Simultaneous Quantitation of Aconitum Alkaloids from You-Gui-Yin in Rat Plasma by UPLC–ESI–MS and Its Application to a Pharmacokinetic Study

JianHua Zhang¹, Ling Wang², Xi Zhu², Xue Bai^{2,3*} and Hua Yin^{2,3*}

¹The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, China

²Standardization of Chinese Medicine Research Laboratory, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, China

³College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou 310053, Zhejiang, China

Received: 25 April 2017; accepted: 23 August 2017

You-Gui-Yin (YGY), a famous traditional Chinese medicine, has been widely used in clinics for the treatment of kidney-yang deficiency, yang deficiency caused by excessive yin, and osteoporosis. A rapid and sensitive ultraperformance liquid chromatography–electrospray ionization–mass spectrometry (UPLC–ESI–MS) method for simultaneous determination of six Aconitum alkaloids including aconitine (AC), hypaconitine (HA), mesaconitine (MA), benzoylaconine (BAC), benzoylhypaconine (BHA), and benzoylmesaconine (BMA) in rat plasma after oral administration of YGY was developed in this study. Chromatographic separation was performed on an ACQUITY UPLC™ BEH C₁₈ column (2.1 × 100 mm, 1.7 μm) using gradient elution with the mobile phase consisting of 2 mmol/L ammonium formate in 0.05% formic acid aqueous solution and 0.05% formic acid methanol solution, at a flow rate of 0.20 mL/min. MS detection was performed in the positive ion mode. The calibration curves were linear in the concentration range of 0.04160–41.60 ng/mL, 0.1070–107.0 ng/mL, 0.07358–73.50 ng/mL, 0.03228–32.28 ng/mL, 0.01809–18.09 ng/mL, and 0.1320–132.0 ng/mL for AC, HA, MA, BAC, BHA, and BMA, respectively. The intra- and inter-day precisions (relative standard deviation [RSD]) were less than 11.6% and 12.6%, respectively. The accuracies Relative Error (RE) ranged from –10.2% to 5.6%, while the recoveries ranged from 70.4% to 99.3%. The method for simultaneous quantitation of Aconitum alkaloids of You-Gui-Yin in rat plasma is accurate and repeatable, and this method was successfully applied to investigate the pharmacokinetics of the six Aconitum alkaloids in rat plasma after oral administration of YGY. For the pharmacokinetic study, the pharmacokinetics of the six Aconitum alkaloids were best described by a two-compartment open model.

Keywords: UPLC–ESI–MS, Aconitum alkaloids, pharmacokinetics, You-Gui-Yin

Introduction

You-Gui-Yin (YGY) is a famous Chinese herbal formula, which was originally recorded in the famous Chinese medical book *Jingyuequanshu* composed by Jingyue Zhang in 1624 A. YGY has been widely used in clinic for the treatment of kidney-yang deficiency, yang deficiency caused by excessive yin, and osteoporosis [1]. YGY is composed of eight herbs including *Radix Aconiti lateralis praeparata*, *Cortex cinnamomi*, *Radix Rehmanniae praeparata*, *Eucommia ulmoides*, *Cornus officinalis*, *Dioscorea opposita*, *Fructus Lycii*, and *Glycyrrhiza uralensis* with a traditional dose ratio of 2:3:3:3:1:2:2:1. It is very effective for warming and enforcing kidney yang and its application in prophylactic therapy [2]. *Radix Aconiti lateralis praeparata* (Fuzi in Chinese), the monarch drug in the YGY prescription, bears hot nature and toxicity. It acts on the heart, kidney, and spleen meridians and plays a role in saving yang for the treatment of collapse, supplementing kidney yang, and eliminating cold to stop pain [3].

Aconitum alkaloids, as the effective parts of Fuzi, are the main components of YGY, which have significant therapeutic effects on analgesia, anti-tumor, anti-inflammatory, and immune disorders [4]. So far, about hundreds of alkaloids have been isolated and identified from Aconitum [5, 6]. These alkaloids have been divided into four groups, i.e., non-ester alkaloids (NEAs), monoester diterpene alkaloids (MDAs), diester diterpene alkaloids (DDAs), and lipo-alkaloids. The pharmacological effects of Aconitum alkaloids were mainly attributed to DDAs and MDAs [7]. However,

DDAs such as aconitine (AC), mesaconitine (MA), and hypaconitine (HA) in YGY have significant toxicities including neurotoxic and cardiotoxic effects [8]. MDAs such as benzoylaconine (BAC), benzoylhypaconine (BHA), and benzoylmesaconine (BMA) in YGY exhibit relatively low toxicity after being hydrolyzed to DDAs analogs [9]. Therefore, Aconitum alkaloids, as the representative bioactive compounds of Fuzi, are directly related to the pharmacological effects and toxicological effects of YGY in vivo.

Pharmacokinetic (PK) studies are useful to elucidate and predict the efficacy and toxicity of drugs and helpful in optimizing the dose regimen and minimize the adverse effects. Currently, few studies on pharmacokinetic behavior of the active components in YGY have been reported [10]. Thus, it is urgent to develop a rapid and sensitive method for the identification and determination of the Aconitum alkaloids from YGY in plasma for investigating its pharmacological and toxicological effects, as well as monitoring therapeutic efficacy in clinic. In this study, we established a sensitive UPLC–ESI–MS method for simultaneous determination of the Aconitum alkaloids in rat plasma. This method was successfully applied to pharmacokinetic study of the six Aconitum alkaloids after oral administration of YGY in rats.

Experimental

Materials. AC, HA, MA, and berberine hydrochloride were obtained from State Food and Drug Administration of China (purity >98%, batch no.: 110720-201002, 110721-201009, 110722-201006, 110713-201212). BAC, BHA, and BMA were purchased

* Authors for correspondence: yinhua@zcmu.edu.cn, bxsy8011@sina.com

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium for non-commercial purposes, provided the original author and source are credited, a link to the CC License is provided, and changes - if any - are indicated.

from Express Technology Co. Ltd. (Beijing, China; purity >98%; confirmed by liquid chromatography [LC]–MS; batch no.: B-010-110316, B-016-110316, B-009-110316). The chemical structures of the determined Aconitum alkaloids are shown in Figure 1. Formic acid was high-performance liquid chromatography (HPLC) grade and obtained from J&K Scientific Ltd. (Shanghai, China, purity >98%, for LC–MS). Ammonium formate was bought from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, analytical grade). HPLC-grade methanol and acetonitrile (ACN) were purchased from Merck (Germany). Ultra-pure water was prepared by a Milli-Q water purification system (Millipore Ltd.).

The crude drugs, *Radix Aconiti lateralis praeparata*, *Cortex cinnamomi*, *Radix Rehmanniae praeparata*, *Eucommia ulmoides*, *Cornus officinalis*, *Dioscorea opposita*, *Fructus lycii*, and *Glycyrrhiza uralensis* were purchased from Huadong Pharmaceutical Co., Ltd., Medicinal Materials Ginseng & Antler Branch (Hangzhou, China) and identified by Professor Ru-Song Zhang (College of Pharmaceutical Science, Zhejiang Chinese Medical University). The crude slices of all drugs met the criterion of the stipulated quality standards in Chinese Pharmacopoeia (2010 edition).

Animals. Female Sprague-Dawley (SD) rats (230–250 g) were obtained from the Experimental Animal Center of Zhejiang Chinese Medical University (Hangzhou, China). Animals were housed in a room with 12-h light–dark cycle (temperature of 22–24 °C and humidity of 50%–60%). All animals were cared for according to the Guide for the Care and Use of Laboratory Animals (NIH Publications, No. 80-23, revised in 1996). Every effort was taken to reduce the number of animals and suffering during the experiment, and all experimental processes were complied with international guidelines on the ethical use of animals.

Preparation of the Extracts of YGY. YGY prescription was soaked in 510 mL (10 times their total weight) 50% ethanol for 1 h and sonicated twice at 45 °C for 30 min. The extracted solution was filtered and concentrated to 12.75 mL (4 g/mL) to prepare YGY decoction at 45 °C and then stored at 4 °C until use.

Liquid Chromatographic and Mass Spectrometric Conditions. A Waters ACQUITY™ UPLC (Waters Corp., Milford, MA, USA) was equipped with a binary pump and a sample manager. An ACQUITY UPLC™ BEH C₁₈ column (1.7 μm, 2.1 mm × 100 mm) was used to separate the compounds and protected by a guard filter. The column temperature was maintained at 40 °C and at a flow rate of 0.20 mL/min with the mobile phases A (ultrapure water with 0.05% formic acid +

2 mmol/L ammonium formate) and mobile phases B (methanol with 0.05% formic acid). The gradient elution was set as follows: 0–3 min, 5% (A); 3–12 min, 5%–40% (A); 12–14 min, 40%–45.5% (A); 14–18 min, 45.5%–51% (A); 18–20 min, 51% (A); 20–32.5 min, 51%–100% (A); 32.5–37.5 min, 100% (A); 37.5–38 min, 100%–5% (A); and 38–43 min, 5% (A).

A Thermo Exactive mass spectrometer equipped with an ESI source (Thermo Fisher Scientific) was set in the positive ion mode for detection. The ESI source was operated in positive ion mode. The parameter of ion source was optimized in order to achieve maximum sensitivity as follows: scan range was set from 100.0 to 900.0 m/z; spray voltage was set at 4.5 kV; heated capillary temperature was set at 350 °C; and capillary voltage, tube lens voltage, and skimmer voltage were set at 60, 120, and 25 V, respectively. Sheath gas and auxiliary gas (N₂) flow rate were set at 35 and 2.0 L/min, respectively.

Preparation of Standard Curve and Quality Control Samples. Stock solutions were prepared in methanol with 0.01% hydrochloric acid (41.60 μg/mL for AC, 107.0 μg/mL for HA, 73.50 μg/mL for MA, 32.28 μg/mL for BAC, 18.09 μg/mL for BHA, and 132.0 μg/mL for BMA). The calibration standards were prepared by spiking an appropriate amount of stock solution in blank plasma (100 μL) to yield final concentrations of 0.0416–41.60 ng/mL for AC, 0.1070–107.0 ng/mL for HA, 0.0735–73.50 ng/mL for MA, 0.03228–32.28 ng/mL for BAC, 0.01809–18.09 ng/mL for BHA, and 0.1320–132.0 ng/mL for BMA.

Three quality control (QC) plasma samples were prepared at low (0.0832 ng/mL for AC, 0.2140 ng/mL for HA, 0.1470 ng/mL for MA, 0.06456 ng/mL for BAC, 0.03618 ng/mL for BHA, and 0.2640 ng/mL for BMA), medium (1.664 ng/mL for AC, 4.280 ng/mL for HA, 2.940 ng/mL for MA, 1.291 ng/mL for BAC, 0.7236 ng/mL for BHA, and 5.280 ng/mL for BMA), and high (33.28 ng/mL for AC, 85.60 ng/mL for HA, 58.80 ng/mL for MA, 25.82 ng/mL for BAC, 14.47 ng/mL for BHA, and 105.6 ng/mL for BMA) concentrations, the same as the plasma for standard curve.

Method Validation

Specificity. The specificity was investigated by comparing the blank plasma and the blank plasma spiked with six Aconitum alkaloids and internal standard (IS) to assess the interference from endogenous substances with these six analytes.

Linearity. The linearity was investigated by constructing standard curves using analysis data from blank plasma samples at 10 concentration levels in line with the range that shown in preparation of standard curve section. The calibration curves were constructed by plotting the peak area against concentrations using the weighted least square method ($w = 1/c^2$).

Precision and Accuracy. The precision of the assay was determined from the QC plasma samples by replicated analyses of the three concentration levels of the six analytes. Accuracy was measured using six determinations per concentration.

Recovery and Matrix Effect. The extraction recovery and matrix effect of the six Aconitum alkaloids through the extraction procedure were determined at three QC levels with six replicates. Extraction recovery was calculated by comparing the peak area obtained from the plasma sample spiked before and after extraction. The matrix effect was evaluated by comparing the peak area of analytes added in to pre-extracted plasma from untreated rats, with analytes dissolved in matrix component-free reconstitution solvent.

Freeze and Thaw Stability. Five QC samples of each concentration at low, medium, and high concentrations were stored at –20 °C for 24 h and thawed unassisted at room temperature. Then, the samples were refrozen for 24 h under the same conditions. The freeze–thaw cycle was repeated three times and then analyzed on the third cycle.

Figure 1. The chemical structures of AC, MA, HA, BAC, BMA and BHA

To extract all of the analytes and the IS with high recovery and no endogenous interference at the retention time, liquid–liquid extraction was investigated in our study. Different types of organic reagents (methanol, acetonitrile, methanol–acetonitrile = 1:1) were tested, and satisfactory recoveries were achieved with methanol–acetonitrile = 1:1. The volume of extracting solvent in extraction process was also very important. A volume of 800 μ L methanol–acetonitrile (1:1) was finally found with high recoveries.

Method Validation

Specificity. UPLC–ESI–MS extracted ion chromatogram of blank plasma spiked with standards and IS, blank plasma, and a sample obtained 45 min after the oral administration of YGY to rats are shown in Figure 2. The retention times of AC, HA, MA, BAC, BHA, BMA, and IS in the extracted ion chromatogram of sample obtained at 45 min are 17.52, 17.22, 16.58, 14.08, 15.10, 14.21, and 13.42 min, respectively, which was consistent with the blank plasma spiked with six Aconitum alkaloids and IS. Compared with extracted ion chromatogram of blank blood sample, no interference of endogenous peaks was observed.

Linearity of Calibration Curve and LLOQs. The calibration curves were all linear with the correlation coefficient (r) higher than 0.992. The LLOQs are appropriate for the quantitative detection of the six analytes in pharmacokinetic studies. The linear ranges, regression equations, LLOQs, and correlation coefficients obtained from the typical calibration curves are shown in Table 1.

Accuracy and Precision. The results of the six analytes in rat plasma for the assessment of the within-day precision and accuracy of the method were acceptable. The overall values for precision were all below 15.0%, and the values for accuracy were all within acceptance. The results are listed in Table 2.

Extraction Recovery and Matrix Effect. The recoveries of the six analytes at low, medium, and high concentrations ranged from 70.4% to 99.3%, with relative standard deviation (RSD) of <14.1%; the mean recovery of IS was 89.7%, and the RSD value was 7.0%, which indicated that the extraction method was consistent and repeatable. The matrix effects ranged from 73.3% to

103.9 % for all of the six analytes, while the mean matrix effect for the IS was 92.8% and the RSD value was 9.6%, all of which indicated a negligible matrix effect on the ionization of the analytes, as detailed in Table 2.

The Freeze and Thaw Stability. The freeze and thaw stability of the six analytes was evaluated by the analysis of QC samples at three concentration levels after three cycles of freeze–thaw. As shown in Table 3, the results of the stability tests indicated that all of the analytes were stable in rat plasma after three freeze–thaw cycles (RSD <11.1%, Relative Error RE –3.4 to –14.2%).

Pharmacokinetic Behaviors of the Six Aconitum Alkaloids.

This is the first study for determining the pharmacokinetic behaviors of six Aconitum alkaloids of YGY in rat. The pharmacokinetics of the six Aconitum alkaloids was confirmed to be two-compartment open model. The plasma concentration–time profiles for the six Aconitum alkaloids are presented in Figure 3, and the main pharmacokinetic parameters are listed in Table 4.

A similar trend was found in the mean concentration of DDAs in plasma versus time curves, which indicated that AC, HA, and MA had similar pharmacokinetic behaviors, while BHA and BMA have similar pharmacokinetic behaviors. Double-absorption peak phenomenon in the concentration–time curves of AC, HA, MA, and BAC was observed from Figure 3, which was the same as the published results [10]. The main reasons for the double-peak phenomenon are widely considered as enterohepatic recirculation, delayed gastric emptying, and variability of absorption [11].

Table 3. The freeze and thaw stability of the six Aconitum alkaloids ($n = 6$)

Compounds	Spiked concentration (ng/mL)	RE (%)	RSD (%)
AC	0.08320	–10.6	9.5
	1.664	–6.7	8.1
	33.28	–3.9	7.2
HA	0.2140	–9.6	10.1
	4.280	–4.9	8.6
	85.60	–6.4	9.9
MA	0.1470	–14.2	9.6
	2.940	–5.5	9.8
	58.80	–6.7	8.7
BAC	0.06456	–6.6	10.0
	1.291	–12.5	9.9
	25.82	–9.2	10.5
BHA	0.03618	–11.8	9.4
	0.7236	–6.1	9.7
	14.47	–3.5	9.8
BMA	0.2640	–12.7	9.9
	5.280	–10.2	9.3
	105.6	–3.4	11.1

Table 1. Calibration curve, correlation coefficient (r), and linear range of the 6 analytes in You-Gui-Yin

Compounds	Calibration curve	Correlation coefficient	Linear range (ng/mL)	LLOQ (ng/mL)
AC	$y = 0.112x - 0.003$	$r = 0.9974$	0.04160–41.60	0.04160
HA	$y = 0.115x + 0.003$	$r = 0.9982$	0.1070–107.0	0.1070
MA	$y = 0.139x - 0.002$	$r = 0.9969$	0.07358–73.50	0.07358
BAC	$y = 0.023x + 0.008$	$r = 0.9933$	0.03228–32.28	0.03228
BHA	$y = 0.012x + 0.106$	$r = 0.9930$	0.01809–18.09	0.01809
BMA	$y = 0.05x - 0.001$	$r = 0.9921$	0.1320–132.0	0.1320

Table 2. The within-day precision, accuracy, extraction recovery, and matrix effect of six Aconitum alkaloids ($n = 6$)

Compounds	Spiked (ng/mL)	Intra-day			Inter-day Precision (RSD%)	Extraction recovery		Matrix effect	
		Test concentration (ng/mL)	Precision (RSD%)	Accuracy (RE%)		Recovery (%)	RSD (%)	Matrix effect (%)	RSD (%)
AC	0.08320	0.08400	9.0	0.96	12.2	99.3	1.1	77.3	16.8
	1.664	1.682	8.6	1.08	10.6	72.0	13.9	92.0	12.0
	33.28	31.68	9.3	–4.81	8.7	71.5	8.3	93.9	11.7
HA	0.2140	0.2260	10.6	5.61	11.3	93.2	9.5	81.5	13.6
	4.280	4.330	9.8	1.21	8.9	75.3	9.0	99.5	7.8
	85.60	83.44	9.2	–2.52	10.2	71.1	3.5	103.9	5.0
MA	0.1470	0.1320	8.2	–10.20	12.6	85.7	4.8	95.4	5.5
	2.940	3.040	10.7	3.4	8.7	74.3	8.8	92.4	5.4
	58.80	57.20	9.5	–2.72	8.9	77.3	3.9	86.7	7.6
BAC	0.06456	0.0608	11.0	–5.82	10.6	84.1	7.1	73.3	6.8
	1.291	1.176	10.8	–8.92	11.2	86.0	5.6	76.8	4.3
	25.82	25.28	10.6	–2.11	9.8	84.8	14.1	85.0	13.8
BHA	0.03618	0.0368	9.4	1.71	12.1	70.4	11.3	96.3	10.5
	0.7236	0.7060	10.5	–2.43	11.7	90.4	10.7	97.4	6.2
	14.47	14.74	8.7	1.85	8.7	82.7	13.8	93.3	9.8
BMA	0.2640	0.2688	11.6	1.82	9.1	80.8	12.1	92.5	9.2
	5.280	5.160	10.9	–2.27	9.4	80.1	5.9	84.3	13.6
	105.6	104.4	10.4	–1.14	10.5	82.3	6.7	83.0	5.1
IS	4.800	–	–	–	–	89.7	7.0	92.8	9.6

Figure 3. The plasma concentration–time profile of six Aconitum alkaloids. Each point was given as the form of mean $\pm$ SEM (A for AC; B for HA; C for MA; D for BAC; E for BHA; F for BMA)

Table 4. The pharmacokinetic parameters of six Aconitum alkaloids ($n = 6$, means $\pm$ SD)

Comp.	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/mL)	$AUC_{(0-t)}$ (h*ng/mL)	MRT_{0-t} (h)	CL (mL/h/kg)
AC	10.6 ± 4.0	3.7 ± 1.5	1.3 ± 0.27	10.2 ± 2.7	7.08 ± 1.8	172.1 ± 59.9
HA	6.5 ± 2.9	3.7 ± 1.5	36.5 ± 7.13	353.9 ± 70.3	8.35 ± 0.79	130.6 ± 37.3
MA	7.3 ± 4.9	3.7 ± 1.51	3.14 ± 0.76	27.3 ± 5.5	7.6 ± 1.0	255.5 ± 62.3
BAC	10.7 ± 3.6	8.4 ± 5.6	18.3 ± 3.8	271.3 ± 39.4	11.5 ± 0.3	51.7 ± 4.1
BHA	9.4 ± 1.9	0.14 ± 0.09	9.7 ± 3.8	12.6 ± 5.2	4.50 ± 1.50	1185.4 ± 211.7
BMA	4.4 ± 1.3	0.14 ± 0.09	81.6 ± 28.2	103.6 ± 35.7	7.00 ± 0.60	1251.8 ± 522.7

The T_{max} of the six Aconitum alkaloids was 3.7 ± 1.5 , 3.7 ± 1.5 , 3.7 ± 1.51 , 8.4 ± 5.6 , 0.14 ± 0.09 , and 0.14 ± 0.09 h, respectively, which indicated that BHA and BMA were rapidly absorbed. The elimination half-life ($T_{1/2}$) of AC, HA, MA, BAC, BHA, and BMA was 10.61 ± 4.00 , 6.5 ± 2.95 , 7.26 ± 4.89 , 10.68 ± 3.62 , 9.37 ± 1.94 , and 4.44 ± 1.35 h, respectively, while the Mean Residence Time (MRT) of the six Aconitum alkaloids was 7.08 ± 1.8 , 8.35 ± 0.79 , 7.6 ± 1.0 , 11.5 ± 0.3 , 4.50 ± 1.50 , and 7.00 ± 0.60 h, respectively. Among the six Aconitum alkaloids, BAC was eliminated slowest and resides longest time in the body. These pharmacokinetic results provide constructive view to understand the absorption mechanism of YGY and to support additional clinical evaluation.

Conclusion

In the present study, a rapid and sensitive UPLC–ESI–MS method for simultaneous determination of AC, HA, MA, BAC, BHA, and BMA in rat plasma was developed and validated. The pharmacokinetic study of the Aconitum alkaloids in rat after oral administration of YGY was conducted by using this efficient and sensitive quantification method for the first time. These results are very useful in further investigating the pharmacologic mechanisms of YGY and expanding its application in clinic.

Conflict of Interest

The authors have declared no conflict of interest.

Acknowledgments. This work was supported by a grant provided by National Natural Science Foundation of China (No. 81273772), the Natural Science Foundation of Zhejiang Province (No. Y2101229, LY17H270011), the Project of Science and Technology for Chinese Medicine of Zhejiang province (No. 2010ZA026), and Zhejiang Provincial Top Key Discipline of Chinese Medicine Open Foundation (No. Yao2016005).

References

1. Zhao, D. D.; Wang, J.; Cui, Y. J.; Wu, X. *J. Tradit. Chin. Med.* **2012**, 32, 308.
2. Zhao, W. J.; Xiao, L. W.; Tong, P. J.; Zhou, H.; Pan, H.; Hu, Q. F. *Chin. J. Trad. Med. Traumat. Orthop. (Chin)* **2000**, 8, 9.
3. Wang, L. L. *Nei. Mongol. J. Trad. Chin. Med.* **2010**, 29, 42.
4. Hikino, H.; Konno, C.; Takata, H.; Yamada, Y.; Yamada, C.; Ohizumi, Y.; Sugio, K.; Fujimura, H. *J. Pharmacobiodyn.* **1980**, 3, 514.
5. Singhuber, J.; Zhu, M.; Prinz, S.; Kopp, B. *J. Ethnopharmacol.* **2009**, 126, 18.
6. Gao, F.; Li, Y. Y.; Wang, D.; Huang, X.; Liu, Q. *Molecules* **2011**, 17, 5187.
7. Xiao, P. G.; Wang, F. P.; Gao, F.; Yan, L. P.; Chen, D. L.; Liu, Y. *Acta Phytotaxon. Sin.* **2006**, 44, 1.
8. Yang, Y.; Chen, J.; Shi, Y. P. *J. Chromatogr. B Biomed. Sci. Appl.* **2010**, 878, 2811.
9. Wang, X. Z.; Li, X. W.; Li, L. J.; Li, M.; Liu, Y.; Wu, Q.; Li, P.; Jin, Y. R. *J. Sep. Sci.* **2016**, 39, 1971.
10. Liu, J. J.; Li, Q.; Yin, Y. D.; Liu, R.; Xu, H. R.; Bi, K. S. *J. Sep. Sci.* **2014**, 37, 171.
11. Godfrey, K. R.; Arundel, P. A.; Dong, Z. M.; Bryant, R. *Comput. Methods Programs Biomed.* **2011**, 104, 62.