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Short communication

HPLC determination of polydatin in rat biological matrices: Application to pharmacokinetic studies

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Abstract

A reversed-phase high-performance liquid chromatographic (RPHPLC) method has been developed for the determination of polydatin (PD) in rat plasma, bile, urine, feces and tissue homogenates using 2,3,5,4′-tetrahydroxychrysophenine-β-D-glucoside as an internal standard. The sample pretreatment included deproteinization for plasma samples and a liquid–liquid extraction for bile, urine, feces and tissue homogenates. Separation was obtained on a C_{18} reversed-phase column with the mobile phase consisting of methanol and water (35:65 v/v). The flow rate was 1 ml/min and the effluent was monitored at 310 nm. The method showed good linearity over the concentration ranges employed for various matrices ($r > 0.998$). The quantification limits of PD in rat plasma, bile, urine, feces and tissue homogenates were 0.0251, 0.126, 0.025 μg/ml, 0.189 and 0.0378 μg/g, respectively. The accuracy and precision of the method were less than 12.0% for the various matrices. No interferences from endogenous substances were found. The method was successfully applied to study the pharmacokinetics of PD in rats after intravenous administration.

Keywords: Polydatin; HPLC; Biological matrices; Pharmacokinetics

1. Introduction

Polydatin (PD), 3,4′,5-trihydroxystibene-3-β-D-mono-D-glucoside (shown in Fig. 1), is one of the active compounds in the Chinese medicinal herb *Polygonum cuspidatum* sieb. et zucc Huzhang Polygonaceae [1]. Modern pharmacological studies have indicated that PD can be used to protect the myocardium [2], inhibit the aggregation of platelets [3], improve microcirculation [4] and protect liver cells [5]. Research works on PD have been focused on the chemical extraction from herbs and pharmacological effects [6]. Very little information is available on the absorption, distribution, metabolism or excretion of PD [7]. The generation of preclinical pharmacokinetic data of PD requires the development of an analytical method in different biological matrices.

This paper describes the development and validation of an HPLC method for the determination of PD in various biological matrices. The plasma pharmacokinetics, tissue distribution and excretion of PD in rats after the intravenous administration of PD injection are also reported.

2. Experimental

2.1. Chemicals and reagents

PD injections and standard reference material were provided by Haiwang Pharmaceutics Co. Ltd. (Guangdong, China). 2,3,5,4′-Tetrahydroxychrysophenine-β-D-glucoside (internal standard, structure in Fig. 1) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol and acetonitrile, both of HPLC grade, were purchased from Merck Company (Darmstadt, Germany). Double-distilled water was used and 0.45 μm pore size filters (Millipore, MA, USA) was used to filter the solutions.

2.2. Standard solutions

A stock standard solution of PD (1 mg/ml) was prepared in methanol and the drug was found to be stable for at least 3

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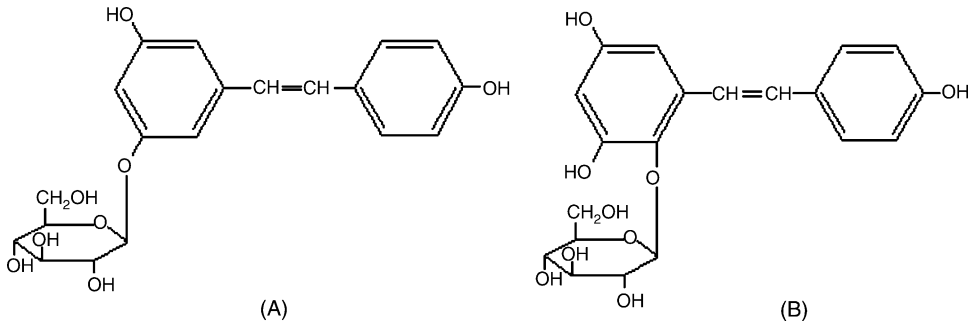

Fig. 1. Chemical structures of polydatin (A) and 2,3,5,4′-tetrahydroxychrysophenine-β-D-glucoside (B, I.S.).

months when stored at 4 °C. Working standard solutions of PD were obtained by diluting the stock solution with water. The internal standard solution was prepared by dissolving 2,3,5,4′-tetrahydroxychrysophenine-β-D-glucoside in methanol to give a final concentration of 5 μg/ml.

2.3. Chromatographic system

The HPLC system (SHIMADZU-10A) consisted of an LC-10AD VP HPLC pump, a SIL-10 AD VP automated sample injector, a thermostatted column compartment CTO-10AS VP, a SPD-10A VP detector and a N2000 chemstation.

Separation was achieved on a Diamonsil™ Ultrabase-C18 reversed-phase column (200 × 4.6 mm i.d., 5 μm particle size) (Dikma, USA), preceded by a Diamonsil™ ODS guard column. The mobile phase consisted of methanol and water (35:65 v/v) at a flow-rate of 1 ml/min. The chromatography was carried out at 40 °C and the effluent was monitored at 310 nm.

2.4. Sample pretreatment

2.4.1. Plasma samples

A 200 μl of acetonitrile containing the internal standard (0.5 μg) was added to a 200 μl aliquot of rat plasma. The mixture was shaken for 30 s and 400 μl of the mobile phase was added. After vortex-mixing, the samples were centrifuged at 10,195 × *g* for 8 min and a 40 μl aliquot of the supernatant was injected directly into the HPLC system.

2.4.2. Tissues, bile, urine and feces samples

Each tissue or feces sample (1.0 g) was homogenized with 3 volumes of 0.9% saline solution using a tissue homogenizer (Fluko, Shanghai, China) and centrifuged at 10,195 × *g* for 10 min. A 1.0 ml aliquot of the supernatant was collected. A simple liquid–liquid extraction was used to isolate PD from tissues, bile, urine and feces samples. A 100 μl of 0.5 mol/l NaH_2PO_4 solution (containing 0.8 μg of the internal standard) was added to 1.0 ml of tissue or feces homogenate, bile or urine, and 5 ml of the mixture of acetonitrile and butan-1-ol (5:1) was used for extraction of PD by vortexing. The mixture was centrifuged at 2891 × *g* for 10 min and the organic layer was evaporated to dryness on a water bath (45 °C). The residue was then reconstituted in a 200 μl aliquot of the mobile phase and a 40 μl aliquot was injected directly into the HPLC system.

2.5. Linearity and limits of quantification

Calibration standards over different ranges were prepared by spiking blank rat plasma (0.1004, 0.251, 0.502, 1.004, 2.51, 5.02 and 10.04 μg/ml), bile (0.126, 0.251, 0.502, 1.255, 2.51, 5.02 and 12.55 μg/ml), urine (0.025, 0.050, 0.126, 0.502, 1.255, 2.510, 5.02 and 12.55 μg/ml), feces (0.189, 0.378, 0.753, 1.88, 3.78 and 7.53 μg/g) or tissue homogenates (0.0753, 0.151, 0.301, 0.753, 1.51, 3.01and 7.53 μg/g) with an appropriate volume of working standard solutions, keeping the volume of organic phase spiked ≤2.5% of the biological matrice.

The limit of quantification was defined as the lowest concentration level, which provided a peak area with a signal-to-noise ratio higher than 10, with precision less than 15% (R.S.D.) and accuracy within ±15% (bias).

2.6. Precision, accuracy and recovery

Precision and accuracy of the replicate assays ($n = 5$) were evaluated by using the following concentrations: 0.251, 1.004 and 5.02 μg/ml for plasma, 0.151, 0.753 and 3.01 μg/g for liver sample, 0.126, 0.502 and 2.510 μg/ml for urine. Precision was assessed by the relative standard deviation (R.S.D.) and accuracy was estimated based on the mean percentage error of the measured concentration to the actual concentration (% bias). The method was extrapolated to other biomatrices like bile, urine and feces by performing partial validation in terms of intra assay precision and accuracy [8].

The recoveries of PD from plasma and liver homogenate were determined ($n = 5$) by spiked samples at three concentrations: 0.251, 1.004 and 5.02 μg/ml for plasma, 0.151, 0.753 and 3.01 μg/g for liver samples. Absolute recoveries were calculated by comparing the peak area ratio of PD/I.S. from spiked samples to those of corresponding concentrations injected directly in HPLC system without extraction.

2.7. Pharmacokinetic studies

Specific pathogen-free Sprague-Dawley rats (200 ± 20 g, male and female), were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. Rats were divided into three groups (65 rats/group) and received PD intravenously at the dose of 10, 20 and 30 mg/kg, respectively. Blood samples were

collected before drug administration and post-dose at 0, 5, 10, 20, 40, 60, 90, 120, 180, 240, 300, 360 and 480 min (five rats/time point). Plasma was separated out by centrifugation at 2124 × *g* for 10 min and was stored at −20 °C until analysis. The plasma concentration–time data were analyzed and pharmacokinetic parameters were estimated by non-compartmental analysis.

For distribution study, rats were treated intravenously with 20 mg/kg of PD and were then sacrificed. Tissues such as heart, liver, kidney, lung, and small intestine were removed at 10, 40, 60, 120, 240 and 360 min after injection (five rats/time point). All tissue samples were stored at −20 °C until analysis.

For the excretion study, the cannulation of the bile duct in rats was performed under general anesthesia. The rats were allowed

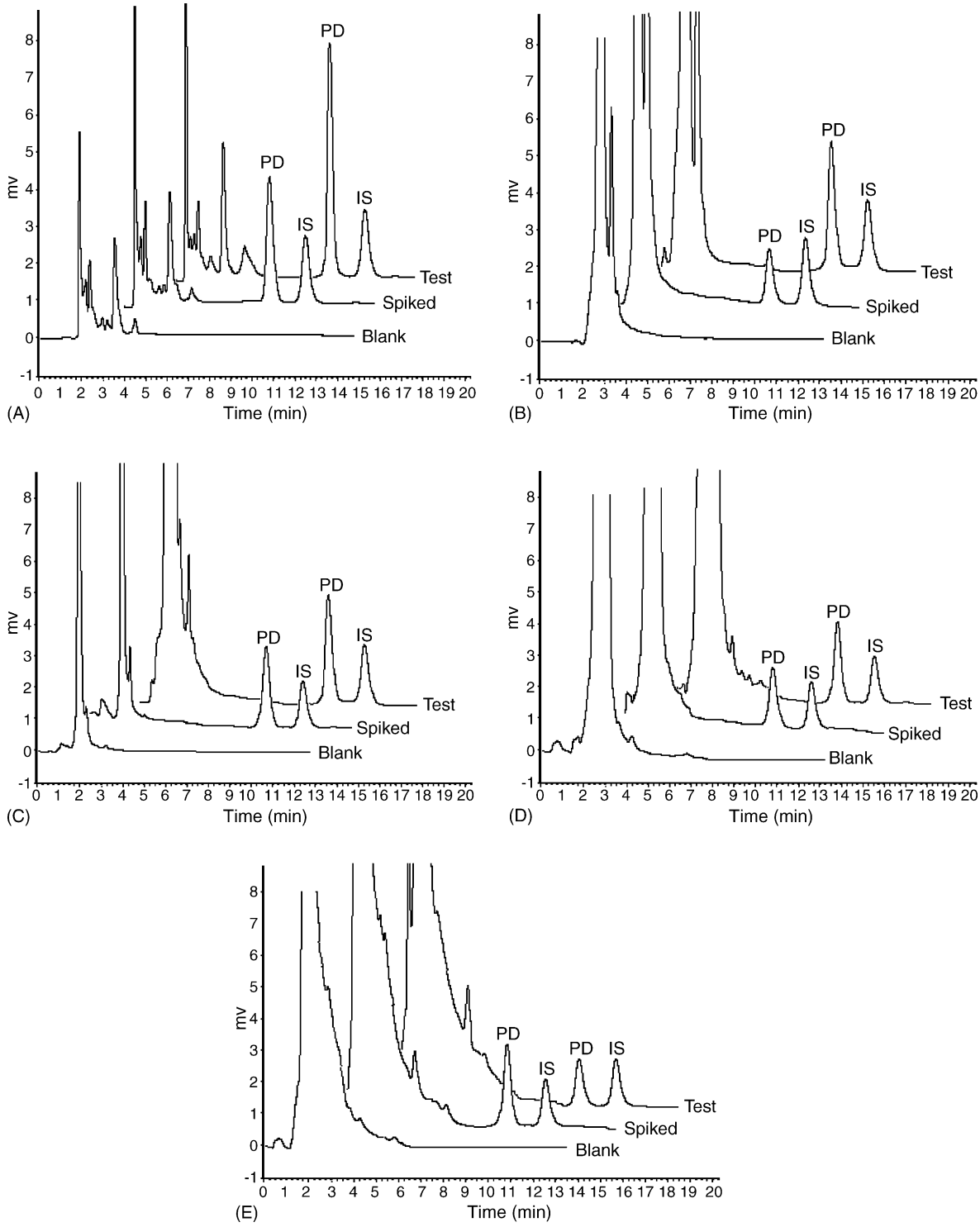

Fig. 2. Representative chromatograms of blank, spiked and test samples of PD in: (A) rat plasma, (B) kidney, (C) bile, (D) urine and (E) feces sample.

Table 1
The calibration functions and correlation coefficients

Biological matrice	Range (μg/ml or μg/g)	Calibration function[a]	Correlation coefficient (r)	Number of data points (n)
Plasma	0.1004–10.04	$y = 0.3084x + 0.0057$	0.9997	7
Bile	0.126–12.55	$y = 0.7222x - 0.0114$	0.9997	7
Urine	0.025–12.55	$y = 0.4874x + 0.0036$	0.9989	8
Feces	0.189–7.53	y=0.4406$x - 0.0060$	0.9996	6
Heart	0.0753–7.53	$y = 0.4649x + 0.0064$	0.9984	7
Liver	0.0753–7.53	$y = 0.4715x + 0.0114$	0.9989	7
Lung	0.0753–7.53	$y = 0.4495x + 0.0206$	0.9997	7
Kidney	0.0753–7.53	$y = 0.4636x + 0.0114$	0.9989	7
Intestine	0.0753–7.53	$y = 0.4537x + 0.0185$	0.9998	7

[a] x, concentration of PD and y, peak area ratio of PD/I.S.

to recover and received PD at a single i.v. dose of 20 mg/kg. Bile was collected over a period of 24 h post-dose. Urine and feces samples were collected over a period of 96 h post-dose. All samples were stored at −20 °C until analysis.

3. Results and discussion

3.1. Method development and validation

Method development was focused on the optimization of sample preparation, chromatographic separation and post-column detection. With regard to the complexity of the various biological matrices, different pretreatment procedures were used. For plasma samples, deproteinization with acetonitrile was adopted, which was simple and fast. For the tissue and excreta samples, liquid–liquid extraction was chosen in order to avoid endogenous interference and thus prolong the lifetime of the column. Various extraction solvents, such as ethyl acetate, methanol, acetonitrile, and butan-1-ol, were studied alone and in combination to assess the recovery of PD from spiked biomatrices. The highest recovery was obtained using the mixture of acetonitrile and butan-1-ol (5:1) as the extraction solvent. Chromatographic separation was performed on a C_{18} reversed-phase column with a mobile phase consisting of methanol and water (35:65 v/v). The λ_{max} value of PD in methanol was 310 nm based on the UV absorption spectra and therefore the effluent was monitored at 310 nm.

3.1.1. Method specificity

Specificity of the assay is defined as no interference in the regions of the compound of interest with endogenous substances or drug metabolites for the accurate determination. Typical chromatograms of the blank, spiked and test samples in rat plasma, kidney, bile, urine and feces are shown in Fig. 2. The retention times for PD and the internal standard in the plasma chromatogram were approximately 7.8 and 9.2 min, respectively. And the retention time for PD and the internal standard in the tissue and excreta samples were approximately 9.0 and 10.8 min, respectively. No endogenous substances in plasma, tissue, bile, urine and feces interfered with the determination of PD.

3.1.2. Linearity and the limits of quantification

An unweighted linear regression was used to perform standard calibration. The peak area ratios (PD/I.S.) were linearly related to the concentration of PD in different biomatrices, with correlation coefficients consistently greater than 0.998. Calibration functions with correlation coefficients in different biomatrices are given in Table 1. The quantification limits of PD in rat plasma, bile, urine, feces and tissue samples were 0.0251, 0.126, 0.025 μg/ml, 0.189 and 0.0378 μg/g, respectively.

3.1.3. Precision and accuracy

The results of precision and accuracy for PD in plasma, urine and liver homogenate are presented in Table 2. All the values

Table 2
Accuracy and precision of PD in spiked rat biomatrice

Biological matrice	Concentration (μg/ml or μg/g)	Accuracy (% bias)		Precision (% R.S.D.)	
		Intra day	Inter day	Intra day	Inter day
Plasma	0.251	−7.17	0.40	3.58	5.43
	1.004	0.10	−0.80	2.68	3.80
	5.020	−0.10	−1.09	0.66	0.72
Urine	0.126	6.34	11.9	4.27	4.44
	0.502	8.16	10.94	3.12	3.76
	2.51	1.87	−1.67	2.91	3.38
Liver	0.151	−2.39	−6.37	3.82	5.43
	0.753	1.19	−1.59	2.33	3.47
	3.01	5.68	−7.94	1.07	2.86

Table 3
Recoveries of polydatin from rat plasma and liver (mean ± S.D., $n = 5$)

Plasma		Liver	
Polydatin (μg/ml)	Recovery (%)	Polydatin (μg/g)	Recovery (%)
0.251	92.92 ± 3.33	0.151	98.44 ± 3.76
1.004	100.05 ± 2.68	0.753	101.26 ± 2.36
5.020	99.91 ± 0.66	3.01	105.68 ± 1.13

were within the acceptable limits of ±12% at three concentration levels and the method was proved to be accurate and precise. During the partial validation of the method in bile and feces, the intra-assay accuracy and precision at all the QC levels were less than 10 and 12%, respectively.

3.1.4. Recovery

Absolute recoveries of PD at the three concentration levels for 5 days in plasma and liver homogenate ranged from 92.92 to 100.05 and 98.44 to 105.68%, respectively (Table 3). Similar recoveries were obtained for bile, urine and feces during partial validation in these biomatrices.

3.2. Application to pharmacokinetic studies in rats

The method described here was successfully applied to study the pharmacokinetics of PD in rats. The mean plasma concentration–time curves in rats after intravenous administration of PD at different doses are shown in Fig. 3. Pharmacokinetic parameters of the three dose groups (10, 20 and 30 mg/kg i.v.) are listed in Table 4, indicating that PD was rapidly eliminated in rats and that the AUC increased in a dose-related manner.

The tissue concentrations of PD were measured after intravenous administration of PD at the dose of 20 mg/kg. The tissue concentrations reached maximum values at 10 min post-dose, which were 1.53 ± 0.13, 5.22 ± 0.46, 4.59 ± 0.59, and 6.41 ± 0.77 μg/g for heart, liver, lung, and kidney, respectively. However, the maximum concentration (6.69 ± 0.41 μg/g) for the intestine was observed at 60 min post-dose, which indicated the possibility of enterohepatic circulation of PD after intravenous administration.

For the excretion study in rats, the accumulated excretion rate in urine and feces within 96 h were 1.51 and 0.035% of the dose given, respectively and that in bile was 1.04% of the administered dose.

Table 4
Pharmacokinetic parameters of polydatin in rats after a single i.v. dose of 10, 20 and 30 mg/kg

Parameters	Dose (mg/kg)		
	10	20	30
$t_{1/2}$ (min)	71.4	90.4	95.0
V_{ss} (l)	0.55	1.01	0.43
Cl/F (l/min)	0.01	0.02	0.02
AUC_{0-480} (μg min/ml)	804.55	944.86	1911.08
$AUC_{0-\infty}$ (μg min/ml)	807.54	953.73	1919.30
MRT (min)	44.1	48.0	27.8

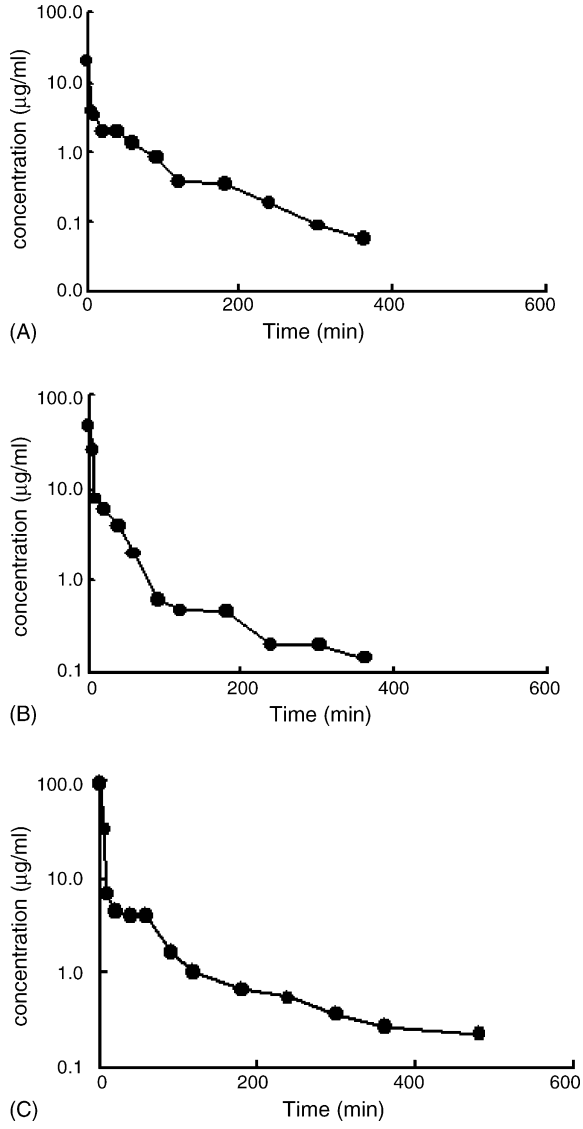

Fig. 3. Mean plasma concentration–time profiles of polydatin after a single dose of i.v. in rats: (A) 10 mg/kg, (B) 20 mg/kg and (C) 30 mg/kg.

4. Conclusion

A reversed-phase HPLC method with UV detection was developed for quantification of PD in rat plasma, bile, urine, feces and tissue homogenates. The chromatographic system provided good separation of PD without endogenous interference. The assay was validated and all results were within the acceptable ranges for bio-analytical purposes. This method was rapid, reproducible and accurate, and has been successfully applied to study the pre-clinical pharmacokinetics of PD in rats.

References

[1] S.Y. Shu, J. Pediatric. Pharm. 8 (2002) 9–11.

[2] C.H. Jin, J. Liu, X.L. Huang, Chin. Pharmacol. Bull. 16 (2000) 400–402.
[3] C.H. Jin, J. Liu, X.L. Huang, Chin. Pharmacol. Bull. 16 (2000) 151–155.
[4] C.W. Shan, Acta Pharm. Sin. 23 (1988) 394–396.
[5] C.W. Shan, S.Q. Yang, Acta Pharm. Sin. 11 (1990) 527–530.
[6] L. Chen, F.Q. Yang, T.Y. Zhang, J. Instrum. Anal. 19 (2000) 60–62.
[7] J.H. Lin, X.H. Li, Y.N. Tang, Chin. J. Pharm. Anal. 21 (2001) 325–327.
[8] V.P. Shah, K.K. Midha, J.W.A. Findlay, H.M. Hill, J.D. Huise, I.J. McGilveray, G. Mckay, K.J. Miller, R.N. Patnaik, M.L. Powel, A. Tonelli, C.T. Viswanathan, A. Yacobi, Pharm. Res. 17 (2000) 1551–1557.