragment ions acquired by TOF-MS may not be easier to confirm, the IT-MS should be a good solution due to its abilities of ion isolation and accumulation, as well as multiple stages analysis. The integration of TOF and IT-MS could facilitate the identification and structure elucidation of target and non-target compounds in complex HM matrices. Furthermore, multiple scans of natural products in MS^n modes and accurate mass measurements can be performed simultaneously using hybrid-IT-TOF/MS, which will achieve an unequivocal confirmation of the target analytes by increasing confidence about the origin of the fragment ions. The analysis procedures will also be simplified and the analysis time will be greatly reduced. In a word, all these features indicate that the strategy of LC coupled with TOF-MS and IT-MS is a powerful tool for the qualitative analysis of HMs and the application of LC-hybrid-IT-TOF/MS in HM analysis can be expected.

References

[1] J.C. Tilburt, T.J. Kaptchuk, Herbal medicine research and global health: an ethical analysis, Bull. World Health Organ. 86 (2008) 594–599.

[2] WHO issues guidelines for herbal medicines, Bull. World Health Organ. 82 (2004) 238.

[3] J.L. Zhou, L.W. Qi, P. Li, Herbal medicine analysis by liquid chromatography/time-of-flight mass spectrometry, J. Chromatogr. A 1216 (2009) 7582–7594.

[4] M. Yang, J. Sun, Z. Lu, et al., Phytochemical analysis of traditional Chinese medicine using liquid chromatography coupled with mass spectrometry, J. Chromatogr. A 1216 (2009) 2045–2062.

[5] S. Lacorte, A.R. Fernandez-Alba, Time of flight mass spectrometry applied to the liquid chromatographic analysis of pesticides in water and food, Mass Spectrom. Rev. 25 (2006) 866–880.

[6] K.R. Jonscher, J.R. Yates 3rd, The quadrupole ion trap mass spectrometer—a small solution to a big challenge, Anal. Biochem. 244 (1997) 1–15.

[7] M.J. Bueno, A. Aguera, M.J. Gomez, et al., Application of liquid chromatography/quadrupole-linear ion trap mass spectrometry and time-of-flight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater, Anal. Chem. 79 (2007) 9372–9384.

[8] M.J. Gomez, M.J. Martinez Bueno, A. Aguera, et al., Evaluation of ozone-based treatment processes for wastewater containing microcontaminants using LC-QTRAP-MS and LC-TOF/MS, Water Sci. Technol. 57 (2008) 41–48.

[9] H. Huang, L. Ji, S. Song, et al., Identification of the major constituents in Xuebijing injection by HPLC–ESI–MS, Phytochem. Anal. (2011).

[10] W. Dai, C. Wei, H. Kong, et al., Effect of the traditional Chinese medicine tongxinluo on endothelial dysfunction rats studied by using urinary metabonomics based on liquid chromatography–mass spectrometry, J. Pharm. Biomedi. Anal. (2011).

[11] J. Zhou, Z. Xu, H. Kong, et al., Comparison of phenolic components among different species of Dendrobium (Shihu fengdou) and determination of their active components—moscatilin and gigantol, Se Pu 28 (2010) 566–571.

[12] G. Tan, Z. Zhu, H. Zhang, et al., Analysis of phenolic and triterpenoid compounds in licorice and rat plasma by high-performance liquid chromatography diode-array detection, time-of-flight mass spectrometry and quadrupole ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 24 (2010) 209–218.

[13] Y.L. Song, R. Yan, W.H. Jing, et al., Characterization of metabolism of (+)-praeruptorin B and (+)-praeruptorin E in human and rat liver microsomes by liquid chromatography coupled with ion trap mass spectrometry and time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 25 (2011) 719–730.

[14] Z.Y. Liu, Y.F. Tao, D.M. Chen, et al., Identification of carbadox metabolites formed by liver microsomes from rats, pigs and chickens using high-performance liquid chromatography combined with hybrid ion trap/time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 25 (2011) 341–348.

[15] P. Kosina, J. Vacek, B. Papouskova, et al., Identification of benzo[c]phenanthridine metabolites in human hepatocytes by liquid chromatography with electrospray ion-trap and quadrupole time-of-flight mass spectrometry, J. Chromatogr. B 879 (2011) 1077–1085.

[16] J.M. Brozinski, M. Lahti, A. Oikari, et al., Detection of naproxen and its metabolites in fish bile following intraperitoneal and aqueous exposure, Environ. Sci. Pollut. Res. Int. 18 (2011) 811–818.

[17] L. Zhang, X. Jia, X. Peng, et al., Development and validation of a liquid chromatography–mass spectrometry metabonomic platform in human plasma of liver failure caused by hepatitis B virus, Acta Biochim. Biophys. Sin. 42 (2010) 688–698.

[18] Z.Y. Liu, L.L. Huang, D.M. Chen, et al., Application of electrospray ionization hybrid ion trap/time-of-flight mass spectrometry in the rapid characterization of quinocetone metabolites formed in vitro, Anal. Bioanal. Chem. 396 (2010) 1259–1271.

[19] X. Chen, Z. Lou, H. Zhang, et al., Identification of multiple components in Guanxinning injection using hydrophilic interaction liquid chromatography/time-of-flight mass spectrometry and reversed-phase liquid chromatography/time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 25 (2011) 1661–1674.

[20] Z.Y. Liu, X.N. Zhou, H.H. Zhang, et al., An integrated method for degradation products detection and characterization using hybrid ion trap/time-of-flight mass spectrometry and data processing techniques: application to study of the degradation products of danofloxacin under stressed conditions, Anal. Bioanal. Chem. 399 (2011) 2475–2486.

[21] Y. Liang, H. Hao, A. Kang, et al., Qualitative and quantitative determination of complicated herbal components by liquid chromatography hybrid ion trap time-of-flight mass spectrometry and a relative exposure approach to herbal pharmacokinetics independent of standards, J. Chromatogr. A 1217 (2010) 4971–4979.

[22] J.V. Sancho, O.J. Pozo, M. Ibanez, et al., Potential of liquid chromatography/time-of-flight mass spectrometry for the determination of pesticides and transformation products in water, Anal. Bioanal. Chem. 386 (2006) 987–997.

[23] Z. Zhu, H. Zhang, L. Zhao, et al., Rapid separation and identification of phenolic and diterpenoid constituents from Radix Salvia miltiorrhizae by high-performance liquid chromatography diode-array detection, electrospray ionization time-of-flight mass spectrometry and electrospray ionization quadrupole ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 1855–1865.

[24] C. Zheng, H. Hao, X. Wang, et al., Diagnostic fragment-ion-based extension strategy for rapid screening and identification of serial components of homologous families contained in traditional Chinese medicine prescription using high-resolution LC–ESI–IT-TOF/MS: Shengmai injection as an example, J. Mass Spectrom. 44 (2009) 230–244.

[25] F. Mbeunkui, M.H. Grace, C. Lategan, et al., Isolation and identification of antiplasmodial N-alkylamides from Spilanthes acmella flowers using centrifugal partition chromatography and ESI-IT-TOF-MS, J. Chromatogr. B 879 (2011) 1886–1892.

[26] H. Huang, L. Ji, S. Song, et al., Identification of the major constituents in Xuebijing injection by HPLC–ESI–MS, Phytochem. Anal. 22 (2011) 330–338.

[27] G. Cao, C. Zhang, Y. Zhang, et al., Global detection and identification of components from crude and processed traditional Chinese medicine by liquid chromatography connected with hybrid ion trap and time-of-flight-mass spectrometry, J. Sep. Sci. (2011).

[28] B. Wang, W.Y. Chu, Z. Li, et al., Investigation on fragmentation pathways of rutaecarpine and its two derivatives using electrospray ionization ion-trap time-of-flight tandem mass spectrometry, Rapid Commun. Mass Spectrom. 24 (2010) 2781–2786.

[29] M. Stobiecki, A. Staszkow, A. Piasecka, et al., LC–MSMS profiling of flavonoid conjugates in wild Mexican lupine, Lupinus reflexus, J. Nat. Prod. 73 (2010) 1254–1260.

[30] J.H. Kennedy, J.M. Wiseman, Direct analysis of Salvia divinorum leaves for salvinorin A by thin layer chromatography and desorption electrospray ionization multi-stage tandem mass spectrometry, Rapid Commun. Mass Spectrom. 24 (2010) 1305–1311.

[31] S. Fu, D. Arraez-Roman, A. Segura-Carretero, et al., Qualitative screening of phenolic compounds in olive leaf extracts by

hyphenated liquid chromatography and preliminary evaluation of cytotoxic activity against human breast cancer cells, Anal. Bioanal. Chem. 397 (2010) 643–654.
[32] S. Fernandez-Arroyo, E. Barrajon-Catalan, V. Micol, et al., High-performance liquid chromatography with diode array detection coupled to electrospray time-of-flight and ion-trap tandem mass spectrometry to identify phenolic compounds from a Cistus ladanifer aqueous extract, Phytochem. Anal. 21 (2010) 307–313.
[33] F. Zhu, Y.Z. Cai, J. Xing, et al., Rapid identification of gallotannins from Chinese galls by matrix-assisted laser desorption/ionization time-of-flight quadrupole ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 23 (2009) 1678–1682.
[34] X. Zhang, L. Shan, H. Huang, et al., Rapid identification of acetophenones in two Cynanchum species using liquid chromatography-electrospray ionization tandem mass spectrometry, J. Pharm. Biomed. Anal. 49 (2009) 715–725.
[35] H. Zhang, C. Gong, L. Lv, et al., Rapid separation and identification of furocoumarins in Angelica dahurica by high-performance liquid chromatography with diode-array detection, time-of-flight mass spectrometry and quadrupole ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 23 (2009) 2167–2175.
[36] M. Takeshita, Y. Ishida, E. Akamatsu, et al., Proanthocyanidin from blueberry leaves suppresses expression of subgenomic hepatitis C virus RNA, J. Biol. Chem. 284 (2009) 21165–21176.
[37] P. Shi, Q. He, Y. Zhang, et al., Characterisation and identification of isomeric dibenzocyclooctadiene lignans from Schisandra Chinensis by high-performance liquid chromatography combined with electrospray ionisation tandem mass spectrometry, Phytochem. Anal. 20 (2009) 197–206.
[38] R. Quirantes-Pine, L. Funes, V. Micol, et al., High-performance liquid chromatography with diode array detection coupled to electrospray time-of-flight and ion-trap tandem mass spectrometry to identify phenolic compounds from a lemon verbena extract, J. Chromatogr. A 1216 (2009) 5391–5397.
[39] L.W. Qi, X.J. Gu, P. Li, et al., Structural characterization of pregnane glycosides from Cynanchum auriculatum by liquid chromatography on a hybrid ion trap time-of-flight mass spectrometer, Rapid Commun. Mass Spectrom. 23 (2009) 2151–2160.
[40] Z. Lou, H. Zhang, C. Gong, et al., Analysis of lignans in Schisandra chinensis and rat plasma by high-performance liquid chromatography diode-array detection, time-of-flight mass spectrometry and quadrupole ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 23 (2009) 831–842.
[41] J. Dong, H. Wang, L. Wan, et al., Identification and determination of major constituents in Polygonum cuspidatum Sieb. et Zucc. by high performance liquid chromatography/electrospray ionization-ion trap-time-of-flight mass spectrometry, Se Pu 27 (2009) 425–430.
[42] J.S. Barnes, H.P. Nguyen, S. Shen, et al., General method for extraction of blueberry anthocyanins and identification using high performance liquid chromatography–electrospray ionization-ion trap-time of flight-mass spectrometry, J. Chromatogr. A 1216 (2009) 4728–4735.
[43] Y. Zhou, S.X. Huang, L.M. Li, et al., Negative electrospray ionization tandem mass spectrometric investigation of ent-kaurane diterpenoids from the genus Isodon, J. Mass Spectrom. 43 (2008) 63–73.
[44] K. Tanaka, T. Tamura, S. Fukuda, et al., Quality evaluation of Astragali Radix using a multivariate statistical approach, Phytochemistry 69 (2008) 2081–2087.
[45] A. Segura-Carretero, M.A. Puertas-Mejia, S. Cortacero-Ramirez, et al., Selective extraction, separation, and identification of anthocyanins from Hibiscus sabdariffa L. using solid phase extraction-capillary electrophoresis–mass spectrometry (time-of-flight /ion trap), Electrophoresis 29 (2008) 2852–2861.
[46] H. Hao, N. Cui, G. Wang, et al., Global detection and identification of nontarget components from herbal preparations by liquid chromatography hybrid ion trap time-of-flight mass spectrometry and a strategy, Anal. Chem. 80 (2008) 8187–8194.
[47] S. Ding, E. Dudley, Q. Song, et al., Mass spectrometry analysis of terpene lactones in Ginkgo biloba, Rapid Commun. Mass Spectrom. 22 (2008) 766–772.
[48] B. Avula, Y.H. Wang, R.S. Pawar, et al., Identification and structural characterization of steroidal glycosides in Hoodia gordonii by ion-trap tandem mass spectrometry and liquid chromatography coupled with electrospray ionization time-of-flight mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 2587–2596.
[49] D. Arraez-Roman, G. Zurek, C. Bassmann, et al., Characterization of Atropa belladonna L. compounds by capillary electrophoresis–electrospray ionization-time of flight-mass spectrometry and capillary electrophoresis–electrospray ionization-ion trap-mass spectrometry, Electrophoresis 29 (2008) 2112–2116.
[50] M. Zehl, E. Pittenauer, L. Jirovetz, et al., Multistage and tandem mass spectrometry of glycosylated triterpenoid saponins isolated from Bacopa monnieri: comparison of the information content provided by different techniques, Anal. Chem. 79 (2007) 8214–8221.
[51] G. Yan, Q. Li, H. Tan, et al., Electrospray ionization ion-trap time-of-flight tandem mass spectrometry of two furofurans: sesamin and gmelinol, Rapid Commun. Mass Spectrom. 21 (2007) 3613–3620.
[52] M. Shibano, A.S. Lin, H. Itokawa, et al., Separation and characterization of active flavonolignans of Silybum marianum by liquid chromatography connected with hybrid ion-trap and time-of-flight mass spectrometry (LC-MS/IT-TOF), J. Nat. Prod. 70 (2007) 1424–1428.
[53] C. Lo, J.C. Le Blanc, C.K. Yu, et al., Detection, characterization, and quantification of resveratrol glycosides in transgenic arabidopsis over-expressing a sorghum stilbene synthase gene by liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 4101–4108.
[54] Q. Li, G. Yan, T. Ge, A fragmentation study of podophyllotoxin and its 4′-demethyl-4beta-substituted derivatives by electrospray ionization ion-trap time-of-flight tandem mass spectrometry, Rapid Commun. Mass Spectrom. 21 (2007) 2843–2852.
[55] H. Li, L. Wan, Y. Hashi, et al., Fragmentation study of a 8-C-glycosyl isoflavone, puerarin, using electrospray ion trap time-of-flight mass spectrometry at high resolution, Rapid Commun. Mass Spectrom. 21 (2007) 2497–2504.
[56] R. Li, Y. Zhou, Z. Wu, et al., ESI-QqTOF-MS/MS and APCI-IT-MS/MS analysis of steroid saponins from the rhizomes of Dioscorea panthaica, J. Mass Spectrom. 41 (2006) 1–22.
[57] R. Li, Z. Wu, F. Zhang, et al., Differentiation of three pairs of aconite alkaloid isomers from Aconitum nagarum var. lasiandrum by electrospray ionization tandem mass spectrometry, Rapid Commun. Mass Spectrom. 20 (2006) 157–170.
[58] Y.Z. Cai, J. Xing, M. Sun, et al., Rapid identification of betacyanins from Amaranthus tricolor, Gomphrena globosa, and Hylocereus polyrhizus by matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF MS), J. Agri. Food Chem. 54 (2006) 6520–6526.
[59] W. Dai, C. Wei, H. Kong, et al., Effect of the traditional Chinese medicine tongxinluo on endothelial dysfunction rats studied by using urinary metabonomics based on liquid chromatography–mass spectrometry, J. Pharm. Biomed. Anal. 56 (2011) 86–92.
[60] Y. Liang, H. Hao, L. Xie, et al., Development of a systematic approach to identify metabolites for herbal homologs based on liquid chromatography hybrid ion trap time-of-flight mass spectrometry: gender-related difference in metabolism of Schisandra lignans in rats, Drug Metab. Dispos. 38 (2010) 1747–1759.
[61] Y. Shen, X. Wang, L. Xu, et al., Characterization of metabolites in rat plasma after intravenous administration of salvianolic acid

A by liquid chromatography/time-of-flight mass spectrometry and liquid chromatography/ion trap mass spectrometry, Rapid Commun. Mass Spectrom. 23 (2009) 1810–1816.

[62] W.D. Zhang, L.W. Qi, X.L. Yang, et al., Identification of the major metabolites of tectorigenin in rat bile by liquid chromatography combined with time-of-flight and ion trap tandem mass spectrometry, Rapid Commun. Mass Spectrom. 22 (2008) 2677–2684.

[63] D.L. Wang, Y. Liang, W.D. Chen, et al., Identification of ginkgolide B metabolites in urine and rat liver cytochrome P450 enzymes responsible for their formation in vitro, Acta Pharmacol. Sin. 29 (2008) 376–384.

[64] J. Liu, X. Wang, Z. Cai, et al., Effect of tanshinone IIA on the noncovalent interaction between warfarin and human serum albumin studied by electrospray ionization mass spectrometry, J. Am. Soc. Mass Spectrom. 19 (2008) 1568–1575.