



## ORIGINAL ARTICLE

# Quantitative and qualitative analysis of common peaks in chemical fingerprint of Yuanhu Zhitong tablet by HPLC-DAD-MS/MS

Dao-Quan Tang<sup>a,b,c,\*</sup>, Xiao-Xiao Zheng<sup>b</sup>, Xu Chen<sup>b</sup>, Dong-Zhi Yang<sup>a,b</sup>, Qian Du<sup>b</sup>

<sup>a</sup>Department of Pharmaceutical Analysis, Xuzhou Medical College, Xuzhou 221004, China

<sup>b</sup>Key Laboratory of New Drug & Clinical Application, Xuzhou Medical College, Xuzhou 221004, China

<sup>c</sup>Environmental Research Institute, National University of Singapore, Singapore 117411, Singapore

Received 27 August 2013; accepted 19 December 2013

Available online 28 December 2013

## KEYWORDS

Yuanhu Zhitong tablet;  
Alkaloids;  
Coumarins;  
Quality control;  
HPLC-DAD-MS/MS

**Abstract** A quality control (QC) strategy for quantitative and qualitative analysis of “common peaks” in chemical fingerprint was proposed to analyze Yuanhu Zhitong tablet (YZT), using high performance liquid chromatography with diode array detector and tandem mass spectrometry (HPLC-DAD-MS/MS). The chromatographic separation was achieved on an Agilent Eclipse plus C<sub>18</sub> column with a gradient elution using a mixture of 0.4% ammonium acetate aqueous (pH 6.0 adjusted with glacial acetic acid) and acetonitrile. In chemical fingerprint, 40 peaks were assigned as the “common peaks”. For quantification of “common peaks”, the detection wavelength was set at 254 nm, 270 nm, 280 nm and 345 nm, respectively. The method was validated and good results were obtained to simultaneously determine 10 analytes (protopine, jatrorrhizine, coptisine, palmatine, berberine, xanthotoxin, bergapten, tetrahydropalmatine, imperatorin and isoimperatorin). For qualification of “common peaks”, 33 compounds including 10 quantitative analytes were identified or tentatively characterized using LC-MS/MS. These results demonstrated that the present approach may be a powerful and useful tool to tackle the complex quality issue of YZT.

© 2014 Xi'an Jiaotong University. Production and hosting by Elsevier B.V.

Open access under [CC BY-NC-ND license](https://creativecommons.org/licenses/by-nc-nd/4.0/).

\*Corresponding author at: Department of Pharmaceutical Analysis, Xuzhou Medical College, Xuzhou 221004, China.  
Tel./fax: +86 516 83262136.

E-mail address: [tdq993@hotmail.com](mailto:tdq993@hotmail.com) (D.-Q. Tang).

Peer review under responsibility of Xi'an Jiaotong University.



Production and hosting by Elsevier

## 1. Introduction

Traditional Chinese medicine (TCM) is well known to generally exert its therapeutic effects through the synergic effects of its multiple active ingredients and multi-targets. However, hundreds of different constituents with diverse physical and chemical properties coexist, making the quality control (QC) of TCM extremely difficult [1].

Multi-ingredients quantification (MIQ) is usually selected as a QC approach of TCM, but these ingredients can hardly stand for

the complex herbal products. Another QC method is fingerprint analysis (FA), which addresses the systematic nature of TCM. However, this strategy can only show results of similarity

calculated on the basis of the relative value using a pre-selected marker compound as a reference [2], and the real content of active ingredients cannot be quantified exactly. Although combination of

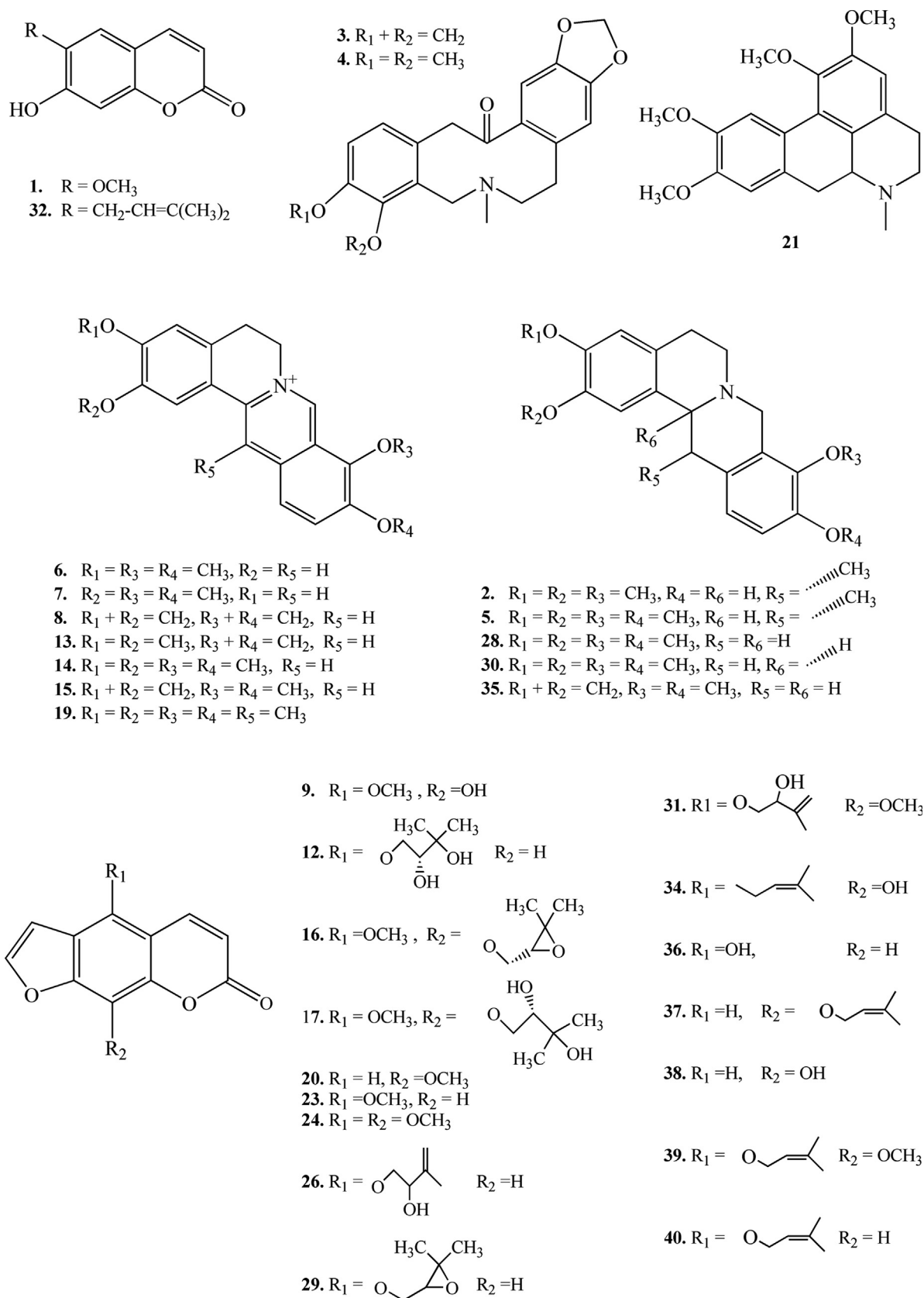


Fig. 1 Chemical structures of 33 identified compounds in Yuanhu Zhitong tablet (YZT).

FA and MIQ may be more effective than FA or MIQ alone for the QC of TCM [3–6], “common peaks” without standard references in FA are usually neglected. The quality difference of medicinal herbs and their products may result from different original production regions, harvest time or manufacturing processes, but existence of “common peaks” may be the basis of their quality consistency, stability and therapeutic effects. Therefore, the quantification and identification of “common peaks” is critically important for controlling the quality of TCM and revealing the material basis of their therapeutic effects. In the present work, a new QC strategy based on quantitative and qualitative analysis of “common peaks” in chemical analysis of TCM was proposed and successfully applied for the QC of Yuanhu Zhitong tablet (YZT).

YZT, a classical TCM prescription consisting of 223 g of Radix *Angelicae dahuricae* and 445 g of Rhizoma *Corydalis* (processed with vinegar), has been clinically used to treat gastralgia, costalgia, headache and dysmenorrhea in China [7]. Alkaloids and coumarins have been generally regarded as the active component of Rhizoma *Corydalis* and Radix *Angelicae dahuricae*, respectively [8,9]. There have been articles describing quantitative determination of a few bioactive components for quality assessment of YZT [10,11]. However, all of these achievements simply focused on one or several components, rather than the overall efficacy and quality of YZT. Moreover, there was no report on the quantitative and qualitative analysis of chemical fingerprint “common peaks” in the quality evaluation of YZT.

This paper describes, for the first time, a high performance liquid chromatography with diode array detector (HPLC-DAD) method for chemical fingerprint of YZT, and 40 “common peaks” were obtained. For those “common peaks”, 10 compounds (protopine, jatrorrhizine, coptisine, palmatine, berberine, xanthotoxin, bergapten, tetrahydropalmatine, imperatorin and isoimperatorin) of them were simultaneously quantified and 33 compounds including 10 quantitative compounds (Fig. 1) were identified or tentatively characterized by electrospray ionization tandem mass spectrometry (ESI-MS/MS).

## 2. Materials and methods

### 2.1. Chemicals, reagents and materials

Acetonitrile (HPLC grade) was purchased from Fisher Scientific (Fisher Scientific, USA). Purified water was used from a Milli-Q system (Millipore, Bedford, MA, USA). All the other reagents

were of analytical grade. The reference compounds of 10 quantitative analytes were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purities of these reference standards were determined to be higher than 98% by HPLC. Commercial products of YZT were collected from 12 pharmaceutical companies in China (Table 1). The YZT from Manufacture A was selected for method optimization and validation of HPLC-DAD-ESI-MS/MS conditions.

### 2.2. HPLC-DAD-ESI-MS/MS

HPLC analysis was performed on an Agilent 1260 series HPLC system. The analytes were isolated on an Agilent Eclipse plus C<sub>18</sub> column (250 mm × 4.6 mm i.d, 5 μm). The separation process followed a gradient elution procedure and used mobile phase A (0.4% ammonium acetate aqueous, pH 6.0 adjusted by glacial acetic acid) and B (acetonitrile) whose ratios changed linearly as follows: 0–25 min, 17–19% B; 25–55 min, 19% B; 55–70 min, 19–25% B; 70–80 min, 25–28% B; 80–95 min, 28–34% B; 95–120 min, 34–35% B; 120–140 min, 35–42% B; 140–160 min, 42–50% B. The flow rate was 1.0 mL/min. The injection volume was 5 μL and the column temperature was 30 °C. Quantitative detection wavelength was set, respectively, at 254 nm (xanthotoxin, bergapten, imperatorin and isoimperatorin), 270 nm (berberine), 280 nm (protopine and tetrahydropalmatine) or 345 nm (jatrorrhizine, coptisine and palmatine), while the wavelength of FA was set at 280 nm.

The above HPLC system was interfaced with an Agilent 6460 Triple Quadrupole mass spectrometer (Agilent Technologies, MA, USA) in a post-column splitting ratio of 4:1. The conditions of ESI source were as follows: source voltage, 3000 V; drying gas (N<sub>2</sub>) flow rate, 10.0 L/min; drying gas temperature, 320 °C; nebulizer, 25 psi. The MS data were acquired from *m/z* 100 to 1000 in positive ion modes.

### 2.3. Preparation of samples and NC solutions

The coatings of YZT samples were removed completely, and the remaining were smashed into fine powder. Pulverized sample (1.0 g) was weighed precisely and ultrasonically extracted using 35 mL methanol for 30 min. After being settled to the volume of 50 mL, the extracted solution was filtered through filter paper and evaporated at 70 °C water bath. The residue was settled with methanol to the volume of 5 mL and centrifuged at 15,000 rpm for 10 min. The supernatant

**Table 1** Summary of the tested YZT commercial samples.

Sample no.	Manufacturers	Batch no.
A	Guangxi Tiantianle Pharmaceutical Co., Ltd., China	100801
B	Foshan Dezhong Pharmaceutical Co., Ltd., China	10012
C	Guangxi Shibiao Pharmaceutical Co., Ltd., China	080901
D	Sichuan Hebang Pharmaceutical Co., Ltd., China	100901
E	Henan Wanxi Pharmaceutical Co., Ltd., China	110502
F	Jiangxi Jiulianshan Pharmaceutical Co., Ltd., China	20101104
G	Shandong Kongfu Pharmaceutical Co., Ltd., China	100301
H	Shandong Lukang Pharmaceutical Co., Ltd., China	20110506
I	Nantong Jinghua Pharmaceutical Co., Ltd., China	090701
J	Shanxi Wanglong Pharmaceutical Co., Ltd., China	20101001
K	Sichuan Shuzhong Pharmaceutical Co., Ltd., China	100906
L	Guangxi Banmu Tianlong Pharmaceutical Co., Ltd., China	101001

was filtered through a 0.45  $\mu\text{m}$  membrane filter and transferred to an autosampler vial for HPLC-DAD-ESI-MS/MS analysis.

According to the prescription and preparation protocol of YZT formula recorded in China Pharmacopoeia (Ch. P.), two negative control (NC) samples without *Radix Angelicae dahuricae* or *Rhizoma Corydalis* were prepared, respectively, to validate the specificity of the method. The medicinal herbs were ground into powder in the particle size of 40–60 mesh and the negative samples were prepared according to the method described above for analysis.

#### 2.4. Preparation of standard solutions

The 10 reference standards were weighed accurately. Jatrorrhizine, palmatine, coptisine and berberine were dissolved in methanol/water (50:50, v/v), and the other standards were dissolved in pure methanol. They were then diluted to appropriate concentrations for establishing calibration curves. All the solutions were stored in a refrigerator at 4  $^{\circ}\text{C}$  until use for analysis.

#### 2.5. Quantitative and qualitative analysis in HPLC fingerprint

##### 2.5.1. Confirmation of common peaks and evaluation of similarity

Data analysis was performed by a professional software named Similarity Evaluation System for Chromatographic Fingerprint of TCM (Version 2004A). The relative retention time (RTT) and relative peak area (RPA) of each common peak related to the reference peak were calculated for quantitative expression of the chemical properties in the chromatographic pattern of YZT. Based on this, the correlation coefficients of entire chromatographic profiles of samples were calculated, while the simulative mean chromatogram was generated.

##### 2.5.2. Quantitative analysis validation of common peaks

The analysis of linearity, repeatability, stability, limit of detection (LOD) and limit of quantification (LOQ), precision, and accuracy were carried out to validate the quantitative method, following the International Conference on Harmonization (ICH) guideline [12].

##### 2.5.3. Qualitative analysis of common peaks

Identification of common peaks in YZT was carried out by LC-ESI-MS/MS analysis. In the full scan mass spectra, most of the constituents exhibited their quasi-molecular ions  $[\text{M}+\text{H}]^{+}$  in positive ion mode under the soft electrospray ionization condition. Precursor ions were subjected to collision-induced dissociation (CID) to generate the fragment ions and the fragmentation patterns were proposed for the structural identification of constituents.

### 3. Results and discussion

In an effort to control the quality of YZT, several excellent studies have been performed. Zhang et al. [11] have determined and quantified 17 constituents in YZT in 9 min using rapid resolution liquid chromatography coupled with a triple quadrupole mass spectrometry. The sensitive and rapid analytical method has made contributions to the QC of YZT or herb medicines. However, this work just focused on the quantification of limited constituents and neglected the contributions of other constituents to YZT's quality and efficacy. Xu et al. [13] employed ultra-performance liquid chromatography coupled with quadrupole time of flight tandem mass

spectrometry (UPLC-Q-TOF-MS) to establish the chromatographic fingerprint and identify 18 common peaks of YZT. Their work gave an overall view of all the components in YZT, but the quantity variation of each ingredient was not revealed. Moreover, their outstanding work cannot be popularized in major laboratories due to costliness and limited application of LC-MS/MS. Our study employed HPLC-DAD to develop the chemical fingerprint and quantified 10 common peaks of YZT and utilized ESI-MS/MS to identify 33 common peaks. This combination of fingerprint with quantitative and qualitative analysis of common peaks for QC of YZT may serve as a significant reference for other laboratories and herb medicines.

#### 3.1. Optimization of extraction conditions

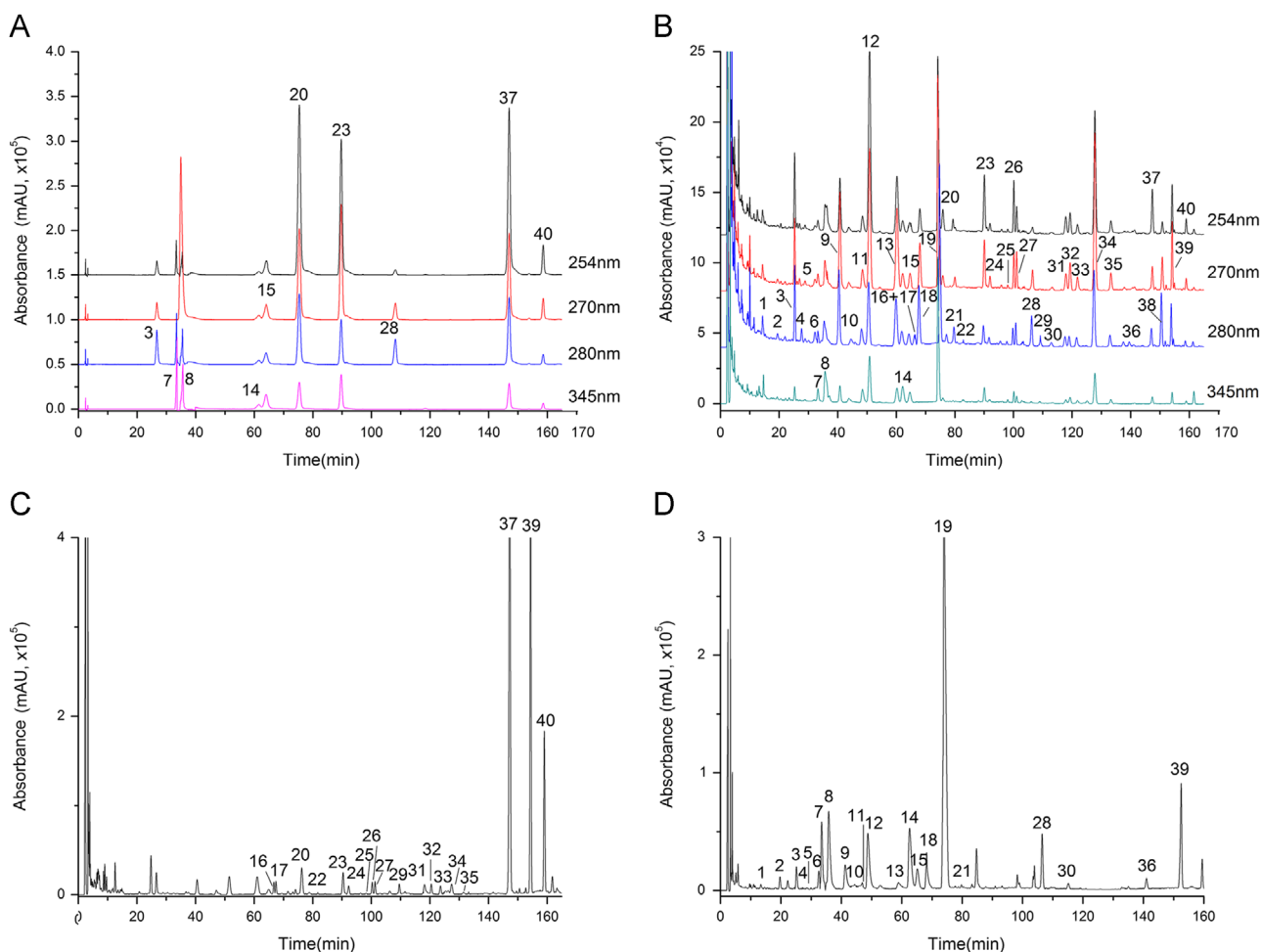
In order to achieve the optimum extraction efficiency, extraction methods, solvents and their volumes, and extraction time were investigated. The results showed that pure methanol was the most efficient extraction solvent. In addition, refluxing extraction and ultrasonic extraction were compared for their popularity in extracting the targets from the matrix. It was found that ultrasonic extraction is more convenient and effective than refluxing extraction and it is difficult to destroy the active ingredient, and is suitable for rapid extraction of a large number of samples. The extraction time and sample-to-solvent ratio were also investigated. The results indicated that an efficient extraction described in the sample preparation was produced.

#### 3.2. Optimization of chromatographic and mass spectrometric conditions

In the present study, different mobile phases, such as acetonitrile or methanol and water containing ammonium acetate, formic acid and acetic acid, were tested. It was found that acetonitrile and ammonium acetate aqueous solution offered a more stable baseline, with more peaks detected and shorter duration of analysis than using other mobile phases. To improve the peak shape, restrain the peak tailing and increase ion response, the concentration and pH value of ammonium acetate aqueous solution were investigated. The findings suggest that the optimal elution was acetonitrile and 0.4% ammonium acetate aqueous solution (pH 6.0 adjusted with glacial acetic acid). In addition, we also evaluated 4 types of columns including Sepax GP-C<sub>18</sub>, Agilent Zorbax SB-C<sub>18</sub>, Kromasil C<sub>18</sub> and Agilent Eclipse plus C<sub>18</sub> columns. The best separation was achieved on the Agilent Eclipse plus C<sub>18</sub> column.

The quantification of constituents in YZT was achieved at 254 nm for xanthotoxin, bergapten, imperatorin and isoimperatorin, 270 nm for berberine, 280 nm for protopine and tetrahydropalmatine and 345 nm for jatrorrhizine, coptisine and palmatine, where the UV spectra of the 10 analytes exhibited maximum absorbance and better response with less interference (Fig. 2A and B). In the FA, the wavelength was set at 280 nm where most chromatograph peaks were detected (Fig. 2B).

By comparing positive- and negative-ion modes, positive-ion mode was selected for MS analysis according to the number and abundance of peaks. Furthermore, optimal MS parameters including source voltage, drying gas ( $\text{N}_2$ ) flow rate and drying gas temperature were developed and the total ion current (TIC) chromatogram was acquired (Fig. 3A and B).



**Fig. 2** Representative HPLC-DAD chromatograms of mixed standard solutions (A) at 254 nm, 270 nm, 280 nm and 345 nm; YZT (B) at 254 nm, 270 nm, 280 nm and 345 nm; the negative sample without *Radix Corydalis* (C) at 280 nm; and the negative sample without *Rhizoma Angelicae dahuricae* (D) at 280 nm. (3) protopine; (7) jatrorrhizine; (8) coptisine; (14) palmatine; (15) berberine; (20) xanthotoxin; (23) bergapten; (28) tetrahydropalmatine; (37) imperatorin; (40) isoimperatorin.

### 3.3. Confirmation of common peaks and evaluation of similarity

According to the recommendation (Drug Administration Bureau of China, 2000), when peaks existed in all chromatograms of the samples and their relative standard deviation (RSD) values of RRT for all the 10 samples were less than 1%, these peaks could be assigned as the same substance and as a “common peak”. Furthermore, the total area of the common peaks must be more than 90% of the whole area in one chromatogram.

Here, 12 YZT samples from different manufacturers were obtained and analyzed to perform FA following the established HPLC-DAD analysis procedure. The average chromatogram from the 12 samples was regarded as the standard fingerprint of YZT. As shown in Fig. 4, 40 peaks of all the peaks observed (>90% of total area, denoted from 1 to 40) were defined as “common peaks”. Peak 19 indicated the highest content in all the 40 peaks and was selected as a reference peak to calculate the RRT and RPA of common peaks. Their RSD values of RRT were less than 2.1%, which demonstrated good stability and reproducibility of the FA by HPLC-DAD. The similarity indexes of 12 samples calculated by fusion vector method were higher than 0.90, which suggested that the samples from different manufacturers shared a similar

chromatographic pattern. However, the RSD values of RPA from the 12 samples were very high (approximately 23.5–130.91%), which might result from different origin, production process, storage conditions and alternative environment.

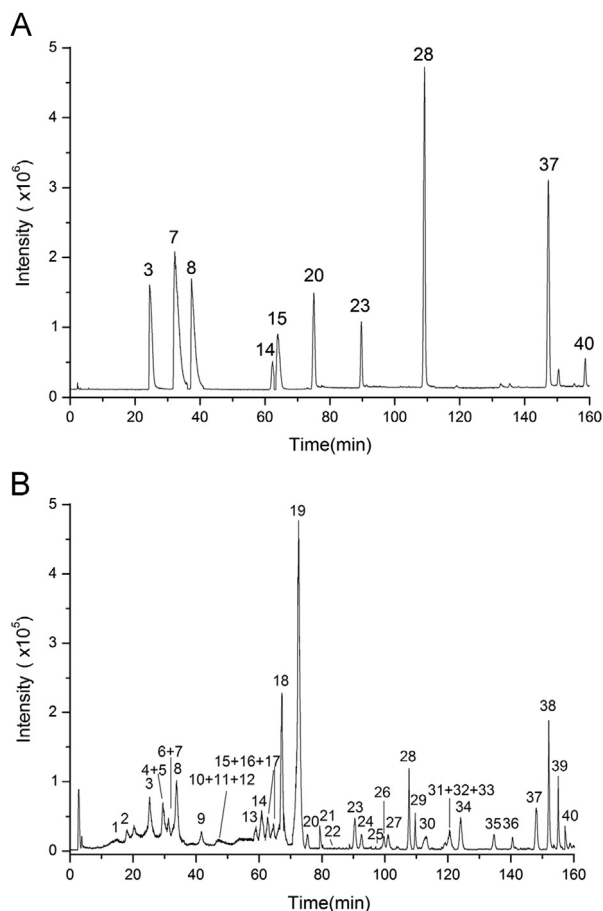
### 3.4. Quantitative analysis of common peaks

#### 3.4.1. Method validation

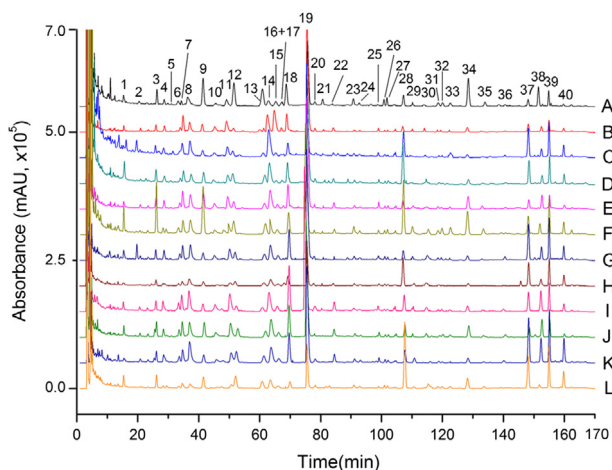
Ten peaks from “common peaks” with reasonable heights and good resolution were chosen as quantitative marker compounds. HPLC profiles of YZT and standard substances detected at 254 nm, 270 nm, 280 nm, and 345 nm are displayed in Fig. 2A and B, respectively.

In order to investigate the specificity of the method, different NC samples were prepared and analyzed, and the chromatograms are shown in Fig. 2C and D. It was noted that there were no interferences for 10 analytes.

Series of standard solutions of the 10 analytes were used to determine linear range. Calibration curves of the 10 analytes were generated by plotting peak areas versus the corresponding concentrations. The peak area values were the average of three replicate injections. Linearity of those calibration curves was



**Fig. 3** HPLC-ESI-MS total ion chromatogram (TIC) in positive ion mode of (A) the mixed standard and (B) YZT.



**Fig. 4** The chromatogram of the investigated 12 samples of YZT.

evaluated through the application of a lack-of-fit test using the software SPSS 16.0. As shown in Table 2, correlation coefficients were better than 0.999 for all analytes with Q values less than 3%. For the lack-of-fit test, the significance levels were greater than 0.05 for all analytes at the 95% confidence level, which indicated that a linear regression model provided a good interpolation of the

experimental data. The LOD and LOQ were determined at S/N ratios of 3 and 10, respectively. The range of LOD for all compounds was from 0.03 to 0.11  $\mu\text{g/mL}$ , and the range of LOQ was from 0.09 to 0.32  $\mu\text{g/mL}$  (Table 2).

The precision of the proposed method was categorized into inter- and intra-day precision that can be determined from RSD for retention time and peak area resulting from the analysis of the studied compounds. In this study, the intra- and inter-day precision was analyzed using six duplicate experiments within 1 day or on 5 separate days. The RSDs of retention time and peak area were used to evaluate precision. The RSDs of intra- and inter-day precision of the 10 compounds were less than 2.0% for peak area and were less than 0.9% for retention time (Table 3).

The analytical repeatability was examined by injecting six different samples, which were prepared according to the same sample preparation procedure. The RSD of retention time and component content of the 10 analytes were used to estimate the repeatability. The results showed that the RSD values of retention time and component content for 10 analytes were less than 2.2% (Table 3), which could meet the need of quantitative analysis.

For the stability test, retention time and peak area of the 10 analytes in a sample solution were analyzed every 8 h for over 2 days, and the sample solution was found to be stable within 48 h (RSD  $\leq$  0.7% for retention time and RSD  $\leq$  1.5% for peak area, Table 3).

The accuracy of the method was determined through recovery measurement using the standard addition method. Three different quantities (low, medium and high) of the authentic standards were added to a sample which was previously analyzed and whose concentrations of the compounds of interest were known. The mixtures were extracted and analyzed using the optimized method. The quantity of each component was subsequently obtained by using the corresponding calibration plots. Each set of additions was repeated three times. The results from determination of recovery are expressed as the percentage of the analytes recovered by the assay. The recovery of the components ranged from 98.9% to 102.3% and all of the RSD were less than 2.5% (Table 4), which indicates the method ensures high accuracy for simultaneous analysis of the 10 compounds.

#### 3.4.2. Quantification of YZT samples

This established analytical method was subsequently applied for simultaneous determination of 10 quantitative analytes in 12 commercial samples of YZT. Each sample was determined in triplicate. Peaks in the chromatograms were identified by comparing the retention times, on-line UV spectra and MS data with those of the standards.

The HPLC-DAD profiles of YZT are illustrated in Fig. 4 and the contents of the 10 analytes are shown in Table 5. It was found that the content of each analyte varied greatly among different samples. According to the provision of Ch. P. [7], the content of tetrahydropalmatine should not be less than 300  $\mu\text{g/g}$ . Although all analyzed samples meet the requirement, the content of tetrahydropalmatine, however, varied from 319.45  $\mu\text{g/g}$  to 1159  $\mu\text{g/g}$  (RSD%=71.5). A similar variation could also be found for the other components such as berberine, xanthotoxin, bergapten, imperatorin, and isoimperatorin. The variation in the content of constituents could certainly lead to the variation of therapeutic effects. Therefore, the detection of a single component or only several components could not effectively control the quality of YZT.

**Table 2** Detection wavelength, linear regression data, LOD, and LOQ for 10 active compounds in YZT analyzed by HPLC-DAD.

Compound	$\lambda$ (nm)	Linearity range ( $\mu\text{g/mL}$ )	Calibration equation $y=ax+b$ <sup>a</sup>	Correlation factor ( $R$ )	$Q$ (%) <sup>b</sup>	$P$ value <sup>c</sup>	LOD <sup>d</sup> ( $\mu\text{g/mL}$ )	LOQ <sup>d</sup> ( $\mu\text{g/mL}$ )
Protopine	280	1.02–101.60	$y=21,104x-36,099$	0.9990	1.03	0.218	0.06	0.19
Jatrorrhizine	345	6.17–267.00	$y=10,661x-59,612$	0.9996	1.20	0.114	0.11	0.32
Coptisine	345	7.70–246.25	$y=21,514x-12,671$	0.9995	1.67	0.157	0.08	0.24
Palmatine	345	1.01–84.00	$y=85,389x-74,742$	0.9997	1.23	0.133	0.03	0.11
Berberine	270	1.00–83.50	$y=51,399x-69,414$	0.9997	1.88	0.055	0.05	0.16
Xanthotoxin	254	1.01–84.67	$y=53,658x-56,538$	0.9996	2.03	0.154	0.04	0.15
Bergapten	254	1.01–84.00	$y=37,976x-43,638$	0.9995	0.87	0.093	0.07	0.20
Tetrahydropalmatin	280	20.00–300.00	$y=13,534x+18,267$	0.9991	1.73	0.223	0.09	0.29
Imperatorin	254	1.01–84.67	$y=40,762x-36,436$	0.9997	0.56	0.078	0.03	0.11
Isoimperatorin	254	1.01–84.67	$y=26,478x-28,165$	0.9996	1.95	0.098	0.05	0.16

<sup>a</sup>In the regression equation  $y=ax+b$ ,  $x$  is the concentration of the compound ( $\mu\text{g/mL}$ ),  $y$  indicates the peak area, and  $R$  is the correlation coefficient of the equation.

<sup>b</sup>Quality coefficient of the regression model.

<sup>c</sup> $P$  value of lack-of-fit test (confidence level at 95%).

<sup>d</sup>The LOD was defined as the concentration for which the signal-to-noise ratio was 3; the LOQ was defined as the concentration for which the signal-to-noise ratio was 10.

**Table 3** Precision, repeatability and stability data of 10 analytes (RSD%,  $n=6$ ).

Compound	Precision				Repeatability		Stability	
	Inter-day		Intra-day		Retention time	Content	Retention time	Peak area
	Retention time	Peak area	Retention time	Peak area				
Protopine	0.4	1.9	0.5	1.2	0.7	1.8	0.4	0.8
Jatrorrhizine	0.5	1.1	0.8	0.7	1.1	1.6	0.5	1.2
Coptisine	0.7	1.7	0.9	1.4	0.9	1.5	0.4	0.9
Palmatine	0.6	1.7	0.7	1.0	1.4	1.7	0.6	1.3
Berberine	0.5	2.0	0.5	1.4	1.6	2.1	0.3	1.4
Xanthotoxin	0.6	1.9	0.4	1.3	1.5	1.7	0.6	1.2
Bergapten	0.5	0.9	0.3	1.0	0.6	2.0	0.5	0.9
Tetrahydropalmatine	0.4	1.3	0.5	1.6	1.2	2.2	0.7	0.8
Imperatorin	0.4	1.1	0.4	0.9	0.8	1.9	0.4	1.5
Isoimperatorin	0.3	1.0	0.6	0.7	1.1	1.6	0.6	1.1

### 3.5. Qualitative analysis of common peaks

Thirty-three common peaks described in the fingerprint chromatogram including 10 quantitative compounds were identified or tentatively characterized, and their ESI-MS/MS data and fragmentations are listed in Table 6.

#### 3.5.1. Identification of fifteen alkaloids from *Rhizoma Corydalis*

For protopine and tetrahydroprotoberberine alkaloids, their characteristic fragmentation pathway was Retro-Diels–Alder (RDA) rupture [14]. Compound 3 gave protonated molecular ion  $[M+H]^+$  ( $m/z$  354) in positive-ion mode. The RDA rupture from  $[M+H]^+$  may produce fragment ions at  $m/z$  149 and  $m/z$  206. The fragment ion at  $m/z$  189 might be due to the loss of OH from the fragment ion at  $m/z$  206. By comparing the UV and MS spectra with those of the reference standard, compound 3 was unequivocally identified as protopine [10,14]. Similarly, compound 4 yielded  $[M+H]^+$  at  $m/z$  370 and a series of ions including  $[M+H-C_{10}H_{12}O_2]^+$  at  $m/z$

206,  $[M+H-C_{10}H_{12}O_2-H_2O]^+$  at  $m/z$  188,  $[M+H-H_2O]^+$  at  $m/z$  352 and  $[M+H-2CH_3O-OH]^+$  at  $m/z$  290, which was tentatively identified as  $\alpha$ -allocryptopine [10,14]. The MS/MS data of compound 5 revealed its main fragmentation, which was the successive losses of  $CH_3$ ,  $C_{10}H_{12}O_2$ ,  $C_{11}H_{15}O_2$ ,  $C_{11}H_{14}O_2N$ , yielding product ions at  $m/z$  354, 206, 192 and 165, corresponding to the structure of corydaline [15,16]. Compound 35 produced  $[M+H]^+$  ion at  $m/z$  340, fragment ions  $[M+H-C_{10}H_{12}O_2]^+$  at  $m/z$  176 and  $[M+H-CH_3-C_{10}H_{10}O_2N]^+$  at  $m/z$  149. Therefore, it can be identified as tetrahydroberberine [10].

Four compounds had the same  $[M+H]^+$  ions at  $m/z$  356 (compounds 2, 21, 28, and 30). Compound 2, 28 and 30 all produced fragment ion  $[M+H-C_{10}O_2H_{12}]^+$  at  $m/z$  192, which resulted from the RDA rupture of tetrahydroprotoberberine alkaloids [14]. Compound 28 was confirmed as tetrahydropalmatine after comparing with a reference standard and literature [14,16]. Compound 30 had the same characteristic fragmentation pathway, but its retention time was different from that of tetrahydropalmatine. Therefore, it was tentatively identified as rotundine. Compound 2 was tentatively identified as yuanhunine

**Table 4** Recovery of each analyte determined by standard addition method ( $n=3$ ).

Compound	Original amount ( $\mu\text{g}$ )	Spiked amount ( $\mu\text{g}$ )	Found amount ( $\mu\text{g}$ )	Recovery (%)	Average recovery (%)	RSD (%)
Protopine	93.53	46.77	139.46	99.4	101.3	1.6
		93.53	190.80	102.0		
		140.00	239.13	102.4		
Jatrorrhizine	6.80	3.40	9.99	97.9	100.7	2.5
		6.80	14.00	102.9		
		10.00	17.00	101.2		
Coptisine	74.00	37.50	112.76	101.1	102.3	1.0
		74.00	152.58	103.1		
		112.00	191.12	102.8		
Palmatine	105.23	52.62	156.82	99.4	98.9	0.4
		105.00	207.78	98.8		
		157.62	259.05	98.6		
Berberine	55.50	27.75	82.98	99.9	100.8	1.0
		55.50	112.27	101.1		
		83.00	140.61	101.5		
Xanthotoxin	4.62	2.32	6.89	99.3	99.9	0.9
		4.70	9.28	99.6		
		7.00	11.73	101.0		
Bergapten	48.84	24.42	72.72	99.3	99.9	0.8
		50.00	99.62	100.8		
		74.42	122.90	99.7		
Tetrahydropalmatine	186.75	93.38	281.74	100.6	101.2	0.5
		187.00	378.71	101.3		
		280.00	474.20	101.6		
Imperatorin	79.83	39.92	119.24	99.6	100.4	0.9
		80.00	161.95	101.3		
		120.00	200.48	100.3		
Isoimperatorin	93.06	46.53	140.82	100.9	101.0	0.1
		93.00	187.99	101.0		
		140.00	235.50	101.1		

referring to the literature [17]. Compound 21 gave an ion  $[\text{M}+\text{H}-2\text{OCH}_3]^+$  at  $m/z$  294 as significant ion with a further fragment  $[\text{M}+\text{H}-2\text{OCH}_3-\text{CH}_3]^+$ . However,  $[\text{M}+\text{H}-\text{OCH}_3-\text{CH}_3]^+$  was observed without any further fragmentation. Thus it was identified as glaucine [16,18].

Quaternary alkaloids gave  $[\text{M}]^+$  ions. For example, compounds 14 and 15 were easily identified as palmatine and berberine by comparing the retention time,  $[\text{M}]^+$  ions and the characteristic fragment ions with those data of the corresponding authentic standards [19,20]. Compound 13 produced  $[\text{M}+\text{H}]^+$  at  $m/z$  336 and had similar characteristic fragment ions at  $m/z$  320 and 292 with berberine, it was tentatively determined as epiberberine [19,20]. Compound 19 produced an  $[\text{M}+\text{H}]^+$  ion at  $m/z$  366,  $[\text{M}-\text{CH}_3]^+$  ion at  $m/z$  350 and  $[\text{M}+2\text{H}-3\text{CH}_3]^+$  ion at  $m/z$  322, and further yielded the fragment ion  $[\text{M}+\text{H}-2\text{CH}_3]^+$  at  $m/z$  336, corresponding to the structure of dehydrocorydaline [9,16].

Compound 6 displayed  $[\text{M}+\text{H}]^+$  ion at  $m/z$  339 and the fragment ions of losing  $\text{CH}_3$ ,  $2\text{CH}_3$  and  $\text{CH}_3\text{CO}$  were observable. Compared with the literature [21], it was identified as columbamine. Compound 7 gave the molecular ion  $[\text{M}+\text{H}]^+$  at  $m/z$  339.  $[\text{M}-\text{CH}_3]^+$  at  $m/z$  323,  $[\text{M}+\text{H}-\text{CH}_3\text{O}]^+$  at  $m/z$  308 and  $[\text{M}+\text{H}-3\text{CH}_3]^+$  at  $m/z$  294 were also observed in the product-ion spectra. The molecular mass of compound 8 was 320. The precursor ion  $[\text{M}]^+$  at  $m/z$  320 gave prominent product ions at  $m/z$  292, 262 and 234. Therefore, compounds 7 and 8 were unequivocally identified as jatrorrhizine and coptisine by comparing the UV and MS spectra with those of the reference standards [19].

### 3.5.2. Identification of two simple coumarin from *Radix Angelicae dahuricae*

Compound 1 provided the fragment ions at  $m/z$  178 by losing methyl group from the  $[\text{M}+\text{H}]^+$ , together with the ions  $[\text{M}-\text{CH}_3-\text{CO}]^+$  at  $m/z$  149 and  $[\text{M}-\text{CH}_3-\text{CO}-\text{OH}]^+$  at  $m/z$  132, which could be tentatively identified as scopoletin [10,22]. Compound 32 was tentatively speculated as 7-demethylsuberosin, which can give  $[\text{M}+\text{H}]^+$  ion at  $m/z$  231. The base peak was the fragment ion  $[\text{M}-\text{C}_4\text{H}_8]^+$  at  $m/z$  175, which may result from the benzyl rupture of  $[\text{M}+\text{H}]^+$  ion. The fragment ions at  $m/z$  203, 187, 159 may be the loss of  $[\text{CO}]$ ,  $[\text{CO}_2]$ ,  $[\text{CO}_2+\text{CO}]$  from  $[\text{M}+\text{H}]^+$ , while  $m/z$  147 may be the loss of  $[\text{CO}]$  from  $[\text{M}-\text{C}_4\text{H}_8]^+$  [23].

### 3.5.3. Identification of 16 furocoumarins from *Radix Angelicae dahuricae*

Sixteen furocoumarins exhibited their quasi-molecular ions  $[\text{M}+\text{H}]^+$  and contained product ions formed by the loss of  $\text{CO}$  and  $\text{CO}_2$  from the furocoumarin skeleton or  $\text{C}_5\text{H}_9\text{O}$ ,  $\text{C}_5\text{H}_8$ ,  $\text{CH}_3$  from the substituent groups at C-5 or C-8. Most substituent groups at C-5 or C-8 of the linear-type furocoumarins were oxysubstituent groups, and different substituent sites possess different fragmentation patterns [24].

If isopentenoxo group was at C-5 and no substituent was at C-8, such as isoimperatorin (compound 40), the ESI-MS spectrum exhibited an  $[\text{M}+\text{H}]^+$  ion of  $m/z$  271 as the base peak. The  $[\text{M}+\text{H}]^+$  ion was further fragmented by neutral loss of a rearranged isopentenyl moiety, leading to the formation of a



**Table 5** Amounts of the 10 compounds in YZT from different manufacturers.

Sample	Amount ( $\mu\text{g/g}$ , mean $\pm$ SD, $n=3$ )									
	Protopine	Jatrorrhizine	Coptisine	Palmitate	Berberine	Xanthotoxin	Bergapten	Tetrahydropalmatine	Imperatorin	Isoimperatorin
A	104.25 $\pm$ 0.30	42.75 $\pm$ 0.06	327.95 $\pm$ 0.04	48.15 $\pm$ 0.005	60.25 $\pm$ 0.24	35.40 $\pm$ 0.03	89.75 $\pm$ 0.05	319.45 $\pm$ 0.15	75.35 $\pm$ 0.06	32.30 $\pm$ 0.003
B	112.85 $\pm$ 0.22	95.05 $\pm$ 0.07	293.10 $\pm$ 0.06	139.70 $\pm$ 0.008	331.00 $\pm$ 0.15	—	31.80 $\pm$ 0.007	346.00 $\pm$ 0.26	80.10 $\pm$ 0.05	47.80 $\pm$ 0.004
C	141.40 $\pm$ 0.06	77.20 $\pm$ 0.004	369.40 $\pm$ 0.005	212.35 $\pm$ 0.002	47.30 $\pm$ 0.07	—	26.05 $\pm$ 0.003	627.50 $\pm$ 0.09	240.20 $\pm$ 0.17	126.25 $\pm$ 0.07
D	121.75 $\pm$ 0.54	68.50 $\pm$ 0.07	219.35 $\pm$ 0.007	148.35 $\pm$ 0.07	34.90 $\pm$ 0.05	6.55 $\pm$ 0.006	32.05 $\pm$ 0.04	977.25 $\pm$ 0.13	197.65 $\pm$ 0.04	1013.50 $\pm$ 0.09
E	120.00 $\pm$ 0.38	89.80 $\pm$ 0.003	376.00 $\pm$ 0.04	149.05 $\pm$ 0.03	57.60 $\pm$ 0.03	8.60 $\pm$ 0.009	29.55 $\pm$ 0.06	418.05 $\pm$ 0.09	82.80 $\pm$ 0.003	46.80 $\pm$ 0.006
F	83.75 $\pm$ 0.08	76.50 $\pm$ 0.005	361.95 $\pm$ 0.005	141.65 $\pm$ 0.06	53.75 $\pm$ 0.009	10.40 $\pm$ 0.14	81.40 $\pm$ 0.12	1297.25 $\pm$ 0.06	191.05 $\pm$ 0.005	137.45 $\pm$ 0.007
G	148.60 $\pm$ 0.07	73.45 $\pm$ 0.01	361.50 $\pm$ 0.006	103.70 $\pm$ 0.21	52.95 $\pm$ 0.004	6.75 $\pm$ 0.05	33.60 $\pm$ 0.04	431.06 $\pm$ 0.002	294.25 $\pm$ 0.02	141.95 $\pm$ 0.004
H	35.90 $\pm$ 0.03	44.30 $\pm$ 0.002	207.55 $\pm$ 0.007	46.40 $\pm$ 0.04	19.95 $\pm$ 0.06	12.25 $\pm$ 0.07	23.35 $\pm$ 0.07	702.10 $\pm$ 0.04	195.50 $\pm$ 0.006	121.25 $\pm$ 0.09
I	170.25 $\pm$ 0.06	80.30 $\pm$ 0.004	512.15 $\pm$ 0.008	140.05 $\pm$ 0.009	59.40 $\pm$ 0.08	6.90 $\pm$ 0.04	26.70 $\pm$ 0.06	382.85 $\pm$ 0.03	160.35 $\pm$ 0.007	83.50 $\pm$ 0.08
J	141.40 $\pm$ 0.07	86.05 $\pm$ 0.14	477.70 $\pm$ 0.02	153.70 $\pm$ 0.04	68.90 $\pm$ 0.02	11.35 $\pm$ 0.06	38.60 $\pm$ 0.10	360.90 $\pm$ 0.03	96.90 $\pm$ 0.003	46.40 $\pm$ 0.001
K	145.10 $\pm$ 0.09	76.70 $\pm$ 0.01	573.65 $\pm$ 0.09	117.40 $\pm$ 0.10	53.20 $\pm$ 0.07	8.40 $\pm$ 0.08	42.90 $\pm$ 0.25	322.35 $\pm$ 0.04	372.05 $\pm$ 0.002	271.75 $\pm$ 0.02
L	33.30 $\pm$ 0.06	45.40 $\pm$ 0.05	140.00 $\pm$ 0.01	58.95 $\pm$ 0.08	27.15 $\pm$ 0.003	21.40 $\pm$ 0.43	54.75 $\pm$ 0.09	1159.00 $\pm$ 0.09	401.65 $\pm$ 0.004	295.90 $\pm$ 0.06
RSD%	38.2	25.1	36.3	40.8	114.6	90.0	51.5	71.5	56.1	137.5

—: Lower than limit of quantification.

RSD: variation of mean amount of each compound.

predominant ion  $[\text{M}+\text{H}-\text{C}_5\text{H}_8]^+$  at  $m/z$  203, produced ions  $[\text{M}+\text{H}-\text{C}_5\text{H}_8-\text{CO}_2]^+$  at  $m/z$  159,  $[\text{M}+\text{H}-\text{C}_5\text{H}_8-2\text{CO}]^+$  at  $m/z$  147 and  $[\text{M}+\text{H}-\text{CH}_4]^+$  at  $m/z$  131 [25]. Its identification was further confirmed by comparison with an authentic compound. Compounds 26 and 29 had the same quasi-molecular and similar fragment ions in MS/MS spectra, in which the consecutive neutral losses of  $\text{C}_5\text{H}_9\text{O}$  and  $\text{C}_5\text{H}_9\text{O}-2\text{CO}$  from  $[\text{M}+\text{H}]^+$  of  $m/z$  288 were observed. Since they showed different retention behavior on column, compound 26 was identified as pabulenol while compound 29 was attributed to oxypeucedanin [24]. Meanwhile, compound 12 had the same produced ions at  $m/z$  203 and 147 with compound 29, the  $[\text{M}+\text{H}]^+$  of which was 305. Therefore, compound 12 was preliminarily identified as oxypeucedanin hydrate [22,25].

Compounds 20 and 23 displayed  $[\text{M}+\text{H}]^+$  ion at  $m/z$  217 and same fragment ion  $[\text{M}+\text{H}-\text{CH}_3]^+$  at  $m/z$  201,  $[\text{M}+\text{H}-\text{CH}_3-\text{CO}]^+$  at  $m/z$  174 and  $[\text{M}+\text{H}-\text{CO}]^+$  at  $m/z$  146. Compared with the standard, compounds 20 and 23 were identified as xanthotoxin and bergapten, respectively [26].

If alkoxy moieties at C-5 and C-8, the compound, like 5-methoxy-8-hydroxypsoralen (compound 9), could give the protonated ion  $[\text{M}+\text{H}]^+$  at  $m/z$  233, which was further fragmented by the losses of methyl group and the successive carbonyl group, leading to the product ions  $[\text{M}+\text{H}-\text{CH}_3]^+$  at  $m/z$  218,  $[\text{M}+\text{H}-\text{CH}_3-\text{CO}]^+$  at  $m/z$  190 and  $[\text{M}+\text{H}-\text{CH}_3-2\text{CO}]^+$  at  $m/z$  162 [24,27]. Similarly, with the parent ion at  $m/z$  247 and fragment ions at  $m/z$  232  $[\text{M}+\text{H}-\text{CH}_3]^+$  and 217  $[\text{M}+\text{H}-2\text{CH}_3]^+$ , compound 24 was identified as isopimpinellin [24,27]. Compound 16 yielded  $[\text{M}+\text{H}]^+$  at 317,  $[\text{M}+\text{H}-\text{C}_5\text{H}_{10}\text{O}]^+$  at  $m/z$  231,  $[\text{M}+\text{H}-\text{C}_5\text{H}_{10}\text{O}-\text{CO}]^+$  at  $m/z$  203 and  $[\text{M}+\text{H}-\text{CO}]^+$  at  $m/z$  175. It was identified as byakangelicol [27,28]. Compound 31 yielded the same  $[\text{M}+\text{H}]^+$  at 317 with compound 16, but had different fragment ions  $[\text{M}+\text{H}-\text{C}_5\text{HO}_8]^+$  at  $m/z$  233 and  $[\text{M}+\text{H}-\text{C}_5\text{HO}_8-\text{CH}_3]^+$  at  $m/z$  218. So it was identified as apaensin [27]. Compound 17 gave the fragment ions  $[\text{M}+\text{H}]^+$  at  $m/z$  335,  $[\text{M}+\text{H}-\text{C}_5\text{H}_{10}\text{O}_2]^+$  at  $m/z$  231 and  $[\text{M}+\text{H}-\text{C}_5\text{H}_{12}\text{O}_2-\text{CH}_3]^+$  at  $m/z$  218, which strongly suggests the presence of byakangelicin [22]. Cnidilin (compound 39) was detected with  $[\text{M}+\text{H}]^+$  at  $m/z$  301, fragment ions at  $m/z$  233 and  $[\text{M}+\text{H}-\text{CH}_3]^+$  at  $m/z$  218 [29].

If isopentenoxo group was at C-8 and no substituent was at C-5, such as imperatorin (compound 37), the protonated molecular ion of  $m/z$  271 was observed in ESI-MS spectrum, and loss of a rearranged isopentenyl fragment ion  $[\text{M}+\text{H}-\text{C}_5\text{H}_8]^+$  at  $m/z$  203 of protonated molecular was also observed. Ion  $[\text{M}+\text{H}-\text{C}_5\text{H}_8-\text{CO}]^+$  at  $m/z$  175 and  $[\text{M}+\text{H}-\text{C}_5\text{H}_8-2\text{CO}]^+$  at  $m/z$  147 were produced in MS/MS analysis. Compounds 36 and 38 both produced  $[\text{M}+\text{H}]^+$  at  $m/z$  203, fragment ions  $[\text{M}+\text{H}-2\text{CO}]$  at  $m/z$  147 and  $[\text{M}+\text{H}-\text{CO}_2-\text{CO}]^+$  at  $m/z$  131.  $[\text{M}+\text{H}-\text{CO}_2]^+$  at  $m/z$  159 was found in product ions of compound 36 while  $[\text{M}+\text{H}-\text{CO}]^+$  at  $m/z$  175 in compound 38, so they were respectively identified as bergaptol and xanthotoxol [26]. Compound 34 exhibited a minor  $[\text{M}+\text{H}]^+$  ion at  $m/z$  271 and predominant fragment ion  $[\text{M}+\text{H}-\text{C}_3\text{H}_6-\text{H}_2\text{O}]^+$  at  $m/z$  223,  $[\text{M}+\text{H}-\text{C}_4\text{H}_8]^+$  at  $m/z$  215 and  $[\text{M}+\text{H}-\text{C}_4\text{H}_8-\text{CO}]^+$  at  $m/z$  187. So it could be tentatively identified as alloimperatorin [30].

#### 4. Conclusion

In the present work, a reliable and efficient HPLC-DAD-ESI-MS/MS method was established for the QC of YZT by quantitative and qualitative analysis of “common peaks” in chemical fingerprint. For 40 common peaks in YZT, 10 analytes were simultaneously

**Table 6** Characterization of 33 identified compounds in YZT by HPLC-DAD-ESI-MS/MS.

Peak No.	RT (min)	Identification	Empirical formula	UV $\lambda_{max}$ (nm)	(+)ESI-MS ( $m/z$ )	MS/MS ( $m/z$ )
1	14.37	Scopoletin	C <sub>10</sub> H <sub>8</sub> O	280	193.0(M+H)	177.6(M+H-CH <sub>3</sub> ); 149.1(M-CH <sub>3</sub> -CO); 132.5(M-CH <sub>3</sub> -CO-OH)
2	19.89	Yuanhunine	C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>	280	356.00 (M+H)	340 (M-CH <sub>3</sub> ); 192 (M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> )
3	25.02	Protopine	C <sub>20</sub> H <sub>19</sub> NO <sub>5</sub>	280	354.0(M+H)	275.0(M+H-CH <sub>3</sub> -NH <sub>2</sub> -CH <sub>2</sub> (OH) <sub>2</sub> ); 189.0(M+2H-149-OH); 148.9(M+2H-C <sub>11</sub> H <sub>13</sub> O <sub>3</sub> N)
4	27.55	$\alpha$ -Allocryptopine	C <sub>21</sub> H <sub>23</sub> NO <sub>5</sub>	280	370.2(M+H)	289.9(M+H-2CH <sub>3</sub> O-OH); 206.0(M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ); 187.9(M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> -H <sub>2</sub> O)
5	30.83	Corydaline	C <sub>22</sub> H <sub>27</sub> NO <sub>4</sub>	270	370.3(M+H)	354.0(M-CH <sub>3</sub> ); 206.2(M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ); 192.1(M+H-C <sub>11</sub> H <sub>15</sub> O <sub>2</sub> ); 164.8(M-C <sub>11</sub> H <sub>14</sub> O <sub>2</sub> N)
6	32.39	Columbamine	C <sub>20</sub> H <sub>20</sub> NO <sub>4</sub> <sup>+</sup>	280	338.9(M+H)	323.3(M-CH <sub>3</sub> ); 308.8(M-2CH <sub>3</sub> ); 294.9(M-CH <sub>3</sub> -CO)
7	33.61	Jatrorrhizine	C <sub>20</sub> H <sub>20</sub> NO <sub>4</sub> <sup>+</sup>	345	338.3(M+H)	322.9(M-CH <sub>3</sub> ); 308.0(M+H-CH <sub>3</sub> O); 294.0(M+H-3CH <sub>3</sub> ); 190.0(M+H-C <sub>9</sub> H <sub>9</sub> O <sub>2</sub> )
8	36.13	Coptisine	C <sub>19</sub> H <sub>14</sub> NO <sub>4</sub> <sup>+</sup>	345	320.1(M)	292.0(M-CO); 262.0(M+H-CO-CH <sub>3</sub> O); 234.0(M-2CO-CH <sub>3</sub> O)
9	41.44	5-Methoxy-8-hydroxyorsolan	C <sub>12</sub> H <sub>8</sub> O <sub>5</sub>	270	233.2(M+H)	218.0(M+H-CH <sub>3</sub> ); 190.0(M-CH <sub>3</sub> -CO); 162.01(M-CH <sub>3</sub> -2CO)
12	51.56	Oxypeucedanin hydrate	C <sub>16</sub> H <sub>16</sub> O <sub>6</sub>	254	305.0(M+H)	203.02(M+H-C <sub>5</sub> H <sub>9</sub> O-OH); 147.0(M+H-C <sub>5</sub> H <sub>9</sub> O-2CO)
13	60.63	Epiberberine	C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub> <sup>+</sup>	270	336.2(M)	319.8(M+H-OH); 292.0(M+H-CO-OH)
14	62.82	Palmatine	C <sub>21</sub> H <sub>22</sub> NO <sub>4</sub> <sup>+</sup>	345	352.4(M)	336.4(M-H-CH <sub>3</sub> ); 322.4(M-2CH <sub>3</sub> ); 308.4(M-H-CH <sub>3</sub> -CO)
15	65.32	Berberine	C <sub>20</sub> H <sub>18</sub> NO <sub>4</sub> <sup>+</sup>	270	335.9(M)	320.0(M-CH <sub>4</sub> ); 292.0(M-CH <sub>4</sub> -CO); 278.0(M-2CH <sub>3</sub> -CO)
16	67.70	Byakangelicol	C <sub>17</sub> H <sub>16</sub> O <sub>6</sub>	280	317.4(M+H)	231.0(M+H-C <sub>5</sub> H <sub>10</sub> O); 202.9(M+H-C <sub>5</sub> H <sub>10</sub> O-CO); 188.1(202.9-CH <sub>3</sub> ); 175.9(202.9-CO)
17	67.70	Byakangelicin	C <sub>17</sub> H <sub>18</sub> O <sub>7</sub>	280	335.3(M+H)	231.5(M+H-C <sub>5</sub> H <sub>10</sub> O); 217.9(M+H-C <sub>5</sub> H <sub>10</sub> O-CH <sub>3</sub> ); 202.9(231.3-CO); 175.2(231.3-2CO)
19	75.70	Dehydrocorydaline	C <sub>22</sub> H <sub>24</sub> NO <sub>4</sub> <sup>+</sup>	270	366.1(M+H)	350.1(M-CH <sub>3</sub> ); 335.9(M+H-2CH <sub>3</sub> ); 321.9(M+2H-3CH <sub>3</sub> )
20	77.84	Xanthotoxin	C <sub>12</sub> H <sub>8</sub> O <sub>4</sub>	254	216.9(M+H)	201.9(M+H-CH <sub>3</sub> ); 173.9(M+H-CO-CH <sub>3</sub> ); 160.9(M+H-2CO); 145.8(M+H-2CO-CH <sub>3</sub> )
21	81.55	Glaucine	C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>	280	356.0(M+H)	325.02(M+H-CH <sub>3</sub> O); 294.0(M+H-2CH <sub>3</sub> O); 279.0(M+H-2CH <sub>3</sub> O-CH <sub>3</sub> )
23	90.05	Bergapten	C <sub>12</sub> H <sub>8</sub> O <sub>4</sub>	254	216.9(M+H)	201.9(M+H-CH <sub>3</sub> ); 173.9(M+H-CO-CH <sub>3</sub> ); 146.0(M+H-2CO-CH <sub>3</sub> )
24	91.97	Isopimpinellin	C <sub>13</sub> H <sub>10</sub> O <sub>4</sub>	270	247.0(M+H)	232.0(M+H-CH <sub>3</sub> ); 217.1(M+H-2CH <sub>3</sub> ); 188.8(M+H-2CH <sub>3</sub> -CO)
26	101.80	Pabulenol	C <sub>16</sub> H <sub>14</sub> O <sub>5</sub>	254	287.6(M+H)	203.0(M+H-C <sub>5</sub> H <sub>9</sub> O); 174.7(M+H-C <sub>5</sub> H <sub>9</sub> O-CO); 146.7(M+H-C <sub>5</sub> H <sub>9</sub> O-2CO)
28	108.05	Tetrahydropalmatine	C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>	280	356.0(M+H)	192.0(M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ); 165.0(M+H-C <sub>11</sub> H <sub>13</sub> O <sub>2</sub> N)
29	112.01	Oxypeucedanin	C <sub>16</sub> H <sub>14</sub> O <sub>5</sub>	280	287.5(M+H)	203.1(M+H-C <sub>5</sub> H <sub>9</sub> O); 147.0(M+H-C <sub>5</sub> H <sub>9</sub> O-2CO)
30	115.29	Rotundine	C <sub>21</sub> H <sub>25</sub> NO <sub>4</sub>	280	356.0(M+H)	191.7(M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ); 164.9(M+2H-C <sub>11</sub> H <sub>13</sub> O <sub>2</sub> N)
31	118.02	Apaensin	C <sub>17</sub> H <sub>16</sub> O <sub>6</sub>	270	317.0(M+H)	233.1(M+2H-C <sub>5</sub> H <sub>9</sub> O); 218.1(M+2H-C <sub>5</sub> H <sub>9</sub> O-CH <sub>3</sub> ); 203.0(M+2H-C <sub>5</sub> H <sub>9</sub> O-2CH <sub>3</sub> ); 175.0(M+2H-C <sub>5</sub> H <sub>9</sub> O-2CH <sub>3</sub> -CO)
32	120.05	7-Demethylsuberosin	C <sub>14</sub> H <sub>14</sub> O <sub>3</sub>	270	231.00 (M+H)	203.00(M+H-CO); 187.90(M+H-CO <sub>2</sub> ); 175.00(M-C <sub>4</sub> H <sub>8</sub> ); 159.90(M+H-CO <sub>2</sub> -CO); 146.90(M-C <sub>4</sub> H <sub>8</sub> -CO)
34	128.46	Alloimperatorin	C <sub>16</sub> H <sub>14</sub> O <sub>4</sub>	270	271.0(M+H)	229.2(M+H-C <sub>3</sub> H <sub>6</sub> ); 214.8(M+H-C <sub>4</sub> H <sub>8</sub> ); 186.8(M+H-C <sub>4</sub> H <sub>8</sub> -CO)
35	131.92	Tetrahydroberberine	C <sub>20</sub> H <sub>21</sub> NO <sub>4</sub>	270	340.20 (M+H)	176 (M+H-C <sub>10</sub> H <sub>12</sub> O <sub>2</sub> ); 149(M+H-CH <sub>3</sub> -C <sub>10</sub> H <sub>10</sub> O <sub>2</sub> N)
36	142.13	Bergaptol	C <sub>11</sub> H <sub>6</sub> O <sub>4</sub>	280	203.00 (M+H)	159(M+H-CO <sub>2</sub> ); 147(M+H-2CO); 131(M+H-CO <sub>2</sub> -CO); 119(M+H-2CO-CO)
37	147.29	Imperatorin	C <sub>16</sub> H <sub>14</sub> O	254	271.0(M+H)	202.9(M+H-C <sub>5</sub> H <sub>8</sub> ); 174.9(M+H-C <sub>5</sub> H <sub>8</sub> -CO); 146.9(M+H-C <sub>5</sub> H <sub>8</sub> -2CO); 130.9(146.9-CH <sub>4</sub> )
38	152.03	Xanthotoxol	C <sub>11</sub> H <sub>6</sub> O <sub>4</sub>	280	203.0(M+H)	175.0(M+H-CO); 146.9(M+H-2CO); 130.8(M+H-CO-CO <sub>2</sub> )
39	154.16	Cnidilin	C <sub>17</sub> H <sub>16</sub> O <sub>5</sub>	270	301.0(M+H)	233.1(M+H-C <sub>5</sub> H <sub>8</sub> ); 218.0(M+H-C <sub>5</sub> H <sub>8</sub> -CH <sub>3</sub> )
40	158.37	Isoimperatorin	C <sub>16</sub> H <sub>14</sub> O <sub>4</sub>	254	271.0(M+H)	202.9(M+H-C <sub>5</sub> H <sub>8</sub> ); 158.8(M+H-C <sub>5</sub> H <sub>8</sub> -CO <sub>2</sub> ); 146.9(M+H-C <sub>5</sub> H <sub>8</sub> -2CO); 130.9(146.9-CH <sub>4</sub> )

quantified and good linearity, precision, repeatability, stability and recovery were obtained. Thirty-three components including the 10 quantitative compounds were successfully identified on the basis of retention time and MS/MS spectra after being compared with those of standards or literature. The present study provided comprehensive information not only for pharmacological researches and clinical applications, but also for quality evaluation of YZT.

### Acknowledgments

The authors acknowledge the support of this work funded by the Priority Academic Program Development of Jiangsu Higher Education Institution and Jiangsu Overseas Research & Training Program for University Prominent Young & Middle-aged Teachers and Presidents.

### References

- [1] S.P. Li, J. Zhao, B. Yang, Strategies for quality control of Chinese medicines, *J. Pharm. Biomed. Anal.* 55 (4) (2011) 802–809.
- [2] C.J. Xu, Y.Z. Liang, F.T. Chau, et al., Pretreatments of chromatographic fingerprints for quality control of herbal medicines, *J. Chromatogr. A* 1134 (1–2) (2006) 253–259.
- [3] L. Xu, X. Han, Y. Qi, et al., Multiple compounds determination and fingerprint analysis of Lidanpaishi tablet and keli by high-performance liquid chromatography, *Anal. Chim. Acta* 633 (1) (2009) 136–148.
- [4] D.Z. Yang, Y.Q. An, X.L. Jiang, et al., Development of a novel method combining HPLC fingerprint and multi-ingredients quantitative analysis for quality evaluation of traditional Chinese medicine preparation, *Talanta* 85 (2) (2011) 885–890.
- [5] D. Tang, D. Yang, A. Tang, et al., Simultaneous chemical fingerprint and quantitative analysis of *Ginkgo biloba* extract by HPLC-DAD, *Anal. Bioanal. Chem.* 396 (8) (2010) 3087–3095.
- [6] H. Wei, L. Sun, Z. Tai, et al., A simple and sensitive HPLC method for the simultaneous determination of eight bioactive components and fingerprint analysis of *Schisandra sphenanthera*, *Anal. Chim. Acta* 662 (1) (2010) 97–104.
- [7] National Commission of Chinese Pharmacopoeia: Pharmacopoeia of Peoples Republic of China. Chemical Industry Press, Beijing, 2010, pp. 525–526.
- [8] K.T. Wang, H.T. Liu, X.G. Chen, et al., Identification and determination of active components in *Angelica dahurica Benth* and its medicinal preparation by capillary electrophoresis, *Talanta* 54 (4) (2001) 753–761.
- [9] J. Zhang, Y. Jin, J. Dong, et al., Systematic screening and characterization of tertiary and quaternary alkaloids from *corydalis yanhusuo W.T. Wang* using ultra-performance liquid chromatography-quadrupole-time-of-flight mass spectrometry, *Talanta* 78 (2) (2009) 513–522.
- [10] G.F. Wang, Z.G. Liao, X.L. Liang, et al., Determination of three active components in Yuanhu Zhitong tablet by HPLC, *China Pharm.* 20 (9) (2009) 672–674.
- [11] Y. Zhang, H. Xu, X. Chen, et al., Simultaneous quantification of 17 constituents from Yuanhu Zhitong tablet using rapid resolution liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry, *J. Pharm. Biomed. Anal.* 56 (3) (2011) 497–504.
- [12] ICH, Guidance for Industry, Q2B Validation of Analytical Procedures: Methodology, 1996.
- [13] H. Xu, Y. Zhang, Y. Tao, et al., Study of chemical fingerprint for yuanhu zhitong tablet by UPLC/Q-TOF-MS, *J. Liq. Chromatogr. Relat. Technol.* 36 (6) (2013) 807–820.
- [14] Q. Liu, B. Zhou, X. Wang, et al., Establishment of a search library about benzylisoquinoline alkaloids based on selective separation on the binaphthyl column and standard analysis on C18 column, *J. Sep. Sci.* 35 (23) (2012) 3317–3325.
- [15] C. Wang, S. Wang, G. Fan, et al., Screening of antinociceptive components in *Corydalis yanhusuo W.T. Wang* by comprehensive two-dimensional liquid chromatography/tandem mass spectrometry, *Anal. Bioanal. Chem.* 396 (5) (2010) 1731–1740.
- [16] B. Ding, T. Zhou, G. Fan, et al., Qualitative and quantitative determination of ten alkaloids in traditional Chinese medicine *Corydalis yanhusuo W.T. Wang* by LC-MS/MS and LC-DAD, *J. Pharm. Biomed. Anal.* 45 (2) (2007) 219–226.
- [17] S.Y. Saito, M. Tanaka, K. Matsunaga, et al., The combination of rat mast cell and rabbit aortic smooth muscle is the simple bioassay for the screening of anti-allergic ingredient from methanolic extract of *Corydalis tuber*, *Biol. Pharm. Bull.* 27 (8) (2004) 1270–1274.
- [18] G.M. Meyer, M.R. Meyer, D.K. Wissenbach, et al., Studies on the metabolism and toxicological detection of glaucine, an isoquinoline alkaloid from *Glaucium flavum (Papaveraceae)*, in rat urine using GC-MS, LC-MS(n) and LC-high-resolution MS(n), *J. Mass Spectrom.* 48 (1) (2013) 24–41.
- [19] D. Wang, Z. Liu, M. Guo, et al., Structural elucidation and identification of alkaloids in *Rhizoma Coptidis* by electrospray ionization tandem mass spectrometry, *J. Mass Spectrom.* 39 (11) (2004) 1356–1365.
- [20] Y. Zhang, Q. Shi, P. Shi, et al., Characterization of isoquinoline alkaloids, diterpenoids and steroids in the Chinese herb Jin-Guo-Lan (*Tinospora sagittata* and *Tinospora capillipes*) by high-performance liquid chromatography/electrospray ionization with multistage mass spectrometry, *Rapid Commun. Mass Spectrom.* 20 (15) (2006) 2328–2342.
- [21] Z.H. Cheng, Y.L. Guo, H.Y. Wang, et al., Qualitative and quantitative analysis of quaternary ammonium alkaloids from *Rhizoma Corydalis* by matrix-assisted laser desorption/ionization Fourier transform mass spectrometry coupled with a selective precipitation reaction using reinecke salt, *Anal. Chim. Acta* 555 (2) (2006) 269–277.
- [22] Y. Xie, W. Zhao, T. Zhou, et al., An efficient strategy based on MAE, HPLC-DAD-ESI-MS/MS and 2D-prep-HPLC-DAD for the rapid extraction, separation, identification and purification of five active coumarin components from *Radix Angelicae Dahuricae*, *Phytochem. Anal.* 21 (5) (2010) 473–482.
- [23] H. Fujiwara, T. Yokoi, S. Tani, et al., Studies on constituents of *Angelicae dahuricae Radix*. I. On a new furocoumarin derivative, *Yakugaku Zasshi* 100 (12) (1980) 1258–1261.
- [24] J. Kang, L. Zhou, J. Sun, et al., Chromatographic fingerprint analysis and characterization of furocoumarins in the roots of *Angelica dahurica* by HPLC/DAD/ESI-MS<sup>n</sup> technique, *J. Pharm. Biomed. Anal.* 47 (4–5) (2008) 778–785.
- [25] S. Vogl, M. Zehl, P. Picker, et al., Identification and quantification of coumarins in *Peucedanum ostruthium (L.) Koch* by HPLC-DAD and HPLC-DAD-MS, *J. Agric. Food Chem.* 59 (9) (2011) 4371–4377.
- [26] G.Q. Liu, J. Dong, H. Wang, et al., Differentiation of four pairs of furocoumarin isomers by electrospray ionization tandem mass spectrometry, *Eur. J. Mass Spectrom.* (Chichester, Eng) 16 (2) (2010) 215–220.
- [27] 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 (14) (2009) 2167–2175.
- [28] J. Lu, L. Jin, Y.S. Jin, et al., Chemical constituents in roots of *Angelica dahurica var. formosana*, *Acad. J. Second Military Med. Univ.* 28 (3) (2007) 294–298.
- [29] H. Zhu, P. Liu, X. Shi, et al., Determination of cnidilin and its two metabolites in rat plasma by high-performance liquid chromatography–electrospray ionization tandem mass spectrometry, *Planta Med.* 79 (1) (2013) 30–36.
- [30] J.N. Cai, P. Basnet, Z.T. Wang, et al., Coumarins from the fruits of *Cnidium monnieri*, *J. Nat. Prod.* 63 (4) (2000) 485–488.