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Review

Herbal medicine analysis by liquid chromatography/time-of-flight mass spectrometry

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ABSTRACT

The fact that the effects of herbal medicines (HMs) are brought about by their chemical constituents has created a critical demand for powerful analytical tools performing the chemical analysis to assure their efficacy, safety and quality. Liquid chromatography coupled to mass spectrometry (LC–MS) is an excellent technique to analyze multi-components in complex herbal matrices. Due to its inherent characteristics of accurate mass measurements and high resolution, time-of-flight (TOF) MS is well-suited to this field, especially for qualitative applications. The purpose of this article is to provide an overview on the potential of TOF, including the hybrid quadrupole- and ion trap-TOF (QTOF and IT-TOF), hyphenated to LC for chemical analysis in HMs or HM-treated biological samples. The peculiarities of LC–(Q/IT)TOF-MS for the analysis of HMs are discussed first, including applied stationary phase, mobile-phase selection, accurate mass measurements, fragmentation and selectivity. The final section is devoted to describing the applicability of LC–(Q/IT)TOF-MS to routine analysis of multi-components, including target and non-target (unknown) compounds, in herbal samples, emphasizing both the advantages and limitations of this approach for qualitative and quantitative purposes. The potential and future trends of fast high-performance liquid chromatography (HPLC) (e.g. rapid resolution LC and ultra-performance LC) coupled to (Q)TOF-MS for chemical analysis of HMs are highlighted.

Contents

1. Introduction

Herbal medicines (HMs) are naturally occurring, plant-derived substances with minimal or no industrial processing that have been used for medicinal purposes [1]. Besides their long history of use, HMs are getting significant attention in global healthcare

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as their great medicinal and economic importance. According to the World Health Organization, about 80% of world's population relies on HMs for some aspect of their primary healthcare [2]. In some countries, herbal medicine is still a central part of the medical system, such as Ayurvedic medicine in India and traditional Chinese medicine (TCM) in China. In Europe, North America and other industrialized regions, herbal medicinal products are becoming increasingly popular, and the worldwide annual market for these products approaches US$ 60 billion [3,4].

Against this background of increasing usage of HMs by the public, a number of major public health issues have raised concerns about these products. The most important one is that the application of herbal medicines is often based on long-term empirical and traditional uses rather than on scientific evidences. This has highlighted the need for up-to-date scientific information on HMs to assure their quality, safety and efficacy. The effects of HMs are, of course, brought about by their chemical constituents; thus, the chemical analysis of HMs is especially important because it helps us to know what chemical components exist in HMs and which ingredients cause a therapeutic effect, and then to establish scientific and rational quality control methods. In most cases, however, medicinal plants comprise hundreds of different constituents that belong to different compound classes with diverse chemical and physical properties. The matrix gets even more complex, when TCM or Ayurvedic formulations are to be analyzed, as they are mixtures of up to 2 or 15 herbal plants or extracts. Therefore, analysis of HMs is a difficult, in some respect even more challenging task.

Different analytical techniques have been employed for analysis of complex constituents in herbal products, including thin layer chromatography, gas chromatography, high-performance liquid chromatography (HPLC), capillary electrophoresis and their hyphenated techniques to mass spectrometry (MS) [5–8]. LC–MS has gained importance in the past decades for chemical analysis of HMs since it can greatly improve the analytical selectivity and sensitivity. For example, LC–MS/MS with triple quadrupole (TQ) in multiple reaction monitoring (MRM, also called selected reaction monitoring, SRM) mode presents excellent sensitivity and selectivity for the quantitation of target compounds in plants or biological matrices so far, as reported extensively in the literature [9–15]. However, LC–MS/MS in MRM as well as single quadrupole (Q) MS in single ion monitoring (SIM) do not permit structural elucidation of non-target (unknown) compounds in herbal products [16,17].

More recently, LC–MS methods that use time-of-flight (TOF) as the detection system are of particular interest in herbal components analysis as TOF analyzers are well-suited to perform structure elucidation or confirmation, especially for non-target compounds, due to its unique features (vide infra). TOF instruments are capable of 5000–10,000 resolving power expressed in terms of FWHM (full peak width at one-half maximum). The high mass resolving power provides better confirmatory ability than Q, TQ or ion trap (IT) MS analyzers, especially when dealing with complex matrix samples. Also, TOF instruments provide accurate mass measurement and full-scan spectral sensitivity. Accurate mass measurement (<5 ppm) gives elemental composition of parent and fragment ions. Therefore, the use of TOF instruments allows the capability of non-target components identification, because the full-spectrum is recorded at all times with accurate mass measurements, which is not possible with standard monitoring practices that use SIM or MRM techniques [18]. When coupled to a quadrupole or ion trap mass filter, QTOF-MS or IT-TOF-MS permit MS/MS or MS^n analysis with accurate mass measurements for both precursor and product ions, which constitutes a higher order mass identification than those afforded by nominal mass measurements obtained by other types of mass analyzers, such as TQ and IT-MS [19]. As a result, the use of TOF instruments in herbal components analysis has increased.

The following review summarizes the potential of TOF, including the QTOF and IT-TOF, hyphenated to LC for analysis of complex constituents in herbal products and HM-treated biological samples, and comprises the following aspects: (1) a brief introduction of general features of LC and TOF analyzers in HM analysis, including the most recent advances in LC separation techniques, that is, fast HPLC with sub-2 μm particle size to improve the separation performance; (2) detailed discussion of LC–(Q/IT)TOF-MS applied to HM analysis, including the advantages and the difficulties encountered when using this technique for qualitative and quantitative analysis of plant extracts or HM-treated biological matrices.

2. General features of LC–(Q/IT)TOF-MS in HM analysis

2.1. LC

2.1.1. Stationary phase

A medicinal plant typically contains a complex mixture of hundreds or more chemical compounds, e.g. alkaloids, glycosides, and flavonoids. For analysis of such a complex mixture, analytical technique with strong separation capability is needed. Currently, reversed-phase HPLC is widely used to analyze the most herbal medicinal products because various chemical constituents in a herbal product can/may obtain different retentions on reversed-phase adsorbent under a definite condition, which confirms the effective separation of each compound. Normal-phase LC is used rather seldom although it can be successfully used to determine highly polar molecules whose retention on the reversed phases is low [20,21]. Silica gel with chemically attached octadecylsilanol groups (C_{18}) is the most frequently used adsorbent in reversed-phase HPLC in HM analysis. Adsorbents with other alkyl groups, such as C_4 [22], C_8 [17,23,24] and C_{30} [25], can also be found in herbal products analysis. The longer the alkyl chain attached to the adsorbent is, the higher the retention of the components is. In some cases, cellulose triacetate supported on silica gel diol was also utilized as an adsorbent to analyze the natural products [26].

The overall goal of analytical chromatography is to achieve sufficient resolution of analytes of interest within the shortest possible time. In HMs analysis field, compounds belong to the same structure class, including the isomers, often need to be separated in a single run. Therefore, to achieve sufficient resolution of analytes, long analysis times are always needed in the case of analysis of multiple structure similar compounds using conventional HPLC columns. For example, the analysis of six isoflavonoids and four saponins in Radix Astragali requires more than 60 min using a 4.6 × 250 mm-column with the particle size of 5.0 μm [27]. Similar results are also can be found in the analysis of multiple alkaloids in *Fritillaria* species [28] or phenolic acids, saponins and diterpenoids in Radix et Rhizoma Salviae Miltiandrhizae and related preparations [29,30]. For shortening the analysis time, several ways can be used, among which are a decrease in the column size and the size of the adsorbent particles and an increase in the eluant flow rate and temperature. However, the use of smaller columns, faster flow rates or higher temperature may sacrifice the resolution for time.

Recently, interest in use of fast HPLC with sub-2 μm particle size has increased because of the dramatic increases in speed of analysis without losing the resolution and sensitivity [31]. According to the van Deemter equation, an empirical formula that describes the relationship between linear velocity (flow rate) and plate height (HETP, or column efficiency), particle size is one of the variables that can influence the chromatographic performance. As the particle size decreases to less than 2.5 μm, not only is there a significant gain in efficiency; but the efficiency does not diminish at increased flow rates. By using smaller particles, speed and peak capacity (number

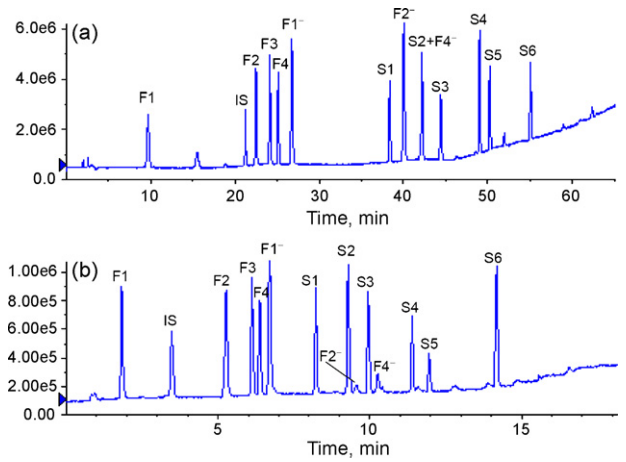

Fig. 1. RRLC vs. HPLC for the analysis of multiple components in Radix Astragali: (a) HPLC separation on a conventional column (4.6 × 250 mm, 5.0 μm). (b) RRLC separation on a shorter column with smaller particle size (4.6 × 50 mm, 1.8 μm). Detection is performed by TOF-MS in ESI (−) scan mode. Peaks are in order: F1 = calycosin-7-*O*-β-D-glucoside; IS (internal standard) = notoginsenoside R1; F2 = ononin; F3 = (6α*R*,11α*R*)-9,10-dimethoxypterocarpan-3-*O*-β-D-glucoside; F4 = (3*R*)-2′-hydroxy-3′,4′-dimethoxyisoflavan-7-*O*-β-D-glucoside; F1⁻ = calycosin; S1 = astragaloside IV; F2⁻ = formononetin; S2 = astragaloside II; F4⁻ = (3*R*)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan; S3 = isoastragaloside II; S4 = astragaloside I; S5 = isoastragaloside I; S6 = acetylastragaloside I. (Reproduced from Ref. [33], Fig. 2, with permission.)

of peaks resolved per unit time) can be extended to new limits [32]. Commercial available fast HPLC systems with sub-2 μm particles, such as ultra-performance LC (UPLC, product of Waters Corporation with 1.7 μm particles) and rapid resolution LC (RRLC, product of Agilent Technologies with 1.8 μm particles), have been used in pharmaceutical or environmental analysis [31,32], and begin to be used in HM analysis field. UPLC or RRLC is an ideal fast separation tool for the analysis of multi-component complex system of HMs. For example, Fig. 1 shows the chromatograms of RRLC on 1.8-μm particles (4.6 × 50 mm) and HPLC on 5.0-μm particles (4.6 × 250 mm) for analysis of Radix Astragali components. It can be seen that the use of RRLC on shorter column with smaller particles (Fig. 1b) provided up to 4 times faster analysis than HPLC on conventional column (Fig. 1a) without sacrificing resolution [33].

2.1.2. Mobile phase

In reversed-phase LC, mixed water–organic phases are usually used in HM analysis. Methanol and acetonitrile are the most often used organic solvents; they combine a good dissolving ability with a low viscosity. Tetrahydrofuran and 2-propanol are used considerably rarely, and as a rule, they are used in mixtures with other organic solvents [26,34]. Separations are performed using mobile phases containing 5–100 vol.% of an organic solvent. Concentrations of the organic solvent lower than 5 vol.% lead to the functional instability of the mobile phase, a change in the properties of the stationary phase, and a poor reproducibility of the results [35]. For analysis of medicinal plants, the use of mobile phases with high concentrations of an organic component (up to 90–100 vol.%) is required because plant extract always contains fat-soluble components of interest or impurities.

Many herbal products are substances with an acid or base character; therefore, a certain pH value of the mobile phase is required. At a low pH, ionization of acidic compounds is suppressed, and thus retention on the reserved-phase adsorbent is optimized. Most of the analyses are performed at pH 2.0–6.0. To maintain this required pH value, a pH modifier is often used in HM analysis. The acidic pH modifier can be selected from formic acid, acetic acid or their ammonium salt solutions, as well as phosphate buffer solution. However, the use of nonvolatile pH modifiers such as above-mentioned phosphate buffer solution is limited when using MS as the detection system because the analyte response in MS will decrease with increasing electrolyte concentration by ion suppression [36].

Solutions with high pH value (>8.0) are also used for analysis of basic compounds to improve peak tailing which is caused by interaction between basic compounds and the residual silanol groups of adsorbent. However, conventional LC columns with silica gel will be decomposed in the solution with such a high pH value. There are adsorbents stable in alkaline media. We used several kinds of these adsorbents to separate different alkaloids at pH 9.0–10.0 and found that the efficiency of the columns did not decrease [28,37]. The required high pH value can be created by one of the solutions containing ammonia, diethylamine or triethylamine. Triethylamine is not recommended to use in LC–MS analysis because strong ion suppression will happen in positive mode.

Elution mode is also an important factor for LC separation. In the separation of herbal products containing many components with different properties, it is difficult to select the conditions for the isocratic determination. It is advisable to use the gradient version, in which the elution capacity of the mobile phase is gradually increased [35]. Most herbal products were analyzed by such an approach. However, special attention should be paid to the strict requirements of gradient mode on the solvent precision the pumps delivered and the quality of the solvents; disregarding these requirements can reduce the accuracy and reproducibility.

2.2. (Q/IT)TOF-MS

2.2.1. Accurate mass measurements

One of the main attributes of TOF instruments that makes it an attractive analytical technique is its accurate mass measurement, which gives the elemental composition of parent and fragment ions. This can be used to identify unknown compounds and differentiation of isobaric compounds (different compounds with the same nominal mass but different elemental composition, and thus, different exact masses). The measurement of accurate masses within 5 ppm is widely accepted for the verification of the elemental composition [19]. To achieve such accurate mass measurement, TOF instruments require frequent tuning and calibration of the spectrometer.

We also had to say that even with very high mass accuracy several chemical formulae candidates might be obtained depending on the mass regions considered. To determine the most probable elemental compositions, different criteria were considered such as the general rule of the number of nitrogen atoms, the DBE index, and isotopic pattern. The DBE parameter, corresponding to the probable molecular formula, was definitive in rejecting those formulae which did not account for the number of unsaturations found. The use of isotopic pattern from the calculator is quite a helpful tool in screening empirical formulae by overlaying the theoretical isotope abundances on the actual spectrum. The use of isotopic abundance patterns as a single further constraint removes >95% of false candidates. Fig. 2 shows an example of elemental composition confirmation of veratridine by accurate mass measurements and isotopic pattern. Using a mass window of 5 ppm, three elemental compositions of the ion 674.3564 are possible. In order to narrow the choices, the use of isotopic pattern from the TOF calculator is quite a helpful tool in screening empirical formulae by overlaying the theoretical isotope abundances on the actual spectrum. According to these criteria, the second formula ($C_{36}H_{52}NO_{11}$) was the only one that had a perfect match. As a result, we got a unique formula, $C_{36}H_{51}NO_{11}$, in this experiment.

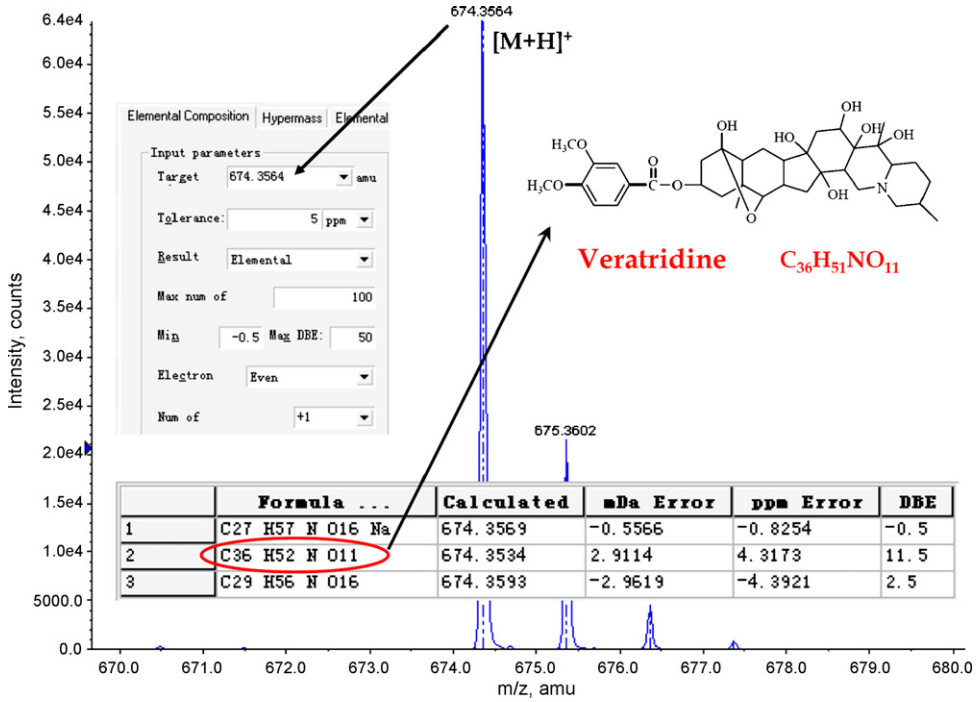

Fig. 2. Elemental composition confirmation of veratridine by accurate mass measurements and isotopic pattern by LC/TOF-MS. (Original data from our lab.)

2.2.2. Fragmentation

The fragmentor role in TOF instruments is crucial for efficient transmission of the ions to obtain the best balance between sensitivity and fragmentation. The fragmentation behavior performed by the fragmentor voltage in TOF plays an important role in the combinative identification or confirmation procedure. Fragmentor voltage is crucial for providing characteristic fragment ions in the MS spectra resulted from collision-induced dissociation (CID) in-source which corresponding to the MS^n fragmentation [38]. As to identification, the more the characteristic fragment ions obtain, the more reliable the structure elucidation or confirmation is. Generally, the fragmentor voltage needs to be adjusted since various types of analytes in HMs produce characteristic fragment ions at different fragmentor voltages. Therefore, with dynamic adjustment of fragmentor voltage (max. ~425 V), TOF can provide valuable structural information by producing various characteristic fragment ions together with their elemental compositions.

Usually, fragmentor voltages of about 200 V can provide significant fragmentation in most chemical components in HMs, such as phenolic acid and saponins, while voltages of 250 V or higher are required for steroidal alkaloids [28] or some kinds of flavonoids [39] because of their relative high stability of chemical structures. In some exceptional cases [33,39], triterpene saponins generated deprotonated molecular ions or solvent additive ions with high abundance, and did not fragment even at the highest fragmentor voltage up to 425 V in negative ion mode. By contrast, in positive mode, they produced rich characteristic fragmentations even at a low fragmentor voltage of 120 V, and may produce different fragment ions at various fragmentor voltages. Although characteristic fragment ions of chemical components in HMs could be produced by dynamic adjustment of fragmentor voltage using TOF, the origin of the fragment ions may not be unequivocal, particularly when analyzing the complex matrix samples. This situation can complicate the identification or confirmation process and affect the deduction of fragmentation pathways for the analytes of interest. When using a QTOF or IT-TOF, however, these drawbacks could be overcome. Tandem or multi-stage MS acquisition produces a product ion spectrum in full scan with accurate mass measurements, which can be used with confidence to identify the chemical components in HMs.

2.2.3. Selectivity with accurate mass

The selectivity of TOF instruments relies on the resolving power of the instrument on the *m*/*z* axis. The higher the resolution provided by the instrument, the better the selectivity for unequivocal identification. Taking into account that the resolving power of a TOF instrument is in the range of 5000–10,000, it can discriminate between "isobaric" interferences within 50 mDa mass difference [18]. The elevated mass resolution of TOF analyzers allows us to reduce the mass window when extracting a specific mass from the full-scan dataset. A smaller mass window leads to a substantial reduction in the chemical noise, facilitating the elimination of the co-eluting interference in complex matrices and elevating the detection sensitivity of the target compound in the extracted ion chromatogram (XIC, also named EIC) [40]. Therefore, the narrow mass windows in XIC of the molecular ion are usually used to quantify the target compounds in HMs [28,30,33,41–45]. In addition and as an alternative, using an optimized fragmentor voltage, the accurate mass of any other characteristic fragment ion could be employed for quantitation in order to avoid the potential isobaric interference.

Compared to other MS instruments, TOF (including QTOF and IT-TOF) can significantly enhance the selectivity since the mass window can be reduced from 1 Da scale (similar to that for Q, TQ or IT analyzers) down to dozens of mDa. When using narrower mass windows, however, the significant mass errors, produced by the unreliability of the mass accuracy attainable by the TOF analyzer or co-eluting isobaric interferents that cannot be resolved by the analyzer, can make the target compound fall out of the extracted mass window. This will affect the result accuracy, especially for quantitative purposes. Thus, when performing quantification in HM samples, one should be cautious and avoid using unreasonably narrow mass windows. As a compromise between improving baseline noise and signal-to-noise ratio and preventing reporting false results, a mass window within 10–50 mDa is recommended when performing quantification of target compounds in herbal samples.

Table 1
LC–(Q/IT)TOF-MS methods for qualitative analysis of natural products in standard solutions, HMs or HM-treated biological matrices.

Analytes	Matrix	LC/adsorbent/particle size (μm)	Detection	Identification	Year	Ref.
C-glycosidic flavonoid isomers, including vitexin, isovitexin, orientin, isoorientin and vitexin-2-*O*″-rhamnoside	Standards (pure flavonoid solutions)	HPLC/C18/no indication	QTOF-MS, IT-MS^n	Accurate mass; elemental composition; MS/MS and MS^n product ions	2001	[51]
Isomeric dicaffeoylquinic acids	*Eleutherococcus senticosus*	HPLC/C8/no indication	TOF-MS, ^{1}H NMR	Accurate mass; elemental composition; retention time; ^{1}H NMR spectra	2002	[52]
Ginsenoside Rg3 and its major metabolites	Rat plasma, urine, and *in vitro* incubation systems	HPLC/C8/3.5	QTOF-MS	Accurate mass; retention time; MS/MS product ions	2003	[24]
Flavonoids	*Hypericum perforatum* and *Rhodiola rosea*	HPLC/C8/3.5	TOF-MS	Accurate mass; retention time; fragment ions	2004	[17]
Cyclic peptides	*Pseudostellaria heterophylla*	HPLC/C18/5	TOF-MS	Accurate mass; elemental composition; retention time	2006	[53]
50 compounds, including 17 monoterpenes, 14 galloyl glucoses, 10 acetophenones, 5 phenolic acids, 3 flavonoids and 1 triterpene	*Paeonia suffruticosa*, *P. delavayi* and *P. decomposita*	HPLC/C18/5	QTOF-MS	Retention time; UV spectra; MS/MS product ions	2006	[54]
Twelve intact glucosinolates, including glucoiberin, glucocheirolin, progoitrin, sinigrin, epiprogoitrin, glucandaphenin, sinalbin, gluconapin, glucosibarin, glucotropaeolin, glucoerucin, and gluconasturtiin	Ten traditional Chinese plants, including *Isatis indigotica*, *Baphicacanthus cusia*, *Patrinia scabiosaefolia*, *Thlaspi arvense* and *Rorippa indica*, etc.	HPLC/C18/5	QTOF-MS	Accurate mass; retention time; MS/MS product ions	2006	[55]
Multiple components, including phenolic acids, iridoids, etc.	*Qingkailing* injection, comprising Radix Isatidis, Flos Lonicerae, Fructus Gardenise, Cornu Bubal, Concha Margaritifera, Baicalinum, Acidum Cholicum and Acidum Hyodesoxy-cholicum	HPLC/C18/5	TOF-MS, IT-MS^n	Accurate mass; elemental composition; retention time; isotopic pattern; MS^n product ions	2006	[38]
Artemisinin, arteannuin B, artemisitene and artemisinic acid	*Artemisia annua*	HPLC/C18/5	QTOF-MS	Accurate mass; retention time; MS/MS product ions	2006	[56]
Flavonoids	*Rhodiola rosea*	HPLC/C18/3.5	TOF-MS and TQ MS	Accurate mass; retention time; MS/MS product ions	2006	[57]
Flavonoids, saponins and phthalides	Incubation systems of Danggui Buxue Tang and HL-7702 cells, RAW 264.7 cells and Caco-2 cells	HPLC/C18/5	TOF-MS	Accurate mass; retention time; DAD spectra	2007	[58]
64 compounds, including flavonoids, triterpene saponins, and monoterpene glycosides	A Chinese medicine formulation PHY906, comprising *Scutellaria baicalensis*, *Paeonia lactiflora*, *Glycyrrhiza uralensis* and *Ziziphus jujuba*	HPLC/C18/3	TOF-MS	Accurate mass; in-source fragmentation; retention time; UV spectra	2007	[59]
Flavonolignans	*Silybum marianum*	HPLC/C18/3	IT-TOF-MS	Accurate mass; elemental composition; MS^n product ions	2007	[60]
Flavonoid *O*-diglycosides	*Fructus aurantii* and *F. aurantii immaturus*	HPLC/C18/5	TOF-MS, IT-MS^n	Accurate mass; elemental composition; MS^n product ions; UV spectra; retention time	2007	[61]
Four saponins, including escin Ia, escin Ib, isoescin Ia and isoescin Ib	*Aesculus chinensis*	HPLC/C18/5	TOF-MS	Accurate mass; isotopic pattern; retention time; UV spectra	2007	[62]
Phenolic acids, including rosmarinic acid, caffeic, chlandogenic, ferulic, gallic, p-coumaric, syringic and vanillic acids	*Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis* and *Thyme vulgaris*	HPLC/C18/3	TOF-MS	Accurate mass; elemental composition; retention time	2007	[63]
Phenolic acids, including danshensu, protocatechuic aldehyde, caffeic acid, salvianolic acid D, lithospermic acid, rosmarinic acid and salvianolic acid B, etc.	Incubation systems of Radix et Rhizoma Salviae Miltiorrhizae and Raw 264.7 cells, Ecv304 cells and HL-7702 cells	HPLC/C18/5	TOF-MS	Accurate mass; elemental composition; retention time	2007	[64]
Eight C_{21} steroids	*Cynanchum* species	HPLC/C18/5	TOF-MS	Accurate mass; elemental composition; retention time	2007	[41]

Six xanthones, including 3-isomangostin, 8-desoxygartanin, gartanin, α-mangostin, 9-hydroxycalabaxanthone and β-mangostin	*Garcinia mangostana*	HPLC/C18/5	TOF-MS	Accurate mass; retention time; PDA spectra	2007	[65]
Rosmarinic acid, caffeic, chlandogenic, ferulic, gallic, p-coumaric, syringic and vanillic acids	*Ocimum basilicum*, *Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis*, *Mentha spicata* and *Thyme vulgaris*	LC × LC (first dimension: SCX*/10 and C18/3; second dimension: amino column/3 and cyano column/3)	TOF-MS	Accurate mass; retention time	2007	[66]
Saponins, including Ginsenosides Rb1, Rc, Rd, Re, Rg1 and notoginsenoside R1	*Panax notoginseng*	UPLC/C18/1.7	TOF-MS	Accurate mass; elemental composition; retention time	2007	[67]
35 polyphenols including 26 isoflavones, 3 flavones, 2 flavanones, 2 aurones and a chalcone	Roots and cell suspension cultures of *Medicago truncatula*	HPLC/C18/5	IT-MS^n, TOF-MS	Accurate mass; elemental composition; MS^n product ions; UV spectra; retention time	2007	[68]
Polyphenols	Grape antioxidant dietary fiber	HPLC/C18/3	ESI-TOF-MS and TQ MS	Accurate mass; elemental composition; MS/MS: precursor ion, neutral loss and product ion scan	2008	[69]
87 non-target components	Mai-Luo-Ning injection	HPLC/C18/4	IT-TOF-MS	Accurate mass; elemental composition; MS^3 product ions; diagnostic ions searching and components classification	2008	[70]
Nine phenolic acids; seven saponins and four diterpenoids	Compound Danshen preparations, mainly comprising *Salvia miltiorrhiza* and *Panax notoginseng*	RRLC/C18/1.8	TOF-MS	Accurate mass; retention time; in-source fragmentation	2008	[42]
15 polyprenylated xanthones	*Garcinia xipshuanbannaensis*	UPLC/BEH C18/1.7	QTOF-MS	Accurate mass; elemental composition; retention time; MS/MS product ions	2008	[71]
Multiple components including flavonoids, saponins, etc.	Chinese herbal prescription Wei-Pi-Tang, comprising *Panax ginseng*, *Glycyrrhiza uralensis*, *Rheum officinale*, *Zingiber officinale* and *Acontium carmichaeli*	HPLC/C18/5 and UPLC/BEH C18/1.7	QTOF-MS	Accurate mass; elemental composition; retention time; MS/MS product ions	2008	[72]
Isomeric malonylated flavonoid glyconjugates	*Lupinus angustifolius*	UPLC/HSS T3/1.8	QTOF-MS	Accurate mass; retention time; MS/MS product ions	2008	[73]
Chlandogenic acid, rutin, and two isomers of isochlandogenic acid, etc.	Flos Lonicerae	HPLC/C18/No indication	TOF-MS	Accurate mass; retention time; DAD spectra	2008	[74]
Saponins, including ginsenoside Rb1, Rg1, Re, Rb2, Rb3, Rc, Rd, F2, Rh2, 20(R)-Rg3, Rg2, F1 and notoginsenoside R1, R2	*Panax ginseng, P. notoginseng* and *P. japonicus*	UPLC/BEH C18/1.7	QTOF-MS	Accurate mass; retention time	2008	[75]
Seven isoflavonoid and six saponins	Radix Astragali	HPLC/C18/5 and RRLC/C18/1.8	DAD–TOF-MS	Accurate mass; retention time; in-source fragmentation; DAD spectra	2008	[33]
25 saponins	*Panax notoginseng*	UPLC/BEH C18/1.7	QTOF-MS	Accurate mass; retention time; MS/MS product ions	2008	[76]
Phenolic acids, phthalides, saponins and isoflavonoids	Danggui Buxue Tang, comprising Radix Astragali and Radix Angelicae Sinensis (5:1)	RRLC/C18/1.8	DAD–TOF-MS	Accurate mass; retention time; in-source fragmentation; DAD spectra	2008	[39]
11 monoterpene glycosides, 11 galloyl glucoses and 4 phenolic compounds	Radix Paeoniae Rubra	RRLC/C18/1.8	TOF-MS	Accurate mass; retention time; in-source fragmentation	2009	[77]
Multiple components including flavonoids, saponins, etc.	Radix Astragali	HPLC/C18/No indication	IT-TOF-MS	Accurate mass; elemental composition; retention time; MS^n product ions	2008	[78]
Flavonoids and saponins	Incubation systems of Radix Astragali and Caco-2 and red blood cells	HPLC/C18/5	TOF-MS	Accurate mass; retention time; in-source fragmentation	2008	[79]
Alkaloids, including berberine, palmatine, and jatrorrhizine, etc.	*Coptis chinensis* Franch	UPLC/BEH C18/1.7 and HPLC/C18/5	PDA–TQ MS and DAD–TOF-MS	Accurate mass; elemental composition; retention time; isotopic pattern; PDA/DAD spectra; MS/MS product ions	2008	[80]

Table 1 (*Continued*)

Analytes	Matrix	LC/adsorbent/particle size (μm)	Detection	Identification	Year	Ref.
Scoparone, capillarisin, rhein, and emodin	Rat urine after oral administration of Yin-Chen-Hao-Tang preparation	UPLC/BEH C18/1.7	QTOF-MS	Accurate mass; retention time; fragment ions	2008	[81]
Multiple components, including cinobufotalin, bufotalin, bufalin and cholic acid, etc.	Liu-Shen-Wan, comprising *Moschus berezovskii*, *Bos Taurus domesticus* and *Bufo gargarizans*, etc.	HPLC/C18/5	IT-MS^n, TOF-MS	Accurate mass; elemental composition; MS^n product ions	2008	[82]
Cyclic peptides including pseudostellarin B	*Pseudostellaria heterophylla*	HPLC/C18/5	TOF-MS	Accurate mass; retention time	2008	[83]
Nine phenolic acids and six diterpenoids	Radix et Rhizoma Salviae Miltiorrhizae	HPLC/C18/5	TOF-MS	Accurate mass; retention time	2008	[29]
26 steroidal alkaloids	*Fritillaria* species	HPLC/C18/5	TOF-MS	Accurate mass; retention time	2008	[28]
One phenolic acids, one phthalides, six saponins and seven isoflavonoids	Danggui Buxue Tang, comprising Radix Astragali and Radix Angelicae Sinensis (5:1)	RRLC/C18/1.8	TOF-MS	Accurate mass; elemental composition; retention time; in-source fragmentation	2008	[43]
Nine phenolic acids, eight saponins and five diterpenoids	Compound Danshen preparations, mainly comprising *Salvia miltiorrhiza* and *Panax notoginseng*	HPLC/C18/5	TOF-MS	Accurate mass; elemental composition; retention time;	2009	[30]
12 saponins, including notoginsenoside R1, ginsenoside Re, Rg1, Rf, Rb1, Rg2, Rh1, Rc, Rb2, Rb3, Rd and notoginsenoside K	Radix et Rhizoma Notoginseng	RRLC/C18/1.8	TOF-MS	Accurate mass; retention time	2008	[44]
32 components, including 6 organic acids, 7 iridoid glycosides, 10 flavonoids and 9 saponins	Flos Lonicerae	HPLC/C18/5	TOF-MS	Accurate mass; elemental composition; retention time; in-source fragmentation	2008	[45]

3. Applications of LC–(Q/IT)TOF-MS for the analysis of herbal medicines

LC–(Q/IT)TOF-MS now has been widely used in many fields, such as foodstuffs, environmental samples, and explosive materials [46,47–50]. For example, the application of LC/(Q)TOF-MS on pesticide residue analysis (PRA) is strongly impressed, and several high impact reviews about the applications on PRA have been published successively [18,19,40]. In spite of its enormous analytical potential, however, not much has been published as yet on the analysis of HMs by LC–(Q/IT)TOF-MS mainly due to the still high costs of the technique and to the initial poor quantitative capabilities of TOF instruments, which fall behind other more commonly used MS such as Q or TQ. It should be noticing that, however, the use of LC–(Q/IT)TOF-MS on HM analysis has increased quickly in last two years (see Tables 1 and 2), which will, in the near future, revolute the field of HM analysis.

3.1. Qualitative analysis

Qualitative analysis of HMs includes the confirmation of target compounds and elucidation of the non-target 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 LC–(Q/IT)TOF-MS. 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 because the monitoring of a specific mass of an analyte is not predefined before data acquisition. This fact allows us to detect the presence of an unlimited number of chemical constituents in a certain herbal product without reanalysis, which is not easily achieved by quadrupole mass analyzers (both Q and TQ, working in SIM or MRM mode, respectively) due to the need to predefine the masses to be monitored and because it is difficult to reduce the dwell time below a threshold value while maintaining a suitable sensitivity [40]. Therefore, LC/TOF-MS is often used for screening of the compounds present in a HM to obtain a fingerprint with the accurate mass of each component. When combining with other instruments, such as ^{1}H NMR, IT or TQ, a practical strategy for confirmation or elucidation of the chemical constituents in HMs is proposed. For example, Zhang et al. [38] screened initially the chemical constituents in *Qingkailing* injection, a well-known TCM formula in China and prepared from eight medicinal materials or their extracts, by using LC/TOF-MS. Thirty-three peaks were found in *Qingkailing* injection and the chemical formula for each compound was given by elemental composition calculator of the TOF software. By combination of the complementary multilevel structural information provided by LC/IT-MS^n, 33 compounds were successfully identified and assigned. In the same way, LC/TOF-MS and TQ analysis were used in the identification of flavonoids in *Rhodiola rosea* [57], and isomeric dicaffeoylquinic acids have been identified with complementary information obtained from ^{1}H NMR and LC/TOF-MS analysis [52]. As can be seen in Table 1, similar applications using LC/TOF-MS and other techniques on various compound identification in HMs [69,80,82] can also be found.

Actually, the LC/TOF-MS itself can also be used to identify and confirm the chemical constituents in HMs in an efficient and reliable manner. An illustrative example has been reported by our research group [39] when analyzing the simplest TCM formula, Dangui Buxue Tang (DBT), by using in-source fragmentation approach. For elucidation of the compositions in DBT, the first step is to propose the fragmentation pathways of the reference compounds based on the accurate masses of the parent ions and fragment ions produced by dynamic adjustment of the fragmentor

Table 2
Quantitative applications of LC–(Q/IT)TOF-MS methods on HMs or HM-treated biological matrices.

Analytes	Matrix	LC/adsorbent/particle size (μm)	Detection	LODs	LOQs	Order of magnitude for linear range	Year	Ref.
Ginsenoside Rg3	Rat plasma	HPLC/C8/3.5	QTOF-MS	0.027 μg/ml	N.A.	1.1	2003	[24]
Flavonoids	*Hypericum perforatum* and *Rhodiola rosea*	HPLC/C8/3.5	TOF-MS	0.04–0.10 μg/ml	0.2 μg/ml	1.3–1.7	2004	[17]
Artemisinin, artemisitene arteannuin B, and artemisinic acid	*Artemisia annua*	HPLC/C18/5	QTOF-MS	0.0001–0.04 μg/ml	~0.1 μg/ml	1.5	2006	[56]
Phenolic acids, including rosmarinic acid, caffeic, chlandogenic, ferulic, gallic, p-coumaric, syringic and vanillic acids	*Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis* and *Thyme vulgaris*	HPLC/C18/3	TOF-MS	3–141 ng/ml	N.A.	0.9	2007	[63]
Eight C_{21} steroids	*Cynanchum* species	HPLC/C18/5	TOF-MS	0.035–2.574 ng	0.068–9.702 ng	2.7	2007	[41]
Rosmarinic acid, caffeic, chlandogenic, ferulic, gallic, p-coumaric, syringic and vanillic acids	*Ocimum basilicum*, *Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis*, *Mentha spicata* and *Thyme vulgaris*	LC × LC (first dimension: SCX/10 and C18/3; second dimension-normal phase: amino column/3 and cyano column/3)	TOF-MS	18–90 ng/ml	N.A.	1.0	2007	[66]
Nine phenolic acids; seven saponins and four diterpenoids	Compound Danshen preparations, mainly comprising *Salvia miltiorrhiza* and *Panax notoginseng*	RRLC/C18/1.8	TOF-MS	0.607–14.6 ng/ml	2.02–53.0 ng/ml	2.3–3.6	2008	[42]
Seven isoflavonoid and six saponins	Radix Astragali	HPLC/C18/5 and RRLC/C18/1.8	DAD–TOF-MS	0.001–0.450 ng (RRLC); 0.09–0.40 ng (HPLC)	0.005–2.250 ng (RRLC); 0.23–1.03 ng (HPLC)	1.6–2.6	2008	[33]
Scoparone, capillarisin, rhein, and emodin	Rat urine after oral administration of Yin-Chen-Hao-Tang preparation	UPLC/BEH C18/1.7	QTOF-MS	3.0–9.0 ng/ml	12.0–33.0 ng/ml	2.0	2008	[81]
26 steroidal alkaloids	*Fritillaria* species	HPLC/C18/5	TOF-MS	0.0139–0.1680 ng	N.A.	1.7–3.4	2008	[28]
One phenolic acids, one phthalides, six saponins and seven isoflavonoids	Danggui Buxue Tang, comprising Radix Astragali and Radix Angelicae Sinensis (5:1)	RRLC/C18/1.8	TOF-MS	0.004–0.08 ng	0.015–0.450 ng	2.2–2.7	2008	[43]
Nine phenolic acids, Eight saponins and five diterpenoids	Compound Danshen preparations, mainly comprising *Salvia miltiorrhiza* and *Panax notoginseng*	HPLC/C18/5	TOF-MS	1.58–10.10 ng/ml	4.85–28.56 ng/ml	N.A.	2009	[30]
12 saponins, including notoginsenoside R1, ginsenoside Re, Rg1, Rf, Rb1, Rg2, Rh1, Rc, Rb2, Rb3, Rd and notoginsenoside K	Radix et Rhizoma Notoginseng	RRLC/C18/1.8	TOF-MS	1.53–5.08 ng/ml	4.07–15.2 ng/ml	1.9–2.2	2008	[44]
32 components, including 6 organic acids, 7 iridoid glycosides, 10 flavonoids and 9 saponins	Flos Lonicerae	HPLC/C18/5	TOF-MS	0.002–0.089 μg/ml	0.006–0.355 μg/ml	1.8–3.0	2008	[45]

N.A., information not available.

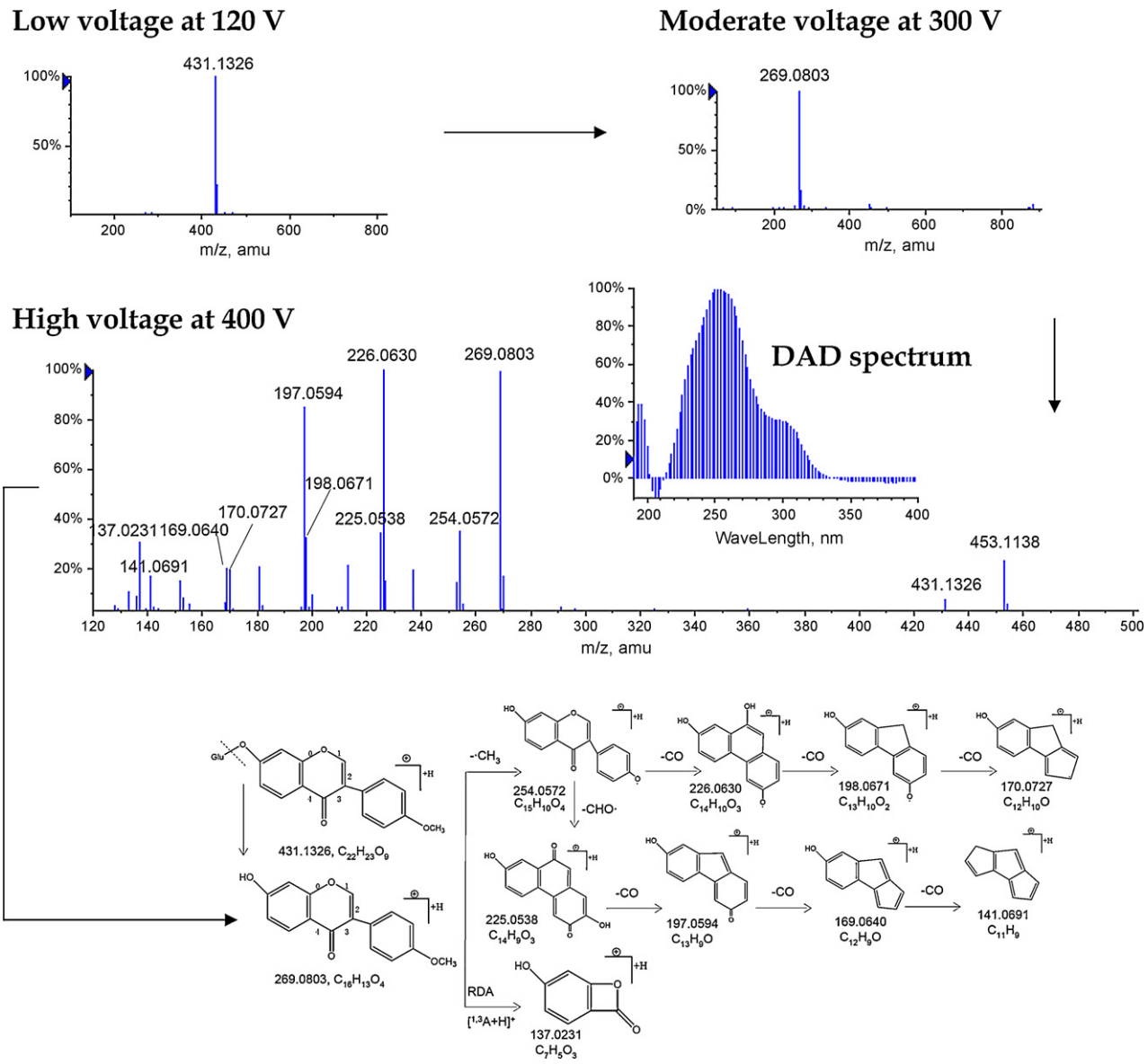

Fig. 3. Identification of ononin (formononetin-7-*O*-β-D-glucoside) by LC/TOF-MS in positive mode using in-source fragmentation approach and DAD spectrum. (Original data from our lab.)

voltage in TOF. Then the molecular and fragment ions of compounds in real samples were obtained under the same conditions for reference compounds. After comparing the molecular and fragment ions with the parent compound, together with retention time, the target compounds can be easily identification. For those without reference compound (non-target compounds), the identification can be performed also by comparing the molecular and fragment ions with those produced by reference compounds because compounds with similar skeletons may coexist in a certain medicinal plant. If similar fragment ions can be found, the compound of interest may have a similar fragmentation pathway with the reference compound compared, which will facilitate the elucidation of this compound. Taking a compound in DBT for instance, Fig. 3 shows its DAD spectrum and ESI-TOF/MS spectrum with low (120 V), moderate (300 V) and high (400 V) fragmentor voltages in positive mode. This compound produces maximum absorption at 195, 254 nm and a shoulder peak at 300 nm, suggesting that it is an isoflavone. It produces a single protonated molecular ion peak at *m*/*z* 431.1326 corresponding to elemental compositions of $C_{22}H_{23}O_9$ at a low 120 fragmentor voltage. When the fragmentor voltage increases to 300 V, it generates a high intensity peak at *m*/*z* 269.0803 ($C_{16}H_{13}O_4$) by the loss of 162 Da ($C_6H_{11}O_5$) from the molecular ion. This neutral loss is typical for *O*-glycosides. When the fragmentor increases up to 400 V, it yields abundant characteristic fragment ions, corresponding to successive or simultaneous losses of $^{\bullet}CH_3$, CO, CH_3OH, $^{\bullet}CHO$, etc., which are attributed to 4′-OCH_3 isoflavone type. This compound was ultimately identified as ononin (formononetin-7-*O*-β-D-glucoside) with related publications and further confirmed by an authentic standard. The characteristic fragmentation pathways are also schemed in Fig. 3. It is worthy mentioning that generally the neutral losses of $^{\bullet}CO_2$ are prominent for isoflavones in tandem MS, and the fragmentation ion at *m*/*z* 225.0538 decreased by 44 Da is naturally assigned as a neutral loss of CO_2 from 269.0803. However, the TOF/MS reveals that the formula composition of 225.0538 is $C_{14}H_9O_3$ from the loss of C_2H_4O rather than CO_2, which suggests that the traditional loss of CO_2 pathway is removed. This product ion is finally proposed by the losses of $^{\bullet}CH_3$ and $^{\bullet}CHO$ ascribed to the 4′-position, but not CO_2, demonstrating the great power of TOF/MS in fragmentation pathways proposition. As a result, using this approach, 10 phenolic acids and phthalides, 13 major saponins

with a 20,24-epoxy-9,19-cyclolanostane-3,6,16,25-tetrol skeleton and 16 isoflavonoids (including 21 non-target compounds) were identified in DBT preparation, and their appropriate fragmentation pathways were also proposed [39]. In the same way, the chemical constituents in Radix Paeoniae Rubra, including 11 monoterpene glycosides, 11 galloyl glucoses and 4 phenolic compounds, were also successfully identified in our research group by in-source fragmentation approach of LC/TOF-MS [77].

Using this in-source fragmentation approach, however, the confirmation or elucidation of compounds at low concentrations might be troublesome due to the lower abundance of fragment ions compared to the precursor ones. Besides, in complex matrices, it can be difficult to obtain information about the origin of the fragment ion since some interferences may share the same mass as the in-source fragment. Some nonisobaric interferences may even produce isobaric fragments that would interfere with the confirmation. When low m/z fragments are selected, this situation is more problematic as this region of the spectrum tends to be noisier when the fragment voltage is increased [40]. For these reasons, the fragmentation pathways proposed by LC/TOF-MS may be equivocal in some cases.

These limitations can be drastically minimized, when QTOF or IT-TOF are used, by increasing confidence about the origin of the ion and also reducing the isobaric interferences. Thus, the elucidation achieved via QTOF or IT-TOF can be considered to be definitive in most cases. At present, the elucidations of natural product metabolites in biological matrices [24] and unknown compounds in herbal samples [54,70,71] by QTOF or IT-TOF have been published. The elucidation procedure by these instruments is similar with that provided by in-source fragmentation approach of LC/TOF-MS except that the fragmentation pathway is proposed based on the product ions in MS/MS or MS^n spectrum. For example, ginsenoside Rg3 metabolites have been elucidated by using LC/QTOF-MS [24]. The fragmentation information obtained from MS/MS and high-resolution MS analyses has successfully elucidated the major metabolites of ginsenoside Rg3 in different incubation systems, such as ginsenoside Rh2 and protopanaxadiol in 0.1 M HCl solution, and a monooxygenated metabolite in rat liver S9 fraction. Zhou et al. [71] identified 12 target polyprenylated xanthones and 3 non-target ones in *Garcinia xipshuanbannaensis* using LC/QTOF-MS. By comparing the product ions with those produced by reference compounds, the skeletons of non-target polyprenylated xanthones can be proposed. With or without the other supporting evidence from literature, these non-target compounds can be ultimately elucidated.

However, the identification or elucidation of the aforementioned compounds, as well as other compounds in various herbal products, by TOF, QTOF or IT-TOF is performed to a great extent with the assistance of reference compounds (see Table 1) and the previous knowledge about the chemical constituents in these HMs. Considering that the reference compounds are always difficult to obtain and most components contained in HMs are unknown, the identification or elucidation of chemical constituents in HMs can be hampered when the samples analyzed without previous knowledge about the possible structures of the investigated compounds. In these cases, the most common approach is to obtain the molecular formulae and then to search in a database. The product ions with accurate masses provide additional structural information which is useful for discriminating between possible isomeric structures, making elucidation feasible in some cases. As an illustrative example of this approach for elucidation in such cases, Hao et al. [70] identified 87 non-target compounds in Mai-Luo-Ning injection, a well-known herbal prescription used in China for the treatment of cerebral thrombosis and vascular occlusion of angeitides, by LC/IT-TOF-MS and a strategy. In this strategy, the components sharing the exact same ions (mass error <5 mDa) were classified into a family. All families were then connected into a coherent network by the bridging components that are present in two or more families. With the benefit from such a network, together with a complementary approach to screening by sequential loss of specific chemical groups, all of the 87 peaks detected have been successfully identified except failed to differentiate some isomers.

3.2. Quantitative analysis

As stated before, each herbal medicine is a complex matrix that contains hundreds of different constituents with diverse chemical structures. Furthermore, in most cases, the chemical constituents of interest may present in HMs with a low concentration levels (<100 μg/g). Thus, for quantitative analysis of the chemical constituents in HMs, the selectivity and sensitivity attainable is possibly the key issue. In this sense, TOF instruments offer high selectivity and sensitivity under full-scan conditions compared to other analyzers, notwithstanding around one order of magnitude less sensitive may happen when in comparison to a TQ instrument used in MRM mode. The selectivity and sensitivity of TOF can be enhanced by narrowing mass window in XIC. Fig. 4 shows an example of the selectivity and sensitivity achieved by HPLC/TOF-MS when analyzing an isoflavonoid, (6α*R*,11α*R*)-9,10-dimethoxypterocarpan-3-*O*-β-D-glucoside, in Radix Astragali extract. When a wide mass window (e.g. 0.3 Da) is selected in the XIC ($[M+HCOO]^-$ m/z 507.1489), interferences might be present (Fig. 4a). When the same window is narrowed (e.g. 0.01 Da), the interferences disappear leading to a more selective identification for the target compound and also to an enhanced signal-to-noise ratio (Fig. 4b). As a result, the narrow mass window in XIC was used to achieve sensitive quantification of this compound with the limits of detection (LOD) of 0.23 ng. This feature reinforces the usefulness of benchtop TOF mass spectrometers for the trace analysis of chemical components in herbal products. Table 2 shows the quantitative applications of LC–(Q/IT)TOF-MS instruments on HMs or HM-treated samples. As can be seen, QTOF has scarcely used and to our knowledge no study quantifying natural products in HMs field by IT-TOF has been performed. When performing quantification by QTOF, the selectivity is much more superior than that provided by TOF as most of the interferences are filtered in the quadrupole, while the sensitivity achieved by a QTOF is of the same order as that achieved by TOF, and normally around 10-fold lower than TQ in MRM mode. However, in all cases, the sensitivity achieved by TOF or QTOF-MS was

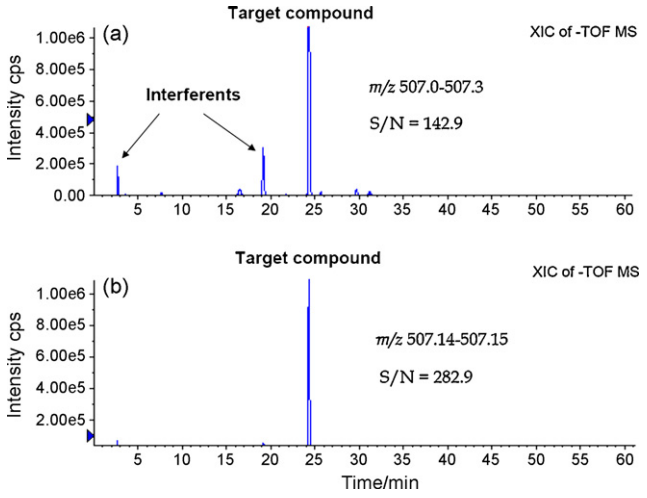

Fig. 4. LC/TOF-MS extracted ion chromatogram for (6α*R*,11α*R*)-9,10-dimethoxypterocarpan-3-*O*-β-D-glucoside at m/z 507 ($[M+HCOO]^-$) using two different extraction windows: (a) 0.3 Da; (b) 0.01 Da. (Original data from our lab.)

Table 3
Comparison of RRLC on 1.8-μm particles with HPLC on 5.0-μm particles for analysis of isoflavonoids and saponins in DBT in terms of analytical time, resolution, sensitivity, and precision. (Reproduced from Ref. [33], Table 1, with permission.).

No.	Compound class	Retention time (min)		Resolution		LOD (ng)		LOQ (ng)		Intra-day RSD of peak area (%) (n = 8)		Inter-day RSD of peak area (%) (n = 3)	
		A[a]	B[b]	A	B	A	B	A	B	A	B	A	B
F1	Isoflavonoid	1.84	9.69	+[c]	+	0.001	0.21	0.005	0.50	0.3	3.9	1.4	4.6
F2	Isoflavonoid	5.27	22.50	+	+	0.003	0.12	0.030	0.29	0.6	4.5	2.0	4.9
F3	Isoflavonoid	6.12	24.16	+	+	0.003	0.23	0.015	0.53	0.4	3.9	1.0	4.6
F4	Isoflavonoid	6.38	25.10	+	+	0.008	0.14	0.030	0.34	0.5	3.3	1.2	3.9
F1$^-$	Isoflavonoid	6.71	26.73	+	+	0.003	0.19	0.030	0.54	1.5	4.0	1.4	4.6
F2$^-$	Isoflavonoid	9.55	40.04	+	+	0.008	0.28	0.015	0.72	2.7	4.1	4.1	5.0
F4$^-$	Isoflavonoid	10.27	42.18	+	_[d]	0.450	0.40	2.250	1.03	2.3	3.9	4.5	3.9
S1	Saponin	8.22	38.37	+	+	0.003	0.16	0.030	0.41	1.0	3.2	1.2	3.6
S2	Saponin	9.29	42.18	+	–	0.008	0.14	0.015	0.37	0.7	2.5	2.3	3.9
S3	Saponin	9.95	44.40	+	+	0.008	0.24	0.015	0.52	1.2	2.6	1.0	4.0
S4	Saponin	11.40	49.04	+	+	0.008	0.10	0.030	0.23	1.2	3.0	1.5	3.2
S5	Saponin	11.96	50.25	+	+	0.011	0.09	0.045	0.24	1.5	2.9	2.0	3.6
S6	Saponin	14.18	55.06	+	+	0.011	0.15	0.045	0.32	1.9	2.7	0.9	3.7

A[a]: performance of Zorbax SB-C_{18} column (4.6 × 50 mm, 1.8 μm); B[b]: performance of Zorbax Extend-C_{18} column (4.6 × 250 mm, 5 μm); +[c]: base-line separation; _[d]: not base-line separation. F1: calycosin-7-O-β-D-glucoside; F2: ononin; F3: (6α*R*,11α*R*)-9,10-dimethoxypterocarpan-3-O-β-D-glucoside; F4: (3*R*)-2′-hydroxy-3′,4′-dimethoxyisoflavan-7-O-β-D-glucoside; F1$^-$: calycosin; F2$^-$: formononetin; F4$^-$: (3R)-7,2′-dihydroxy-3′,4′-dimethoxyisoflavan; S1: astragaloside IV; S2: astragaloside II; S3: isoastragaloside I; S4: astragaloside I; S5: isoastragaloside I; S6: acetylastragaloside I.

sufficient regarding the required detection limit of each application.

It should be mentioned that the selectivity and sensitivity also can be enhanced by improving the chromatographic separation between the analyte and the interference using RRLC or UPLC. As an example, our research group compared the performances of RRLC on 1.8-μm particles (4.6 × 50 mm) with HPLC on 5.0-μm particles (4.6 × 250 mm), using TOF as the detector, for quantitative analysis of 13 isoflavonoids and saponins in Radix Astragali and related preparations [33], and the results are listed in Table 3. RRLC system provides fourfold faster analysis and 1–210 times more sensitive by comparing the LODs than HPLC without sacrificing resolution and precision. Therefore, UPLC or RRLC coupled with TOF analyzers will have a good prospect in the HM field.

In addition to selectivity and sensitivity, the feasible linear dynamic range of the TOF response is of paramount importance when applied for quantitative purposes. Previous TOF instruments usually suffered from narrow dynamic ranges, requiring mathematical algorithms, such as the "time to digital correction", in order to obtain a longer linear dynamic range, and this thus severely limited the usability of TOF instruments [18]. This disadvantage was overcome in new instruments, by using an analog-to-digital convertor (ADC) [18,28] or a voltage applied to the Z-focus lens of TOF instruments [46], which offered a linear dynamic range of about 2–4 orders of magnitude. This is enough to make possible successful quantitative applications in routine analyses of chemical compounds in herbal samples (see Table 2). For example, multicomponents in Flos Lonicerae, including 6 organic acids, 7 iridoid glycosides, 10 flavonoids and 9 saponins were successfully quantified using the linear dynamic range of about 1.8–3.0 orders of magnitude, although some components cover a large range of different concentration levels (2–3 orders of magnitude) in different medicinal plants used for Flos Lonicerae [45].

Usually, the precision and accuracy of LC/(Q)TOF-MS methods for quantification purposes is not an issue to be considered, because in most cases LC/(Q)TOF-MS can provide satisfactory precision and accuracy which is comparable to that produced by other techniques. However, during the quantitative analysis of 26 steroidal alkaloids in *Fritillaria* species by LC/TOF-MS [28], a parameter, called bunching factor, was found to affect the precision and accuracy of quantitative method. The bunching factor is the number of data points that are grouped to form one bunched point. Ideally, this value should be set so that there are approximately 12 data points for each peak, from start to end (setting path: *Tools* → *Settings* → *Processing Options* → *TurboChrom Integration* in a data analysis software, Applied Biosystems/MDS-Sciex Analyst QS software). The value of bunching factor can influence the integration results of chromatographic peaks of steroidal alkaloids. Taking four steroidal alkaloids for instance, the peak areas of these compounds are significantly different at various values of bunching factor (Fig. 5 and Table 4). The bigger the value is, the larger the peak area obtained is. However, the increase of peak areas was not significant when the value was more than 3. After optimizing the

Table 4
Peak areas of four steroidal alkaloids in XICs from Fig. 5 at different values of bunching factor.

Value of bunching factor	Peak area			
	19[a]	IS	21	24
Bunching factor = 1	1.4e6	4.5e5	3.1e5	8.0e6
Bunching factor = 2	6.1e6	6.4e6	8.0e6	9.1e6
Bunching factor = 3	7.8e6	6.9e6	9.0e6	9.7e6
Bunching factor = 4	8.0e6	6.9e6	9.2e6	9.8e6
RSD (%)	52.7	61.0	64.0	9.03

[a] Peak number listed in Fig. 5.

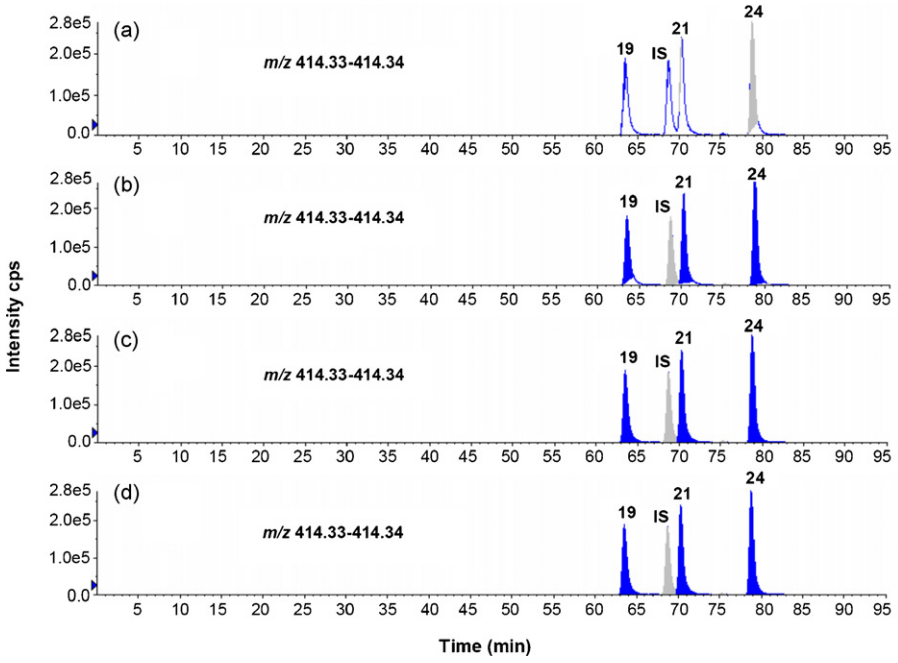

Fig. 5. Integration results of four steroidal alkaloids at different values of bunching factor in the extracted ion chromatograms (XICs) by LC/TOF-MS analysis: (a) bunching factor = 1; (b) bunching factor = 2; (c) bunching factor = 3; (d) bunching factor = 4; 19 = ebeienine; IS (internal standard) = solasodine; 21 = puqiedinone; 24 = ebeiedinone. (Reproduced from Ref. [28], Fig. 3, with permission.)

value of bunching factor, satisfactory precision and linearity were achieved. The ranges of RSD values of intra-day and inter-day variability for all alkaloids were decreased remarkably from 41.8–159% and 13.2–140% to 0.32–7.98% and 2.37–16.1%, when the value of bunching factor was optimized from 1 to 3 [28]. Therefore, the value of bunching factor should be optimized when the peak area integration is incomplete at the default value of bunching factor of 1 in the TOF methods, to ensure the result precision and accuracy.

4. Conclusion

The quality, safety and efficacy of herbal medicines and preparations thereof are usually assured by chemical analysis, whether an analytical technique is suitable for this purpose depends on its capability for characterization and determination of the target and non-target (unknown) chemical constituents in a herbal sample. The inherent characteristics of TOF in accurate mass measurements and high resolution make this analyzer very attractive in the qualitative analysis of chemical constituents in herbal samples. Acquisition of full-scan spectra with high sensitivity and mass accuracy (elemental composition), combined with dynamic fragment voltage adjustment (fragmentation pattern), facilitates the identification of target even the non-target compounds in complex plant matrices by LC/TOF-MS. Furthermore, the use of hybrid TOF instruments, such as QTOF and IT-TOF, will achieve an unequivocal confirmation of the analyte identity by increasing confidence about the origin of the fragment ions and also reducing the isobaric interferences.

In terms of quantification, the increase in sensitivity and linear dynamic range in recent TOF instruments also contribute to the suitability of LC/(Q)TOF-MS in achieving lower LODs with high precision and accuracy which are comparable to quadrupole methods (SIM and MRM). Moreover, the high selectivity provided by narrowing mass window in XIC make the matrix interferences are less important in quantitative purposes because the number of coincident ions between matrix and analytes can be considered negligible for mass-accuracy levels higher than 5 mDa. In addition, the employment of RRLC or UPLC can tremendously increase the sensitivity and analytical speed without sacrificing the resolution. All these features indicate that a further increase in the popularity of "classical" LC/TOF-MS and its more recent developments like RRLC/TOF-MS or UPLC/QTOF-MS in HM analysis can be expected.

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