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High-performance liquid chromatographic determination and pharmacokinetic study of vitexin-2″-*O*-rhamnoside in rat plasma after intravenous administration

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Abstract

A simple and specific high-performance liquid chromatographic (HPLC) method was developed for the pharmacokinetic study of vitexin-2″-*O*-rhamnoside (VOR) in rat after intravenous administration. The plasma samples were deproteinized with methanol after addition of internal standard (i.s.) hesperidin. HPLC analysis was performed on a Diamonsil ODS C18 analytical column, using acetonitrile-0.3% formic acid (20:80, v/v) as the mobile phase with UV detection at 270 nm. The standard curve was linear over the range of 0.1070–21.41 μg/mL in rat plasma. The average extraction recovery of VOR was 97.9 ± 3.1%, and the relative standard deviations (R.S.D.s) of the intra- and inter-day precisions were no more than 7.4 and 8.5%, respectively. The lower limit of quantification (LLOQ) was 0.1070 μg/mL. The AUC of VOR was proportional to the dose after intravenous administration of 15, 30, 60 and 120 mg/kg body weight, and the elimination half-life ($t_{1/2\beta}$), systemic clearance (Cl) and apparent volume of distribution (V_c) were not significantly different among the four doses, and all the results indicated that the pharmacokinetics of VOR in rat obeyed first-order kinetics.

Keywords: Vitexin-2″-*O*-rhamnoside; Pharmacokinetics; High-performance liquid chromatography; Rat plasma

1. Introduction

Flavonoids are polyphenolic compounds that are present in many plants, and exhibit a variety of biological and pharmacological activities, such as the prevention of coronary heart disease [1], inhibition of tumor promotion [2], anti-oxidation [3,4] and anti-inflammation [5] effects. Vitexin-2″-*O*-rhamnoside (VOR) is one of the main flavonoid components of the leaves of *Crataegus pinnatifida* Bge. var *major* N. E. Br. [6], which has been demonstrated to inhibit DNA synthesis in MCF-7 cells [7].

To date, there are no published reports of VOR in rat plasma after intravenous administration and so it was necessary to develop an assay to fully evaluate the pharmacokinetics of VOR. In recent years, many papers have appeared dealing with electrophoretic [8,9] and LC–UV/LC–MS [10–13] methods for VOR or vitexin analysis in natural extracts of *Crataegus* species, however, no data have so far been published on VOR analysis in biological fluids. In this study, a high-performance liquid chromatographic (HPLC) method was developed and validated for the determination of VOR in rat plasma. The method was applied to a pharmacokinetic study following intravenous administration of VOR at four different doses.

2. Experimental

2.1. Chemicals and reagents

The internal standard (i.s.), hesperidin (Fig. 1a) was provided by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC grade acetonitrile was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), and the water used in all experiments was purified by a Milli-Q® Biocel Ultrapure Water System

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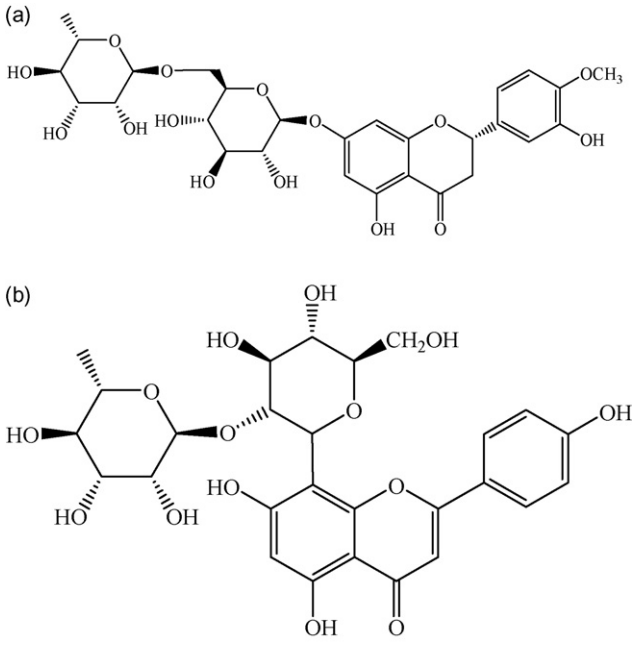

Fig. 1. Chemical structures of hesperidin (a) and vitexin-2″-*O*-rhamnoside (b).

(Millipore, Bedford, MA, USA). All other chemicals were of analytical reagent grade purchased from Sinopharm Chemical Reagent Co., Ltd.

2.2. Isolation and identification

A sample (3 kg) of the leaves of *C. pinnatifida* Bge. var *major* N. E. Br. collected in Shenyang, Liaoning Province, China was extracted three times with 24 L of 70% aqueous ethanol. The crude extract was concentrated and then passed through a porous-polymer resin (AB-8, Tianjin, China). The fraction eluted with 60% ethanol was evaporated under reduced pressure to obtain an extract which was then fractioned with chloroform and *n*-butanol successively. The *n*-butanol layer (100 g) was chromatographed on a silica–gel column with chloroform–methanol as the gradient eluent to afford 24 fractions. Fractions 7–10 (chloroform–methanol 4:1, 10 g) were subjected to silica–gel column chromatography and 1.0 g of VOR was obtained from the fraction eluted with ester acetic acid:butanone:formic acid:water (4:3:1:1). The purity (99%) was checked by HPLC (Fig. 2). The structure of VOR (Fig. 1b) was fully characterized by ^{1}H NMR(δ): 6.78 (1H, s, 3-H), 6.25 (1H, s, 6-H), 10.89 (1H, brs, 7-OH), 8.05 (2H, d, J = 8.7, 2′6′-H), 6.91 (2H, d, J = 8.7, 3′5′-H), 10.38 (1H, brs, 4′-H), 4.77 (1H, d, J = 9.9, 1″-H-Glu), 4.98 (1H, s, 1‴-H-rha), 0.463 (3H, d, CH_3-rha) and ESI-MS *m*/*z* 577 $[M–H]^-$ compared with the data in literature [14].

2.3. Chromatographic system and conditions

The analysis was carried out on Shimadzu HPLC system (Kyoto, Japan), consisting of an LC-10AT pump and an SPD-10A VP UV–vis spectrophotometric detector. Data collection and integration were accomplished using a Chromato-Solution

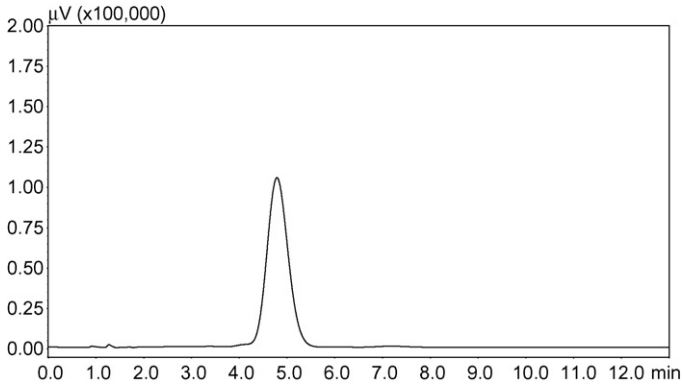

Fig. 2. Chromatogram of vitexin-2″-*O*-rhamnoside.

Light workstation (Shimadzu). The analytes were determined at room temperature on an analytical column (Diamonsil ODS C18, 150 mm × 4.6 mm, i.d., 5 μm) (Dikma Technologies, Bejing, China). The mobile phase consisted of a mixture of acetonitrile–0.3% formic acid (20:80, v/v). The mobile phase was passed under vacuum through a 0.45 μm membrane filter and degassed before use. The analysis was carried out at a flow rate of 1 mL/min with the detection wavelength set at 270 nm.

2.4. Preparation of calibration standards and quality control samples

Stock standard solutions of VOR and i.s. were prepared with methanol. Eight calibrators of VOR with i.s. were prepared by dilution of stock solutions followed by spiking with drug-free plasma. The calibration range was 0.1070–21.41 μg/mL VOR. Quality control (QC) samples were prepared at low (0.185 μg/mL), medium (1.85 μg/mL), and high (18.5 μg/mL) concentrations in bulk and aliquots were stored frozen before use.

2.5. Plasma sample preparation

To 200 μL plasma, 20 μL acetic acid, 100 μL i.s. (38 μg/mL), and 1 mL methanol were added, followed by vortex mixing for 1 min and centrifuging at 3000 × *g* for 15 min. The supernatant was collected and evaporated to dryness at 50 °C under a gentle stream of nitrogen. The residue was reconstituted with 200 μL mobile phase, and centrifuged at 15,000 × *g* for 5 min, and an aliquot (20 μL) of the supernatant was injected into the HPLC system.

2.6. Method validation

To determine the selectivity of this method, blank rat plasma, plasma spiked with known amounts of VOR and i.s. (38 μg/mL) and plasma samples from rats after intravenous doses of VOR were analyzed.

The linearity was evaluated over the concentration range of 0.1070–21.41 μg/mL at eight levels (0.1070, 0.2141, 0.4282, 1.070, 2.676, 5.352, 10.70 and 21.41 μg/mL). The calibration

curves for VOR in plasma were generated by plotting the peak area ratio of VOR to i.s. versus the nominal concentrations in the standard plasma samples. The regression equation was obtained by weighted ($1/c^2$) least square linear regression. The lower limit of quantification (LLOQ) was defined as the lowest concentration of VOR in the calibration curves, giving an acceptable accuracy (RE) within ±20% and a precision (R.S.D.) that did not exceed 20%.

The intra- and inter-assay precisions were evaluated by analyzing the quality control samples at three concentration levels of VOR (0.185, 1.85 and 18.5 μg/mL). For the intra-day validation, five replicates of the QC plasma samples were analyzed on the same day. For the inter-day validation, five replicates of the QC plasma samples were analyzed on three different days. The precision was expressed as the R.S.D. which should be less than 15%, except at the LLOQ where it should not exceed 20%, and the accuracy of the assay was determined by comparing the means of the determined VOR concentrations with the nominal concentrations. The mean percentage deviation from the nominal values expressed as the RE which should be within ±15% of the nominal value, except at the lower limit of quantification where it should not exceed ±20%.

The extraction recovery was determined by comparing the peak areas of VOR obtained for the QC samples (0.185, 1.85 and 18.5 μg/mL, $n = 6$) that were subjected to the extraction procedure with those obtained from blank plasma extracts that were spiked post-extraction at the corresponding concentrations.

The freeze–thaw stability was tested after three freeze (24 h storage, −20 °C) and thaw (room temperature for 2–3 h) cycles and the long-term stability of VOR in plasma was studied using QC samples at two concentration levels (0.185 and 18.5 μg/mL) stored at −20 °C for 2 weeks. The post-preparation stability was assessed by analyzing six replicates of the reconstituted QC samples stored at 25 °C.

2.7. Animals and pharmacokinetic study

All animal studies were performed according to the Guidelines for the Care and Use of Laboratory Animals that was approved by the Committee of Ethics of Animal Experimentation of Liaoning University of TCM.

Male Wistar rats, weighing 250–300 g, were obtained from the Laboratory Animal Center of Liaoning University of TCM (Shenyang, China). They were kept in an environmentally controlled breeding room for 1 week before the experiments and fed with standard laboratory food and water ad libitum and fasted overnight before the experiment. Four groups (five rats/group) were randomly assigned to receive VOR solution via a tail vein injection at doses of 15, 30, 60 and 120 mg/kg, respectively. Blood samples (0.5 mL) were collected into heparinized tubes from the vena orbitalis at times of 3, 5, 8, 12, 17, 23, 30, 38, 47, 57 and 90 min after intravenous administration and then centrifuged at 3000 × g for 15 min. The obtained plasma was stored at −20 °C until analysis. Physiological saline (0.5 mL) was administered to compensate for the blood loss after each blood was withdrawn.

3. Results and discussion

3.1. Chromatography and extraction procedure

Under optimized HPLC conditions, 0.3% formic acid was added to the mobile phase consisting of a mixture of acetonitrile–water (20:80, v/v), to improve the peak shape. There were two absorption maxima at 270 and 340 nm in the UV spectrum of VOR. The i.s. had a maximum absorption at 280 nm. Therefore, the detection wavelength of 270 nm proved to be the most suitable and was selected for the assay.

The sample was deproteinized with methanol which was advantageous because methanol not only extracted VOR but also i.s. simultaneously; and it also has a high extraction efficiency and is inexpensive. Then, 20 μL acetic acid was added to the plasma, which resulted in a high extraction efficiency for VOR.

3.2. Method validation

3.2.1. Selectivity

Typical chromatograms of blank, spiked plasma and plasma sample are given in Fig. 3 which show no interfering peaks in the region of the location of the peaks of the analyte and i.s. The retention times of VOR and i.s. were approximately 4.6 and 10.9 min, respectively, with complete baseline resolution between the peaks of interest, and the total run time was 13.0 min.

3.2.2. Linearity, LOD and LLOQ

The evaluation of the linearity was performed with an eight-point calibration curve over the concentration range of 0.1070–21.41 μg/mL. The slope and intercept of the calibration graphs were calculated by weighted ($1/c^2$) least squares linear regression. The regression equation of the calibration curves was typically: $y = 0.1053x + 0.001525$, and r was 0.9965, where y is the peak area ratio of VOR to i.s., and x is the plasma concentration of VOR.

The limit of detection (LOD) was 0.04022 μg/mL which was determined by a signal-to-noise ratio (S/N) of 3. The lower limit of quantification defined as the lowest concentration on the calibration curve, was 0.1070 μg/mL, with the precision and accuracy within 15% verified by repeated analysis.

3.2.3. Precision and accuracy

The accuracy and precision of the method were evaluated with QC samples at three concentrations and using five replicates. The results are shown in Table 1. The intra- and inter-day precisions were satisfactory with R.S.D.s less than 7.4 and 8.5%, respectively. The REs of intra- and inter-day accuracy were within −4.9 to 0.86%. The precision and accuracy study indicated that the developed HPLC method was reproducible and accurate.

3.2.4. Extraction recovery

The extraction recoveries of VOR at three concentrations (0.185, 1.85 and 18.5 μg/mL) are shown in Table 2. The aver-

Table 1
Precision and accuracy of vitexin-2″-*O*-rhamnoside determination in rat plasma (intra-day: $n = 5$; inter-day: $n = 3$ days with 5 replicates per day)

Added concentration (μg/mL)	Intra-day			Inter-day		
	Mean detected concentration (μg/mL)	R.S.D. (%)	RE (%)	Mean detected concentration (μg/mL)	R.S.D. (%)	RE (%)
0.185	0.1762 ± 0.012	7.3	−4.9	0.1790 ± 0.014	7.9	−3.2
1.85	1.830 ± 0.135	7.4	−1.1	1.828 ± 0.154	8.5	−1.6
18.5	18.52 ± 0.853	4.6	0.13	18.66 ± 1.34	7.2	0.86

age extraction recovery of VOR and i.s. were 97.9 ± 3.1% and 96.9 ± 4.5% which suggested that there was negligible loss during extraction. The high recovery could be attributed to the high solubility of VOR in methanol and the one-step protein precipitation used in the sample preparation.

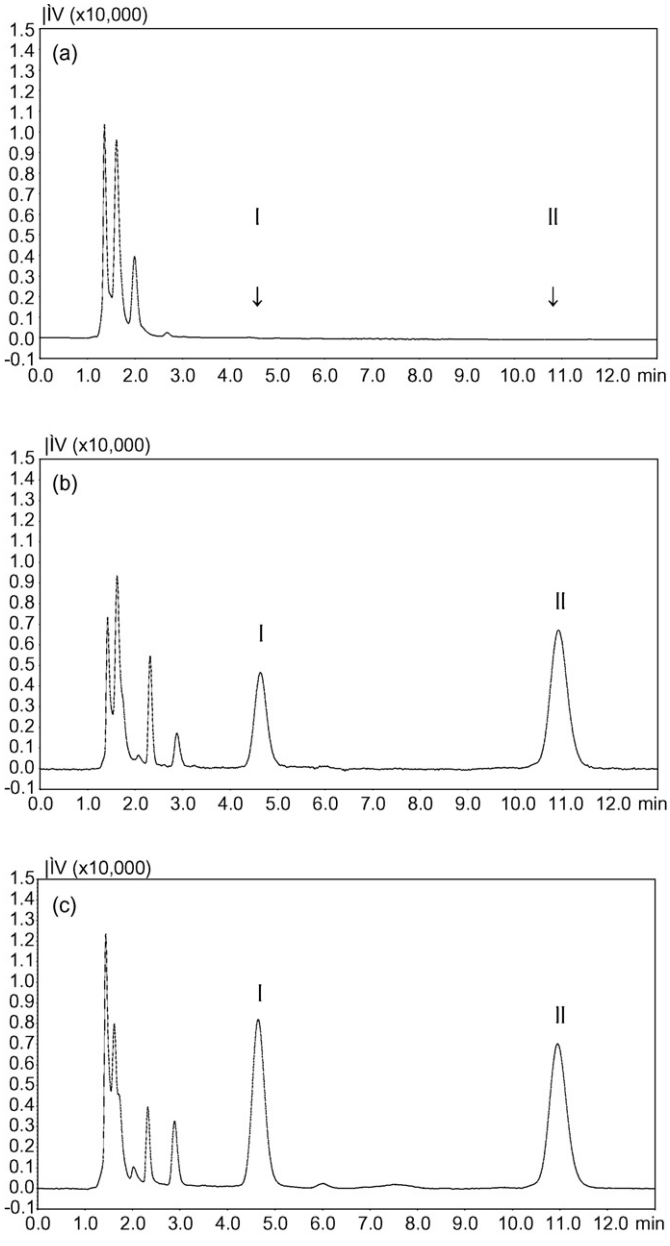

Fig. 3. Representative chromatograms of blank plasma (a), plasma spiked with vitexin-2″-*O*-rhamnoside and hesperidin (b) and plasma sample at 8 min after an intravenous administration of vitexin-2″-*O*-rhamnoside at dose of 30 mg/kg (c). Peak I: vitexin-2″-*O*-rhamnoside; Peak II: hesperidin.

Table 2
Recovery of vitexin-2″-*O*-rhamnoside in rat plasma ($n = 6$)

Added concentration (μg/mL)	Mean detected concentration (μg/mL)	Recovery (%)	R.S.D. (%)
0.185	0.1776 ± 0.009	96.0 ± 4.64	4.8
1.85	1.779 ± 0.118	96.2 ± 6.38	6.6
18.5	18.78 ± 0.847	101.5 ± 5.94	6.0

3.2.5. Stability

The results of the stability study involving QC samples at two levels (0.185 and 18.5 μg/mL, $n = 6$) are presented in Table 3. The R.S.D.s were below 9.0% and the REs were within −4.1 to 3.1%, which confirmed the high stability of VOR throughout the determination.

3.3. Pharmacokinetic studies

Pharmacokinetic data were processed by a DAS program (Drug And Statistics, Beijing Bosom Science and Technology Co., Ltd.). The plasma concentration–time curves of VOR in rats following intravenous injection of 15, 30, 60 and 120 mg/kg body weight are shown in Fig. 4, demonstrating that VOR is eliminated rapidly from the plasma. The plasma concentration of VOR was detectable only up to 90 min in rats using this analytical method. A two-compartment open model (Weight = $1/x$) gave the best fit to the plasma concentration–time curves obtained in rats. The systemic clearance (Cl) ranged from 0.290 ± 0.047 to 0.343 ± 0.037 L/min/kg, the elimination half-life ($t_{1/2\beta}$) ranged from 45.34 ± 24.0 to 61.69 ± 13.2 min and the apparent volume of distribution (V_c) ranged from 5.587 ± 5.58

Table 3
Stability of vitexin-2″-*O*-rhamnoside in rat plasma ($n = 6$)

Stability	Added concentration (μg/mL)	Mean detected concentration (μg/mL)	R.S.D. (%)	RE (%)
Freeze–thaw	0.185	0.1775 ± 0.012	7.2	−4.1
	18.5	19.07 ± 0.968	5.1	3.1
Long-term	0.185	0.1786 ± 0.014	8.1	−3.5
	18.5	18.77 ± 0.687	3.7	1.5
Post-preparative	0.185	0.1798 ± 0.016	9.0	−2.8
	18.5	19.04 ± 0.841	4.4	2.9

Table 4
Pharmacokinetic parameters of vitexin-2″-*O*-rhamnoside in rats (mean ± S.D., $n = 5$) after intravenous administration of doses of 15, 30, 60 and 120 mg/kg

Parameter	15 (mg/kg)	30 (mg/kg)	60 (mg/kg)	120 (mg/kg)
$t_{1/2\alpha}$ (min)	8.21 ± 8.01	7.46 ± 3.73	9.35 ± 2.44	8.72 ± 1.36
$t_{1/2\beta}$ (min)	45.34 ± 24.0	56.47 ± 18.7	61.57 ± 13.4	61.69 ± 13.2
V_c (L/kg)	5.587 ± 5.58	7.532 ± 1.89	8.885 ± 0.837	11.33 ± 1.55
Cl (L/min/kg)	0.290 ± 0.047	0.315 ± 0.062	0.335 ± 0.037	0.343 ± 0.037
$AUC_{0–90}$ (mg/L min)	41.08 ± 3.17	74.48 ± 9.29	141.4 ± 3.48	244.2 ± 37.6
$AUC_{0–\infty}$ (mg/L min)	53.09 ± 9.08	98.54 ± 16.5	181.8 ± 21.3	354.1 ± 38.0
K_{10} (1/min)	0.078 ± 0.011	0.049 ± 0.028	0.038 ± 0.004	0.031 ± 0.002
K_{12} (1/min)	0.083 ± 0.008	0.10 ± 0.130	0.031 ± 0.012	0.038 ± 0.011
K_{21} (1/min)	0.024 ± 0.0098	0.049 ± 0.050	0.023 ± 0.011	0.026 ± 0.010

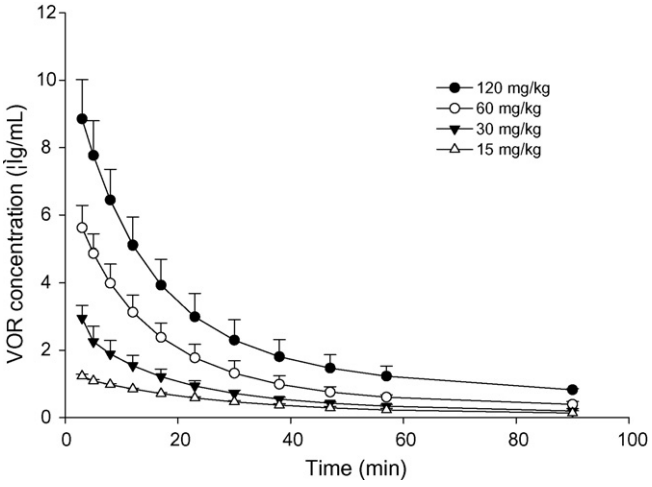

Fig. 4. Mean plasma concentration–time curves of vitexin-2″-*O*-rhamnoside in rats (mean ± S.D., $n = 5$) after an intravenous administration of doses of 15, 30, 60 and 120 mg/kg.

to 11.33 ± 1.55 L/kg, and all these pharmacokinetic parameters are given in Table 4. The $AUC_{0–90}$ values versus dose were linear over the administered dose range ($r = 0.9976$) and the $AUC_{0–\infty}$ values versus doses ($r = 0.9999$). VOR exhibited linear pharmacokinetic characteristics in rats after intravenous administration of 15, 30, 60 and 120 mg/kg body weight.

4. Conclusion

A simple and specific HPLC method for the determination of VOR in rat plasma was developed, and successively applied to an in vivo kinetic study in rats. Using this method, allowed us for the first time to investigate the pharmacokinetics of VOR in rat after intravenous administration of doses of 15, 30, 60 and 120 mg/kg body weight. This investigation contributes not only to the determination VOR in rat plasma by HPLC but also to our understanding of the linear pharmacokinetic characteristics of VOR over the dose range studied in rats after intravenous administration.

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References

[1] M. Dell'Agli, A. Busciala, E. Bosisio, Cardiovasc. Res. 63 (2004) 593–602.
[2] J.A. Bomser, K.W. Singletary, M.A. Wallig, M.A. Smith, Cancer Lett. 135 (1999) 151–157.
[3] A.M. Haque, M. Hashimoto, M. Katakura, Y. Tanabe, Y. Hara, O. Shido, J. Nutr. 136 (2006) 1043–1047.
[4] T. Lapidot, R. Granit, J. Kanner, J. Agric. Food Chem. 53 (2005) 3391–3396.
[5] I. Rahman, S.K. Biswas, P.A. Kirkham, Biochem. Pharmacol. 72 (2006) 1439–1452.
[6] X. Ding, Y. Jiang, Y. Zhong, C. Zuo, China J. Chin. Mater. Med. 15 (1990) 295–297, 31.
[7] P. Ninfali, M. Bacchiocca, A. Antonelli, E. Biagiotti, A.M. Di Gioacchino, G. Piccoli, V. Stocchi, G. Brandi, Phytomedicine 14 (2007) 216–222.
[8] A. Urbonaviciute, V. Jakstas, O. Kornysova, V. Janulis, A. Maruska, J. Chromatogr. A 1112 (2006) 339–344.
[9] R. Sladkovský, M. Urbánek, P. Solich, Chromatographia 58 (2003) 187–192.
[10] H. Sakakibara, Y. Honda, S. Nakagawa, H. Ashida, K. Kanazawa, J. Agric. Food Chem. 51 (2003) 571–581.
[11] A. Rehwald, B. Meier, O. Sticher, J. Chromatogr. A 677 (1994) 25–33.
[12] G.C. Kite, E.A. Porter, F.C. Denison, R.J. Grayer, N.C. Veitch, I. Butler, M.S. Simmonds, J. Chromatogr. A 1104 (2006) 123–131.
[13] J. Su, W.D. Zhang, Y. Zhou, J. Zhou, Z.B. Gu, China J. Chi. Mater. Med. 29 (2004) 525–527.
[14] J.Y. Sun, S.B. Yang, H.X. Song, G.H. Li, H.X. Qiu, Chin. Tradit. Herbal Drugs 6 (2002) 483–486.