Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 674–680

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Simultaneous qualification and quantification of baccharane glycosides in Impatientis Semen by HPLC–ESI-MSD and HPLC–ELSD

Hui-Jun Li, Jun-Jie Yu, Ping Li*

Key Laboratory of Modern Chinese Medicines (China Pharmaceutical University), Ministry of Education, No. 24 Tongjia Lane, Nanjing, 210009, China

ARTICLE INFO

Article history:
Received 7 August 2010
Received in revised form 12 October 2010
Accepted 17 October 2010
Available online 27 October 2010

Keywords:
Baccharane glycosides
HPLC–ESI-MSD
HPLC–ELSD
Impatientis Semen
Quality control

ABSTRACT

This study presents a high performance liquid chromatography (HPLC) with electrospray ionization mass spectrometric detection (ESI-MSD) and evaporative light scattering detection (ELSD) method for the simultaneous qualification and quantification of eight major baccharane glycosides, namely hosenlosides A, B, C, F, G, K, L, and M in Impatientis Semen, a Chinese herbal medicine derived from the seeds of *Impatiens balsamina* L. In order to achieve optimum performance, several extraction parameters (including extraction solvent, extraction mode, extraction time) were optimized. The baccharane glycosides were separated on a Shim-pack CLC-ODS column with gradient elution of water and methanol. Temperature for the ELSD drift tube was set at 98 °C and the nitrogen flow rate was 2.7 l/min. The unambiguous identities of the analytes were realized by comparing retention times and mass data with those of reference compounds. The developed method was fully validated in terms of linearity, sensitivity, precision, repeatability, recovery as well as robustness, and subsequently applied to evaluate the quality of 14 batches of Impatientis Semen commercial samples from different collections.

1. Introduction

Impatiens balsamina L. (Fam. Balsaminaceae), an indigenous plant of southern Asia in India and Myanmar, has been largely cultivated in China for ornamental and medicinal purpose. Different parts of this herb possess different functions: the juice of its flower petals has been used as a natural purple dye for staining fingernails; the aerial parts (including stem and leaf) have been utilized locally in some areas of China for the treatment of inflammatory, rheumatic and pruritic diseases [1]; and most importantly, the dried ripe seeds (Latin name 'Impatientis Semen') have been officially recorded in Chinese Pharmacopoeia as a traditional Chinese herbal medicine for the treatment of amenorrhea, abdominal mass, bone choking throat and sores [2].

Although various types of chemical constituents, including fatty acids [3], flavonoids, diterpenes and sterols [4], triterpenes [5] were separated and identified from Impatientis Semen, the isolation of baccharane glycosides [6–8] showed chemotaxonomic significance due to their rare presence in the field of natural products. To the best of our knowledge, baccharane derivatives were found only in other few species in Cucurbitaceae (*Actinostemma lobatum* Maxim. [9]), Fabaceae (*Glycine max* (L.) Merr. [10]), and Asteraceae (*Baccharis halimifolia* L. [11], etc. Consequently, the apparently restricted occurrence of baccharane glycosides in plants conferred upon this type of secondary metabolites great value as chemical markers for quality control of Impatientis Semen. To date, however, there is only one publication [12] that has been reported on analysis of this herbal drug. In this published article, owing to substantial shortage of reference compounds, merely two major baccharane glycosides, viz. hosenlosides A and K were analyzed by high performance liquid chromatography with evaporative light scattering detection (HPLC–ELSD). In addition, the two analytes in sample solutions were identified solely by means of retention time, which was proved to be insufficient for an extremely complex matrix when the co-eluting chromatographic behavior was taken into consideration [13–16].

Thus, the primary goal of this study was to set up a baccharane glycosides-based qualitative and quantitative method for quality control of Impatientis Semen. To achieve this goal, we firstly separated the baccharane glycosides by a series of phytochemical procedures. Then, we employed mass spectrometric detection (MSD) as a reliable tool to unambiguously identify the chromatographic peaks in analysis of complex sample solution, and selected ELSD to quantify the non-chromophoric analytes as literature [12] did. The developed method was fully validated and subsequently applied to analyze 14 batches of Impatientis Semen commercial samples from different collections.

* Corresponding author. Tel.: +86 25 83271382; fax: +86 25 83271379.
E-mail addresses: liping2004@126.com, fleude@126.com (P. Li).

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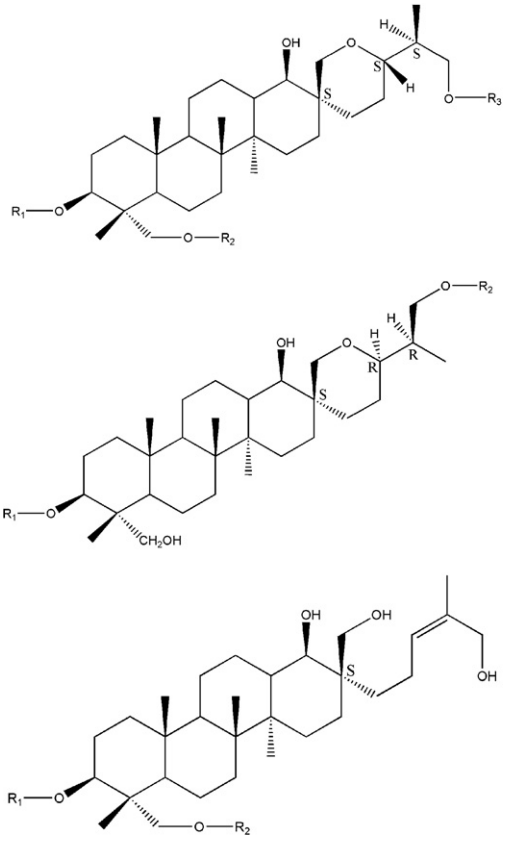
doi:10.1016/j.jpba.2010.10.014



No.	Compound	R_1	R_2	R_3
4	Hosenkoside K	-Glc(2-1)Glc	-Glc	-Glc
6	Hosenkoside M	-Glc(2-1)Xyl	-Glc	-Glc
7	Hosenkoside A	-Glc(2-1)Glc	-Glc	H
8	Hosenkoside L	-Glc(2-1)Xyl	-Glc	H

No.	Compound	R_1	R_2
1	Hosenkoside B	-Glc(2-1)Glc	-Glc
2	Hosenkoside F	-Glc(2-1)Xyl	-Glc

No.	Compound	R_1	R_2
3	Hosenkoside C	-Glc(2-1)Glc	-Glc
5	Hosenkoside G	-Glc(2-1)Xyl	-Glc

Fig. 1. Chemical structures of eight baccharane glycosides from Impatientis Semen. Glc, (-D-glucose; Xyl, (-D-xylose.

2. Materials and methods

2.1. Chemicals, reagents and materials

Eight reference baccharane glycosides including hosenlosides A, B, C, F, G, K, L and M (Fig. 1) were isolated and purified from Impatientis Semen in our laboratory. Their structures were elucidated by comparison of spectroscopic data (HR–ESI-MS, ^{1}H- and ^{13}C-NMR) with the literature values [6–8]. The purity of each reference was determined to be over 98% by normalization of the peak area detected by HPLC–ELSD, and showed good short-term (one week) and long-term (one month) stability at room temperature as well as thermal stability (90 °C for 2 h) in methanol solution.

Methanol (Merck, Germany) was of HPLC-grade. Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). Petroleum ether (60–90 °C) and ethanol were of analytical grade and purchased from Nanjing Chemical Factory (Nanjing, China).

A total of 14 batches of Impatientis Semen commercial samples (No. 1–14) were collected from different sources in China. All samples were authenticated as the seeds of *I. balsamina* L. by one of the authors (Prof. Ping Li) and the corresponding voucher specimens were deposited in the Key Laboratory of Modern Chinese Medicines (China Pharmaceutical University), Ministry of Education, China Pharmaceutical University.

2.2. Apparatus and chromatographic conditions

2.2.1. HPLC–ELSD analysis

The analyses were performed using an Agilent 1100 Series HPLC system, equipped with a binary pump, an auto-sampler, a column oven, and a ChemStation Software Version A.10.02 (Agilent Technologies, USA). An Alltech ELSD 2000 instrument, operated by the ELSD 2000 control software (Alltech, USA), was connected to the liquid chromatography for detection of baccharane glycosides. The separation was carried out on a Shim-pack CLC-ODS column (6.0 mm × 150 mm, 5 μm) (Shimadzu Corporation, Japan) at a column temperature of 25 °C. The gradient elution was employed using water (solvent A) and methanol (solvent B), and eluted by the following program at the flow rate of 0.8 ml/min: 0–10 min (53% B), 10–25 min (53–60% B), 25–35 min (60–80% B), 35–37 min (80–100% B), 37–45 min (100% B), 45–46 min (100–53% B), 46–80 min (53% B). The drift tube temperature for ELSD was set at 98 °C and the nitrogen flow rate was 2.7 l/min.

2.2.2. HPLC–ESI-MSD analysis

MSD was used to confirm the identification of chromatographic peaks of interest. The HPLC–ESI-MSD analyses were conducted on an Agilent 1100 series HPLC system (Agilent Technologies, USA) coupled with a SL G1946D quadruple mass spectrometer equipped with an electrospray ionization (ESI) source (Agilent Technologies, USA), and the HPLC conditions were identical to those used for HPLC–ELSD analyses mentioned above. Mass spectra were acquired in both positive and negative mode using full scan mode with scan range from *m/z* 100 to 3000. The conditions of ESI source were as followed: drying gas (N_2) flow rate, 10.0 l/min; temperature, 325 °C; pressure of nebulizer, 30 psi; capillary voltage, 4.0 kV, and fragmentor voltage, 120 V. Data were acquired and analyzed by Agilent ChemStation Software Version A.01.00 (Agilent Technologies, USA).

2.3. Preparation of standard solutions

Mixed standard stock solution was prepared by accurately weighing 8 baccharane glycosides, i.e., hosenlosides A (6.49 mg), B (6.35 mg), C (4.38 mg), F (4.29 mg), G (4.64 mg), K (14.96 mg), L

Table 1
Retention times, mass spectra for each analyte.

No.	t_R[a] (min)	Analyte	M.W.[b]	Characteristic ions (m/z)	
				Positive ions	Negative ions
1	8.8	Hosenkoside B	978	979[M+H]$^+$, 1001[M+Na]$^+$	977[M−H]$^-$, 1023[M+HCOO]$^-$
2	11.1	Hosenkoside F	948	949[M+H]$^+$, 971[M+Na]$^+$	947[M−H]$^-$, 993[M+HCOO]$^-$
3	16.3	Hosenkoside C	978	979[M+H]$^+$, 1001[M+Na]$^+$	977[M−H]$^-$, 1023[M+HCOO]$^-$
4	18.2	Hosenkoside K	1140	1141[M+H]$^+$, 1163[M+Na]$^+$	1139[M−H]$^-$, 1185[M+HCOO]$^-$
5	21.7	Hosenkoside G	948	949[M+H]$^+$, 971[M+Na]$^+$	947[M−H]$^-$, 993[M+HCOO]$^-$
6	23.5	Hosenkoside M	1110	1111[M+H]$^+$, 1133[M+Na]$^+$	1109[M−H]$^-$, 1155[M+HCOO]$^-$
7	29.0	Hosenkoside A	978	979[M+H]$^+$, 1001[M+Na]$^+$	977[M−H]$^-$, 1023[M+HCOO]$^-$
8	33.1	Hosenkoside L	948	949[M+H]$^+$, 971[M+Na]$^+$	947[M−H]$^-$, 993[M+HCOO]$^-$

[a] Retention time.
[b] Molecular weight.

(5.02 mg) and M (8.31 mg), and dissolved them in 5 ml methanol. The working standard solution was prepared by diluting the mixed standard solution with methanol to a series of proper concentrations. The standard stock and working solutions were all stored at 4 °C until use.

2.4. Preparation of sample solutions

The dried powders of Impatientis Semen (1.0 g, 60 mesh) were accurately weighed and defatted with petroleum ether (60–90 °C) in a Soxhlet extractor for 4 h. After that, the residue was transferred into a stopper conical flask containing 30 ml 70% ethanol, weighed accurately, immersed at the room temperature for 6 h, and then extracted by ultrasonication for 30 min. Finally, the resultant mixture was made up to the original weight with 70% ethanol. The supernatant was filtered and 15 ml of the successive filtrate was evaporated to dryness with a rotary evaporator. After that, the residue was reconstituted in 2 ml methanol with a volumetric flask. The resultant solution was filtered through a 0.45 μm syringe filter (Type Millex-HA, Millipore, USA), an aliquot of 10 μl of the filtrate was injected into the HPLC system.

2.5. Calibration curves

The working standard solutions were brought to room temperature and an aliquot of 5 μl was injected into HPLC for the construction of calibration curves. At least six concentrations in triplicate were analyzed, and the calibration curves were calculated by linear regression of the double logarithmic plots of the peak area versus the amount of baccharane glycoside injected.

2.6. Limits of detection and quantitation

The limits of detection (LODs) and quantification (LOQs) under the present chromatographic conditions were determined by diluting the standard solution when the signal-to-noise ratios (S/N) of analytes were almost 3 and 10, respectively. The S/N was calculated as the peak height divided by the background noise value. The background noise was measured from the background start to background end time (about a 60-s section of the baseline where no analyte peak occurred and the baseline was stable).

2.7. Precision, repeatability and accuracy

Intra- and inter-day variations were chosen to determine the precision of the developed method. For intra-day variability test, the working standard solutions (at low, medium and high levels of concentration) were analyzed in triplicate three times within one day, whereas for inter-day variability test, the working solutions were examined in triplicate for consecutive 3 days. Variations of the peak area were taken as the measures of precision and expressed as percentage relative standard deviations (R.S.D.).

For repeatability test, five independent analytical sample solutions from the same batch of sample (No.5) were prepared in same procedures noted in Section 2.4. R.S.D. (%) values of the obtained contents of each analyte were used to estimate repeatability.

Standard addition method was performed to evaluate the accuracy of this method. The procedure was summarized as follows: 0.5 g of Impatientis Semen (sample No.5) was defatted with petroleum ether, the residue was transferred into a stopper conical flask, and appropriate amount of working standard solution was then spiked, the residue was subsequently extracted and analyzed as described in Section 2.4. For comparison, an unspiked sample was concurrently prepared and analyzed simultaneously. The recovery was determined by the formula: recovery (%) = (observed amount − original amount)/spiked amount × 100%.

3. Results and discussion

3.1. Development of sample preparation procedure

Since the Impatientis Semen sample contains very high amount of fatty acids (15–20%, w/w), defattening will be a critical step

Table 2
Calibration curves, LODs and LOQs of the eight analytes.

Analyte[a]	Calibration curve[b]	r^2	Linear range (μg/mL)	LOD[c] (μg/mL)	LOQ[d] (μg/mL)
1	$Y = 1.7331X + 2.7893$	0.9986	76.20–762.00	12.70	38.10
2	$Y = 1.7791X + 2.6102$	0.9953	50.48–504.80	14.20	40.38
3	$Y = 1.7571X + 2.8004$	0.9982	52.56–525.60	15.80	37.60
4	$Y = 1.7009X + 2.6810$	0.9988	179.52–1795.20	11.06	43.82
5	$Y = 1.7017X + 2.7636$	0.9979	55.68–556.80	19.35	46.27
6	$Y = 1.7220X + 2.5394$	0.9977	99.72–997.20	29.20	59.80
7	$Y = 1.7306X + 2.7110$	0.9991	77.88–778.80	15.74	45.05
8	$Y = 1.3645X + 2.6868$	0.9984	60.24–602.40	12.20	39.55

[a] The notation for analyte refers to Table 1.
[b] Y and X stand for logarithmic values of peak area and concentration (μg/mL), respectively.
[c] Limit of detection (S/N = 3).
[d] Limit of quantification (S/N = 10).

Table 3
Precisions, repeatabilities and recoveries of the eight analytes.

Analyte[a]	Precision[b] (n = 3)						Repeatability[c] (n = 3)		Recovery (n = 3)	
	Intra-day R.S.D. (%)			Inter-day R.S.D. (%)			Mean Content (mg/g)	R.S.D. (%)	Mean Recovery (%)	R.S.D. (%)
	Low	Medium	High	Low	Medium	High				
1	1.65	2.18	2.41	2.07	2.82	3.34	0.61	3.02	100.4	3.04
2	1.81	1.35	2.69	1.50	3.10	2.90	0.44	2.82	98.8	4.68
3	1.54	1.94	3.31	2.46	1.59	4.47	0.47	2.41	104.7	2.55
4	1.79	0.39	1.31	2.92	2.53	3.56	1.41	3.18	100.8	1.97
5	2.03	0.52	2.26	1.89	4.33	4.14	0.49	4.21	101.9	2.78
6	0.96	1.23	2.09	1.22	2.65	3.95	0.89	4.36	103.2	2.36
7	2.23	1.94	2.46	2.08	2.09	4.87	0.65	3.84	97.4	2.88
8	1.41	2.62	2.59	1.78	2.79	3.52	0.39	2.70	98.1	4.93

[a] The notation for analyte refers to Table 1.
[b] The low, medium and high concentrations of working standard solutions were 0.06, 0.3, and 0.6-fold of stock solution, respectively.
[c] Sample No.5 was analyzed, and contents of each analyte were used to access repeatability.

for the successive extraction and chromatographic separation. It was found that the fatty acids could be extracted exhaustively by petroleum ether in a Soxhlet extractor for 4 h, and in return this clean-up step effectively protected the analytical column and ensured high reproducibility of retention times.

Three important factors, namely, extraction solvents, extraction modes and extraction time which might influence the extraction efficiency of baccharane glycosides, were optimized. The different levels of each factor including extraction solvent (50% methanol, 100% methanol, 70% ethanol and 100% ethanol), extraction mode (ultrasonic extraction versus heat reflux extraction), extraction time (30, 45 and 60 min) after immersion for 6 h were investigated individually by using univariate approach. And three baccharane glycosides, i.e., hosenlosides B, C, and K, were chosen as representatives for evaluation of extraction efficiencies. Eventually, 70% ethanol, ultrasonic extraction for 30 min were adopted because this combination yielded higher extraction efficiencies for the three representatives.

3.2. Optimization of ELSD parameters

Nowadays, ELSD is increasingly being a routine technique connected with HPLC as a quasi-universal detector in the analysis of non-chromophoric and non-volatile natural products, such as triterpenoid saponins [14,17–19], terpene trilactones [20], steroidal alkaloids [21,22], sugars [23], etc. For ELSD applications, nevertheless, selection of operational parameters is essential and should be paid careful attention. S/N was used as the key criteria for optimization of two principal parameters, drift tube temperature and nebulizing gas flow rate.

Similar to the optimization of extraction procedure, hosenlosides B, C, and K were selected as model compounds. The drift tube temperature and nebulizing gas flow rate were evaluated systematically from 90 to 110 °C, and from 2.5 to 3.0 l/min, respectively. Although hosenlosides B, C, and K belonged to different sub-types of baccharane glycosides, their S/N values shared nearly the same tendency toward variations of operational parameters. Finally, maximum S/N values for hosenlosides B, C, and K were obtained by using a drift tube temperature of 98 °C and nitrogen flow of 2.7 l/min.

3.3. Qualitative analysis of eight baccharane glycosides in Impatientis Semen by HPLC–ESI-MSD

The previous chromatographic conditions for determination of two baccharane glycosides in Impatientis Semen by HPLC–ELSD [12] were used as the basis for mobile phase selection and optimization. Unfortunately, the reported gradient elution of methanol–water could not be applied to the separation of eight baccharane glycosides, although five of them obtained baseline separation. Under this circumstance, the gradient elution program was carefully adjusted and after several trials the new gradient program was selected until it permitted the best separation ability for all the analytes investigated.

Table 4
Contents of eight analytes in Impatientis Semen.

Sample No.	Source	Content of baccharane glycosides[a,b] (mg/g)								
		1	2	3	4	5	6	7	8	Sum
1	Qingdao, Shandong	0.98 ± 0.06	0.29 ± 0.01	0.72 ± 0.08	1.66 ± 0.08	0.38 ± 0.02	0.55 ± 0.04	0.95 ± 0.05	0.35 ± 0.03	5.88
2	Bozhou, Anhui	0.86 ± 0.05	0.45 ± 0.03	0.72 ± 0.05	1.88 ± 0.10	0.55 ± 0.08	0.91 ± 0.07	0.87 ± 0.06	0.46 ± 0.02	6.70
3	Wuhan, Hubei	0.78 ± 0.06	0.45 ± 0.08	0.65 ± 0.02	1.69 ± 0.09	0.49 ± 0.03	0.97 ± 0.08	0.69 ± 0.03	0.40 ± 0.02	6.12
4	Bozhou, Anhui	0.77 ± 0.09	0.23 ± 0.02	0.51 ± 0.01	1.09 ± 0.03	0.23 ± 0.01	0.39 ± 0.01	0.89 ± 0.07	0.27 ± 0.04	4.38
5	Bozhou, Anhui	0.61 ± 0.08	0.44 ± 0.05	0.47 ± 0.0	1.41 ± 0.07	0.49 ± 0.08	0.89 ± 0.02	0.65 ± 0.01	0.39 ± 0.03	5.35
6	Chengdu, Sichuan	0.83 ± 0.09	0.57 ± 0.04	0.57 ± 0.07	1.56 ± 0.05	0.58 ± 0.01	0.98 ± 0.10	0.95 ± 0.07	0.61 ± 0.03	6.65
7	Nanjing, Jiangsu	0.99 ± 0.07	0.53 ± 0.03	0.79 ± 0.04	2.06 ± 0.13	0.56 ± 0.02	1.04 ± 0.08	0.89 ± 0.04	0.43 ± 0.01	7.29
8	Nanjing, Jiangsu	0.74 ± 0.05	0.42 ± 0.01	0.58 ± 0.05	1.52 ± 0.09	0.53 ± 0.08	0.75 ± 0.07	0.88 ± 0.09	0.46 ± 0.06	5.88
9	Anguo, Hebei	0.72 ± 0.06	0.22 ± 0.03	0.44 ± 0.02	0.83 ± 0.07	0.21 ± 0.04	0.24 ± 0.04	0.92 ± 0.05	0.29 ± 0.02	3.87
10	Hangzhou, Zhejiang	0.51 ± 0.02	0.23 ± 0.02	0.37 ± 0.01	0.86 ± 0.08	0.22 ± 0.02	0.34 ± 0.01	0.51 ± 0.06	0.19 ± 0.01	3.23
11	Nanjing, Jiangsu	0.76 ± 0.09	0.38 ± 0.03	0.45 ± 0.03	1.18 ± 0.08	0.44 ± 0.01	0.80 ± 0.06	0.62 ± 0.05	0.49 ± 0.02	5.12
12	Nanjing, Jiangsu	0.91 ± 0.08	0.27 ± 0.02	0.80 ± 0.04	1.54 ± 0.09	0.41 ± 0.06	0.52 ± 0.04	0.87 ± 0.05	0.31 ± 0.01	5.63
13	Nanjing, Jiangsu	0.84 ± 0.05	0.55 ± 0.03	0.72 ± 0.09	1.77 ± 0.11	0.65 ± 0.04	0.27 ± 0.01	0.92 ± 0.07	0.52 ± 0.06	6.24
14	Nanjing, Jiangsu	0.76 ± 0.04	0.20 ± 0.02	0.34 ± 0.02	0.73 ± 0.05	0.23 ± 0.01	0.43 ± 0.03	0.63 ± 0.05	0.37 ± 0.02	3.69
Average content[c] (mg/g)		0.79 ± 0.13	0.37 ± 0.13	0.58 ± 0.15	1.41 ± 0.41	0.43 ± 0.15	0.65 ± 0.29	0.80 ± 0.15	0.40 ± 0.11	5.43 ± 1.23

[a] The notation for analyte refers to Table 1.
[b] The values are expressed as mean ± standard deviation (n = 3).
[c] The values are expressed as mean ± standard deviation (n = 14).

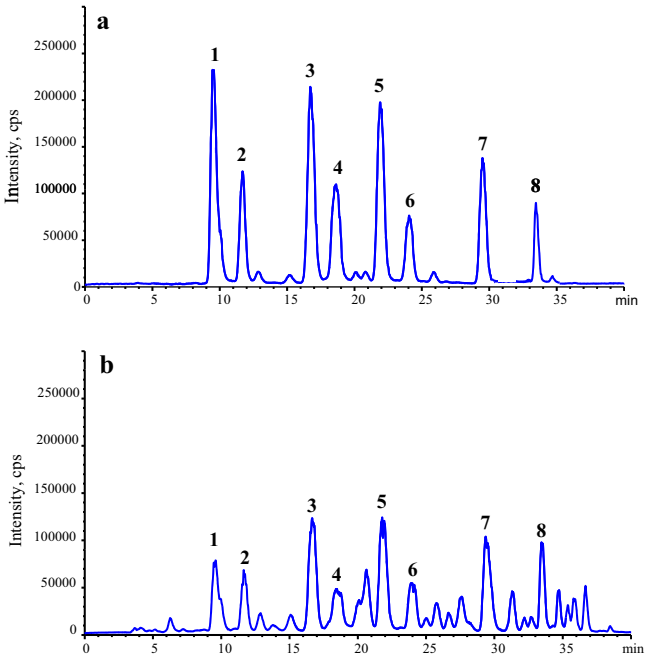


Fig. 2. Representative total ion chromatograms of (a) standard solution and (b) 70% ethanol extract of Impatientis Semen (sample No. 5) analyzed by HPLC–MS in negative ion mode. Peak assignments: 1, hosenkoside B; 2, hosenkoside F; 3, hosenkoside C; 4, hosenkoside K; 5, hosenkoside G; 6, hosenkoside M; 7, hosenkoside A; 8, hosenkoside L.

For the purpose of correct identification, a HPLC–ESI-MS analysis was performed both on standard and sample solutions under the HPLC–ESI-MS conditions described in Section 2.2.2. The mass spectra data of eight baccharane glycosides in both negative and positive ion modes are listed in Table 1. In positive ion mode, the compounds of interest exhibited mainly protonated ions and sodium adduct ions. In negative ion mode, $[M-H]^-$ ions and $[M+HCOO]^-$ ions were the most abundant ions. Finally, eight investigated analytes in the 70% ethanol extract of Impatientis Semen were comprehensively identified by comparing their retention times and MS data with those of reference compounds (Fig. 2).

3.4. Validation of the method

3.4.1. Linearity, LOD and LOQ

As shown in Table 2, acceptable results of the regression analysis, the correlation coefficients (r^2), LODs and LOQs were obtained for all the analytes: all calibration curves showed good linear regression ($r^2 > 0.9953$) within the test ranges; the LODs and LOQs of the eight baccharane glycosides were in the range of 11.06–29.20 μg/ml and 37.60–59.80 μg/ml, respectively.

3.4.2. Precision, repeatability and recovery

The intra- and inter-day variations were less than 5% and the percentage recoveries were in the range of 97–105% with R.S.D. less than 5% (Table 3). The results of the repeatability test shown in Table 3 demonstrated that the developed assay was reproducible (R.S.D. < 5%).

3.4.3. Method robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small changes in method parameters ensuring that the analytical method is reliable during use [24]. In terms of robustness study for an HPLC assay, analytical column is one of the most typical changing vari-

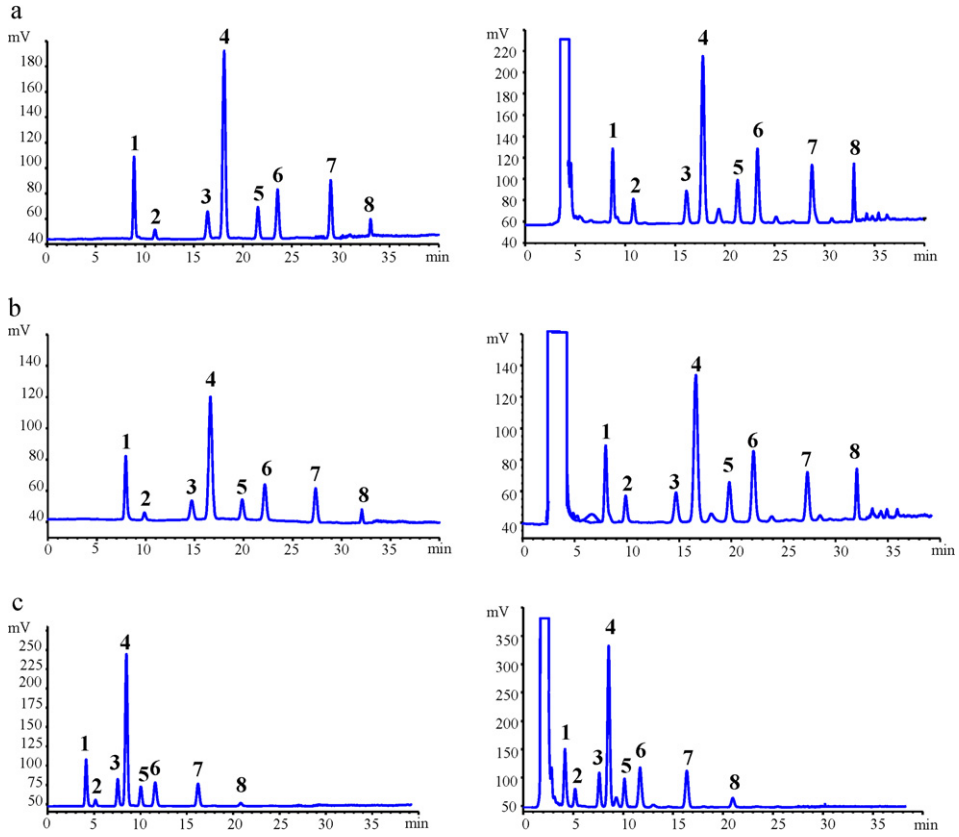


Fig. 3. HPLC–ELSD separation of standard solution (left chromatogram) and sample solution (right chromatogram) using different columns: (a) Shim-pack CLC-ODS; (b) Hedera ODS-3; (c) Hypersil ODS2. Peaks see Fig. 2.

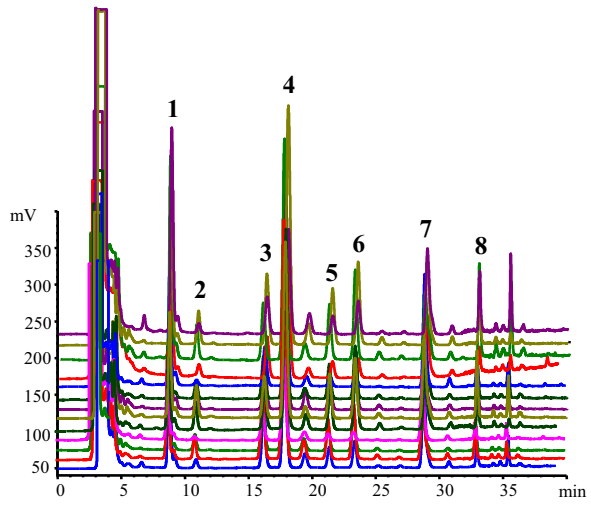


Fig. 4. HPLC–ELSD chromatograms from 70% ethanol extracts of 14 batches of Impatientis Semen collected from different sources. The overlapped chromatograms stand for sample Nos. 1–14 from bottom to up, respectively. Peaks see Fig. 2.

ables. To give some freedom in the method, different columns from various manufacturers were tested, i.e., Shim-pack CLC-ODS (6.0 mm × 150 mm, 5 μm) (Shimadzu Corporation, Kyoto, Japan), Hedera ODS-3 (4.6 mm × 250 mm, 5 μm) (Hanbon Science & Technology, Huai'an, China) and Hypersil ODS2 (4.6 mm × 150 mm, 5 μm) (Elite Corporation, Dalian, China). As shown in Fig. 3, by applying the same chromatographic conditions, baseline separations of the eight baccharane glycosides tested were independent of column brand, demonstrating good robustness of the method.

The above results were considered to be satisfactory for subsequent quantitative analysis of Impatientis Semen commercial samples.

3.5. Quantitative analysis of eight baccharane glycosides in 14 batches of Impatientis Semen commercial samples by HPLC–ELSD

The proposed HPLC–ELSD method was successfully applied to simultaneous determination of eight baccharane glycosides in 14 batches of Impatientis Semen from different sources in China. The quantitative analyses were performed by means of the external standard methods, the analytical results are summarized in Table 4 and the overlaid HPLC–ELSD chromatograms of all samples are presented in Fig. 4. From the view of average content, hosenkoside K (average concentration of 1.41 mg/g) was the predominant constituent among the eight baccharane glycosides in Impatientis Semen, followed by hosenkosides B and A, and hosenkosides F, C, G, M and L were the minor constituents. On the other hand, from the view of total content (levels from 3.23 to 7.29 mg/g), the result indicated the inhomogeneous quality of the 14 batches of commercial samples, which might be ascribed to multiple factors such as environmental conditions, harvesting time, primary process, etc. Thus, the establishment of a quality control method so as to ensure its efficacy and safety is essential.

4. Conclusion

Just like other Chinese herbal medicines, the effective constituents of Impatientis Semen still remain unknown. Under this circumstance, the very limited distribution of baccharane glycosides in plants gives this type of metabolites the priority over other compounds as chemical markers for quality control of Impatientis Semen. In this study, a HPLC coupled with ESI-MSD and ELSD method has been developed for simultaneous qualification and quantification of eight baccharane glycosides in Impatientis Semen. This method is validated for good accuracy, repeatability, precision and robustness, and could be used to evaluate the quality of Impatientis Semen.

Acknowledgements

This work was financially supported by Program for Changjiang Scholars and Innovative Research Team in University (No. IRT0868) and Program for New Century Excellent Talents in University (NCET-07-0850) awarded to Hui-Jun Li, National Science and Technology Major Project 'Creation of Major New Drugs' from China (No. 2009ZX09502-020) to Ping Li, and the Research Program of Chinese Pharmacopoeia (2010 Edition) (No. YD-061).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2010.10.014.

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