Talanta 120 (2014) 167–172

Contents lists available at ScienceDirect

Talanta

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

Rapid determination of eight bioactive alkaloids in *Portulaca oleracea* L. by the optimal microwave extraction combined with positive–negative conversion multiple reaction monitor (+/−MRM) technology

Xiao Liang [a,b], Jinlong Tian [a,b], Lingzhi Li [a,b], Jun Gao [c], Qingyi Zhang [c], Pinyi Gao [a,b], Shaojiang Song [a,b,*]

[a] Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China
[b] School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China
[c] Liaoning Chengda Biotechnology Co., Ltd., Shenyang 110179, China

ARTICLE INFO

Article history:
Received 1 October 2013
Received in revised form
18 November 2013
Accepted 24 November 2013
Available online 3 December 2013

Keywords:
UPLC–(+/−)MRM
Portulaca oleracea L.
Alkaloids
Microwave extraction
Quantitative analysis

ABSTRACT

A rapid and reliable microwave extraction and the triple quadrupole-linear ion trap mass spectrometry method was developed and validated for the determination of eight alkaloids in *Portulaca oleracea* L. The optimal microwave extraction (MWE) condition was performed at 60 °C for 12 min with ethanol–water (70:30, v/v) as the extracting solvent, and the solvent to solid ratio was 30:1. The alkaloids were first detected simultaneously by electrospray ionization tandem mass spectrometry under positive–negative conversion multiple reaction monitor ((+/−)MRM) technique. With investigating three different columns, samples were separated in only 8 min on a Waters ACQUITY UPLC HSS T3 (50×2.1 mm^2, 1.8 μm) column using acetonitrile and formic acid–water solution as a mobile phase with a flow rate at 0.2 mL/min. All calibration curves showed good linearity ($r > 0.999$) within the test ranges. The method developed was validated with acceptable sensitivity, intra- and inter-day precision, reproducibility, and extraction recoveries. It was successfully applied to the determination of eight alkaloids in *Portulaca oleracea* L. from different sources and different harvest periods. The method also provide a reference for extraction and determination of alkaloids in other complex systems.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Portulaca oleracea L. (*P. oleracea* L., purslane) is an annual green herbaceous plant widespread in temperate and tropical regions of the world [1]. It is extensively used not only as an edible plant [2,3], but also as a traditional Chinese herbal medicine. Phytochemical investigations of purslane revealed that it mainly contained a variety of alkaloids, including aurantiamideacetate (1), aurantiamide (2), 1,5-dimethyl-6-phenyl-1,6,3,4,-tetrahydro-1,2,4-2(1H)-triazin (3), trollisine (4), cyclo(L-tyrosinyl-L-tyrosinyl) (5), 3,5-bis(3-methoxy,4-hydroxyphenyl)-5,6-dihydr-o,2(1H)-pyridinone (6), N-feruloyl normetanephrine (7), N-trans-feruloyl tyramine (8) (Fig. 1) [4,5]. Due to the presence of these alkaloids, the extracts of purslane have been reported to possess various pharmacological activities, such as antioxidant, anti-platelet, anti-inflammatory, and antitumor effects [6–14]. Therefore, the detection of these alkaloids is essential for understanding the pharmacological basis of their activity as well as enhancing the product quality control of purslane.

In conventional methods, the extraction of alkaloids from herbs may be carried out by means of heat reflux extraction (HRE), infusion extraction (IE), or ultrasound extraction (USE). However, with the increasing energy prices and environmental concerns to reduce CO_2 emissions, there is compelling interest in developing new techniques for the extraction of natural product that use less energy with minimal solvent volumes. Recently, microwave extraction (MWE) had been advanced as a means to separate bioactive compounds from plant matrices [15–20]. In this paper, the extraction yields of eight alkaloids using four distinct methods were compared to microwave extraction.

There have been no reports in the literature involving the quantitative determination of alkaloids in purslane, probably due to inherent structural similarity of alkaloids and complex sample matrices, which causes interference in detection. As a separation and detection method, ultra performance liquid chromatography

* Corresponding author at: Key Laboratory of Structure-Based Drug Design & Discovery, Ministry of Education, Shenyang Pharmaceutical University, Shenyang 110016, China. Tel./fax: +86 24 2398 6088.
E-mail address: songsj99@gmail.com (S. Song).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.11.067

coupled with mass spectrometry (UPLC–MS) is recognized for its short analysis time, high throughput, greater resolution, higher peak capacity, less solvent consumption, and extremely high sensitivity. Furthermore, tandem mass spectrometry (MS/MS) with multiple reaction monitor (MRM) provides an advanced degree of certainty in analyte identification due to its high level of selectivity. The UPLC–MRM techniques enable the simultaneous determination of alkaloids at relatively low concentration levels.

The sensitivity of detection of alkaloids is usually higher with the positive ion mode, so the reported detection method is often in this mode [21,22]. However, in this experiment, the detection of five alkaloids has higher sensitivity in the negative ion mode. In order to obtain higher sensitivity, eight alkaloids were simultaneously determined under positive–negative conversion multiple reaction monitor ((+/−)MRM). Not only was higher sensitivity and specificity obtained but also the analysis time was significantly shortened. Thereafter, the MWE–UPLC–(+/−)MRM method was successfully used to extract and quantify alkaloids from ten different natural sources of purslane. As such, this approach appears promising for use in the quality control of purslane. This method also has great reference value for the extraction and determination of alkaloids in other complex systems.

2. Materials and methods

2.1. Chemicals

The standards of compounds 1–8 were isolated and purified from *Portulaca oleracea* L. in the authors' laboratory, and their identities were confirmed by 1D-NMR, 2D-NMR, and MS analyses. The purity of the compounds was determined to be more than 98% by normalization of the peak areas detected by UPLC.

2.2. Reagents

HPLC grade acetonitrile (Fisher, USA) was used for LC–MS analysis. Formic acid (50% in water) was HPLC grade and purchased from Sigma-Aldrich (USA), and ultra-pure water was purified using a Milli-Q system (Millipore, USA). Analytical-grade methylene dichloride, acetone, ethyl acetate, methanol and ethanol were obtained from Tianjin Chemical Corporation (PR China).

2.3. Materials

Samples of purslane were collected from different regions in China; Liaoning (Shenyang), Jilin (Baicheng), Heilongjiang (Yushu), Anhui (Wuhu), Sichuan (Leshang), Hubei (Yichang), Hebei (Cangzhou). Hebei samples 1–4 were collected at March, June, August, and November, correspondingly. They were identified by Professor Jincai Lu, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University (Shenyang, China). The voucher specimens of these samples were deposited in the Laboratory of Structure-Based Drug Design & Discovery, Shenyang Pharmaceutical University (Shenyang, China).

2.4. Extraction procedures

The overground parts of purslane (10 g) were extracted with different techniques (heat reflux extraction, infusion extraction, ultrasound extraction, and microwave extraction) individually for obtaining the optimized extraction procedure as described in detail below.

2.4.1. Heat reflux extraction (HRE)

HRE was conducted in a water bath at 80 °C. The materials (10 g) was placed into a 250 mL glass flask with 100 mL methanol and extracted twice with 2 h cycles.

2.4.2. Infusion extraction (IE)

The materials (10 g) was placed into a 250 mL conical flask and extracted with 100 mL methanol twice with 2 h cycles at room temperature.

2.4.3. Ultrasound extraction (UE)

The materials (10 g) was weighed and put into a conical flask. Then, 100 mL methanol was added to the flask and extracted in an ultrasonic bath (Kunshan Ultrasonic Instrument Co. Ltd., China) at 60 °C with two 30 min cycles with ultrasonic frequency of 40 KHz.

2.4.4. Microwave extraction (MWE)

MWE was performed with an Ethos A Microwave Extraction System (Milestone, Italy). The materials (10 g) were placed into closed vessels, and the optimum extraction conditions (extraction solvent, extracting temperature, extraction time, solvent to solid ratio) were studied systematically through orthogonal experiment in this work.

All extracts were filtered. The filtrate was evaporated to dryness and the residue was dissolved in acetonitrile in a 50 mL volumetric flask, which was then diluted tenfold with acetonitrile–water (v/v, 1:1). The solution was filtered through a 0.22 μm organic microporous membrane (Agilent, USA), the injection volume was 2 μL.

2.5. Preparation of standard solutions

A mixed stock solution was prepared in acetonitrile (the concentrations of compound 1–8: 166.5, 36.90, 37.00, 1254, 9.760, 255.6, 4640, 3355 ng/mL). A series of working standard solutions were prepared by successive dilution of the stock solution with acetonitrile–water (v/v, 1:1). The concentration ranges of compound 1–8: 3.330–166.5 ng/mL, 0.369–36.90 ng/mL, 0.074–37.00 ng/mL,

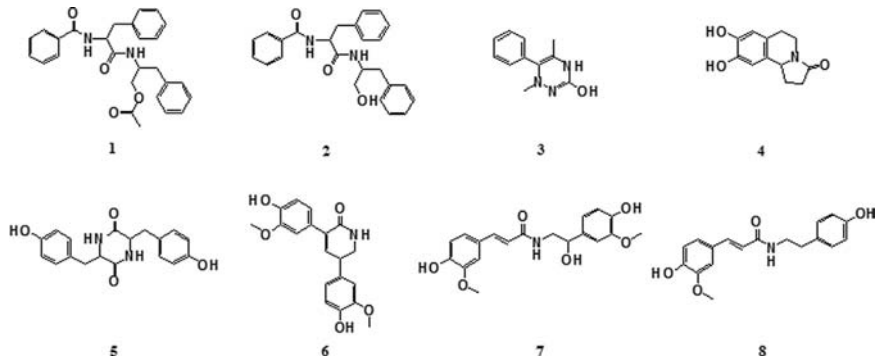

Fig. 1. Structures of the eight alkaloids.

20.90–1254 ng/mL, 0.244–9.760 ng/mL, 0.852–255.6 ng/mL, 23.20–4640 ng/mL, 67.10–3355 ng/mL. All the solutions were stored at 4 °C until use.

2.6. UPLC conditions

Final separation was performed on a Waters ACQUITY UPLC™ system (Waters Co., MA, USA) equipped with an autosampler, a column compartment, and a Waters ACQUITY UPLC HSS T3 (50×2.1 mm^2, 1.8 μm) column at 30 °C. The flow rate was 0.2 mL/min. The other two columns were also evaluated for separation; a Waters ACQUITY UPLC BEH C18 (50×2.1 mm^2, 1.7 μm) and a Waters ACQUITY UPLC BEH Shield RP18 (50×2.1 mm^2, 1.7 μm). With these columns, elution was achieved with a gradient program of acetonitrile (A) and water–formic acid (100:0.05, v/v. B): 0–5 min, 10–30% A; 5–7 min, 30–100% A; 7.5 min, 100% A; and 8 min, 10% A.

2.7. MS conditions

The mass spectrometer was operated using a 4000 Qtrap mass spectrometer (Applied Biosystems/MDS SCIEX, Foster City, CA, USA). Applied Biosystems/MDS Sciex Analyst software (versions 1.5.1) was used for the data acquisition and processing. The ion source was an electrospray ionization (ESI) in positive and negative ion mode. The ion spray voltage was set to 4.5 and −4.5 kV, and the turbo spray temperature was maintained at 550 °C. Both nebulizer gas (gas 1) and heater gas (gas 2) pressures were set at 50 psi. The curtain gas was maintained at 30 psi. Nitrogen was used as nebulizer and auxiliary gas. The precursor-to-product ion pair, the optimized collision cell exit potential (CXP), declustering potential (DP), and collision energy (CE) for each analyte are provided in Table 1.

3. Results and discussion

3.1. Optimization of extraction procedure

To obtain satisfactory extraction efficiency, four types of extraction methods and different solvents were evaluated by the sample from Hebei 2.

3.1.1. Selection of the extraction method

The extraction efficiency for the eight alkaloids was evaluated by HRE, IE, UE, and MWE. The results indicated that the yield of total alkaloids using MWE with solvent:methanol, extracting temperature of 60 °C, solvent to solid ratio of 20 mL/g, and extraction time of 10 min was much higher than those achieved by use of HRE, IE, and UE (Fig. 2A). MWE also had the advantage of a shorter extraction time. Therefore MWE was selected as the extraction method in the subsequent experiments.

3.1.2. Selection of extraction solvent

The evaluation of different solvents (methylene dichloride, ethyl acetate, acetone, methanol, ethanol, ethanol–water (30:70, v/v), ethanol–water (70:30, v/v), and water) (Fig. 2B) was made on the basis of the total content of eight alkaloids from purslane. It was showed that ethanol–water (70:30, v/v) was the most effective solvent.

3.1.3. Optimization of MWE conditions

An orthogonal experiment was employed in order to optimize the MWE conditions. There are many factors affecting the extraction yields including the impact of concentration of ethanol (A), extracting temperature (B), solvent to solid ratio (C), and extraction time (D). The experiment factors that were investigated, corresponding levels, and orthogonal designs $L_9(3^4)$ are presented in Table 2. The total content of the eight alkaloids in purslane was used as the criterion for selecting the optimal extraction conditions.

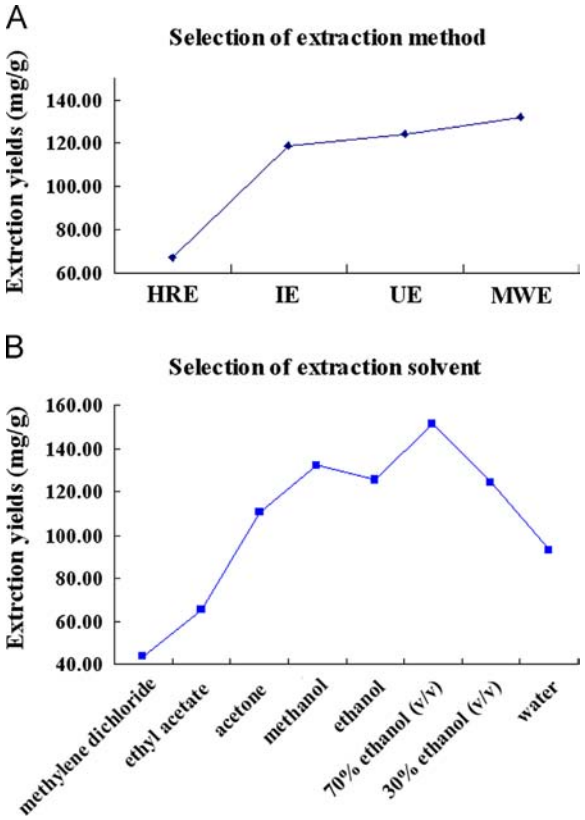

Fig. 2. Evaluation of the extraction procedures. (A) Evaluation of extraction methods (MWE: methanol, 60 °C, 10 min, 20 mL/g, other extraction methods reference on "2.4."), (B) Evaluation of different solvents (extraction method: MWE, 60 °C, 10 min, 20 mL/g).

Table 1
Retention time, MRM transitions, declustering potential (DP), collision energy (CE) and collision cell exit potential (CXP) of the eight alkaloids in purslane.

Compound no.	Compounds	MW	Retention times (min)	MRM	DP (V)	CE (eV)	CXP (V)
1	Aurantiamideacetate	444	7.31	445.3/194.0	80	19	12
2	Aurantiamide	402	6.99	403.3/152.2	70	14	6
3	1,5-dimethyl-6-phenyl-1,6,3,4,-tetrahydro-1,2,4-2(1H)-triazin	203	1.63	204.1/56.1	59	46	10
4	trollisine	219	2.41	218.0/162.0	−103	−41	−6
5	Cyclo(L-tyrosinyl-L-tyrosinyl)	326	2.28	325.1/219.0	−77	−24	−3
6	3,5-bis(3-methoxy,4-hydroxyphenyl)-5,6-dihydro,2(1H)-pyridinone	341	4.67	340.1/324.9	−104	−27	−6
7	N-feruloyl normetanephrine	359	4.53	358.0/340.1	−95	−21	−8
8	N-trans-feruloyl tyramine	313	6.04	312.0/147.9	−114	−40	−6

Table 2
Factors in the orthogonal design for the optimization of MWE extraction conditions.

Run no.	A: concentration of ethanol (%)	B: extracting temperature (°C)	C: solvent to solid ratio (mL/g)	D: extraction time (min)	The total content of 8 alkaloids (μg/g)
1	60	60	20	8	145.2
2	60	70	30	10	142.3
3	60	80	40	12	138.9
4	70	60	30	12	152.3
5	70	70	40	8	147.2
6	70	80	20	10	138.5
7	80	60	40	10	148.6
8	80	70	20	12	143.4
9	80	80	30	8	140.9
k_1[a]	142.1	148.7	142.4	144.4	
k_2	146.0	144.3	145.2	143.1	
k_3	144.3	139.4	144.9	144.9	
Range	3.9	9.3	2.8	1.7	
Optimized scheme	A2	B1	C2	D3	

[a] *k* represents the average values of the same level of the same factor.

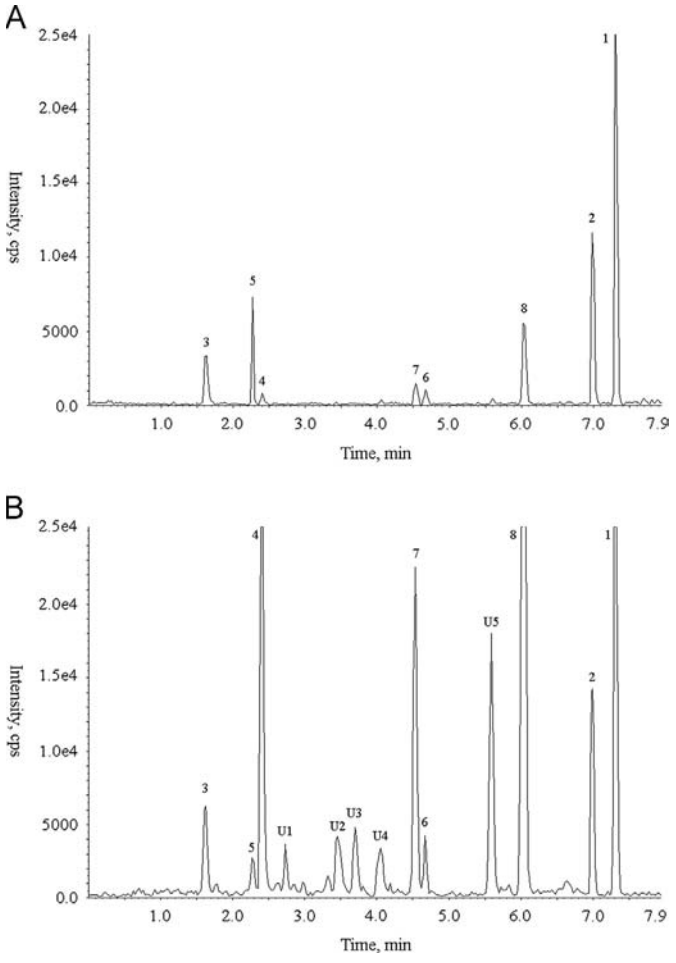

Fig. 3. Total ion chromatorgraphy of a standard mixture of eight alkaloids (A) and an extract from a purslane sample (B). Peaks: (1) aurantiamideacetate; (2) aurantiamide; (3) 1,5-dimethyl-6-phenyl-1,6,3,4,-tetrahydro-1,2,4-2(1H)-triazin; (4) trollisine; (5) cyclo(L-tyrosinyl-L-tyrosinyl); (6) 3,5-bis(3-methoxy,4-hydroxyphenyl)-5,6-dihydr-o,2(1H)-pyridinone; (7) N-feruloyl normetanephrine; and (8) N-trans-feruloyl tyramine; U1, U2 U3 U4, and U5 are unknown components in purslane.

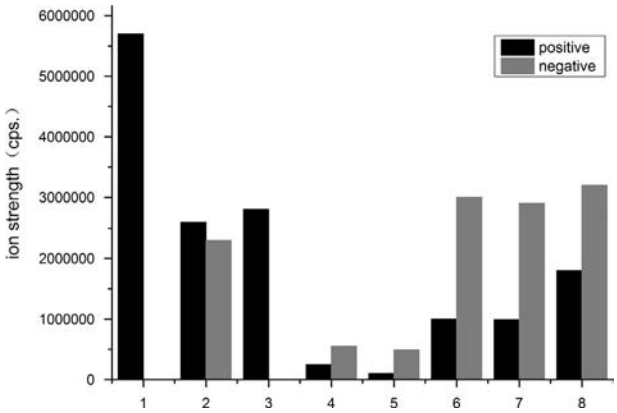

Fig. 4. The ion strength of the eight alkaloids in the positive and negative ion mode.

According to the statistical analysis shown in Table 2, the largest range of the three levels was 9.3 for factor B, and the smallest was 1.7 for factor D. This suggested that factor B was the primary factor in the extraction conditions of alkaloids in purslane. The second level of factor A had the largest average value (k_2=146.0) compared to the other two levels. This indicated that the second level had the best condition for factor A. By analogy, the optimal conditions of MWE were 70% ethanol as extraction solvent, extracting temperature of 60 °C, extraction time of 12 min, solvent to solid ratio of 30 mL/g.

3.2. Optimization of UPLC conditions

Different kinds of mobile phases with a variety of modifiers were tested. Acetonitrile was found to have a shorter duration of analysis in comparison to methanol. Several mobile phase additives, including formic acid, glacial acetic acid, ammonia and ammonium acetate, were used to optimize resolution, peak shape, and mass spectrometric ionization intensity of the analytes. Finally, 0.05% formic acid was chosen as the mobile phase additives, which has higher ionic strength and better separation efficiency.

In comparing chromatographic columns, the best resolution among the alkaloids was achieved by Waters ACQUITY UPLC HSS T3 column (50 × 2.1 mm^2, 1.8 μm). (Fig. 3) The excellent retention as well as the good column efficiency may be attributed to the triple bond of C18 alkyl, which is used for T3 bonding technology. The stationary phase is better reserved for hydrophilic compounds, especially the alkaloids. Along with these advantages, improved column shelf life, peak shape and stability were found.

Table 3
Calibration parameters, precision, accuracy, reproducibility and average recoveries of UPLC–(+/−)MRM analysis for the eight alkaloids of purslane.

Alkaloids	Linear regression data			LOD (ng/ml)	LOQ (ng/ml)	Intra-day precision (RSD%)	Inter-day precision (RSD%)	Reproducibility (RSD%)	Recovery	
	Linear range (ng/ml)	Regression equation	r						Mean (%)	R.S.D. %
1	3.330–166.5	$y=66{,}000x+638$	0.9990	0.011	0.022	2.34	2.39	2.61	97.9	2.47
2	0.369–36.90	$y=8360x+4030$	0.9993	0.037	0.111	1.73	1.95	2.16	100.2	1.93
3	0.074–37.00	$y=3510x+600$	0.9997	0.037	0.074	1.86	2.21	2.33	100.1	2.45
4	20.90–1254	$y=845x-63.2$	0.9998	0.836	2.090	1.55	2.01	2.07	99.2	2.01
5	0.244–9.760	$y=3290x+0.572$	0.9993	0.122	0.244	1.42	1.87	1.99	101.2	1.70
6	0.852–255.6	$y=704x+834$	0.9993	0.426	0.852	2.19	2.58	2.89	99.7	2.40
7	23.20–4640	$y=600x+187$	0.9998	0.773	3.090	1.46	1.57	2.03	97.4	1.96
8	67.10–3355	$y=964x-369$	0.9995	0.672	2.240	0.88	0.98	1.83	100.4	1.78

Table 4
The contents of the eight alkaloids in purslane from different sources ($n=3$, μg/g).

Alkaloids	Sources									
	Liaoning	Jilin	Heilongjiang	Anhui	Sichuan	Hubei	Hebei 1	Hebei 2	Hebei 3	Hebei 4
1	2.105	0.730	1.680	2.145	1.620	2.165	6.750	2.170	0.735	1.745
2	0.525	0.185	0.535	0.590	0.600	0.550	1.645	0.427	0.190	0.805
3	0.008	0.006	0.034	0.024	[a]	0.006	0.038	0.037	0.042	0.048
4	56.50	15.70	24.15	53.00	32.40	51.00	23.25	28.10	15.70	28.20
5	0.500	0.022	0.123	0.434	0.145	0.363	0.339	0.026	0.030	0.049
6	9.250	6.250	9.500	8.900	8.300	12.50	0.990	7.800	5.750	11.50
7	149.5	108.0	104.5	148.5	149.0	136.0	23.15	32.05	110.5	188.5
8	114.5	86.50	71.00	86.50	111.0	88.50	46.20	81.00	109.0	156.0
Total	332.9	217.4	211.5	300.1	303.1	291.1	102.4	151.6	242.0	386.9

[a] Not detected.

3.3. Optimization of MS conditions

With the ESI source of the mass spectrometer, compounds 1–3 had high sensitivity in the positive ion mode, but compounds 4–8 had little or no response sensitivity. In contrast, these latter compounds had a higher sensitivity in the negative ion mode (Fig. 4). Given this discrepancy, a positive–negative conversion multiple reaction monitor was used to determine the content of all eight alkaloids. The precursor-to-product ion pair, declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) for each analyte were also optimized (Table 1).

3.4. Method validation

With the ESI source of the mass spectrometer, compounds 1–3 had high sensitivity in the positive ion mode, but compounds 4–8 had little or no response sensitivity. In contrast, these latter compounds had a higher sensitivity in the negative ion mode (Fig. 4). Given this discrepancy, a positive–negative conversion multiple reaction monitor was used to determine the content of all eight alkaloids. The precursor-to-product ion pair, declustering potential (DP), collision energy (CE), and collision cell exit potential (CXP) for each analyte were also optimized (Table 1).

The intra- and inter-day precision for each compound were determined by the mixed standard solution on the same day and on three sequential days, respectively. Based on the results, the present method was found to have acceptable precision and accuracy, with the intra-day precision RSD values between 0.88% and 2.34%, and the inter-day precision RSD values between 0.98% and 2.58%. The repeatability was assessed using five different solutions prepared from the same sample (Hebei 2). The RSDs of repeatability were less than 2.89%. The recovery test was performed using the standard addition approach. Accurately weighted 0.5 g of purslane (Hebei 2) which the contents of the eight alkaloids were known. Nine samples were spiked with three concentration levels of the standards (approximately equivalent to 0.5, 1.0, and 1.5 times of the concentration of the sample). The spiked samples were extracted following the optimum procedure for sample preparation as described above. The recovery was determined by comparing the amount of analyte added to the sample and the amount of analyte detected during UPLC–(+/−) MRM analysis. The recoveries of the eight analytes were in the range of 97.4–101.2%, with RSDs less than 2.47%. The results from validation of the method showed satisfactory linearity, sensitivity, precision, reproducibility, and average recovery for the eight alkaloids (Table 3).

3.5. Quantitative analysis

The MWE–UPLC–(+/−)MRM method, which was developed above, was applied to quantify the eight alkaloids in different purslane samples from ten different batches (seven Provinces). The quantitative analyses were performed by means of the external standard method. Each sample was extracted and analyzed in triplicate. The contents of eight constituents in purslane from different sources are listed in Table 4. The quantitative analytical results indicated that the compounds **7** and **8** were abundant in purslane, and the content of compound **3** was lower than other alkaloids. Our results demonstrate that the content of eight alkaloids are significantly different among the ten batches. Generally, the variations might be based on internal factors such as genetic variation and plant origin as well as external factors including season, cultivation, harvest time, storage, and processing of the herb.

4. Conclusions

Compared with routine analytical methods, which usually takes hours to extract and identify eight kinds of alkaloids, the

MWE–UPLC–(+/−)MRM method only required 12 min to extract eight different alkaloids and 8 min for identification. This first time use of MWE for extraction of alkaloids in purslane represents a significant improvement in methodology. The application of (+/−)MRM technique for simultaneous determination of multiple alkaloids also provides an important advantage over currently available approaches. The validation data indicates that this time efficient method can be reliably applied to determine the contents of eight alkaloids in 10 batches of purslane. The ability to quantify the variability in the content of these pharmacologically active constituents in purslane is essential for the quality assessment and can provide a basis for understanding the pharmacological activity and thereby useful guidance in the clinical use of purslane. The proposed method also offers a practical and efficient approach for extraction and determination of alkaloids in other complex systems.

Acknowledgments

The present study was supported by a Key Laboratory of Structure-Based Drug Design & Discovery from the Ministry of Education of China.

We appreciate Professor Timothy Wiedmann from Pharmaceutics, University of Minnesota, for revising this paper.

References

[1] H. Zhu, Y. Wang, H. Liang, Q. Chen, P. Zhao, J. Tao, Talanta 81 (2010) 129–135.
[2] A.N. Rashed, F.U. Afifi, A.M. Disi, J. Ethnopharmacol. 88 (2003) 131–136.
[3] Y.Y. Lim, E.P.L. Quah, Food Chem. 103 (2007) 734–740.
[4] L. Xiang, D. Xing, W. Wang, R. Wang, Y. Ding, L. Du, Phytochemistry 66 (2005) 2595–2601.
[5] Z.J. Yang, C.J. Liu, L. Xiang, Y.N. Zheng, Phytother Res. 23 (2009) 1032–1035.
[6] D. Liu, T. Shen, L. Xiang, Helv. Chim. Acta. 94 (2011) 497–501.
[7] Z. Yang, C. Liu, L. Xiang, Y. Zheng, Phytother Res. 23 (2009) 1032–1035.
[8] Y.C. Tsai, S.Y. Chiang, M. El-Shazly, C.C. Wu, L. Beerhues, W.C. Lai, S.F. Wu, M.H. Yen, Y.C. Wu, F.R. Chang, Food Chem. 140 (2013) 305–314.
[9] R. Suhas, D. Channe Gowda, Chem. Biol. Drug Des. 79 (2012) 850–862.
[10] K. Chan, M.W. Islam, M. Kamil, R. Radhakrishnan, M.N.M. Zakaria, M. Habibullah, A. Attas, J. Ethnopharmacol. 73 (2000) 445–451.
[11] M.O. Ullah, M. Haque, K.F. Urmi, A.H. Zulfiker, E.S. Anita, M. Begum, K. Hamid, S.J. Uddin, Asian Pac. J. Trop. Biomed. 3 (2013) 1–7.
[12] A.N. Rashed, F.U. Afifi, A.M. Disi, J. Ethnopharmacol. 88 (2003) 131–136.
[13] Y.Y. Lim, E.P.L. Quah, Food Chem. 103 (2007) 734–740.
[14] H. Shen, G. Tang, G. Zeng, Y.J. Yang, X.W. Cai, D.L. Li, H.C. Liu, N.X. Zhou, Carbohydr. Polym. 93 (2013) 395–400.
[15] Q. Zhou, Y. Liu, X. Wang, X. Di, Talanta 99 (2012) 932–938.
[16] S.N. Tan, J.W. Yong, C.C. Teo, L. Ge, Y.W. Chan, C.S. Hew, Talanta 83 (2011) 891–898.
[17] W. Ma, Y. Lu, R. Hu, J. Chen, Z. Zhang, Y. Pan, Talanta 80 (2010) 1292–1297.
[18] Z. Jiang, F. Liu, J.J. Goh, L. Yu, S.F. Li, E.S. Ong, C.N. Ong, Talanta 79 (2009) 539–546.
[19] F.L. Hu, C.H. Deng, Y. Liu, X.M. Zhang, Talanta 77 (2009) 1299–1303.
[20] Z.B. Li, D.N. Huang, Z.X. Tang, C.H. Deng, X.M. Zhang, Talanta 82 (2010) 1181–1185.
[21] Y. Shen, C. Han, Y. Jiang, X. Zhou, Z. Zhu, X. Lei, Talanta 84 (2011) 1026–1031.
[22] F. Liu, S.Y. Wan, Z. Jiang, S.F. Li, E.S. Ong, J.C. Osorio, Talanta 80 (2009) 916–923.