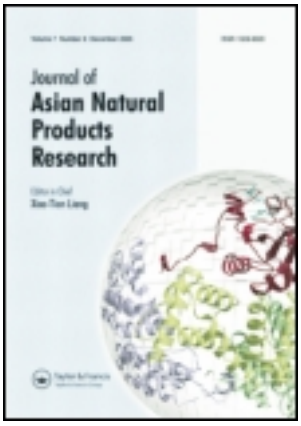

Journal of Asian Natural Products Research

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/ganp20

Identification of the metabolites of baicalein in human plasma

Xiao-Yu Guo [a], Lin Yang [a b], Ying Chen [a], Qi-Fang Wang [c], Qi-Shi Sun [c], Yi-Xin Che [a] & Qing-Ming Che [a]

[a] Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing, 100191, China

[b] Center of Cardiovascular Diseases, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing, 100091, China

[c] Shenyang Pharmaceutical University, Shenyang, 110016, China

Available online: 10 Aug 2011

To cite this article: Xiao-Yu Guo, Lin Yang, Ying Chen, Qi-Fang Wang, Qi-Shi Sun, Yi-Xin Che & Qing-Ming Che (2011): Identification of the metabolites of baicalein in human plasma, Journal of Asian Natural Products Research, 13:9, 861-868

To link to this article: http://dx.doi.org/10.1080/10286020.2011.599321

Journal of Asian Natural Products Research
Vol. 13, No. 9, September 2011, 861–868

Identification of the metabolites of baicalein in human plasma

Xiao-Yu Guo[a], Lin Yang[ab], Ying Chen[a], Qi-Fang Wang[c], Qi-Shi Sun[c], Yi-Xin Che[a] and Qing-Ming Che[a]*

[a]*Department of Natural Medicines, School of Pharmaceutical Sciences, Peking University, Beijing 100191, China;* [b]*Center of Cardiovascular Diseases, Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing 100091, China;* [c]*Shenyang Pharmaceutical University, Shenyang 110016, China*

(*Received 17 March 2011; final version received 19 June 2011*)

The metabolites of baicalein in human plasma were investigated after taking baicalein, which is one of the main bioactive flavones in *Scutellaria baicalensis* Georgi. Five metabolites (**M1**–**M5**) together with the parent drug baicalein (**P**) were detected and identified by the HPLC-diode-array detector (DAD) and LC-MS/MS methods. Among them, 7-methoxybaicalein 6-*O*-glucuronide (**M5**) is a new metabolite. Based on the results, the proposed metabolic pathway of baicalein in humans was inferred.

Keywords: baicalein; flavones; metabolites; human plasma; LC-MS/MS; HPLC-DAD

1. Introduction

Baicalin and its aglycon, baicalein are both the major bioactive flavones in *Scutellaria baicalensis* Georgi, which is widely used as a traditional Chinese medicine for the treatment of upper respiratory tract infection, hepatitis, etc. [1]. Previous studies showed that baicalein and baicalin had similar bioactivities, such as anti-inflammatory, antivirus, anti-oxidation, anti-anaphylaxis, anticancer, and anti-hepatitis activities [2]. Baicalin has low bioactivity after oral administration which has been used in clinic to treat fever and inflammation, because baicalin is mainly hydrolyzed by the intestinal microbial flora into baicalein before being absorbed into the blood [3–5]. Thus, baicalin is the prodrug of baicalein. Our previous researches showed that baicalin was the main metabolite of baicalein in rats after oral administration of baicalein [6,7], and the bioactivity of baicalin in rats after oral administration was lower than that of baicalein by comparing the concentration of baicalin in rat plasma by the HPLC-ECD method [7]. Based on the above achievements, we considered that baicalein might be better than baicalin for oral administration.

Our previous research has reported the metabolites of baicalein in human urine after taking baicalein [8]. The present study is the first time on the identification of the metabolites of baicalein in human plasma after taking baicalein. Five metabolites and the parent drug baicalein were detected and identified by the HPLC-diode-array detector (DAD) and LC-MS/MS methods in human plasma, and

*Corresponding author. Email: cheqingming2008@sina.com

ISSN 1028-6020 print/ISSN 1477-2213 online

DOI: 10.1080/10286020.2011.599321
http://www.informaworld.com

the metabolic pathway of baicalein in human plasma was proposed.

2. Results and discussion

2.1 Identification of the metabolites (M1–M5) in human plasma

The prepared plasma samples were analyzed by the established HPLC-DAD and LC-MS/MS methods. Five metabolites (**M1**–**M5**) together with the parent drug were detected by comparing the human plasma after taking baicalein with the blank plasma in the HPLC-DAD chromatograms (Figure 1) and by the total ion current (TIC) and extracted ion chromatograms (Figure 2). The metabolites were marked as **M1**–**M5** in order of their retention times in the extracted ion chromatograms. The UV and mass spectral (Figure 3) data were further used to identify the structures of the metabolites.

The retention time of **M1** was at 3.75 min in the HPLC-DAD chromatogram. The UV spectrum revealed the absorption maxima at 270 and 308 nm,

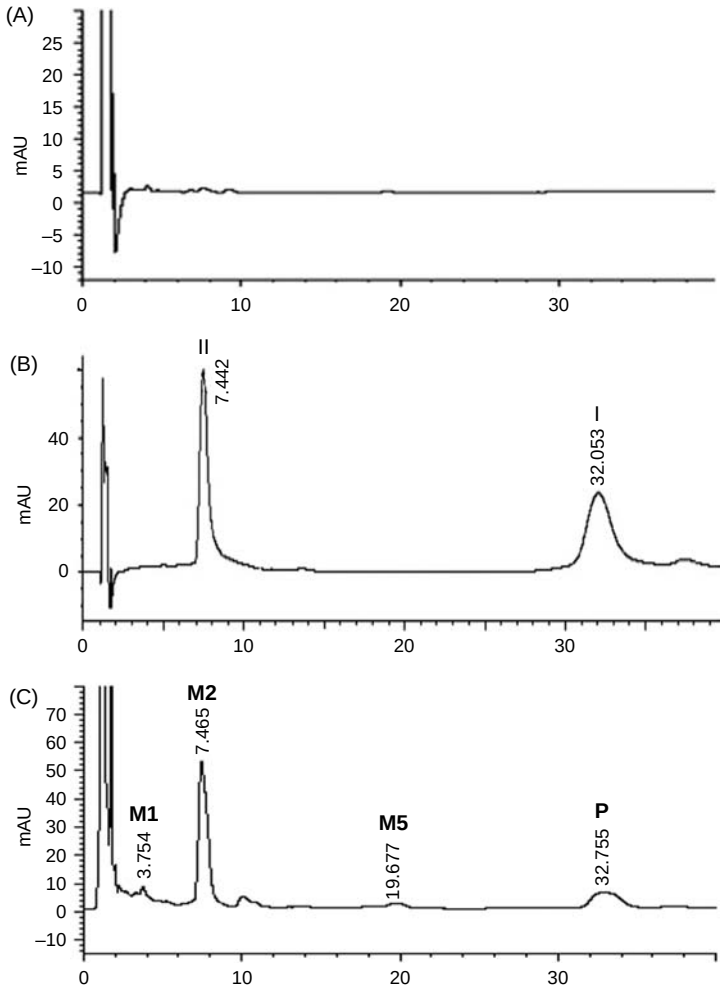

Figure 1. Representative HPLC-DAD chromatograms at 274 nm of (A) blank plasma sample, (B) authentic samples of baicalein (I) and baicalin (II), and (C) plasma sample obtained at 3 h after taking baicalein: **P**, baicalein; **M1**, **M2**, and **M5**, metabolites.

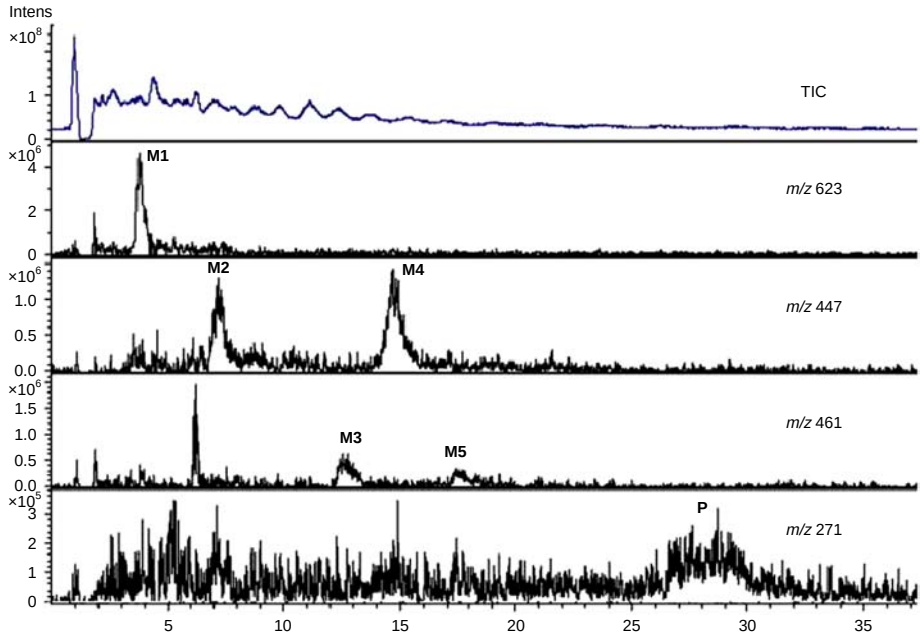

Figure 2. TIC and extracted ion chromatograms of baicalein (**P**) and its metabolites (**M1**–**M5**) in human plasma obtained at 3 h after taking baicalein.

which showed the characteristics of the flavone skeleton. The MS showed the pseudomolecular ions at *m/z* 623 $[M + H]^+$ and the corresponding MS^2 gave the fragment ions at *m/z* 447 and 271 which derived from the neutral loss of one (− 176 Da) and two glucuronic acid moieties (− 176 × 2 Da), respectively, indicating that **M1** should be the conjugate of baicalein with two molecular glucuronic acids. There are three hydroxyl groups in the structure of baicalein that can be conjugated with glucuronic acids. Regarding the chemical inertia of the 5-hydroxyl group which formed a hydrogen bond with the ketone carbonyl group in the structure of flavone, the glycosidation on the 5-hydroxyl group is impossible. Thus, the conjugation points of **M1** should be on 6- and 7-hydroxyl groups, which were supported by the HPLC behavior and UV spectrum of baicalein 6,7-di-*O*-glucuronide in previous reports [9,10]. Thus, **M1** was identified as baicalein 6,7-di-*O*-glucuronide.

The HPLC-DAD chromatogram showed the retention time of **M2** at 7.47 min, and the UV spectrum of **M2** exhibited the absorption maxima at 277 and 316 nm. The MS showed the pseudomolecular ion peak at *m/z* 447 $[M + H]^+$ and the corresponding MS^2 gave the fragment ion at *m/z* 271, suggesting that **M2** was a monoglucuronide of baicalein. The retention time and the UV spectrum of **M2** were coincident with those of the authentic sample of baicalin. Thus, **M2** was identified as baicalin (baicalein 7-*O*-glucuronide) by directly comparing with the authentic sample.

Metabolite **M3** ($t_R = 12.80$ min) gave $[M + H]^+$ at *m/z* 461 in the MS, which was 14 Da higher than that of **M2**, indicating that **M3** was a methylate of baicalin (**M2**). The corresponding MS^2 showed the fragment ion peak at *m/z* 285, which indicated that a glucuronyl group (− 176 Da) was conjugated with a free hydroxyl group in the structure of baicalein. For the same reason as mentioned above, the probable

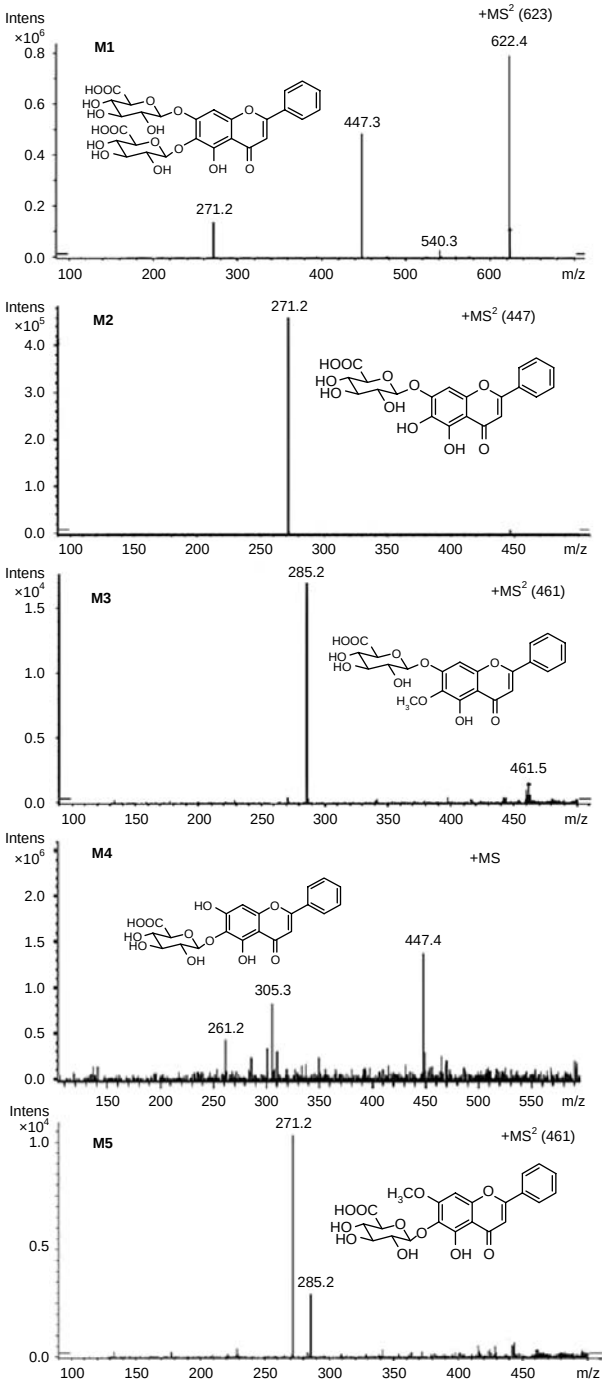

Figure 3. Respective MS and MS^2 of the metabolites (**M1**–**M5**) of baicalein in human plasma obtained at 3 h after taking baicalein.

conjugation points of **M3** should be on 6- or 7-hydroxyl groups. So there were two possible structures for **M3**, 6-methoxybaicalein 7-*O*-glucuronide, or 7-methoxybaicalein 6-*O*-glucuronide. Comparing with the references [9,10] and our previous study [8,11], the HPLC behavior, UV spectrum, and MS data of **M3** were coincident with those of 6-methoxybaicalein 7-*O*-glucuronide. Thus, **M3** was identified as 6-methoxybaicalein 7-*O*-glucuronide.

The full mass of **M4** ($t_R = 14.80$ min) showed the pseudomolecular ion peak at *m/z* 447 $[M + H]^+$, suggesting that **M4** should be another monoglucuronide of baicalein. Since the chemical inertia of the 5-hydroxyl group in the structure of baicalein, the conjugation point of a glucuronyl group in the structure of **M4** should be on the 6-hydroxyl group of baicalein. Thus, **M4** was identified as baicalein 6-*O*-glucuronide, which was supported by our previous study [8,11].

Metabolite **M5** ($t_R = 19.68$ min) showed the UV absorption maxima at 276 and 314 nm in the HPLC-DAD experiment. The MS showed the pseudomolecular ion peak at *m/z* 461 $[M + H]^+$ and the corresponding fragment ion peaks were at *m/z* 285 $[M + H-176]^+$ and 271 $[M + H-176-14]^+$ in the MS^2, suggesting that **M5** was the isomer of **M3** and a glucuronyl and a methyl group were present in the structure of **M5**. Comparing with the structure of **M3** and the UV spectra in the literature [12], **M5** was inferred as 7-methoxybaicalein 6-*O*-glucuronide, which is a new metabolite.

In the HPLC-DAD chromatogram, the retention time of **P** was at 32.76 min. The absorption maxima were at 276 and 320 nm in the UV spectrum. The LC-MS showed the pseudomolecular ion peak at *m/z* 271 $[M + H]^+$. The HPLC behavior, UV spectrum and the MS data of **P** were the same as those of the authentic sample of baicalein. Thus, **P** was identified as baicalein.

2.2 Metabolic pathway of baicalein in humans

Five metabolites, together with the parent drug baicalein, were mainly detected in human plasma obtained at 3 and 5 h after taking baicalein. However, the main metabolite **M2** could be obviously detected at any time point in human plasma. The chromatographic and mass spectrometric data of the metabolites are shown in Table 1. On the basis of the identification of these metabolites, the proposed metabolic pathway of baicalein in human plasma was inferred (Figure 4). Baicalein is absorbed directly into the blood in the gastrointestinal tract, and then it was mainly metabolized to its monoglucuronides, baicalin (**M2**), and baicalein 6-*O*-glucuronide (**M4**) by the UDP-glucuronyltransferase. Parts of **M2** and **M4** were further catalyzed by UDP-glucuronyltransferase to form baicalein 6,7-di-*O*-glucuronide (**M1**) or by methyltransferase to form 6-methoxybaicalein 7-*O*-glucuronide (**M3**) and 7-methoxybaicalein 6-*O*-

Table 1. Chromatographic and mass spectrometric characteristics of metabolites of baicalein in human plasma.

Metabolite	t_R (min)	UV λ_{max} (nm)	$[M + H]^+$ (*m/z*)	MS^2 (*m/z*)
M1	3.75	270,308	623	447,271
M2	7.47	277,316	447	271
M3	12.80	–	461	285
M4	14.80	–	447	–
M5	19.68	276,314	461	285,271

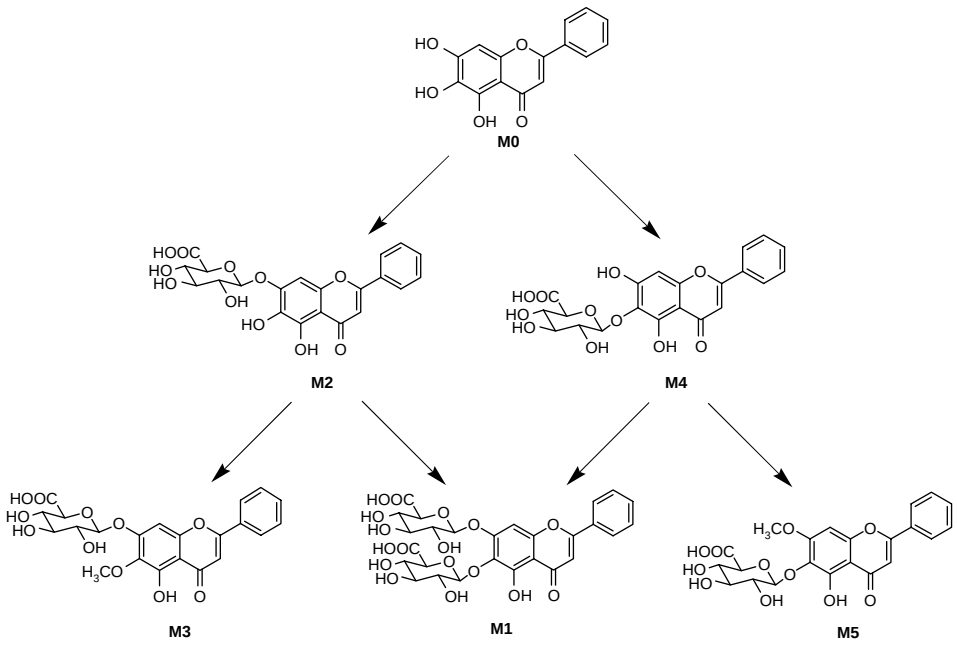

Figure 4. The proposed metabolic pathway of baicalein in human plasma after taking baicalein.

glucuronide (**M5**). Among them, baicalin is the main metabolite of baicalein in human plasma.

3. Conclusion

The methods of HPLC-DAD and LC-MS/MS have been established to detect and identify the metabolites of baicalein in human plasma. Five metabolites (**M1**–**M5**) together with the parent drug baicalein (**P**) were identified by directly comparing their HPLC behaviors, UV spectra, and MS data with those of the authentic samples and the literatures. Among them, 7-methoxybaicalein 6-*O*-glucuronide (**M5**) is a new metabolite *in vivo*. It is suggested that baicalein is quickly metabolized in humans after taking baicalein and the main metabolite of baicalein in humans is baicalin. This study gives the proposed metabolic pathway of baicalein in humans and provides important methods for further pharmacokinetic study of baicalein in humans.

4. Experimental

4.1 Materials and reagents

Baicalein was prepared from baicalin by enzymatic hydrolysis in our laboratory. The chemical structure of baicalein was identified by comparison of its ^{1}H NMR, ^{13}C NMR, and MS data with those in the literature [13]. The purity of baicalein was above 98% by HPLC analysis. Acetonitrile, methanol (Fisher, Fairlawn, NJ, USA), and sodium dihydrogen phosphate (J.T. Baker, Phillipsburg, NJ, USA) were of HPLC grade. The isopropanol, phosphoric acid, formic acid, and acetic acid were of analytical grade (Tianjin, China). The distilled water was purchased from Peking University Health Sciences Center (Beijing, China).

4.2 HPLC-DAD and LC-MS/MS analysis conditions

The HPLC-DAD detection was performed with an Agilent 1100 series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a quaternary pump

and a DAD. The data were processed using Agilent Chemstation for LC 3D software (version Rev. A.08.03). The chromatography analysis was carried out on a BISCHOFF C_{18} column (2.0 × 150 mm, 5 μm) at 35°C. The mobile phase was acetonitrile/water/formic acid (24:76:0.5, v/v/v) and the flow rate was 0.3 ml/min. The detection wavelength was set at 274 nm, together with wavelength scanning from 200 to 400 nm. The sample injection volume was 10 μl.

The LC-MS/MS analysis was performed with an Agilent 1100 series LC/MSD Trap SL system (Agilent Technologie, Palo Alto, CA, USA) equipped with an electrospray ionization (ESI) source and connected with an Agilent 1100 series HPLC system. The ESI-MS was acquired in positive ion mode and the mass range was set from *m/z* 100 to 1000 for full-scan MS analysis. Ultrahigh-purity helium (He) was used as the collision gas and high-purity nitrogen (N_2) as the nebulizing gas. The ESI parameters were set as follows: capillary voltage, 3.5 kV; nebulizer pressure, 35.0 psi; dry gas flow rate, 8 l/min; and dry gas temperature at 330°C. The most abundant ions were selected for MS^2 analysis and the fragmentation amplitude for MS^2 was 1.0 V. The recorded mass spectra were evaluated using the MSD Trap Control software (version 5.1).

4.3 Subjects

Three healthy male volunteers were involved in these studies, who were nonsmokers with no history of significant medical illness and medicine hypersensitiveness. All volunteers provided written informed consent. They did not consume any other drugs and alcohol within the last 2 weeks. Each of the volunteers took 360 mg of baicalein powder orally with 250 ml of water. During the period of study, strenuous physical and mental activities were limited and the use of alcohol, tea, coffee, cola, and fruit juice was forbidden.

4.4 Plasma sample collection and preparation

The blood samples were collected into heparinized vacuum tubes before dosing and at 0.33, 0.67, 1, 1.5, 2, 3, 5, 9, 13, 17, and 24 h after dosing and the volume was 4 ml each time. The blood samples were centrifuged at 3000 rpm for 10 min to obtain the plasma samples. The plasma samples were stored immediately at −20°C until analysis.

The varian C_{18} solid-phase extraction (SPE) cartridge was used to purify the plasma sample. Before the plasma sample was purified, the SPE cartridge was treated with 2 ml of isopropanol, 20% acetic acid/isopropanol, methanol, distilled water, and 0.05 mol/l potassium dihydrogen phosphate–phosphoric acid buffer (pH = 4) consequently. The frozen plasma sample was thawed to room temperature and was vortexed, and then 0.6 ml of the plasma sample was added on the prepared SPE cartridge. The SPE cartridge carrying sample was eluted with 2 ml of distilled water, 1% of phosphoric acid, and 20% acetic acid/isopropanol consequently. The fraction of 20% of acetic acid/isopropanol was collected and evaporated to dryness under the nitrogen flow at 45°C. The residue was then dissolved in 100 μl of methanol and centrifuged at 5000 rmp for 10 min, and 10 μl of the supernatant was injected for HPLC-DAD and LC-MS/MS analyses.

Acknowledgements

This research was financially supported by the program of National Natural Science Foundation of China (Grant No. 30225049).

References

[1] Jiangsu New Medicinal College, *Dictionary of Chinese Materia Medica* (Shanghai Scientific and Technical Publisher, Shanghai, 1986), pp. 2017–2019.
[2] X.P. Zhang, Z.F. Li, and X.G. Liu, *Chin. Pharm. Bull.* **17**, 711 (2001).

[3] J. Xing, X.Y. Chen, and S.Q. Zhang, *J. Chin. Mass Spectrom. Soc.* **25**, 129 (2004).
[4] M.Y. Lai, S.L. Hisu, S.Y. Tsai, Y.C. Hou, and P.D.L. Chao, *J. Pharm. Pharmacol.* **55**, 205 (2003).
[5] T. Akao, K. Kawabata, E. Yanaginsawa, K. Ishihara, Y. Mizuhara, Y. Wakui, Y. Sakashita, and K. Kobashi, *J. Pharm. Pharmacol.* **52**, 1563 (2000).
[6] Q.M. Che, L. Yang, Y. Chen, and X.Y. Guo, *Chin. New Drugs J.* **16**, 604 (2007).
[7] X.Y. Guo, L. Yang, Y. Chen, and Q.M. Che, *Chin. Pharm. J.* **43**, 524 (2008).
[8] Y.J. Zhou, Q.M. Che, S.X. Xu, and Q.S. Sun, *Pharmazie* **55**, 626 (2000).
[9] K. Abe, O. Inoue, and E. Yumioka, *Chem. Pharm. Bull.* **38**, 208 (1990).
[10] N.P. Feng, B. Di, and W.Y. Liu, *Chem. Pharm. Bull.* **53**, 978 (2005).
[11] Q.M. Che, X.L. Huang, Y.M. Li, K. Zhang, T. Akao, and M. Hattori, *China J. Chin. Mater. Med.* **26**, 768 (2001).
[12] R. Singh, B.J. Wu, L. Tang, Z.Q. Liu, and M. Hu, *J. Agric. Food Chem.* **58**, 9384 (2010).
[13] H.Y. Wang, L.H. Xiao, L. Liu, S.X. Xu, and J. Shenyang, *Pharm. Univ.* **20**, 339 (2003).