Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 45 (2007) 510–515
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Simultaneous determination of salidroside and tyrosol in extracts of *Rhodiola* L. by microwave assisted extraction and high-performance liquid chromatography

Yu Mao [a,*], Yan Li [b], Ning Yao [b]

[a] *Center of Evaluation for Drug Safety, Second Military Medical University, Shanghai 200433, China*
[b] *Department of Chemistry, Fudan University, Handan Road, No. 220, Shanghai 200433, China*

Received 15 February 2007; received in revised form 22 May 2007; accepted 24 May 2007
Available online 2 June 2007

Abstract

Rhodiola L. has a long history of use in traditional Chinese medicine (TCM) and has shown great promise in recent clinical trials. Salidroside and tyrosol are two important active compounds present in this TCM. In this work, microwave-assisted extraction (MAE) followed by high performance liquid chromatography (HPLC) with a photodiode array detector (DAD) was developed for quantitative analysis of salidroside and tyrosol in *Rhodiola* L. samples. After systematical investigation, the optimal experimental parameters of soak time (60 min), extraction solvent volume (1 g sample, 5 mL), extract solvent composition (50% methanol/water), microwave power (400 W) and extraction time (5 min) were investigated. The optimized method provided satisfactory precision (R.S.D. values less than 7.5%), good recovery (from 94.4 to 123%), and good linear relation in the range of 5.0–500 μg/mL for salidroside and 1.0–100 μg/mL for tyrosol ($R^2 > 0.999$). The proposed method was applied to quantitative analysis of salidroside and tyrosol in *Rhodiola* L. samples from five different growing areas. To demonstrate the method feasibility, recirculation was also used to analyze salidroside and tyrosol in *Rhodiola* L. samples. The proposed MAE–HPLC–DAD is a simple, rapid and low-cost method for quantitative analysis of salidroside and tyrosol, and a potential tool for quality assessment of *Rhodiola* L. sample.

Keywords: *Rhodiola* L.; Microwave-assisted extraction; Salidroside; Tyrosol; High performance liquid chromatography

1. Introduction

Rhodiola L. (Crassulaceae, Hongjingtian in Chinese) has been known as a medicinal plant for a long time and has been used in Chinese traditional medicine to maintain body health. This precious perennial herbaceous plant is distributed at high altitudes in the Arctic and mountainous regions throughout Europe and Asia. As a drug of "source of adaptation to environment", the dried root of *Rhodiola* L. has been used in such special occupations as diver, astronaut, pilot and mountaineer to enhance the body's ability to survive in adverse environments. In recent years, many biological activities have been reported for *Rhodiola* species, such as adaptogenic [1,2], antihypoxia [3], antifatigue [4,5], involving in cell antiapoptosis process [6], antioxidant [7], anticancer [7] and enhancement in learning and memory [8,9].

The major active constituent of this medicinal herb is salidroside and tyrosol. Concentration of these two components in *Rhodiola* L. is one of the standard indexes to appraise the quality of *Rhodiola* L. [10]. Therefore, it is very interesting to quantitatively determined concentration of these two in TCMs. Their structures are shown in Fig. 1.

Some previously published methods, such as capillary zone electrophoresis [11] and high performance liquid chromatography [12–14], were developed to determinate the salidroside or tyrosol in *Rhodiola* L. samples. Prior to the analyses, it was required to isolate and extract salidroside from the TCMs. Various extraction techniques can be used for that purpose, e.g., hydro-distillation, heat recirculation, Soxhlet extraction and solvent extraction [15–18]. However, low extraction efficiency and high ratio of organic solvent may be encountered using these methods. Moreover, the procedures are time-consuming. These

* Corresponding author. Tel.: +86 21 25074387; fax: +86 21 25074386.
E-mail address: maoyu701115@yahoo.com.cn (Y. Mao).

0731-7085/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2007.05.031

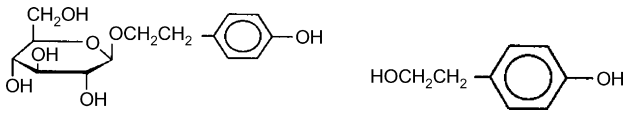

Fig. 1. The structural representation of salidroside (left) and tyrosol (right).

shortcomings have led to the consideration of the new technique in salidroside and tyrosol extraction, which typically use less organic solvent, time and energy (such as supercritical fluids, ultrasound and microwave). There has recently been widespread interest in the application of microwave heating to the analysis of active compounds in plant herbs [19–35]. The main virtue of MAE is the reduction of extraction time and organic solvent.

In this work, for the first time, MAE extract followed by HPLC method was developed for the rapid analysis of salidroside and tyrosol in *Rhodiola* L. samples. The experimental parameters: soak time, extraction solvent volume, extraction solvent composition, microwave power and extraction time were investigated. The method was tested by the application to the determination of salidroside and tyrosol in *Rhodiola* L. samples from different growing areas.

2. Experimental

2.1. Materials and chemicals

Five commercial herb samples of *Rhodiola* L. dried root were purchased from drug stores or markets in different provinces of China (Tibet, Yunnan, Qinghai, Jilin) and authenticated by department of Pharmacognosy, School of Pharmaceutical Science, Second Military Medical University. Standard of salidroside and tyrosol were obtained from the National Institute for the Control of Pharmaceuticals and Biological Products, Beijing, China. Methanol (HPLC grade) was obtained from Merk (Darmstadt, Germany). Ultra pure water with a resistance greater than 18 MΩ was collected from a certified Millipore Milli-Q system (Bedford, MA, USA).

2.2. Chromatography

The HPLC system HP 1100 series (Agilent Technologies, Waldbronn, Germany), equipped with the ChemStation software (Agilent Technologies) and comprised a binary high-pressure pump, an online vacuum degasser, an auto-sampler, a thermostated column compartment and a photodiode array detector using a maximum plot in the range 190–800 nm, was used for the chromatographic analysis. All separations were carried out on a Zorbax XDB-C18 column (150 mm × 4.6 mm i.d., 5.0 μm particle size) with a guard column (4.6 mm i.d. × 12.5 mm, packed with SB-C18) from Agilent Technologies. The mobile phase was 80% aqueous methanol (methanol/water = 20/80, v/v). The flow rate of mobile phase was set at 1.0 mL/min. The injection volume was 10 μL, and the column temperature was maintained at 25 °C.

2.3. Preparation of standard solutions

Twenty-five milligrams of salidroside and 5 mg tyrosol were accurately weighed and placed in a 5 mL volumetric flask. Eighty percent aqueous methanol was used as the solvent to give a 5000 mg/L salidroside and 1000 mg/L tyrosol mixed standard solution. An appropriate quantity of the mixed standard solution were diluted with 80% aqueous methanol in separate 5 mL volumetric flasks and made up to give a series of standard solutions (5, 25, 50, 100, 200 and 500 mg/L for salidroside and 1, 5, 10, 20, 40 and 100 mg/L for tyrosol) for plotting the calibration curve.

2.4. Preparation of sample solutions with microwave-assisted extration

A commercially available microwave oven (Haier, Qingdao, China) equipped with a magnetron of 2450 MHz with a nominal maximum power of 700 W was modified.[1] A hole (60 mm i.d.) was made through the top wall plate of the microwave oven, and through this a condensation tube was connected to the sample cartridge vessel located in the microwave irradiation zone. The whole system was open and run at atmospheric pressure. The apparatus was tested to be safe without potential danger by continuously irradiating the pure solvent (water or methanol) in the sample cartridge vessel for more than 1 h.

Samples from different area were powdered by an electrical blender and sieved through a 120-mesh sieve. The powder (1000 mg) was transferred into a 100 mL flask and 5 mL of 50% aqueous methanol was added. After the sample soaked up the solution (10, 30, 60, 120, 90, 120 and 150 min), the flask was put into microwave oven. The flask with the sample was heated by a microwave at the power of 200–700 W for 1–8 min, and a condenser with a continuous flow of freezing water was used to condense the vapor so that the solvent could take part in the extraction process repeatedly. Upon the termination of the microwave irradiation, the flask was cooled in the air. After cooling, the mixture was filtered under vacuum, and the residue was washed with 10mL extract solvent. The filtrate was metered volume to a 100 mL volumetric flask. An aliquot of 10 μL of the final solution filtered through a 4.5 μm Millipore filter membrane (Bedford, MA, USA) was injected for the HPLC analysis. Five extractions were performed for each sample following the above procedures. Each extract was determined in triplicate.

2.5. Preparation of solutions for recovery test

Rhodiola crenulata from Tibet (China) was selected for recovery test. Sample was treated as described in Section 2.4. The powder (1000 mg) was transferred into a 100 mL flask with 0, 0.5, 1.0 and 2.0 mL of the mixed standard solution(5000 mg/L

[1] The modification of commercially supplied laboratory apparatus should only be carried out by thoroughly qualified personnel and must not render the equipment unsafe for use as defined by local regulations. The modifications described in this paper are not advocated by *Journal of Pharmaceutical and Biomedical Analysis*.

salidroside and 1000 mg/L tyrosol, solvent was 50% aqueous methanol), and then 5.0, 4.5, 4.0 and 3.0 mL of 50% aqueous methanol was added. The resultant four solutions spiked by different amount of standard (0, 2.5, 5.0 and 10.0 mg of salidroside and 0, 0.5, 1.0 and 2.0 mg of tyrosol) were pretreated and analyzed as Section 2.4. The original drug concentrations present in the *Rhodiola* L. powder (first solution with no standard added) were subtracted from the total concentration of the other three solutions, thus only the added drug was determined and compared with the known added amounts of salidroside and tyrosol to calculate the recovery. For every concentration, six replicate analyses were conducted in 1 day or in consecutive 6 day to evaluate the intra-day and inter-day method precision.

3. Results and discussion

3.1. Optimization of extraction parameters

3.1.1. Soak time and extraction solvent volume

The sample used to optimize extraction condition was *Rhodiola crenulata* from Tibet. Because the herb was dry powder, enough soak time was indispensable to absorb sufficient microwave energy when carry out the MAE. In the experiment, 1 g sample was soaked into 5 mL solvent (50% aqueous methanol) for 10, 30, 60, 120 and 150 min before MAE. When soak time <60 min, extract efficiency increased with soak time. But longer soak time did not significantly increase the peak area of salidroside and tyrosol. There may be dynamic equilibrium between solvent and components. Hence 60 min was chosen as soak time.

After soaking time was settled, 1 g sample was soaked and extracted with 1, 2, 5, 10 and 20 mL 50% aqueous methanol, respectively. It is obviously that 5 mL solvent already has the satisfactory behavior for both salidroside and tyrosol. More extract solvent did not markedly increase the extraction efficiency.

3.1.2. Extract solvent

Nine different ratio of methanol-water mixture (10, 20, 30, 40, 50, 60, 80 and 90%, v/v, methanol) were tested as the extraction solvent of MAE with soak time of 60 min and solvent volume of 5 mL. The signal increases with water ratio up to 50%. When water ratio increased up to 60% or more, the peak areas did not increased any more or even decreased. Additionally, too high water ratio leads to more impurities like polycose, which may interfere with the detection of salidrolide and tyrosol. So 50% aqueous methanol solvent was selected to extract samples.

3.1.3. Microwave power and extraction time

The MAE parameters of microwave power and extraction time were studied. *Rhodiola crenulata* sample was extracted with optimized condition mentioned above at different microwave power (200, 400 and 700 W) and different irradiation time (1–8 min). As seen from Fig. 2, the extraction efficiency increased with the exposure time. However, the extraction efficiency decreased when the irradiation time was more than 5 min. This may be due to that high microwave power and long irradiation time can lead to decomposition of the analyte. The best extraction efficiencies of salidroside was achieved at the 400W for 5 min, and the extraction efficiency of tyrosol at 400 W for 5 min was only a little less than that obtained at 700 W for 5 min. So in the followed work, MAE was performed at microwave power of 400 W and irradiation time 5 min.

3.2. HPLC analysis

Using the Zorbax XDB C18 column and methanol–water as the solvent mixture, good separation was achieved within 12 min, with the retention times of salidroside and tyrosol determined as 6.11 ± 0.10 and 7.82 ± 0.08 min, respectively. Addition of acetic or phosphoric acids to the solvent system was tested but gave no benefit over water alone. The typical chromatographic profiles of the standard solution and the real sample solution were shown in Fig. 3. The target components in the chromatographic profile of samples were identified by comparing the retention times and the characteristic of the UV spectra of these peaks with those presented in the chromatogram of the mixture standard solution. Almost no interference was presented in the chromatographic separation, and each target peak had a good resolution. The system suitability was conducted by using the standard solutions and evaluated by making five replicate injections. The system was deemed to be suitable for use if the tailing factor was less than 1.2, the resolution was greater than 1.5 and column plate number was more than 7000 for each analyte.

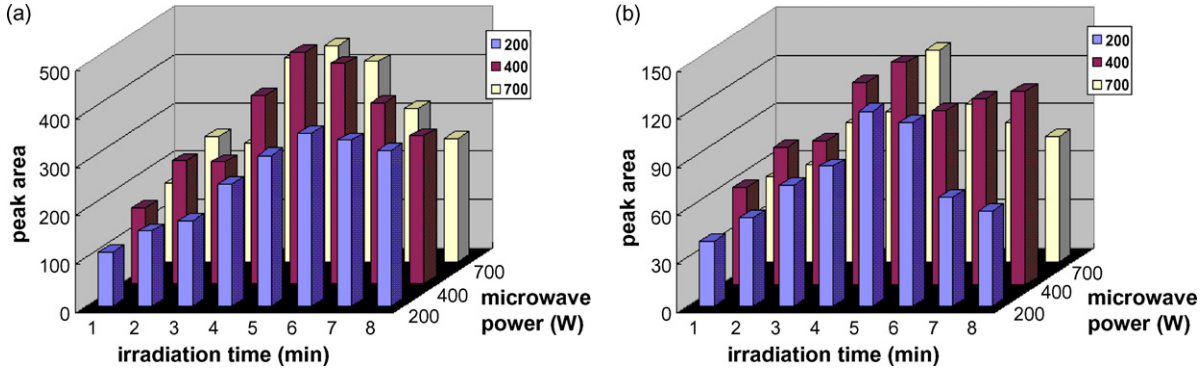

Fig. 2. The effect of microwave power and extraction time on the extraction efficiencies of salidroside (a) and tyrosol (b) in *Rhodiola crenulata* sample from Tibet.

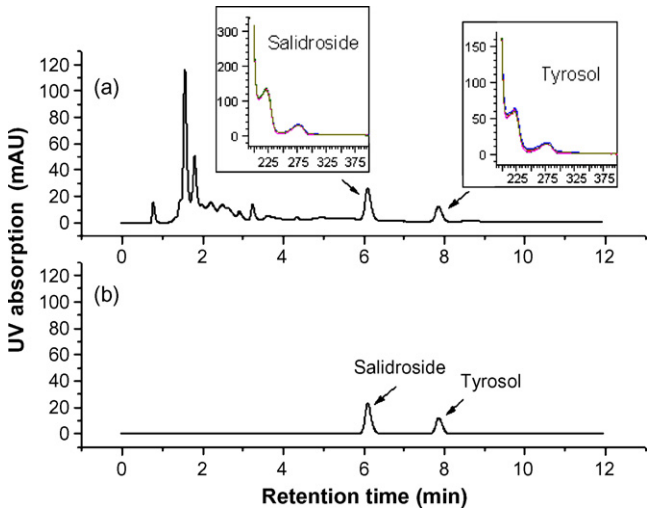

Fig. 3. Representative chromatography profile of (a) *Rhodiola crenulata* from Tibet. (b) Mixed standard of salidroside (0.050 mg/mL) and tyrosol (0.010 mg/mL). (Inset) The UV spectra of salidroside (left) and tyrosol (right). HPLC conditions: column: Eclipse XDB-C18 column (150 mm × 4.6 mm i.d., 5.0 μm particle size) with a guard column (4.6 mm i.d. × 12.5 mm, packed with SB-C18) from Agilent Technologies; the mobile phases: methanol/water = 20/80 (v/v); flowing rate: 1.0 mL/min; detection: 278 nm; temperature: 25 °C; injection: 10 μL; wavelength: 278 nm.

3.3. Method validation

The analytical method described above was used to analyze the *Rhodiola* L. samples. This method was validated for its specificity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ) with the following results.

The retention times and the UV spectra of the eluted components from the samples agreed well with those of the standards (Fig. 3), indicating the identity and purity of the peaks eluted from the samples. Therefore, the specificity of the method was validated.

For quantification of salidroside and tyrosol, calibration curves were constructed and tested for linearity. The plot of peak area (Y) against the concentration (X, mg/L) was evaluated using linear regression analysis. The regression equations and correlation coefficients (R^2) of salidroside and tyrosol were derived as $Y = 2.63X - 3.70$ ($n = 6$, $R^2 = 0.9999$, 5–500 mg/L) and $Y = 7.03X - 1.78$ ($n = 6$, $R^2 = 0.9996$, 1–100 mg/L), respectively. The limit of detection (LOD), defined as the lowest sample concentration which can be detected (S/N > 3), was 2.52 ng for salidroside and 1.20 ng for tyrosol, and the limit of quantification (LOQ), defined as the lowest sample concentration which can be quantitatively determined with suitable precision and accuracy (S/N > 10), was 8.39 ng for salidroside and 4.00 ng for tyrosol (R.S.D. < 10%).

The injection repeatability was determined by the injection of continuous six times using the same mixture standard solution. All the R.S.D. values were lower than 0.2% (data not shown). The intra-day and inter-day precision was examined by performing six replicate injections of the mixture standard solutions used above. The intra-day precision was performed with the interval of 4 h in 1 day, while the inter-day precision was performed over 6 days. It was shown that the R.S.D. values of retention time were lower than 0.54%, while the R.S.D. values of peak area were lower than 3.51% both for the intra-assay and inter-assay precision. The precision result for salidroside and tyrosol was presented in Table 1.

The sample preparation procedure was evaluated by the recovery of salidroside and tyrosol added to the *Rhodiola crenulata* before extraction (described in Section 2.5). Average recovery was summarized in Table 2. From the table, it showed that all the average recoveries were more than 94% and R.S.D. less than 7.5%. The results of intra- and inter-day were similar

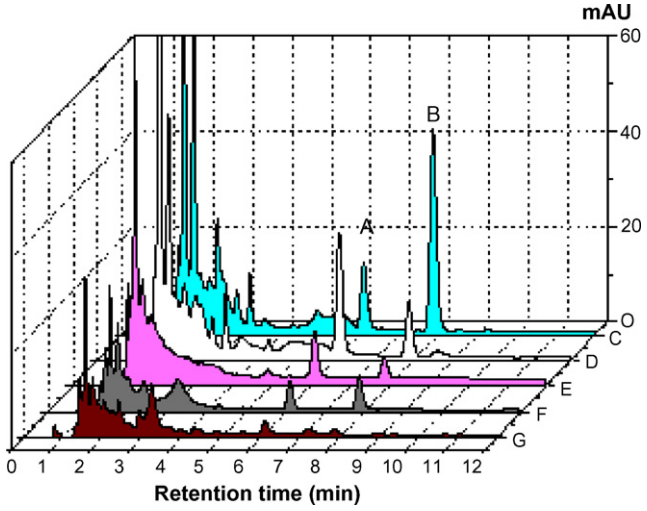

Fig. 4. HPLC chromatograms of salidroside (A) and tyrosol (B) from different sources. Sample: (C) *Rhodiola sachalinensis* from Jilin province; (D) *Rhodiola crenulata* from Tibet; (E) *Rhodiola coocinea* from Qinghai province; (F) *Rhodiola yunnanensis* from Yunnan province; (G) *Rhodiola fassiglea* from Yunnan province; wavelength: 278 nm.

Table 1
Intra-day ($n = 6$) and inter-day ($n = 6$) precision of the developed method

Analytes	Concentration (mg/L)	Retention time R.S.D. (%)		Peak area R.S.D. (%)	
		Intra-day	Inter-day	Intra-day	Inter-day
Salidroside	5.0	0.13	0.54	0.82	2.10
	50.0	0.21	0.11	0.18	0.36
	500.0	0.16	0.15	0.25	0.15
Tyrosol	1.0	0.13	0.14	0.45	3.51
	10.0	0.18	0.19	0.16	1.44
	100.0	0.06	0.20	0.09	1.78

Table 2
Recovery of salidroside and tyrosol from *Rhodiola Rosea* L. sample ($n = 6$)

Analytes	Spiked (mg)	Inter-day			Intra-day		
		Concentration found		Recovery (%)	Concentration found		Recovery (%)
		Mean	R.S.D. (%)		Mean	R.S.D. (%)	
Salidroside	2.5	2.59	4.1	103.6	2.43	4.6	97.2
	5.0	4.72	3.5	94.4	5.59	5.6	111.8
	10.0	9.51	4.4	95.1	9.62	4.8	96.2
Tyrosol	0.5	0.48	2.6	96.0	0.52	6.6	104.0
	1.0	1.23	7.5	123.0	1.19	5.3	119.0
	2.0	1.89	0.6	94.5	1.92	2.9	96.0

Table 3
The relative content of salidroside and tyrosol in five TCM samples by MAE and recirculation

Sample	Salidroside (%)		Tyrosol (%)	
	MAE	Recirculation	MAE	Recirculation
Rhodiola sachalinensis	0.296 ± 0.013	0.274 ± 0.020	0.458 ± 0.048	0.426 ± 0.032
Rhodiola crenulata	0.617 ± 0.054	0.605 ± 0.068	0.208 ± 0.035	0.210 ± 0.049
Rhodiola coocinea	0.320 ± 0.035	0.331 ± 0.041	0.149 ± 0.010	0.112 ± 0.008*
Rhodiola yunnanensis	0.350 ± 0.028	0.361 ± 0.021	0.429 ± 0.012	0.411 ± 0.023
Rhodiola fassiglea	0.179 ± 0.006	0.172 ± 0.016	0.077 ± 0.002	0.065 ± 0.006

* $p < 0.05$, compare to MAE.

which demonstrated good reproducibility and accuracy within the concentration range selected.

3.4. Determination of salidroside and tyrosol in five Rhodiola L. samples

The optimized method was used to determine the relative content (%) of the salidroside and tyrosol in five *Rhodiola* L. samples. The HPLC/DAD profiles were illustrated in Fig. 4, and the results of determination are presented in Table 3. The results of the analyses showed that there was a wide variation in the content of salidroside and tyrosol in different samples. *Rhodiola crenulata* from Tibet was found to contain the highest amount of salidroside, while *Rhodiola sachalinensis* from Jilin province contained the highest amount of tyrosol. Salidroside and tyrosol were very low in *Rhodiola fassiglea* from Yunnan province. Apparently the TCM with the best quality was regarded to be from Tibet and Jilin. The analytical method developed can be used for the quality control of *Rhodiola* L. sample.

3.5. Analysis of salidroside and tyrosol in Rhodiola L. by recirculation

To demonstrate the feasibility of this method, salidroside and tyrosol *in Rhodiola crenulata* was also isolated by the conventional recirculation method. The analytical results are listed in Table 3. As seen from Table 3, the relative contents by MAE were close to those by recirculation. There was no statistic difference between two methods expect for *Rhodiola coocinea* sample except for *Rhodiola coocinea*. The conventional recirculation method required long time (6 h). In the proposed method, the isolation of salidroside and tyrosol *in Rhodiola* L. was rapidly completed with small sample amount (1.0 g), little solvent (5 mL) and short time (5 min). MAE–HPLC is a simple, rapid and low-cost method for the determination of salidroside and tyrosol in *Rhodiola* L. samples.

4. Concluding remarks

In this study, a simple, accurate and rapid MAE–HPLC method was developed and this is the first report of MAE–HPLC simultaneous determination of salidroside and tyrosol in *Rhodiola* L. samples. The assay is reproducible, sensitive and has been fully validated. Furthermore, it was successfully applied in analysis of five different *Rhodiola* L. samples. The results indicate that herbals from different places show a specific and similar HPLC chromatogram and the evaluation of data might be useful in quality assurance as well as for determination of adulteration of the crude drug.

Acknowledgement

The study was supported by grants from National Basic Research Priorities Programme (Project no. 2001CB510202).

References

[1] A.A. Spasov, G.K. Wikman, V.B. Mandrikov, I.A. Mironova, V.V. Neumoin, Phytomedicine 7 (2000) 85–89.
[2] N.N. Rege, U.M. Thatte, S.A. Dahanukar, Phytother Res. 13 (1999) 275–291.
[3] H.Q. Ming, Chin. J. Pharm. Bull. 21 (1986) 373–375.
[4] M. Furmanova, E. Skopinska-Rozewska, E. Rogala, M. Hartwich, Acta Soc. Bot. Pol. 67 (1998) 69–73.

[5] V. Darbinyan, A. Kteyan, A. Panossian, E. Gabrielian, G. Wikman, H. Wagner, Phytomedicine 7 (2000) 365–370.
[6] I. Mook-Jung, H. Kim, W. Fan, Y. Tezuka, S. Kadota, H. Nishijo, M.W. Jung, Biol. Pharm. Bull. 25 (2002) 1101–1104.
[7] W. Zheng, S. Wang, J. Agric. Food Chem. 49 (2001) 5165–5170.
[8] Y. Tezuka, W. Fan, R. Kasimu, S. Kadota, Phytomedicine 6 (1999) 197–203.
[9] W. Fan, Y. Tezuka, K.M. Ni, S. Kadota, Chem. Pharm. Bull. 49 (2001) 396–401.
[10] S.G. Kang, J. Wang, J. Zhang, F.Y. Liu, Z.J. Xu, China J. Chin. Mater. Med. 23 (1998) 365–368.
[11] H.L. Wang, Y.F. Ming, Y.L. Lee, et al., Chin. J. Anal. Lab. 24 (2005) 40–42.
[12] W.F. Bao, X. Li, J. Shengyang Pharm. U. 12 (1995) 263–265.
[13] X. Han, T. Zhang, Y. Wei, et al., J. Chromatogr. A 971 (2002) 237–241.
[14] Y.C. Lu, P.C. Lin, S.H. Bao, J. Qinghai. Normal U. 1 (2005) 34–37.
[15] H.B. Li, F. Chen, J. Chromatogr. A 932 (2001) 91–95.
[16] F.Q. Guo, Y.Z. Liang, C.J. Xu, X.N. Li, L.F. Huang, J. Pharmaceut. Biomed. 35 (2004) 469–478.
[17] J.Q. Yu, J.C. Lei, H.D. Yu, X. Cai, G.L. Zou, Phytochemistry 65 (2004) 881–884.
[18] L.F. Huang, B.Y. Li, Y.Z. Liang, F.Q. Guo, Y.L. Wang, Anal. Bioanal. Chem. 378 (2004) 510–517.
[19] K. Robards, J. Chromatogr. A 1000 (2003) 657–691.
[20] S.J. Stout, A.R. Dacunha, G.L. Picard, M.M. Safarpour, J. Agric. Food Chem. 44 (1996) 3548–3553.
[21] J.R.J. Pare, J.M.R. Belanger, S.S. Stafford, Trends Anal. Chem. 13 (1994) 176–184.
[22] X.J. Pan, G.G. Niu, H.Z. Liu, J. Chromatogr. A 922 (2001) 371–375.
[23] Y. Yang, L. Chen, X.X. Zhang, Z.K. Guo, J. Liq. Chromatogr. R. T. 27 (2004) 3203–3211.
[24] H. Li, B. Chen, L. Nie, S.Z. Yao, Phytochem. Anal. 15 (2004) 306–312.
[25] C.Z. Liu, H.Y. Zhou, Y. Zhao, Anal. Chim. Acta 581 (2007) 298–302.
[26] F.Y. Du, X.H. Mao, G.K. Li, J. Chromatogr. A 1140 (2007) 56–62.
[27] C.Z. Liu, H.Y. Zhou, Q. Yan, Anal. Chim. Acta 582 (2007) 61–68.
[28] Z.L. Liu, J. Wang, P.N. Shen, et al., Sep. Purif. Technol. 52 (2006) 18–21.
[29] M. Zhu, Y. Cao, G.R. Fan, J. Liq. Chromatogr. R. T. 30 (2007) 123–133.
[30] L.Y. Zhao, G.H. Zhao, F. Chen, et al., J. Agric. Food Chem. 54 (2006) 8346–8351.
[31] C.W. Huie, Anal. Bioanal. Chem. 373 (2002) 23–30.
[32] J.T. He, Z.H. Shi, W.B. Chang, J. Liq. Chromatogr. R. T. 27 (2004) 1769–1784.
[33] Z.K. Guo, Q.H. Jin, G.Q. Fan, et al., Anal. Chim. Acta 436 (2001) 41–47.
[34] L.G. Chen, H.Y. Jin, L. Ding, et al., J. Chromatogr. A 1140 (2007) 71–77.
[35] H.Y. Zhou, C.Z. Liu, J. Chromatogr. B 835 (2006) 119–122.