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Chemical profiling of Radix Paeoniae evaluated by ultra-performance liquid chromatography/photo-diode-array/quadrupole time-of-flight mass spectrometry

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ABSTRACT

In this study, an ultra-performance liquid chromatography/photo-diode-array/quadrupole time-of-flight mass spectrometry (UPLC-PDA-QTOFMS) based chemical profiling method was established for rapid global quality evaluation of Radix Paeoniae. By virtue of the high resolution, high speed of UPLC and the accurate mass measurement of TOFMS, a total of 40 components including 29 monoterpene glycosides, 8 galloyl glucoses and 3 phenolic compounds were simultaneously separated within 12 min, and identified through the matching of empirical molecular formulae with those of published components in the in-house library, and were further elucidated by adjusted lower energy collision-induced dissociation (CID) mass spectra. Among forty components, five monoterpene glycoside sulfonates were identified as novel components. The established method was successfully applied to rapidly and globally compare the quality of Radix Paeoniae Alba and Radix Paeoniae Rubra, two post-harvesting handled products of Radix Paeoniae. Together with paeoniflorin sulfonate, five newly assigned monoterpene glycoside sulfonates were characteristic markers to detect non-official sulfur dioxide gas fumigated Radix Paeoniae Alba samples. It could be concluded that UPLC-PDA-QTOFMS based chemical profiling is a powerful approach for the global quality evaluation of Radix Paeoniae as well as other herbal medicines.

1. Introduction

It is widely accepted that the efficacy of herbal medicines is contributed by the holistic actions of multi-components in either single herb or multi-herb preparations. This challenges the conventional quality control strategy with qualitative or quantitative determination of one or a few bioactive marker components. Nevertheless, fingerprint techniques, such as chromatographic fingerprint, were introduced for the global quality control of herbal medicines [1–4]. Among various chromatographic fingerprint approaches, high performance liquid chromatography–mass spectrometry (HPLC–MS) has been increasingly investigated and applied in this area [4–8]. However, aiming to simultaneously elute and separate more components with diverse chemical structures in the herbal medicines, most of HPLC–MS fingerprinting analysis had a long analysis time of over 60 min [5,7–9]. On the other hand, different mass analyzers such as triple quadruple and ion traps provide the online mass information, which is useful in the identification of components eluted. However, both kinds of two mass analyzers provide nominal mass.

Ultra-performance liquid chromatography (UPLC) is a newly developed chromatographic technique. Compared to conventional liquid chromatography, UPLC using short column packed with 1.7–1.8 μm porous particles holds enhanced retention time reproducibility, high chromatographic resolution, improved sensitivity and increased operation speed [10]. In addition, time-of-flight mass spectrometry (QTOFMS) allows the generation of mass information with higher accuracy and precision. For the identification of unknown compounds, these accurately measured mass values can be used to produce candidate empirical formulae which, at the mass accuracy less than 5 ppm, significantly reduce the number of possible structures of putative compounds [11]. Furthermore, the use of hybrid quadrupole time-of-flight mass spectrometry (QTOFMS), with low and high collision-energy full-scan acquisitions simultaneously performed, offers more possibilities in screening and identification, resulting in valuable fragmentation information for use in elucidation and confirmation of unknowns [12]. Consequently UPLC-QTOFMS has been proved to be a powerful hyphenated technique for characterizing a wide variety of compounds in complex samples [11–14].

Radix Paeoniae (Shaoyao in Chinese), the root of plant *Paeonia lactiflora* Pall. (Family Ranunculaceae), is a commonly used medicinal herb in traditional Chinese medicine (TCM). Modern

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phytochemical and pharmacological studies have shown that the major bioactive components of Radix Paeoniae are monoterpene glycosides, galloyl glucoses and phenolic compounds [15–17]. Traditionally, the roots of *P. lactiflora* have been post-harvesting handled in two ways for different medicinal purposes. One way is to directly sun-dry the whole fresh root to produce the red peony root (Chishao, Radix Paeoniae Rubra), which is used for "eliminating blood stasis and relieving pain". The other way is to sun-dry the whole fresh root after boiling it in water and peeling off the bark to produce the white peony root (Baishao, Radix Paeoniae Alba), which is used for "subduing hyperactivity of the liver and nourishing blood and regulating menstruation, etc." [18].

As traditionally prescribed for different medicinal purposes, the quality difference between Radix Paeoniae Rubra and Radix Paeoniae Alba has always been a concern. Although many studies on the quality evaluation of these two kinds of Radix Paeoniae were conducted using ELISA [19], HPCE-UV [20], HPLC-UV [21–26] and LC–MS [27,28], these published methods dealt with only one or a few main components of Radix Paeoniae [19–26]. In the LC–MS or MS studies only monoterpene glycosides were investigated [27–29]. Moreover, the operation time for some studies lasted up to 100 min in a single analysis [27]. To our knowledge, few studies have been reported for the global chemical comparison of Radix Paeoniae Rubra and Radix Paeoniae Alba by LC–MS.

In this paper, for the first time, an ultra-performance liquid chromatography/photo-diode-array/quadrupole time-of-flight mass spectrometry (UPLC-PDA-QTOFMS) method was developed for rapid chemical profiling of Radix Paeoniae, and used for global quality evaluation of different kinds of Radix Paeoniae.

2. Experimental

2.1. Chemicals, solvents and herbal materials

Gallic acid, (+)-catechin, albiflorin, paeoniflorin and paeonol were purchased from National Institute for Control of Pharmaceutical and Biological Products (Beijing, China). The purity of these compounds was determined to be more than 98% by HPLC-UV analysis.

Radix Paeoniae Rubra (CMED-0481-02) and Radix Paeoniae Alba (CMED-0481-05) were collected from Pan-An county, Zhejiang Province, one of its indigenous cultivating regions in China. They were produced respectively from the fresh roots of *P. lactiflora* according to the methods described in Chinese Pharmacopoeia [18]. In addition, some commercial Radix Paeoniae Alba samples (CMED-0481-04, CMED-0481-06, -07, -08, -09, -10, -11, -12 and -13) were purchased from different herbal shops in China. The voucher specimens were deposited in Chinese Medicine Laboratory, Hong Kong Jockey Club Institute of Chinese Medicine (Table 1).

HPLC–MS grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany), MS grade formic acid from Sigma–Aldrich (Cat. No. 94318), other chemicals and solvents were of analytical grade. Purified water was prepared in-house with Millipore (Bedford, MA, USA).

2.2. Liquid chromatography

UPLC was performed with a Waters ACQUITY UPLC™ system (Waters Corp., MA, USA), equipped with a binary solvent delivery system, auto-sampler, and a PDA detector. The separation was performed on a Waters ACQUITY HSS T3 (100 mm × 2.1 mm, 1.8 μm) column. The mobile phase consisted of (A) 0.1% formic acid in purified water and (B) acetonitrile containing 0.1% formic acid. The linear gradient elution was optimized as follows: 2–5% B (0–2 min), 5–12% B (2–4 min), 12–20% B (4–8 min), 20–30% B (8–9 min), 30–50% B (9–10 min), and 50–100% B (10–12 min). The flow rate was 0.5 ml/min. The column and auto-sampler were maintained at 35 and 10 °C, respectively. The monitoring UV wavelength was set at 270 nm, and the scan rang for PDA was 190–400 nm.

For comparison of UPLC column with conventional column, an XTerra MS C18 (150 mm × 2.1 mm, 3.5 μm) column was selected and eluted with the same mobile phase, except that the gradient was optimized as follows: 3% B (0–10 min), 3–20% B (10–85 min), 20–85% B (85–95 min), 85% B (95–100 min). The flow rate was 0.8 ml/min.

2.3. Mass spectrometry

Mass spectrometry was performed on a Waters Q-TOF Premier (Micromass MS Technologies, Manchester, UK) mass spectrometer. The nebulization gas was set to 650 l/h at 300 °C, the cone gas set to 50 l/h, the source temperature set to 90 °C. The capillary voltage and sample cone voltage were set to 2700 and 35 V, respectively. The Q-TOF Premier acquisition rate was set to 0.3 s, with a 0.05 s interscan delay. Argon was employed as the collision gas at a pressure of 5.3×10^{-5} Torr.

The energies for collision-induced dissociation (CID) were set at 5 and 45 eV respectively for fragmentation information.

2.4. Accurate mass measurement

All MS data were acquired using the LockSpray™ to ensure mass accuracy and reproducibility. The $[M–H]^-$ ion of Leucine-enkephalin at m/z 554.2615 was used as the lock mass in negative electrospray ionization mode. The concentration of leucine-enkephalin was 50 pg/μl and the infusion flow rate was 10 μl/min. Centroided data were acquired for each sample from 100 to 1000 Da, and dynamic range enhancement (DRE™) was applied throughout

Table 1
Samples of Radix Paeoniae and monoterpene glycoside sulfonates detection.

Code	Name	Collection (Purchase) site	Collection (Purchase) time	Monoterpene glycoside sulfonate derivatives
CMED-0481-02	Radix Paeoniae Rubra	Pan-An County, Zhejiang province	2008-03-25	–
CMED-0018-05	Radix Paeoniae Alba	Pan-An County, Zhejiang province	2008-03-25	–
CMED-0018-04	Radix Paeoniae Alba	Bai Cao Tang herbal shop, Nanjing	2004-05-02	+
CMED-0018-06	Radix Paeoniae Alba	Xing Wang herbal shop, Guangzhou	2008-04-16	+
CMED-0018-07	Radix Paeoniae Alba	An Le Wan Guo herbal shop, Luo Yang	2008-04-16	+
CMED-0018-08	Radix Paeoniae Alba	Xian Sheng herbal shop, Nanjing	2008-05-26	+
CMED-0018-09	Radix Paeoniae Alba	Lei Xi herbal shop, Shanghai	2008-05-26	+
CMED-0018-10	Radix Paeoniae Alba	Lei Yun Shang herbal shop, Shanghai	2008-05-26	+
CMED-0018-11	Radix Paeoniae Alba	Fu Xing herbal shop, Shanghai	2008-05-26	+
CMED-0018-12	Radix Paeoniae Alba	Jin Ling herbal shop, Nanjing	2008-05-26	+
CMED-0018-13	Radix Paeoniae Alba	Ben Cao herbal shop, Nanjing	2008-05-26	+

+: Detectable; –: undetectable.

the MS experiment to ensure accurate mass measurement over a wide dynamic range.

2.5. Sample preparation

A standard mixture containing gallic acid, (+)-catechin, albiflorin, paeoniflorin and paeonol was prepared in 50% (v/v) methanol.

The pulverized samples of Radix Paeoniae from different collections were accurately weighed (approximately 0.5 g), and ultrasonic-extracted with 5.0 ml 50% (v/v) methanol for 25 min. The extracted solutions were filtered through a 0.2 μm PTFE syringe filter, and an aliquot (2 μl) of each filtrate was subjected to UPLC-PDA-QTOFMS analysis.

Blank 50% (v/v) methanol (2 μl) was injected between selected analyses to validate inter-sample cross-talking effect.

2.6. Establishment of in-house library and molecular formula matching

By searching from such databases as PubMed of the U.S. National Library Medicine and the National Institutes of Health, SciFinder Scholar of American Chemical Society and Chinese National Knowledge Infrastructure (CNKI) of Tsinghua University, all components reported in the literatures on *P. lactiflora* and other *Paeonia* species were summarized in a Microsoft Office Excel table to establish a in-house library, which includes the name, molecular formula, UV maximum wavelength, chemical structure and literatures of

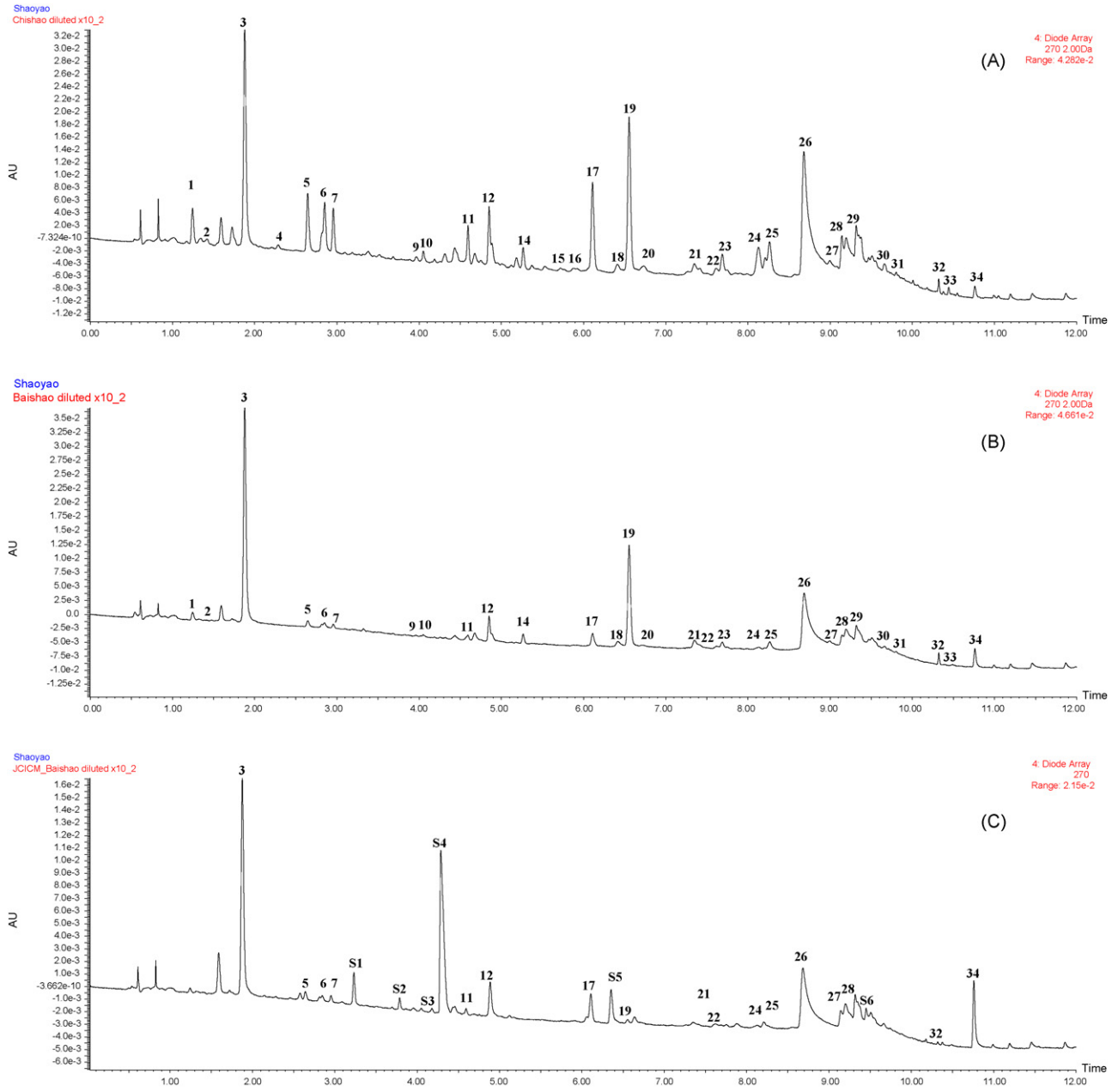


Fig. 1. Representative chromatograms of Radix Paeoniae analyzed on Waters ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) column. (A, D) Radix Paeoniae Rubra (CMED-0481-02); (B, E) Radix Paeoniae Alba (CMED-0018-05); (C, F) Radix Paeoniae Alba (CMED-0018-04); (A–C) monitored at UV 270 nm; (D–F) total ion chromatogram. The peak numbers represent the same meanings as in Table 2. Mobile phase: (A) 0.1% formic acid in purified water; (B) acetonitrile containing 0.1% formic acid. Linear gradient elution: 2–5% B (0–2 min), 5–12% B (2–4 min), 12–20% B (4–8 min), 20–30% B (8–9 min), 30–50% B (9–10 min), and 50–100% B (10–12 min). Flow rate: 0.5 ml/min. Column and auto-sampler temperature: 35 and 10 °C. Detect wavelength: 270 nm; scan rang: 190–400 nm. Nebulization gas: 650 l/h and 300 °C, cone gas: 50 l/h, source temperature: 90 °C. Capillary voltage and sample cone voltage: 2700 and 35 V. The Q-TOF Premier acquisition: 0.3 s, inter-scan delay: 0.05 s. Collision gas pressure: 5.3×10^{-5} Torr.

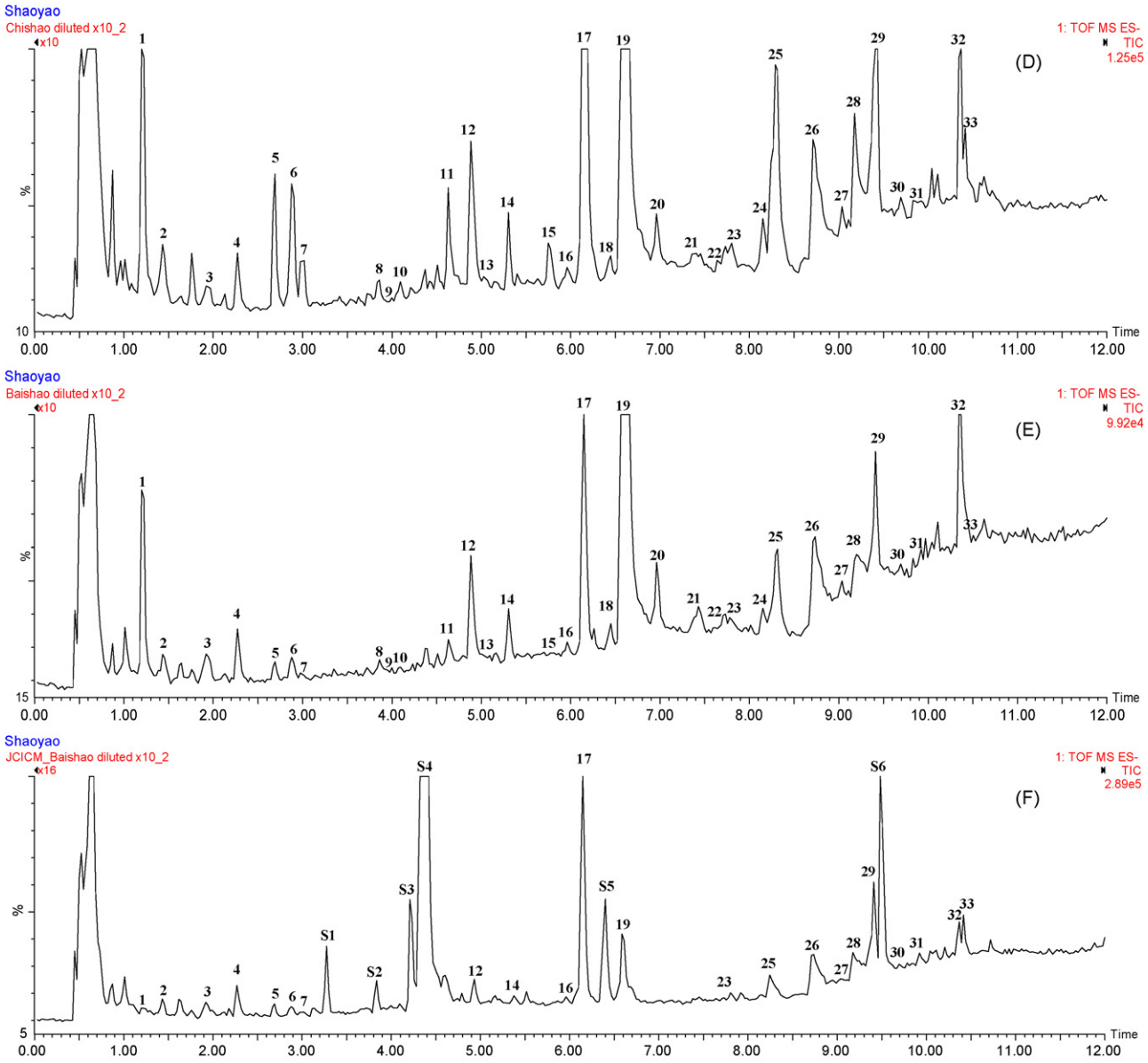


Fig. 1. (*Continued*).

each published known compound. The "*Find*" function of Microsoft Office Excel was used to match the empirical molecular formula with that of published known compounds in the library. The empirical molecular formula was short listed by comparing the accurately measured mass value to the exact mass value of putative molecules at the mass accuracy less than 5 ppm.

3. Results and discussion

3.1. Chromatographic conditions and QTOFMS method development

In our preliminary studies, two UPLC columns ACQUITY HSS T3 (100 mm × 2.1 mm, 1.8 μm) and ACQUITY BEH C18 (100 mm × 2.1 mm, 1.7 μm) were tested. It was found that the ACQUITY HSS T3 (100 mm × 2.1 mm, 1.8 μm) column has more peak capacity and stronger retention ability as well as better resolution of major components, especially for those hydrophilic components of Radix Paeoniae under the optimized conditions. ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) column was thus selected for this study. Isocratic elution offered no obvious separation because of the similar polarity and structure of most monoterpene glycosides; gradient elution was used instead to obtain better separation. Under the optimized conditions, the main components were separately eluted within 12 min on this column. The representative UV and TIC chromatograms of different kinds of Radix Paeoniae were illustrated in Fig. 1A–F. When compared to conventional LC column, such as XTerra MS C18 (150 mm × 2.1 mm, 3.5 μm) column, on which the same sample (ICM-0018-05) was analyzed under optimized chromatographic conditions with the analysis time up to 100 min per run, higher resolution, increased sensitivity and peak capacity, and significantly decreased run time were obviously demonstrated for UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) column (Fig. 2A and B).

Both positive and negative ion modes were tested, and it was found that the sensitivities for all three kinds of components in Radix Paeoniae were higher in the negative ion mode. In mass spectra determination, for most components, in particular those unmatchable in the in-house library, collision-induced dissociation (CID) with the collision-energy of 45 eV was conducted so as to get

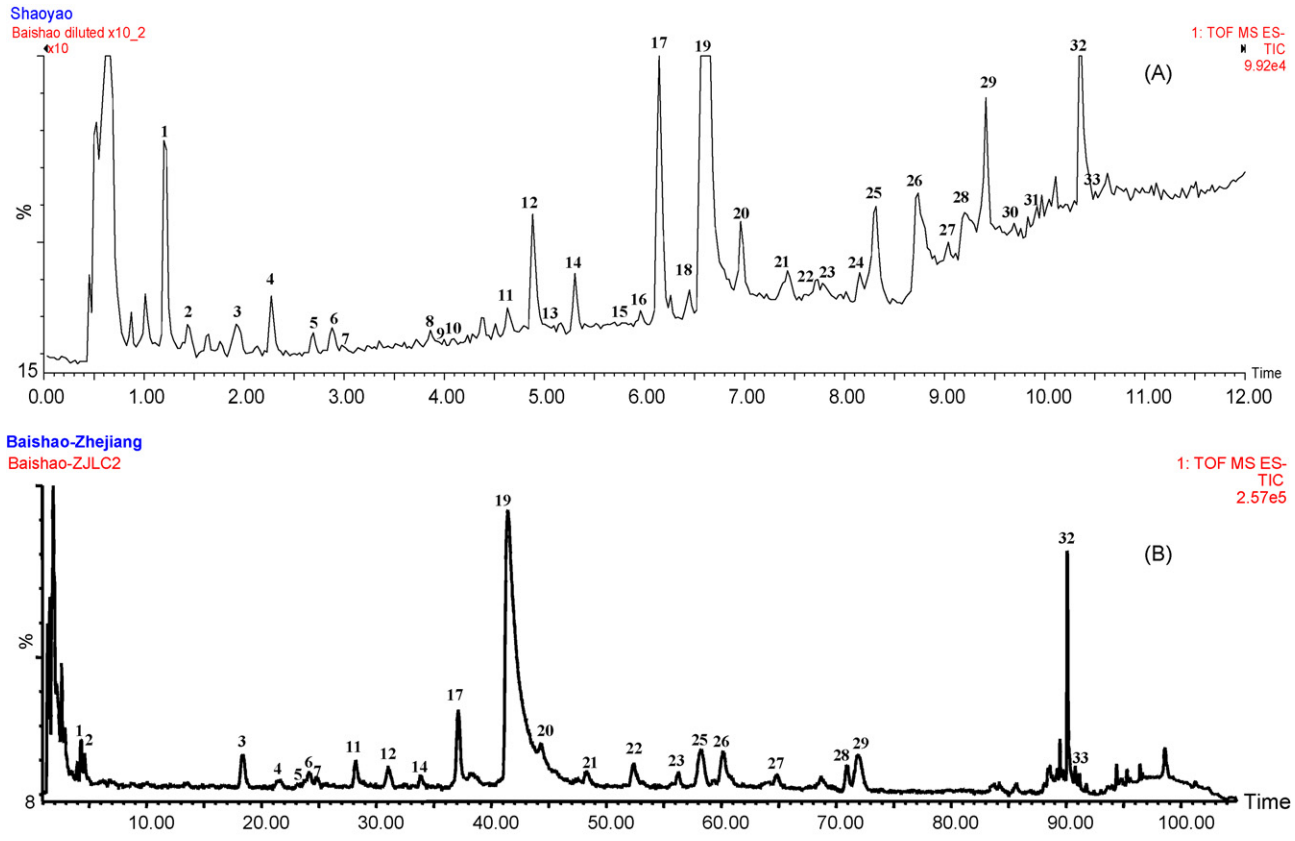


Fig. 2. Representative chromatograms of Radix Paeoniae Alba (CMED-0018-05) analyzed on different columns. (A) On Waters ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) column; (B) on Waters XTerra MS C18 (150 mm × 2.1 mm, 3.5 μm) column. For Waters ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) column, conditions were the same as in Fig. 1. For Waters XTerra MS C18 (150 mm × 2.1 mm, 3.5 μm) column, all conditions were the same as for Waters ACQUITY UPLC HSS T3 (100 mm × 2.1 mm, 1.8 μm) column except the gradient elution: 3% B (0–10 min), 3–20% B (10–85 min), 20–85% B (85–95 min), 85% B (95–100 min), and flow rate: 0.8 ml/min.

more fragmentation information for further elucidation of putative components.

3.2. Identification of major components in the samples of Radix Paeoniae

Forty components belonging to monoterpene glycosides, galloylglucoses and phenolic compounds respectively were identified in the samples of Radix Paeoniae. The chemical structures of the identified components were shown Fig. 3. The identity of each component was confirmed by matching the empirical molecular formula with that of the published components, and was further elucidated by lower energy CID mass spectra, especially for those unmatchable components in the in-house library. In addition, some data such as retention times and UV spectra of available reference compounds, or UV data in the literatures were used as complementary data for the identity confirmation. The details of identified components were summarized in Table 2.

As shown in Table 2, the mass accuracy for all assigned components was less than 5 ppm. When several empirical molecular formulae matched the same formula, those isomeric components previously reported from *P. lactiflora* or other *Paeonia* species would be preferentially selected as the putative components with chemotaxonomy considerations. Formic acid was added to the mobile phase as a modifier, adduct ions $[M+HCOO]^-$ could be observed in the mass spectra of most components. In addition, dimmer ions $[2M-H]^-$ were found in the mass spectra of most components. Interestingly, doubly charged ions $[M-2H]^{2-}$ could only be found in the mass spectra of galloylglucoses. All these quasi-molecular ions could help the confirmation of $[M-H]^-$ ions and sometimes structure types of the components.

3.2.1. Monoterpene glycosides

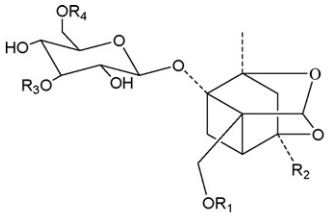
In the present study, a total of 29 monoterpene glycosides were identified in the extracts of Radix Paeoniae. Compound **17** (t_R = 6.15 min) and **19** (t_R = 6.59 min) were identified to be albiflorin and paeoniflorin by comparison with reference compounds. To facilitate identification and confirmation of other monoterpene glycosides, the MS fragmentation of these two reference compounds were investigated by low energy (45 eV) CID. For albiflorin, the feature fragment ions at *m/z* 121.0319, 283.0826, 327.1093 and 357.1202 were produced, and the ion at *m/z* 283.0826 was proposed to be produced via six-membered cyclic transition through connection of the glucosyl group with the benzoate group [29]. For paeoniflorin, the feature ions at *m/z* 121.0316, 165.0579, 317.1117and 449.1473 were observed, consistent with the data reported in the literature [27]. Compound **11/12** and **20** shared the same empirical molecular formula $C_{23}H_{27}O_{12}$, matched to that of oxypaeoniflorin [30] and *ortho*-oxypaeoniflorin [31]. Considering oxypaeoniflorin has higher polarity than *ortho*-oxypaeoniflorin, compound **11/12** and **20** were assigned to be oxypaeoniflorin/oxypaeoniflorin isomer and *ortho*-oxypaeoniflorin, respectively. Similarly compound **32** and **33** had the same empirical molecular formula $C_{30}H_{31}O_{12}$, matched to that of benzoylpaeoniflorin [30] and isobenzoylpaeoniflorin [32]. Because of the fact that benzoylpaeoniflorin is more frequently isolated or detected from Radix Paeoniae [25,26,28–30,32], and the peak area of compound **32** is larger than that of compound **33** (Fig. 1A, B, D and E), these two compounds were assigned as benzoylpaeoniflorin and isobenzoylpaeoniflorin, respectively.

Compound **1**/**2**, **4**, **8**, **9**, **10**, **14**, **24**, **30** and **31** were assigned to be desbenzoylpaeoniflorin or its isomer [33] 1-*O*-β-D-glucopyranosyl-paeonisuffrone [34], 6-*O*-β-D-glucopyranosyl

lactinolide [34], mudanpioside F [33], 6′-*O*-galloyl desbenzoylpaeoniflorin [33], mudanpioside E [33], 1-*O*-β-D-glucopyranosyl-8-*O*-benzoylpaeonisuffrone [35], 3′, 6′-di-*O*-galloylpaeoniflorin [36,37] and benzoyloxypaeoniflorin [37] respectively based on the molecular formulae matching and the comparison of their UV with reported data in the literature, and were further confirmed by the low energy (45 eV) CID fragmentation information (Table 2). The mass spectrum and proposed fragmentation of 3′,6′-di-*O*-galloylpaeoniflorin were shown in Fig. 4A.

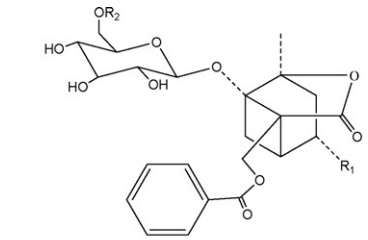
Compound **16** has the empirical molecular formula $C_{29}H_{37}O_{16}$, matched to that of isomaltopaeoniflorin [33] and 6′-*O*-β-D-glucopyranosylalbiflorin [38], two components previously isolated from *P. lactiflora*, so this component was assigned as isomaltopaeoniflorin (**16**) or 6′-*O*-β-D-glucopyranosylalbiflorin (**16′**). The empirical formula of compound **23** and **29** was $C_{23}H_{27}O_{11}$. Except for paeoniflorin and albiflorin, two other monoterpene glycosides isopaeoniflorin [32] and albiflorin R1 [39], which was previously reported from *P. lactiflora*, share the molecular formula $C_{23}H_{27}O_{11}$. These two components were therefore identified as isopaeoniflorin and albiflorin R1. The empirical molecular formulae of compound **25**, **27** and **28** were all short listed to be $C_{30}H_{31}O_{15}$, matched to that of galloylpaeoniflorin [36] or galloylalbiflorin [37]. Considering the reported co-existence of paeoniflorin with its isomer isopaeoniflorin in this herb [32], these three components were assigned to be galloylpaeoniflorin, galloylablbiflorin and their isomers respectively.

Compared with samples directly collected from Pan-An county, Zhejiang Province, six extra components **S1**–**S6** were detected in commercial samples (Fig. 1C and F). However, only the empirical molecular formula ($C_{23}H_{27}O_{13}S$) of **S4** could be matched in the in-house library, which was identical to that of paeoniflorin sulfonate, a sulfonate derivative whose molecular weight is 64 Da more than the main monoterpene glycoside paeoniflorin [25,40]. This component was assigned as paeoniflorin sulfonate rationally supported by the fragment ions at *m*/*z* 121.0313, 213.0263, 259.0315, 375.0792, 421.0829 and 497.1160 in the low energy CID mass spectrum. The mass spectrum and proposed fragmentation of paeoniflorin sulfonate was demonstrated in Fig. 4B. For **S1**, **S2**, **S3**, **S5** and **S6**, no published components could be matched in the in-house library. However, **S5** had the empirical molecular formula $C_{30}H_{31}O_{17}S$, with 64 Da more than galloylpaeoniflorin. In the low energy CID mass spectra of this compound, fragment ions at *m*/*z* 121.0303, 169.0168, 259.0317, 313.0557 and 543.0203 were observed, so this compound was presumed to be galloylpaeoniflorin sulfonate. The mass

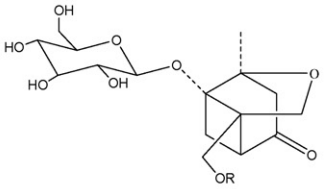


	R_1	R_2	R_3	R_4
1/2	H	OH	H	H
10	H	OH	H	G
11/12	HB	OH	H	H
14	Va	OH	H	H
16	B	OH	H	Glc
19	B	OH	H	H
20	*ortho*-HB	OH	H	H
25 (27/ 28)	B	OH	H	G
30	B	OH	G	G
31	HB	OH	H	B
32/33	B	OH	H	B
S1	HB	SO_3H	H	H
S2	Va	SO_3H	H	H
S3	B	SO_3H	H	Glc
S4	B	SO_3H	H	H
S5	B	SO_3H	H	G
S6	B	SO_3H	H	B

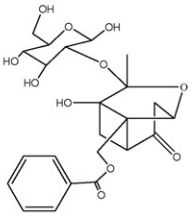
Fig. 3. Chemical structures of major components identified from Radix Paeoniae. The compound numbers represent the same meanings as in Table 2.



	R_1	R_2
16'	OH	Glc
17	OH	H
25(27/28)	OH	G



	R
4	H
24	B



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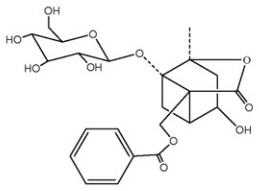

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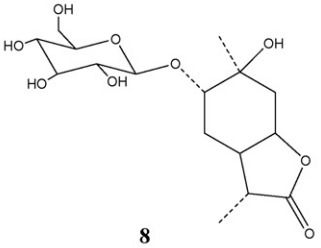

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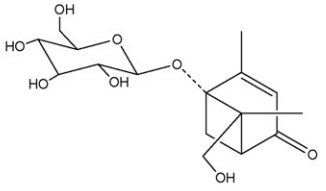

9

	R_1	R_2	R_3
15	G	H	H
5/6/7	H	G	H
	H	H	G
	B	H	H

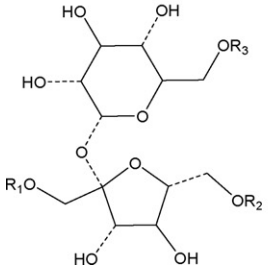

Fig. 3. (*Continued*)

spectrum and proposed fragmentation of galloylpaeoniflorin sulfonate were shown in Fig. 4C. Similarly for compound **S1**, **S2**, **S3** and **S6**, their empirical molecular formulae were $C_{23}H_{27}O_{14}S$, $C_{24}H_{29}O_{15}S$, $C_{29}H_{37}O_{18}S$ and $C_{30}H_{31}O_{14}S$, respectively, all with 64 Da difference to oxypaeoniflorin, mudanpioside E, isomaltopaeoniflorin and benzoylpaeoniflorin, respectively. The fragment ions in the low energy CID mass spectra were observed at *m*/*z* 213.0232, 259.0299, 375.0787, 421.0808 for **S1**, *m*/*z* 167.0376, 259.0293, 421.0835 for **S2**, *m*/*z* 121.0311, 213.0244, 259.0306, 421.0786, 479.0904, 543.1183 for **S3**, and *m*/*z* 121.0320, 213.0259, 259.0310, 497.1044, 525.1092 for **S6** respectively, all supporting the assignment of these components to be oxypaeoniflorin sulfonate,

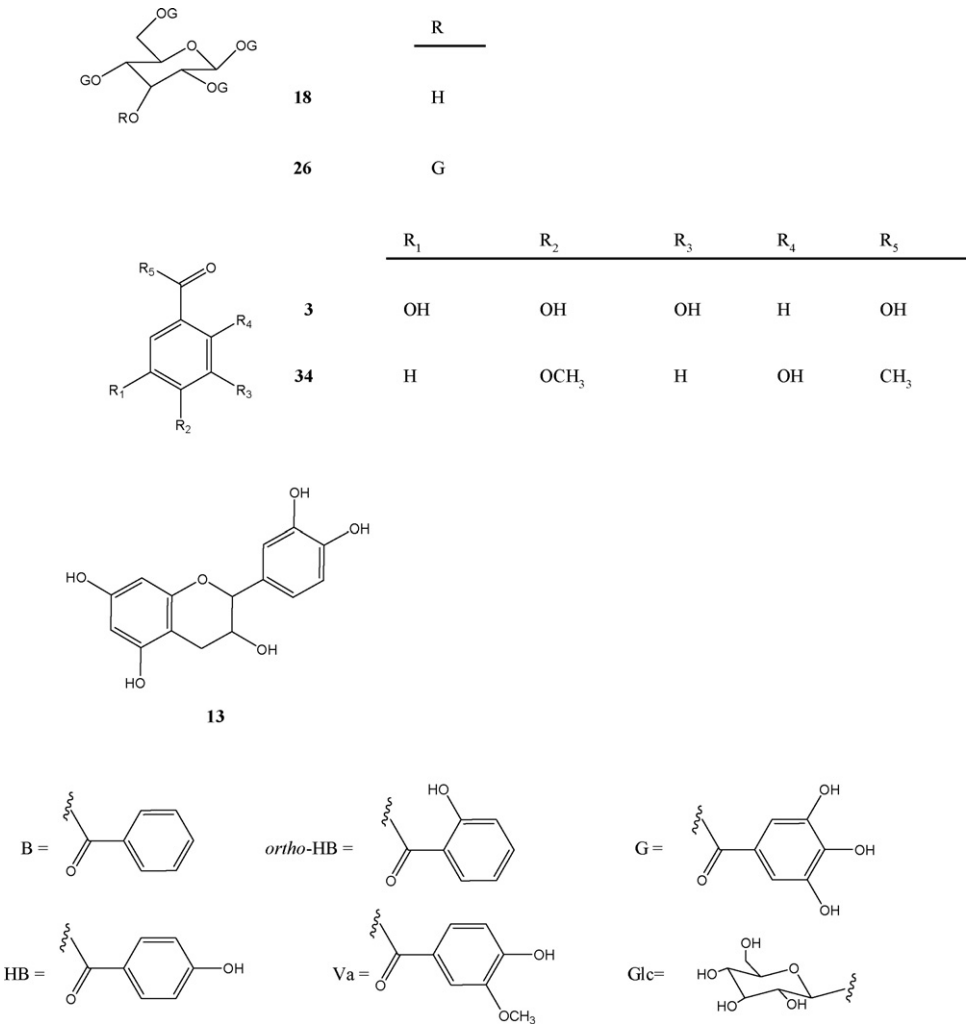

Fig. 3. (*Continued*).

mudanpioside E sulfonate, isomaltopaeoniflorin sulfonate or 6′-*O*-β-glucopyranosylalbiflorin sulfonate and benzoylpaeoniflorin sulfonate, respectively (Table 2).

3.2.2. Galloyl glucoses

A total of 8 galloylglucoses were identified in Radix Paeoniae in this study. Compound **5**, **6** and **7** had the same empirical molecular formula $C_{19}H_{25}O_{15}$, matched to that of 1′-*O*-galloylsucrose, 6′-*O*-galloylsucrose and 6-*O*-galloylsucrose, three galloylsucroses previously isolated from *P. lactiflora* [33]. Their fragment ions further indicated the loss of glucose moiety (180 Da) and gallic acid moiety (170 Da) from the precursors of $[M–H]^-$, thus these three compounds were assigned as 1′-*O*-galloylsucrose, 6′-*O*-galloylsucrose and 6-*O*-galloylsucrose.

The empirical molecular formula of compound **26** was $C_{41}H_{31}O_{26}$, matched to that of pentagalloylglucose, a major galloylglucose previously reported in Radix Paeoniae [41]. The $[M–2H]^{2-}$ ion at *m*/*z* 469.0482 was found in the mass spectrum, and the fragment ions at *m*/*z* 169.0173, 295.0527, 313.0465, 447.0607, 465.0642, 617.0794, 769.0937 and 787.2085 were observed in the low energy CID mass spectrum, indicating the successive neutral losses of gallic acids (170 Da) and/or galloyl radicals (152 Da), supporting the assignment of this compound to be pentagalloylglucose. The mass spectrum and proposed fragmentation were shown in Fig. 4D.

Compound **18**, **21** and **22** had the same empirical molecular formula $C_{34}H_{27}O_{22}$, and all exhibited the $[M–2H]^{2-}$ ions in their mass spectra (Table 2). In low energy CID mass spectra, they all displayed several fragment ions that indicated the successive neutral losses of gallic acids (170 Da) and/or galloyl radicals (152 Da). These compounds were tentatively identified as tetragalloyl glucose, but their structures could not be elucidated by the MS data due to the limited information as to link position of the galloyl groups to the glucose unit. Compound **15** had an empirical molecular formula $C_{19}H_{25}O_{12}$, which is identical to that of 1′-*O*-benzoylsucrose, a component previously isolated from *P. lactiflora* [34], and was thus assigned as 1′-*O*-benzoylsucrose.

3.2.3. Phenolic compounds

Compound **3** (t_R = 1.92 min) and **13** (t_R = 5.02 min) were identified as gallic acid and (+)-catechin respectively by comparison of their retention time, UV and MS data with those of reference compounds. For compound **34** (t_R = 10.76 min), no signal could be observed in total ion chromatograms owing to the possible suppression effect under present conditions. This compound, however had the same retention time and UV spectrum as that of reference compound paeonol and was thus identified as paeonol (Fig. 1A–C).

3.3. Quality evaluation of Radix Paeoniae

Eleven samples of Radix Paeoniae from different collections were analyzed by the newly established UPLC-PDA-QTOFMS method. The representative UV and TIC chromatograms were shown in Fig. 1, and the detection results were summarized in Table 1.

Table 2
Components identified from Radix Paeoniae.

Peak no.	t_R (min)	Assigned identity	Molecular formula	UV λ_{max} (nm)	$[M-H]^-$ m/z			$[M+HCOO]^-$ m/z	$[2M-H]^-$ m/z	$[M-2H]^{2-}$ m/z	Fragment ions of $[M-H]^-$ at low energy (45 eV) CID
					Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)				
1	1.20	Desbenzoylpaeoniflorin or Desbenzoylpaeoniflorin isomer	$C_{16}H_{24}O_{10}$	209	375.1218	375.1291	−2.4	421.1346			165.0577 345.1180
2	1.43			211	375.1317	375.1291	−3.5	421.1399			294.9478 345.1208
*3	1.92	Gallic acid	$C_7H_5O_5$	216, 271	169.0143	169.0137	3.5		339.0596		-
4	2.27	1-*O*-β-D-glucopyranosyl-paeonisuffrone	$C_{16}H_{23}O_9$	210, 271	359.1347	359.1342	1.4	405.1394	719.2748		179.0686 197.0843
5	2.69	1′-*O*-galloylsucrose, 6′-*O*-galloylsucrose and 6-*O*-galloylsucrose	$C_{19}H_{25}O_{15}$	216, 274	493.1190	493.1193	−0.6		987.2393		169.0187 313.0610
6	2.88			216, 274	493.1196	493.1193	0.6		987.2329		169.0177 313.0574
7	3.02			215, 275	493.1201	493.1193	1.6		987.2169		169.0161 313.0626
8	3.86	6-*O*-β-D-glucopyranosyl lactinolide	$C_{16}H_{25}O_9$	210	361.1491	361.1499	−2.2	407.1559			241.9003 275.0407
9	4.09	Mudanpioside F	$C_{16}H_{23}O_8$	211, 264	343.1392	343.1393	−0.3	389.1396			223.9562 266.9580
10	4.21	6′-*O*-galloyl desbenzoylpaeoniflorin	$C_{23}H_{27}O_{14}$	212, 275	527.1407	527.1401	1.1				451.1306 497.1256
11	4.63	Oxypaeoniflorin or Oxypaeoniflorin isomer	$C_{23}H_{27}O_{12}$	259	495.1500	495.1503	−0.6		991.2695		289.0762 379.1673
12	4.88			260	495.1505	495.1503	0.6		991.2876		443.1888 289.0752 431.0847 465.1431
*13	5.02	(+)-catechin	$C_{15}H_{13}O_6$	209, 260	289.0699	289.0712	−4.5				205.0502 245.0820
14	5.30	Mudanpioside E	$C_{24}H_{29}O_{13}$	217, 264	525.1619	525.1608	2.1				223.9582 294.9517 429.1161 495.1422
15	5.75	1′-*O*-benzoylsucrose	$C_{19}H_{25}O_{12}$	217, 277	445.1350	445.1346	0.9	491.1443	891.2713		283.0928 323.1012 411.0450
16 (16′)	5.96	Isomaltopaeoniflorin or 6′-*O*-β-D-glucopyranosylalbiflorin	$C_{29}H_{37}O_{16}$	213, 277	641.2059	641.2082	−3.6	687.2151			341.1197 415.1746 495.1474 525.1912 593.1892 611.1992
*17	6.15	Albiflorin	$C_{23}H_{27}O_{11}$	233, 275	479.1553	479.1553	0	525.1638	959.3168		121.0319 283.0826 327.1093 357.1202

Table 2 (*Continued*)

Peak no.	t_R (min)	Assigned identity	Molecular formula	UV λ_{max} (nm)	$[M-H]^-$ m/z			$[M+HCOO]^-$ m/z	$[2M-H]^-$ m/z	$[M-2H]^{2-}$ m/z	Fragment ions of $[M-H]^-$ at low energy (45 eV) CID
					Mean measured mass (Da)	Theoretical exact mass (Da)	Mass accuracy (ppm)				
18	6.45	Tetragalloyl glucose	$C_{34}H_{27}O_{22}$	215, 278	787.0989	787.0994	−0.6			393.0431	169.0158 295.0297 313.0570 447.1352 465.1383 483.0638
*19	6.59	Paeoniflorin	$C_{23}H_{27}O_{11}$	234	479.1556	479.1553	0.6	525.1621	959.3211		121.0316 165.0579 327.1117 449.1473
20	6.96	*ortho*-Oxypaeoniflorin	$C_{23}H_{27}O_{12}$	209, 259	495.1500	495.1503	−0.6				137.0247 165.0578 465.1412
21	7.38	Tetragalloylglucose	$C_{34}H_{27}O_{22}$	216, 278	787.0970	787.0994	−3.0			393.0327	169.0459 295.1015 313.1027 477.1354 465.1488 617.1949 635.2112
22	7.64	Tetragalloylglucose	$C_{34}H_{27}O_{22}$	216, 278	787.0972	787.0994	−2.8			393.0668	169.0364 295.0952 447.1354 465.1488 617.1949 635.1928
23	7.80	Isopaeoniflorin or Albiflorin R1	$C_{23}H_{27}O_{11}$	234	479.1562	479.1553	1.9	525.1561			327.1095 433.1129 449.1474
24	8.15	1-*O*-β-D-glucopyranosyl-8-*O*-benzoylpaeonisuffrone	$C_{23}H_{28}O_{10}$	229	463.2252	463.1604	13.99	509.2230			327.1159 359.0367 449.1432
25	8.29	Galloylpaeoniflorin or Galloylalbiflroin or their isomers	$C_{30}H_{31}O_{15}$	219, 275	631.1656	631.1663	−1.1				169.0364 313.0962 479.1886 491.7770 509.2184 613.2386
26	8.71	Pentagalloylglucose	$C_{41}H_{31}O_{26}$	223, 280	939.1104	939.1104	0			469.0482	169.0173 295.0527 313.0465 447.0609 465.0642 617.0794 769.0937 787.2085
27	9.04			218, 279	631.1644	631.1663	−3.0				169.0412 313.0560
28	9.18	Galloylpaeoniflorin or Galloylalbiflroin or their isomers	$C_{30}H_{31}O_{15}$	217, 279	631.1667	631.1663	0.6				479.1886 169.0459 313.1092

29	9.41	Isopaeoniflorin or Albiflorin R1	$C_{23}H_{27}O_{11}$	220, 276	479.1555	479.1553	0.4	525.1644	959.3220		479.1886 283.0847 357.1225
30	9.69	3′,6′-di-*O*-galloylpaeoniflorin	$C_{37}H_{35}O_{19}$	217, 277	783.1781	783.1773	1.0				169.0180 313.0608 465.1286 509.1049 631.1753
31	9.86	Benzoyloxypaeoniflorin	$C_{30}H_{31}O_{13}$	215, 275	599.1780	599.1765	2.5				293.1025 491.2348 509.1945
32	10.34	Benzoylpaeoniflorin	$C_{30}H_{31}O_{12}$	218, 275	583.1840	583.1816	4.1	629.1882			121.0318 165.0581 431.1364 553.1740
33	10.42	Isobenzoylpaeoniflorin		219, 275	583.1837	583.1816	3.6	629.1870			431.1628 553.1743
*__34__	10.76	Paeonol	$C_9H_{10}O_3$	217, 275							–
S1	3.27	Oxypaeoniflorin sulfonate	$C_{23}H_{27}O_{14}S$	257	559.1129	559.1122	0.9				213.0232 259.0299 375.0787 421.0808
S2	3.83	Mudanpioside E sulfonate	$C_{24}H_{29}O_{15}S$	263	589.1208	589.1227	−3.2				167.0376 259.0293 421.0835
S3	4.21	Isomaltopaeoniflorin sulfonate	$C_{29}H_{37}O_{18}S$	218	705.1735	705.1701	4.8				121.0311 213.0244 259.0306 421.0786 497.0904 543.1183
S4	4.35	Paeoniflorin sulfonate	$C_{23}H_{27}O_{13}S$	232	543.1172	543.1172	0				121.0313 213.0263 259.0315 375.0792 421.0829 497.1160
S5	6.40	Galloylpaeoniflorin sulfonate	$C_{30}H_{31}O_{17}S$	275	695.1280	695.1282	−0.3			347.1202	121.0303 169.0168 259.0317 313.0557 543.1203
S6	9.48	Benzoylpaeoniflorin sulfonate	$C_{30}H_{31}O_{14}S$	276	647.1421	647.1435	−2.2				121.0320 213.0259 259.0310 479.1044 525.1092

-: No fragment ion found.

–: No response under present conditions.

* Identified with reference compounds.

As shown in Fig. 1, samples of Radix Paeoniae Rubra and Radix Paeoniae Alba directly collected from the same indigenous cultivating region had similar chemical profiles (Fig. 1A and B, D and E) with differences in the peak areas of most individual components. This indicated that the post-harvest handling by boiling in water and peeling off the bark did not significantly change the chemical compositions of Radix Paeoniae. The rationality behind using Radix Paeoniae Rubra and Radix Paeoniae Alba for different medical purposes might be the quantitative differences of components, but this requires further investigation.

However, significant differences in their chemical profiles were found between commercial Radix Paeoniae Alba samples (Fig. 1C and F) and those collected directly from indigenous cultivating site (Fig. 1B and E). Six monoterpene glycoside sulfonate derivatives

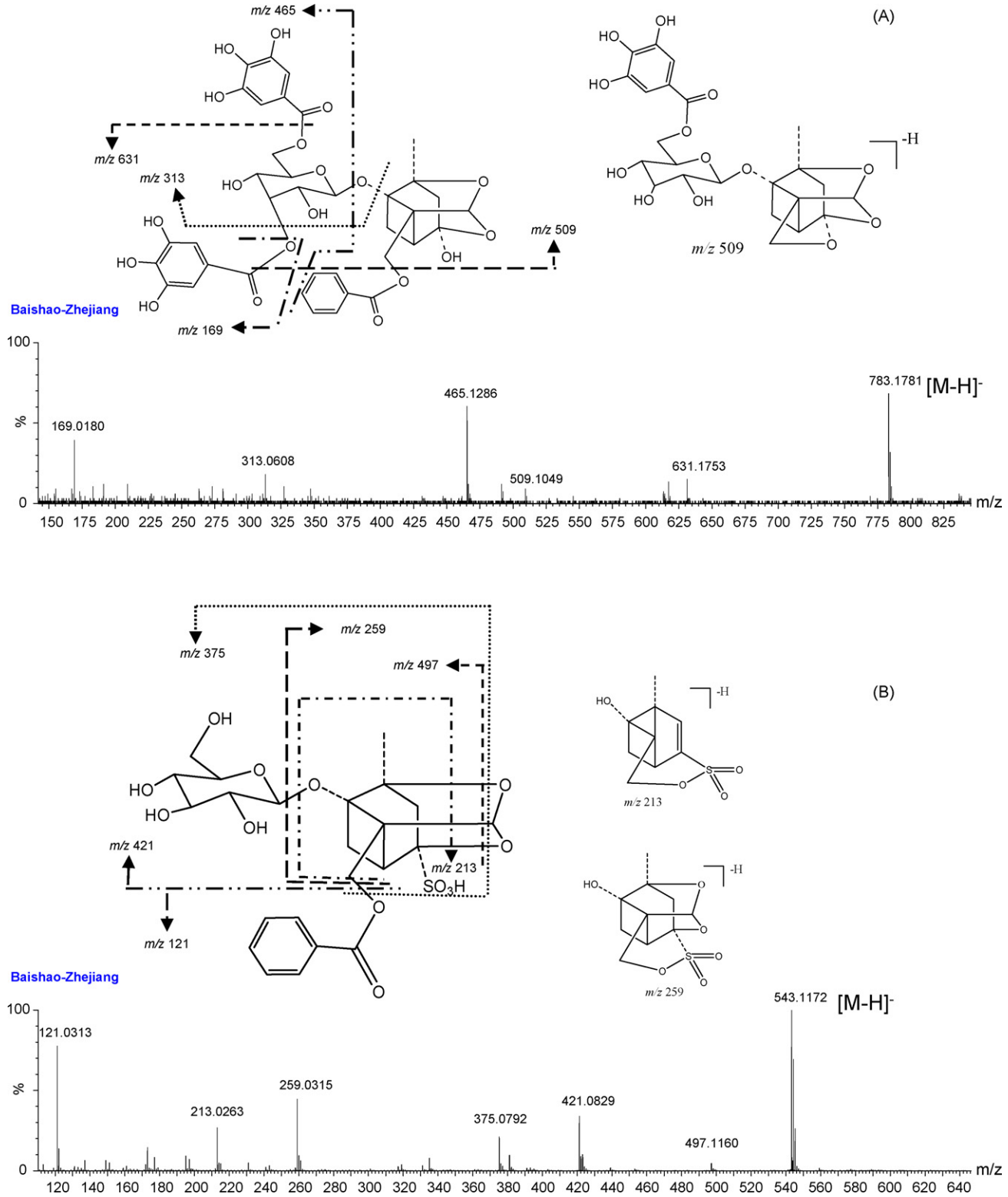

Fig. 4. Low energy (45 eV) CID mass spectra and proposed major fragmentation of selected components identified from Radix Paeoniae. (A) 3′,6′-di-*O*-galloylpaeoniflorin; (B) paeoniflorin sulfonate; (C) galloylpaeoniflorin sulfonate; (D) pentagalloylglucose.

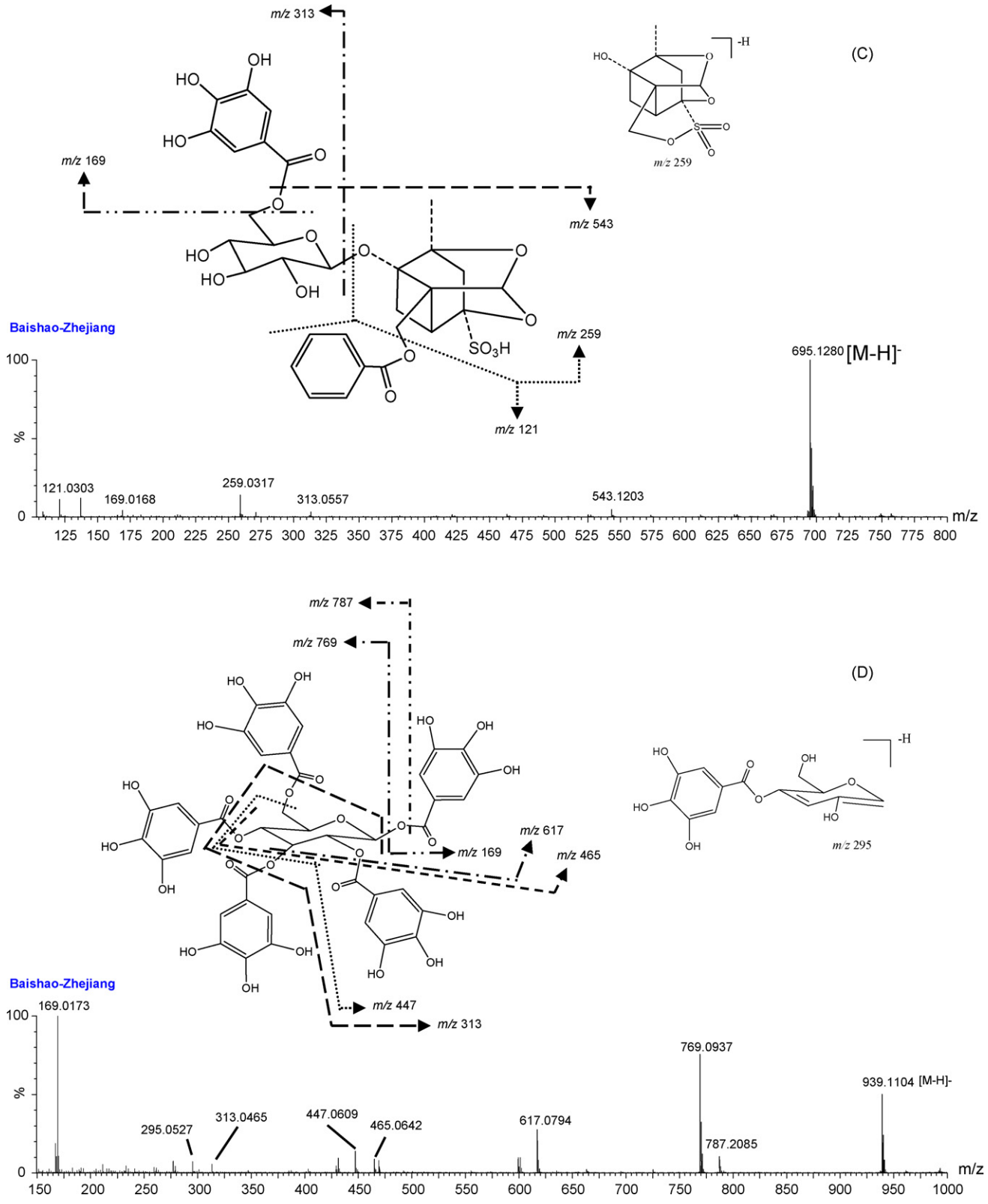

Fig. 4. (*Continued*).

(**S1**–**S6**) were detected in all nine commercial Radix Paeoniae Alba samples analyzed (Table 1). It was reported that during the storage and marketing process, in order to preserve its white appearance, Radix Paeoniae Alba was often non-officially fumigated with toxic sulfur dioxide gas, which is generated by burning sulfur [24]. This non-official post-harvest handling method was recently revealed leading to the conversion of the main component paeoniflorin into its sulfonate derivative paeoniflorin sulfonate [25,40]. In the present study, five other monoterpene glycoside sulfonate derivatives were newly detected and identified along with paeoniflorin sulfonate, suggesting that other main monoterpene glycosides, such as galloylpaeoniflorin, mudanpioside E, oxypaeoniflorin and

benzoylpaeoniflorin, could also be easily converted into their sulfonate derivatives, resulting in the significant change of chemical profiles of Radix Paeoniae Alba (Fig. 1C and F). Furthermore, the fact that monoterpene glycoside sulfonate derivatives could be detected in all nine commercial samples indicated that the non-official sulfur dioxide gas fumigation is a common processing method for Radix Paeoniae Alba during storage and marketing. However, up to now, there have been few comprehensive pharmacological and toxicological studies on the monoterpene glycoside sulfonate derivatives. Therefore, to ensure the safety and effectiveness of Radix Paeoniae Alba, it is strongly recommended that chemical profiles based global quality evaluation should be conducted before Radix Paeoniae Alba being prescribed for remedy purposes. Furthermore, the pharmacological and toxicological studies on these sulfonate derivatives are also warranted.

4. Conclusion

An UPLC-PDA-QTOFMS method was developed for rapid chemical profiling of Radix Paeoniae. 40 components belonging to monoterpene glucosides, galloylglucose and phenolic compounds were separately eluted and identified simultaneously from the extract of Radix Paeoniae with analysis time lasting less than 12 min per run. By comparison of empirical molecular formula with those of published components, as well as elucidation of CID fragmentation pathways, five new monoterpene glycoside sulfonates were assigned, which, together with paeoniflorin sulfonate, could be used as characteristic markers to detect non-official sulfur dioxide gas fumigated Radix Paeoniae Alba samples. It can be concluded that, with high speed, high resolution and high sensitivity of UPLC, coupled with the accurate molecular mass measurement of QTOFMS, UPLC-PDA-QTOFMS based chemical profiling is an effective strategy for rapid global quality evaluation of Radix Paeoniae as well as other herbal medicines.

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