Acta Chromatographica 26(2014)4, 727–737
DOI: 10.1556/AChrom.26.2014.4.13

Multi-Composition Analysis in *Radix Aconiti Lateralis* by Single Marker Quantitation

H. YANG[1,†], H. YIN[1*,†], Z. WANG[2], L. FAN[1], Q. LI[1], AND X. ZHU[1]

[1]College of Pharmaceutical Sciences, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China
[2]Traumatology Institute, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, China
*Corresponding author: Hua Yin, The Standardization of Chinese Materia Medica in Zhejiang, Zhejiang Chinese Medical University, Hangzhou, PR China.
E-mail: maryyinhua@163.com
†These authors contributed equally to this work

Summary. Traditional Chinese medicine (TCM) has been widely used in many countries for thousands of years and played an indispensable role in the prevention and treatment of diseases, especially the complicated and chronic ones. However, the application of TCM in diseases is still not fully recognized by people around the world, the main reason is that Chinese herb is a very complex mixture containing hundreds of different components. Thus, it is essential to make quality control and evaluation of TCM. A new quality evaluation method, quantitative analysis of multi-components by single marker (QAMS), was developed to the quality control of alkaloids in TCM, a case study on *Radix aconiti lateralis,* named Fuzi in Chinese. Six alkaloids, including aconitine, hypaconitine, mesaconitine, benzoylaconine, benzoylmesaconine, and benzoylhypaconine, were selected as main components to evaluate the quality of *Radix aconiti lateralis*. The feasibility and accuracy of QAMS were checked by the external standard method, and various high-performance liquid chromatographic instruments and chromatographic conditions were investigated to verify its applicability. Using aconitine as the internal reference substance and the content of aconitine was calculated according to relative correction factors by high-performance liquid chromatography. The present results showed that there was no significant difference observed between the QAMS method and the external standard method with the relative average deviations less than 3.0%, and QAMS is an effective way to control the quality of herbal medicines and seems to be a convenient and accurate approach to analyze multi-composition when reference substances are unavailable.

Key Words: quantitative analysis of multi-components by single marker, relative correction factor, *Radix aconiti lateralis*, quality control

Introduction

The lateral roots of *Aconitum carmichaeli* Debx is named "Fuzi," which is widely distributed across Asia and North America. It has been used to warm meridians to dissipate cold and treat rheumatic diseases for over two thousand years, and it is called the first medicine for restoring Yang to rescue collapse [1–3]. According to the different ways of processing, it is com-

0231–2522 © 2013 Akadémiai Kiadó, Budapest

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

monly converted into Fuzi preparations, including Heishunpian (HSP), Baifupian (BFP), Yanfuzi (YFZ), which are recorded in Chinese Pharmacopoeia (2010 editions) [4]. Four major groups of alkaloids were found in the roots of *Aconitum carmichaeli*, which are monoester diterpene alkaloids (MDAs), amine diterpenoid alkaloids (ADAs), diester diterpene alkaloids (DDAs), and lipoalkaloids. The toxicity of *Aconitum carmichaeli* mainly derives from the DDAs, including aconitine, mesaconitine, and hypaconitine [5–8]. As we know, traditional Chinese medicine (TCM) herbal processing approaches, namely, "Paozhi," by means of the transformation of secondary plant metabolites, help to reduce the toxicity of the crude drug and might exert a large maximal therapeutic efficacy with minimal adverse effects. As a traditional processing form, Paozhi could remarkably reduce the toxicity of Fuzi by decomposing the DDAs to the relatively lower toxic MDAs [9].

Alkaloids can cause cardiotoxicity and neurotoxicity. Although the Chinese Pharmacopoeia (2010 editions) has made a specific limitation of total aconite alkaloids and DDAs, the total alkaloids cannot represent a single alkaloid for efficacy and toxicity between alkaloids since the function of each alkaloid is quite different. Even if the total quantity of two types of alkaloids (DDAs and MDAs) remains the same, but with different ratios, they can exhibit different toxicity. Therefore, the determination of DDAs and MDAs is very important to the toxicity and pharmacodynamics evaluation of alkaloids, not only to ensure the safety and effectiveness but also to reduce the incidence of clinical poisoning. There were some reports about the quality control of Fuzi with the determination of six or three alkaloids in plants and herbal preparation, even *in vivo* or in body fluids [10–16], but the methods mentioned in reports showed no reproducibility in practical work and required six or three kinds of reference alkaloids whose prices are very expensive and also hard to isolate. In this experiment, a new quality evaluation method, quantitative analysis of multi-components by single marker (QAMS) [17–19], was established and validated with Fuzi. Aconitine was made as internal reference material, and the contents of other alkaloids were calculated with the relative correction factor based on aconitine. This method, which is simple and practical, can achieve multi-index components quality control by measuring one single component.

Experiments and Results

Chemicals and Materials

Acetonitrile and methanol (HPLC grade) were purchased from Merck (Germany). Deionized water was prepared using a PURELAB Classic

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

(ELGA, UK) ultrapure water system. Other chemicals, except as noted, were of analytical grade. All solvents and solutions were filtered through a Milipore filter (0.45 μm) before use.

Fuzi and its processed products were collected from Jiangyou in Sichuan province (South, China) in July 2012 and identified by Prof. Rusong Zhang of Zhejiang Chinese Medical University. Reference substances of aconitine, mesaconitine, and hypacomitine were purchased from National institutes for Food and Drug Control; reference substances of benzoylaconitine, benzoylmesaconine, and benzoylhpacoitine were purchased from Yip Reese Biological Products Co. Ltd. (Beijing, China), HPLC purity >98%.

Sample Preparation

Standard Solution Preparation

Primary stock standard solutions of the six compounds were prepared by dissolving them with 0.05% HCl-methanol, respectively, to get a concentration of 1.040 mg mL^{-1} (aconitine), 1.070 mg mL^{-1} (hypacomitine), 0.735 mg mL^{-1} (mesaconitine), 1.345 mg mL^{-1} (benzoylaconitine), 1.100 mg mL^{-1} (benzoylmesaconine) and 1.005 mg mL^{-1} (benzoylhpacoitine). Working mixed standard solutions were prepared by mixing and diluting the stock solutions with 0.05% HCl–methanol. The standard stock and working solutions were all prepared in calibration flasks and stored at 4°C. The solutions were filtered through a 0.45-μm membrane prior for injection.

Sample Solution Preparation

The crude and processed Fuzi were pulverized into powder, passed through a 0.3-mm sieve, accurately weighed to approximately 2.0 g. Each weighted sample was stored in a 150-mL Erlenmeyer flask, infiltrated in 28% ammonia (2 mL) for 30 min, and then soaked in 75-mL diethyl ether overnight. Each sample was extracted by sonication (KQ5200B ultrasonicator) for 30 min at 40°C, and then filtered by the quantitative filter paper, the residues were washed with diethyl ether (20 mL), and the filtrates were combined and evaporated to dryness under 40–50°C water bath. Finally, the residues were dissolved with 0.05% HCl–methanol and stored in a 10-mL calibration flask. The solutions were filtered through a 0.45-μm membrane prior for injection.

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

Instrument and Chromatographic Conditions

A Waters e2695 HPLC system controlled with quaternary pump 2998 diode array detector and Empower chromatography workstation, and an Aglient 1200 HPLC system consisting of a G1322A online degasser machine, G1311A quarternary pumps, G1315D diode array detector and Agilent chromatography workstation were employed in the experiment.

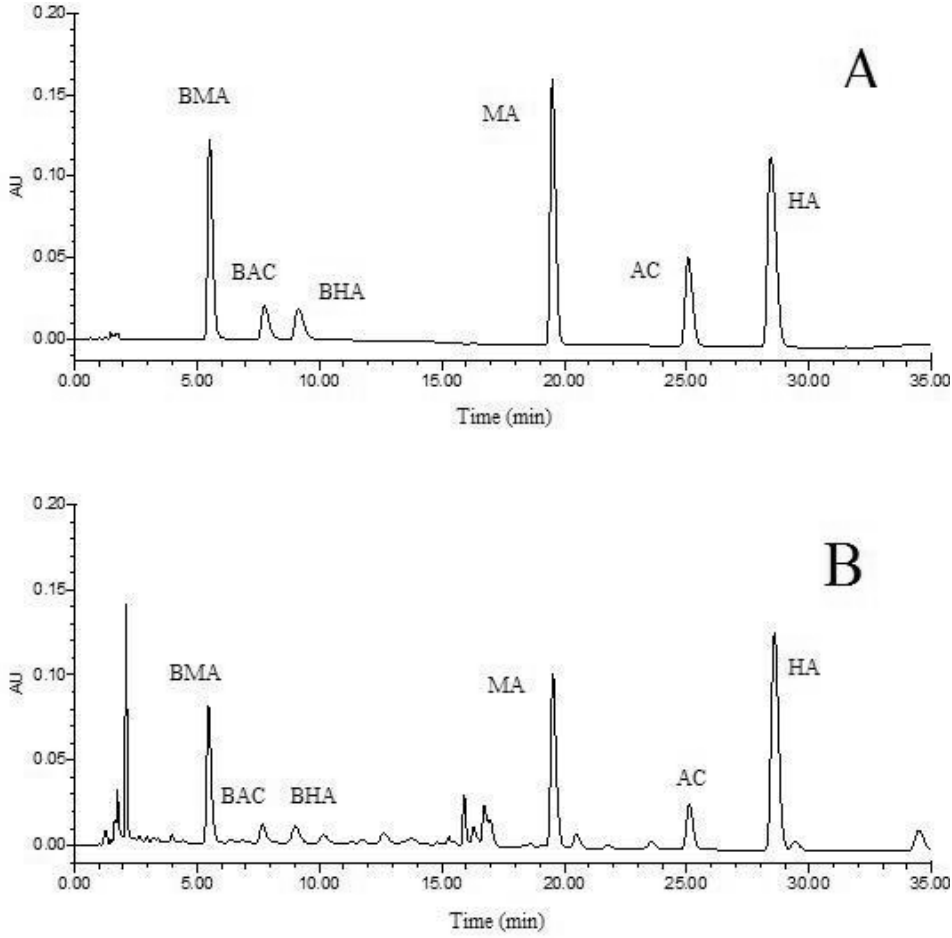

Fig 1. Typical HPLC chromatograms of six alkaloids in crude aconite roots *Radix aconiti lateralis* (B) and Reference substances (A). BMA is the abbreviation for benzoylmesaconine, BAC for benzolyaconine, BHA for benzoylhypaconine, AC for aconitine, MA for mesaconitine and HA for hypaconitine. The chromatographic conditions: monolithic separation system Agilent Extend-C_{18} (4.6 mm × 150 mm，5 μm), mobile phase pH = 10.0 with flow rate 1.0 mL min^{-1} and column temperature of 30°C. Detection was carried out at the wavelength of 235 nm

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

The measurements of the alkaloids were performed on the Agilent Extend-C_{18} （4.6 mm × 150 mm，5 μm) and Waters × Bridge-C_{18} (4.6 mm × 250 mm，5 μm). The flow rate was set at 1.0 mL min^{-1} with column temperature of 30°C. The mobile phase consisted of a linear gradient system of A (acetonitrile) and B (40 mmol L^{-1} ammonium acetate, pH = 10.0, regulated by ammonia), 0–7 min, 28%A; 7–12 min, 28–32%A; 12–13 min, 32–40%A; 13–20 min, 40–43%A; 20–30 min, 43–50% A; 30–35 min, 50–28%. Detection was carried out at the wavelength of 235 nm *(Fig. 1)*.

Validation of the Analytical Method

Linearity Study

Linear range was evaluated by injecting different volumes of working solutions (1, 2, 4, 6, 8, 10, 20, 30 μL) under the selected optimal chromatographic conditions. Each volume was injected for three times, then calculated the average of area, made the injection volume *X* as the abscissa and the average peak area *Y* as the vertical axis, and calculated the linear regression. The regression equation was shown in *Table I*.

Table I. Correlation and linear ranges of six alkaloids

Constituent	Linear equation[a]	Linear range (mg)	*r*
Benzoylmesaconine	$y = 1.58 \times 10^6 x - 37952$	0.066–1.980	0.9999
Benzoylaconine	$y = 1.63 \times 10^6 x - 15547$	0.016–0.484	0.9998
Benzoylhypconine	$y = 1.54 \times 10^6 x - 14496$	0.018–0.543	0.9999
Mesaconitine	$y = 1.98 \times 10^6 x - 48140$	0.074–2.205	0.9999
Aconitine	$y = 1.61 \times 10^6 x - 25875$	0.042–1.250	0.9999
Hypaconitine	$y = 1.54 \times 10^6 x - 58785$	0.107–3.210	0.9999

Stability

Stability was evaluated by injecting one sample solution into the chromatograph at the time interval of 0 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 48 h. The relative standard deviations (RSDs) of the peak areas of benzoylmesaconine,

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

benzoylaconine, benzoylhypconine, mesaconitine, aconitine, and hypaconitine were 1.25%, 2.58%, 0.53%, 0.87%, 0.99%, and 1.21% respectively, which indicated that the test solution was stable within 48 h.

Repeatability

Six batches of crude aconitine powder were passed through a 0.3-mm sieve, each powder was accurately weighed to approximately 2.0 g and transferred into a 150-mL Erlenmeyer flask. Sample solutions were made according to "Sample Solution Preparation". The average contents of benzoylmesaconine, benzoylaconine, benzoylhypconine, mesaconitine, aconitine, and hypaconitine in six groups of samples were 0.2108 mg g^{-1}, 0.0238 mg g^{-1}, 0.0257 mg g^{-1}, 0.7140 mg g^{-1}, 0.1768 mg g^{-1}, 0.5401 mg g^{-1}, respectively, and RSDs were 2.33%, 2.73%, 1.97%, 0.30%, 1.66%, and 0.88%, respectively, which indicated that the established method has good repeatability.

Recovery

Accurately weighed six batches of Fuzi powder (passed through a 0.3-mm sieve), where the contents of the six alkaloids were already known, and added with a certain amount of reference substance. The prepared samples (n = 6) above were then processed in accordance with "Sample Solution Preparation" in parallel and quantified in accordance with the selected method. The average recoveries and RSDs of benzoylmesaconine, benzoylaconine, benzoylhypconine, mesaconitine, aconitine, and hypaconitine were 98.96% (1.18%), 97.28% (2.88%), 99.38% (1.28%), 101.48% (1.62%), 100.87% (0.87%), and 99.82% (1.68%), respectively.

Correction Factor Calculation

The related correction factors were calculated, aconitine as the internal reference material, by comparing the peak areas between aconitine and other alkaloids including benzoylmesaconine (BMA), benzoylaconine (BAC), benzoylhypconine (BHA), mesaconitine (MA), and hypaconitine (HA) in Fuzi. The related correction factors were used to obtain the alkaloids contents in Fuzi. The results were shown in *Table II*.

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

Table II. Relative correcting factors of five alkaloids

Injection volume (μL)	Relative correction factor[a]				
	$f_{BMA/AC}$	$f_{BAC/AC}$	$f_{BHA/AC}$	$f_{HA/AC}$	$f_{MA/AC}$
1	0.993	1.065	1.107	0.784	1.024
2	1.009	1.079	1.118	0.795	1.035
4	1.017	1.042	1.083	0.802	1.034
6	1.019	1.032	1.068	0.804	1.036
8	1.020	1.028	1.062	0.807	1.040
10	1.020	1.017	1.058	0.807	1.039
20	1.022	1.028	1.051	0.811	1.042
30	1.022	1.023	1.048	0.813	1.043
Average	1.015	1.039	1.074	0.803	1.037
RSD%	0.98%	2.09%	2.42%	1.17%	0.58%

[a]BMA is the abbreviation for benzoylmesaconine, BAC for benzolyaconine, BHA for benzoylhypaconine, AC for aconitine, MA for mesaconitine and HA for hypaconitine.

Reproducibility Study of Correction Factor

Study of Column and HPLC

The working mixed standard solution (1 μL, 2 μL, 4 μL, 8 μL, 10 μL, 20 μL), which was made in accordance with "Standard Solution Preparation" was injected into HPLC for measuring. The relative correction factors of BMA, BAC, BHA, MA and HA were calculated according to "Correction Factor Calculation". Two kinds of columns were investigated in the experiment, which were Agilent Extend-C_{18} (4.6 mm × 150 mm，5 μm) and Waters × Bridge-C_{18} (4.6 mm × 250 mm，5 μm). The two chromatographs used in the experiment were Waters 2695 and Agilent 1200. The results are shown in *Table III*.

Table III. Relative correction factors of different instruments and columns (n = 3)

Instrument column[a]		Relative correction factor				
		$f_{BMA/AC}$	$f_{BAC/AC}$	$f_{BHA/AC}$	$f_{MA/AC}$	$f_{HA/AC}$
Waters 2695	1	1.015	1.039	1.074	0.803	1.037
	2	1.013	0.994	1.038	0.811	1.050
Agilent 1200	1	1.011	1.005	1.071	0.803	1.034
	2	1.018	0.999	1.018	0.820	1.056
Average		1.014	1.009	1.050	0.809	1.044
RSD%		0.29%	2.02%	2.57%	1.00%	1.00%

[a]1 denotes Agilent Zorbax Extend-C_{18} column (4.6 × 150 mm, 5 μm); 2 denotes Waters × Bridge-C_{18} column (4.6 × 250 mm, 5 μm).

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

Positioning of the Peaks of the Component to be Tested

The relative retention time of other alkaloids in respect of the internal reference material aconitine were used to get the accurate position of the targeted peaks. The results were shown in *Table IV*.

Table IV. Relative retention times of the target components under different instruments and different columns

Instrument column[a]		Relative retention time				
		$Rt_{BMA/AC}$	$Rt_{BAC/AC}$	$Rt_{BHA/AC}$	$Rt_{MA/AC}$	$Rt_{HA/AC}$
Waters 2695	1	0.258	0.360	0.432	0.780	1.128
	2	0.286	0.373	0.456	0.789	1.160
Agilent 1200	1	0.258	0.365	0.421	0.762	1.208
	2	0.266	0.387	0.454	0.795	1.130
Average		0.267	0.371	0.441	0.782	1.157
RSD%		4.95%	3.17%	3.87%	1.84%	3.23%

[a]1 denotes Agilent Zorbax Extend-C_{18} column (4.6 × 150 mm, 5 μm); 2 denotes Waters × Bridge-C_{18} column (4.6 × 250 mm, 5 μm).

Results

To compare the results obtained from QAMS and conventional method, i.e. external standard method. Sample solutions of 13 batches of Fuzi and its herbal medicine were prepared in accordance with "Sample Solution Preparation", and injected 10 μL into the chromatograph for three times in parallel. The contents of the six alkaloids were acquired by external standard method and QAMS. The results are shown in *Table V*, which demonstrated that there is higher similarity and lower relative error between external standard method and QAMS. As a conclusion, it was feasible to use QAMS in the quality evaluation of *Radix aconite lateralis*.

Discussion

In this investigation, the relative standard deviations of the relative correction factors were found all very low compared with aconitine as the internal reference material. The results of QAMS and the external standard method are similar with relative average deviations (RADs) below 3% (RADs were 0.09%, 2.66%, 0.55%, 1.24%, and 0.05%, respectively), in regard of the real contents of the other five alkaloids. When different columns and HPLC instruments [20–22] were used to inspect the adaptability and duality of the

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

system, the relative correction factors showed no significant difference with RSD below 3%. A QAMS method was established to the quality control of Fuzi and it has a universal applicability.

Table V. Determination of the contents of six alkaloids by external standard method and QAMS (n = 3) μg g^{-1} separation conditions of target analytes: monolithic separation system Agilent Extend-C_{18} (4.6 mm × 150 mm，5 μm), mobile phase pH = 10.0 with flow rate 1.0 mL min^{-1} and column temperature of 30°C, Detection was carried out at the wavelength of 235 nm

Sample[a]	BMA		BAC		BHA		MA		AC	HA	
	A	B	A	B	A	B	A	B	A	A	B
HSP	46.4	46.4	14.7	15.1	33.4	33.8	0.9	0.9	4.5	17.1	17.1
BFP	92.6	92.7	22.5	23.1	24.6	24.9	309.0	307.3	121.4	710.1	710.4
Crude	36.4	36.5	6.5	6.7	19.9	20.2	267.3	265.8	112.6	854.2	854.6
Jangyou1	47.4	47.5	5.4	5.6	28.3	28.6	378.5	376.4	147.6	981.7	982.2
Jangyou2	39.1	39.2	6.6	6.8	20.3	20.6	270.5	269.0	129.6	749.5	749.8
Jangyou3	31.9	31.9	9.5	9.8	12.9	13.0	266.4	264.9	100.4	735.4	735.8
Jangyou4	50.0	50.0	4.9	5.0	26.8	27.1	308.2	306.4	71.9	946.1	946.6
Jangyou5	56.2	56.3	10.4	10.7	32.4	32.8	334.9	333.0	82.4	708.2	708.5
Jangyou6	22.9	23.0	2.1	2.2	7.8	7.9	444.2	441.7	126.9	732.4	732.7
Jangyou7	32.3	32.3	1.8	1.9	13.2	13.3	367.8	365.7	52.4	853.8	854.2
Jangyou8	65.0	65.1	8.4	8.7	13.7	13.9	336.4	334.6	81.9	444.0	444.2
Jangyou9	220.2	220.4	41.5	42.6	48.2	48.8	241.3	240.0	102.4	567.7	568.0
Jan-gyou10	190.0	190.1	35.6	36.6	37.0	37.5	153.9	153.0	70.2	469.3	469.5
RAD%	0.09		2.66		0.55		1.24			0.05	

[a]A denotes external standard method, B denotes quantitative analysis of multi-components by single marker (QAMS)

Mobile phases of acetonitrile-tetrahydrofuran–0.1 mol L^{-1} ammonium acetate [4], methanol–water, acetonitrile–water, and acetonitrile–40 mmol L^{-1} ammonium acetate (the aqueous ammonia pH 8.0, 9.0, 10.0) had been investigated in the experiment, and it turned out that acetonitrile–40 mmol L^{-1} ammonium acetate (the aqueous ammonia pH 10.0) was appropriate with the best gradient elution effect, sharp peaks, symmetry, and smooth baseline. Aconitine, hypoconitine, and mesaconitine are diester diterpene alkaloids and can be easily hydrolyzed to monoester diterpene alkaloids and amine diterpenoid alkaloids when they were dissolved in methanol [23]. The stability of aconitine, mesaconitine, and hypoconitine in methanol, 0.05% and 0.1% HCl–methanol, as well as acetonitrile, was studied at 4°C. One week later, peaks of hydrolyzates were detected by HPLC in the reference substance dissolved by methanol. However, with 0.05% and 0.1% HCl–methanol and acetonitrile as the solvent, aconitine, hypoconitine, me-

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

saconitine were stable within 30 days. Finally, 0.05% HCl–methanol was chosen as the solvent because acetonitrile has a high toxicity.

The result of different batches of Fuzi showed that the content of total alkaloids in HSP was much lower than that in crude Fuzi, especially the content of DDAs, resulting in a lower toxicity. It was more appropriate to evaluate the quality of Fuzi by a comprehensive determination of the six alkaloids since the content of each single alkaloid from total alkaloids of 11 batches of crude Fuzi differed a lot. QAMS was proven to be feasible and available in quality control of TCM by the experiment, but it still needs to be improved in the future.

Acknowledgments

The authors would like to thank Prof. Rusong Zhang, Prof. Daozong Xia and Mr Cao Gang. This work was mainly supported by Natural Science Foundation of Zhejiang Provincial (no. Y2101229) as well as the National Natural Science Foundation of China (no. 81273772), Analysis and Testing Technology projects (no. 2012C37066) and the Zhejiang Provincial Project of Traditional Chinese Medicine Science and Technology Plan (NO.2010ZA026).

References

[1] M.Q. Xiong, Shanghan Lun, People's Medical Publishing House, Beijing, 2009, pp. 945
[2] J.P. Chen, Jingui Yaolue, People's Medical Publishing House, Beijing, 2009, pp. 106–111
[3] L. Tang, Y. Gong, C. Lv, Ch. Lv, L. Ye, L. Liu, and Zh.Q. Liu, J. Ethnopharmacol., **141**, 736–741 (2012)
[4] C.P. Commission, Chin. Pharm, vol. 1, China Medical Science Publisher, Beijing, 2010, pp. 177–178
[5] H. Sun, B. Ni, A.H. Zhang, M. Wang, H. Dong, X.J. Wang, Analyst., **137**, 170 (2012)
[6] F. Gao, Y.Y. Li, D. Wang, X. Huang, Q. Liu, Molecules., **17**, 5187–5194 (2012)
[7] G.H. Lu, Zh.Q. Dong, Q. Wang, G.S. Qian, W.H. Huang, Zh.H. Jiang, K.S. YinLeung, and Zh.Zh. Zhao, Planta. Med., **76**, 825–830 (2010)
[8] K. Chan, Chemosphere., **52**, 1361–1371 (2003)
[9] M.G. Huang and Y.M. Zhang, J. Tradit. Chin. Herb., **32,** 2143–2145 (2007)
[10] D. Csupor, E.M. Wenzig, I. Zupkó, K. Wölkart, J. Hohmann, and R. Bauer, J. Chromatogr., A, **1216**, 2079–2086 (2009)
[11] Y. Xie, Z.H. Jiang, and H. Zhou, J. Chromatogr., A, **1093**, 195–203 (2005)
[12] Sh.G. Zhao, Zh.W. Pan, X.G. Chen, and Zh.D. Hu, Biomed. Chromatogr., **18**, 381–387 (2004)

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC

[13] X. Liu and R.B. Chao, Chromatogr. Phar. J., **41**, 365–369 (2006)
[14] L. Sun, H.Y. Zhou and R.H. Zhao, J. Herbs., **40**, 131–134 (2009)
[15] J.S. Wang, R.v.d. Heijden, G. Spijksma, Th. Reijmers, M. Wang, G.W. Xu, Th. Hankemeier, and J.v.d. Greef, J. Chromatogr., A, **1216**, 2169–2178 (2009)
[16] L.Ye, S. Gao, Q. Feng, W. Liu, Zh. Yang, M. Hu, and Zh.Q. Liu. Xenobiotica, **42**, 518–525 (2012)
[17] Y.H. Kuang, J.J. Zhu, Zh.M. Wang, and Q.W. Zhang, Chin. Pharm. J., **44**, 390–394 (2009)
[18] Zh.M. Wang, Zh.Zh. Qian, Q.W. Zhang, J.J. Zhu, H.M. Gao, and Zh.T. Wan, China J. Chin. Mater. Med., **36,** 657–658 (2011)
[19] Zh.M. Wang, H.m. Gao, X.T. Fu, and W.H. Wang, China J. Chin. Mater. Med., **31**, 1925–1928 (2006)
[20] H.X. Cai, J. Chen, and P. Li, China J. Chin. Mater. Med., **35,** 2712–2717 (2010).
[21] J.J. Zhu, Zh.M. Wang, Q.W. Zhang, J.W. Niu, and F. Li, China J. Chin. Mater. Med., **34**, 3229–3234 (2009)
[22] X. Yu, J. Song, Zh.L. Xiong, and F.M. Li, China J. Chin. Mater. Med., **35**, 3310–3313 (2010)
[23] X.K. She, X.X. Jian, D.L. Chen, Q.H. Chen, and F.P. Wang, J. Asian Nat. Prod. Res., **14**, 665–677 (2012)

Accepted by MWH

Unauthenticated | Downloaded 03/22/25 12:21 PM UTC