Journal of Chromatography B, 878 (2010) 1199–1203

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Validated liquid chromatography–tandem mass spectrometry method for quantitative determination of dauricine in human plasma and its application to pharmacokinetic study

Xiaoying Liu[a], Qian Liu[a], Dongmei Wang[a], Xueya Wang[b], Peng Zhang[a], Haiyan Xu[a], Hui Zhao[a], Huaiqing Zhao[a,*]

[a] School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenyang 110016, PR China
[b] Vocational and Technical College, Liaoning University of Traditional Chinese Medicine, Shenyang 110101, PR China

ARTICLE INFO

Article history:
Received 20 November 2009
Accepted 15 March 2010
Available online 20 March 2010

Keywords:
LC–MS/MS
Pharmacokinetics
Dauricine

ABSTRACT

A highly sensitive and selective LC–MS/MS method was developed and validated for the determination of dauricine in human plasma, using protopine as internal standard (IS). The analyte and IS were extracted by liquid–liquid extraction and analyzed by LC–MS/MS. Chromatographic separation was performed on Agilent TC-C_{18} column with a mobile phase of methanol–water–glacial acetic acid (60:40:0.8, v/v/v) at a flow rate of 0.7 mL/min. Detection was performed on a triple quadrupole tandem mass spectrum by multiple reaction monitoring (MRM) mode using the electrospray ionization technique in positive mode. The method was linear over the concentration range of 1–200 ng/mL. The lower limit of quantification (LLOQ) was 1 ng/mL in human plasma with acceptable precision and accuracy. The intra- and inter-day precision was less than 5.9% determined from quality control (QC) samples at concentrations of 2.0, 20.0 and 160 ng/mL, and the accuracy was within ±9.9%. This method was successfully applied for the evaluation of pharmacokinetics of dauricine after oral doses of 100, 300 and 600 mg phenolic alkaloids of menispermum dauricum tablet (PAMDT) to 12 Chinese healthy volunteers.

1. Introduction

Dauricine (Dau, Fig. 1) is one type of bisbenzyl-tetrahydro-isoquinoline alkaloid derivative isolated from the root of the traditional Chinese medicine Menispermum dauricum D.C. Dauricine has good anti-arrhythmic effect, it can prolonged the action potential duration (APD), and was considered to be a promising anti-arrhythmic agent [1]. Several analytical methods [2–6] based on reversed phase HPLC methods with UV detection have been reported for the determination of dauricine in plants and biological samples, in which the assays were not sensitive enough for the pharmacokinetic study of dauricine. Due to the low concentration of dauricine in human plasma, the high sensitivity of the analytical method is very important for the investigation of the pharmacokinetics of dauricine in human. Han et al. made use of HPLC/ESI-ITMS^n method to identify dauricine and its metabolites in rat urine [7]. In this paper, we report a LC–MS/MS method which provides sensitive and rapid quantitation of dauricine in human plasma to a LLOQ of 1 ng/mL using 200 μL of plasma. This assay method was successfully applied to the pharmacokinetic study of dauricine in healthy male Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

Dauricine (purity >98%) was provided by Shenzhen Meihe Technology Co. Ltd. (Shenzhen, PR China), and protopine (purity >98%) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, PR China). Methanol and glacial acetic acid were of HPLC grade, and other reagents used were of analytical grade.

2.2. Instrumentation and LC–MS/MS conditions

The LC–MS/MS equipment consisted of a G1311A quaternary pump, a G1379A vacuum degasser, a G1313A autosampler, a G1316A column heater and an Agilent API2000 triple quadrupole mass spectrometer (Agilent Corporation, MA, USA). The data acquisition was performed with Analyst 1.4.1 software.

Chromatography was performed on an Agilent TC-C_{18} column (150 mm × 4.6 mm, 5 μm, Agilent, USA), using the mobile phase of

* Corresponding author. Tel.: +86 24 2398 6250; fax: +86 24 2398 6250.
E-mail address: zhaohq1955@sina.com (H. Zhao).

1570-0232/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2010.03.028

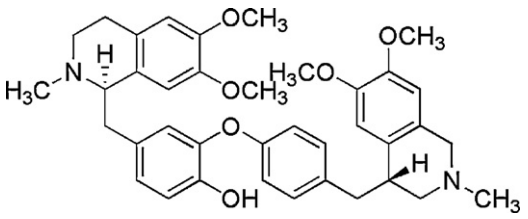

Fig. 1. The structure of dauricine.

methanol–water–glacial acetic acid (60:40:0.8, v/v/v) at a flow rate of 0.7 mL/min. The column temperature was maintained at 30 °C and the injection volume was 20 μL.

The HPLC system was connected to the mass spectrometer via an ESI source. The mass spectrometer was operated in the positive ion detection mode. The instrument was operated with an ion spray voltage at 5500 V, heater gas temperature at 450 °C, nebulizer gas (Gas 1) of 60 psi, heater gas (Gas 2) of 70 psi, curtain gas of 10 psi and collision gas of 3 psi. All gases used were nitrogen. Declustering potential (DP) was set at 80 V for dauricine, 30 V for IS, respectively. Quantitation was performed using multiple reaction monitoring (MRM) of the transitions of m/z 625.3 → m/z 206.2 for dauricine, m/z 354.1 → m/z 188.1 for protopine, respectively, with a scan time of 200 ms per transition.

2.3. Preparation of standard and quality control (QC) samples

The stock solution of dauricine (200 μg/mL) was prepared by dissolving 10.0 mg in methanol–water (50:50, v/v) in a 50 mL amber volumetric flask. Dauricine standard solutions (4, 8, 20, 40, 80, 200, 400 and 800 ng/mL) were prepared by dilution of this stock solution with methanol–water (50:50, v/v). QC solutions (8.0, 80.0 and 640 ng/mL) were prepared independently in the same way. The stock (200 μg/mL) and working solution (20 ng/mL) of the IS were prepared by dissolving an accurately weighed quantity of protopine in methanol–water (50:50, v/v) and serial dilution with methanol–water (50:50, v/v). All the solutions were stored at 4 °C and were brought to room temperature before use.

Both the calibration standard samples and the QC samples, which were used in the pre-study validation and during the pharmacokinetic study, were prepared by spiking 200 μL blank plasma with 50 μL working solutions correspondingly.

2.4. Sample preparation

A 200 μL aliquot of plasma sample was added with 50 μL IS (20 ng/mL) and 50 μL methanol–water (50:50, v/v). After vortexing, 5 mL of ether–dichloromethane (4:1, v/v) was added, and the mixture was vortexed for 5 min and centrifuged at 8000 × g for 8 min. The 4 mL of organic layer was removed and evaporated to dryness at 40 °C under a stream of nitrogen. The residue was dissolved in 200 μL of mobile phase, and 20 μL of the supernatant liquid was injected into the LC–MS/MS system.

2.5. Assay validation

Selectivity was studied by comparing the chromatograms of six different batches of blank human plasma with the corresponding spiked plasma. Each blank plasma sample was tested using the proposed extraction procedure and LC–MS/MS conditions to ensure no interference of dauricine and IS from plasma.

Matrix effects were evaluated by comparing peak areas of analytes dissolved in the blank plasma sample's reconstituted solution (the final solution of blank plasma after extraction and reconstitution) with that dissolved in mobile phase. Three different concentration levels of dauricine at 2.0, 20.0 and 160 ng/mL were evaluated by analyzing three samples at each level.

Linearity was assessed by assaying calibration curves in human plasma in duplicate in three separate runs. And the curves were fitted by a linear weighted ($1/x^2$) least-squares regression method through the measurement of the peak area ratio of the analyte to IS. The LLOQ was defined as the lowest concentration on the calibration curve, and it was established using six samples independent of standards.

QC samples were prepared and analyzed on 3 consecutive days (one batch per day) to evaluate the accuracy and the intra- and inter-day precision. The accuracy as well as the intra- and inter-day precision of the method was determined by analyzing six replicates at 2.0, 20.0 and 160 ng/mL of dauricine. Assay precision was calculated using the relative standard deviation (RSD). The assay accuracy was expressed as relative error (RE).

Recovery of dauricine was evaluated by comparing the mean peak areas of the regularly prepared samples (n = 6) at 2.0, 20.0 and 160 ng/mL with the mean peak areas of spiked-after-extraction samples, which represented the 100%. To prepare the spiked-after-extraction samples, blank human plasma was processed according to the sample preparation procedure as described above. The supernatant was evaporated to dryness, and were reconstituted with the mobile phase by addition of appropriate standards at concentrations corresponding to the final concentration of the pretreated plasma samples.

The stability of dauricine in human plasma was evaluated by analyzing replicates (n = 3) of plasma samples at concentrations of 2.0, 20.0 and 160 ng/mL, which were exposed to different conditions. Stability of two freeze–thaw cycles, reconstitution extract at ambient temperature for 8 h and storage at −20 °C for 30 days was assessed by analyzing replicates (n = 3) of QC samples. The results were compared with those QC samples freshly prepared, and the percentage concentration deviation was calculated.

2.6. Pharmacokinetic study

Twelve healthy male volunteers who gave written informed consent participate in the study according to the principles of the Declaration of Helsinki. The doses of dauricine chosen for this study were based on findings from preclinical studies. A total of 12 volunteers were randomly divided into three groups: A, B and C. After an overnight fasting, groups A, B and C were administered a single dose of PAMDT 100, 300 and 600 mg (equivalent to 60, 120 and 180 mg dauricine, respectively) with 200 mL water, respectively. 4 mL of blood samples were withdrawn from antecubital vein immediately before (0 h) and at 0.33, 0.67, 1.0, 1.33, 1.67, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10 and 12 h after dosing. Samples were centrifuged in heparinised tubes and plasma was separated and stored at −20 °C until analyzed. Volunteers were administrated PAMDT 100, 300, or 600 mg in three periods in a crossover manner separated by 5 days of drug free wash-out period.

Pharmacokinetic parameters of dauricine were calculated. The terminal elimination rate constant (k_e) is determined by least-square regression analysis of terminal logarithm-linear portions of the plasma concentration-time profile ($k_e = -2.303 \times$ slope). The elimination half-life ($t_{1/2}$) is calculated as $0.693/k_e$. The pharmacokinetic parameters, namely maximum plasma concentration (C_{max}), time point of maximum plasma concentration (T_{max}), area under the plasma concentration–time curve from 0 h to the last measurable concentration (AUC_{0-t}), area under the plasma concentration–time curve from 0 h to infinity ($AUC_{0-\infty}$), apparent volume of distribution (V/F) and mean residence time (MRT) were calculated using Drug and Statistics software (Version. 2.0, University of Science and Technology, Hefei, PR China).

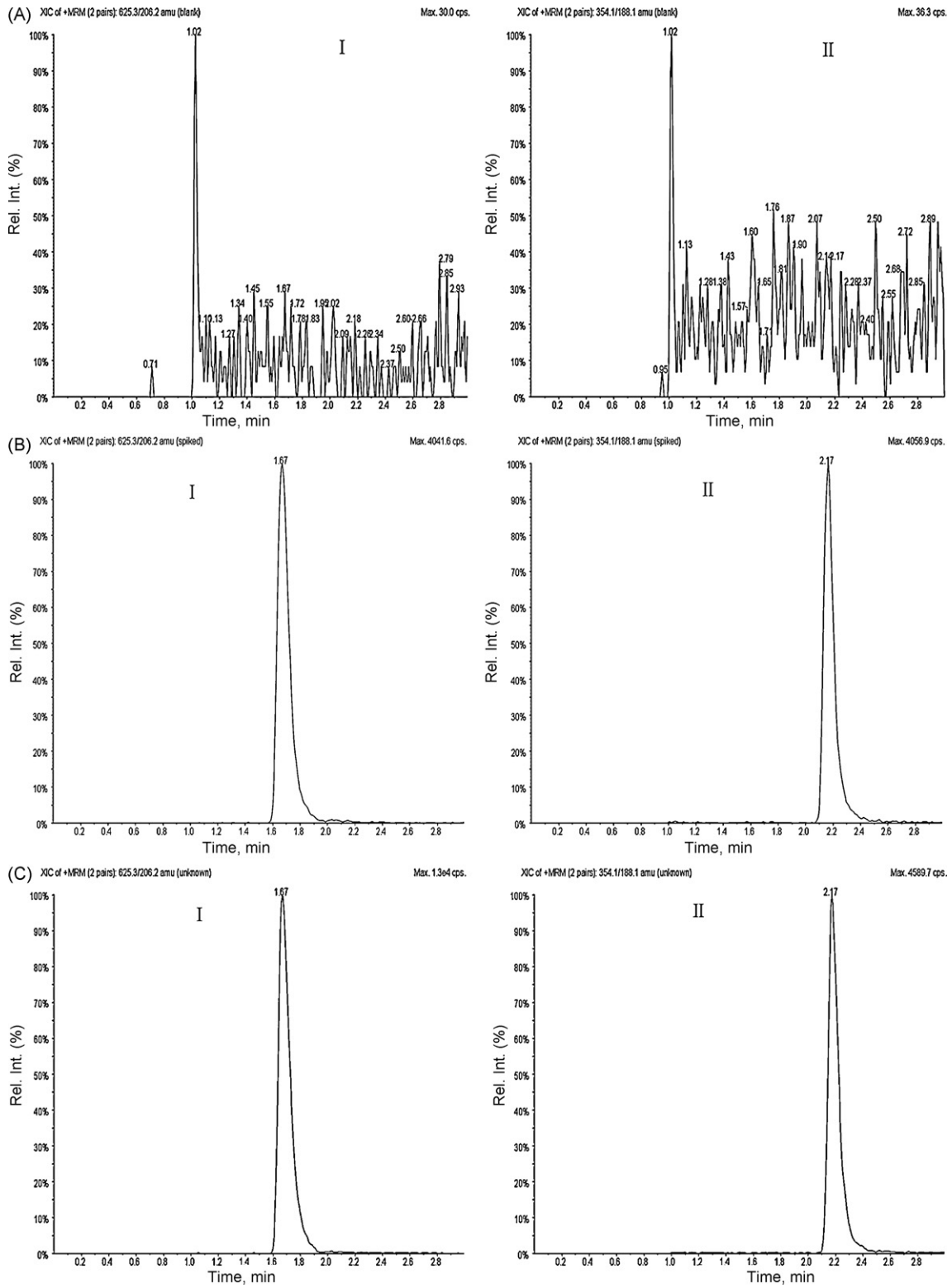

Fig. 2. Representative MRM chromatograms of dauricine (I) and protopine (IS, II) in human plasma samples: (A) blank plasma sample; (B) blank plasma sample spiked with dauricine (20 ng/mL) and IS (5 ng/mL); (C) volunteer plasma sample 1.67 h after an oral administration of 300 mg of dauricine.

3. Results and discussion

3.1. Optimization of the mass spectrometric conditions

Dauricine and protopine (IS) were scanned with ESI and APCI positive and negative ion modes using injection standard solutions. In different ionization modes, the base peak intensity of positive ion was higher than that of the negative ion, and the efficiency of ionization in ESI was higher than APCI. The molecular ions with an m/z 625.3 $[M+H]^+$ for dauricine were produced. Protopine (IS) molecule was protonated to form molecule ions with m/z 354.1 $[M+H]^+$. Therefore, these ions were chosen as parent ions for frag-

Table 1
Precision and accuracy of the LC–MS/MS method to determine dauricine in human plasma (3 days, 6 replicates per day).

Added, C (ng/mL)	Found, C (ng/mL)	Intra-run RSD (%)	Inter-run RSD (%)	Relative error (%)
2.00	2.01	5.8	5.7	0.11
20.0	20.0	4.8	5.9	0.93
160	153.3	5.7	3.5	8.37

mentation in the MRM mode. Finally, m/z 206.2 and m/z 188.1 were selected as the target ions of dauricine and IS, respectively. The collision energy in the product LC–MS/MS mode was investigated to optimize the sensitivity, and the optimal values were found to be 45 V for both of dauricine and IS.

3.2. Optimization of the chromatographic conditions

The compositions of mobile phase were optimized with varying percentages of organic solvent. It was found that high organic solvent contents (about 60%) in HPLC system decreased the background noise and provided rapid separation and stable MS signal. Methanol was chosen as the organic solvent because it was less toxic and cheaper than acetonitrile while providing the same sensitivity as acetonitrile. The addition of acidic modifiers (glacial acetic acid) to the mobile phase improved the sensitivity by promoting the ionization of the analytes. To achieve symmetrical peak shapes, and a short chromatographic run time, a mobile phase consisting of methanol–water–glacial acetic acid (60:40:0.8, v/v/v) was used in the experiment.

3.3. Sample preparation

Sample preparation is a critical step for accurate and reliable LC–MS/MS assays. Liquid–liquid extraction (LLE) was a widely adopted method and often achieved satisfactory extraction recoveries of analytes from biological samples. In this study, four organic extraction solvents were evaluated: diethyl ether, dichloromethane, acetoacetate and ether–dichloromethane (4:1, v/v). Among them, ether–dichloromethane (4:1, v/v) yielded a good clean-up of the plasma samples and adequate recovery values.

3.4. Assay validation

Fig. 2 shows the typical MRM chromatograms of a blank plasma, a spiked plasma sample with dauricine (20 ng/mL) and IS (5 ng/mL), and a plasma sample from a healthy volunteer 1.67 h after an oral administration of 300 mg of PAMDT. There were no significant endogenous interferences observed at the retention times of the analyte and IS.

The matrix effect would be caused by ionization competition between the analytes and co-eluents existing when using LC–MS/MS for analysis. The absolute matrix effects for dauricine at concentrations of 2.0, 20.0 and 160 ng/mL were 99.6%, 86.5% and 95.3%, respectively. The results showed that there was no matrix effect of the analytes observed from the matrix of plasma in this study.

A calibration curve was established on each validation day. The calculated peak area ratios of dauricine to the internal standard versus the nominal concentration displayed a good linear relationship over the concentration range from 1.0 to 200 ng/mL in human plasma. A typical standard curve was as follows: $y = 5.12 \times 10^{-2}\,x + 8.04 \times 10^{-3}$, $r = 0.9982$, where y represents the ratio of dauricine peak area to that of the IS and x represents the plasma concentration of dauricine.

The LLOQ was established at 1 ng/mL, which was sensitive enough to investigate the pharmacokinetics of low dose (100 mg) of dauricine in human. The precision and accuracy at this concentration level were acceptable, with 8.2% of the RSD and −2.1% of the RE.

The intra- and inter-day precision and accuracy were calculated by analyzing QC samples. The results are summarized in Table 1. The results demonstrated that the precision and accuracy of this assay are within the acceptable range and the method is accurate and precise.

Mean extraction recoveries of dauricine at 2.0, 20.0 and 160 ng/mL were 65.3 ± 3.2%, 73.4 ± 8.7% and 68.6 ± 9.9%, respectively ($n = 6$). Mean extraction recovery of the internal standard (5 ng/mL in plasma) was 78.8 ± 9.8% ($n = 6$).

The analytes were found to be stable in human plasma after two freeze–thaw cycles and at least 8 h at ambient temperature. The analytes were also shown to be stable for at least 30 days under −20 °C freezer conditions. All RE values between post-storage and initial QC samples were within ±15%.

3.5. Pharmacokinetic study

The validated LC–MS/MS method was applied to investigate the pharmacokinetic profiles of dauricine after single oral doses of 100, 300 and 600 mg PAMDT. Profiles of the mean plasma concentration versus time are shown in Fig. 3. And the corresponding pharmacokinetic parameters are presented in Table 2. At 100–600 mg dose levels, plasma exposure to dauricine appeared increasing dose-proportionally.

Individual profiles showed double peaks usually at 1 and 4 h with a clear elimination phase after 6 h. The T_{max} could be at the time of the first peak. The appearance of a second peak has also been observed in previous study in rats [5] and in dogs [11], suggesting an erratic absorption. There was at most threefold variability in AUC and C_{max} between subjects, which is in agreement with published pharmacokinetic studies [8–10]. The high sensitivity of the present method allowed us to measure the plasma concentration up to 12 h after a single dose of 100, 300 and 600 mg administration.

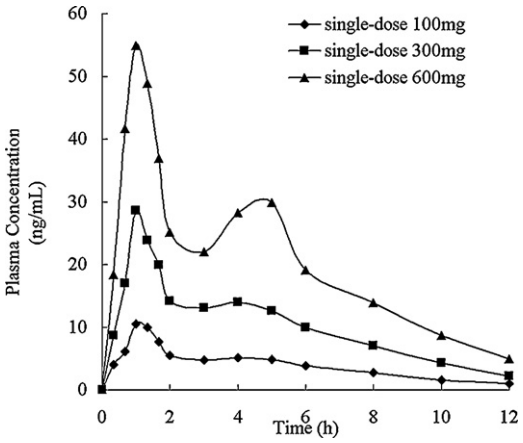

Fig. 3. Time course of mean concentrations of dauricine in plasma after an oral administration of the test formulations of dauricine (100, 300 and 600 ng/mL, respectively) to 12 volunteers ($n = 12$).

Table 2
The main pharmacokinetics parameters for dauricine after single doses of 100, 300 and 600 mg PAMDT to 12 healthy volunteers, respectively (mean ± SD).

Parameters	Dose groups		
	100 mg (n = 12)	300 mg (n = 12)	600 mg (n = 12)
T_{max1} (h)	1.08 ± 0.25	1.14 ± 0.30	1.0 ± 0.24
T_{max2} (h)	4.17 ± 0.94	4.25 ± 0.97	4.08 ± 0.67
C_{max1} (ng/mL)	12.3 ± 3.26	33.6 ± 12.5	69.8 ± 24.5
C_{max2} (ng/mL)	6.26 ± 1.53	16.9 ± 4.17	36.2 ± 14.3
$t_{1/2}$ (h)	3.57 ± 1.28	2.87 ± 0.86	3.07 ± 0.85
$AUC_{(0-12\,h)}$ (ng h/mL)	44.4 ± 9.39	121 ± 28.1	243 ± 76.7
$AUC_{(0-\infty)}$ (ng h/mL)	53.0 ± 10.2	132 ± 29.9	267 ± 85.9
MRT (h)	4.01 ± 0.33	4.28 ± 0.41	4.34 ± 0.30
CL/F (L/h)	1168 ± 210	1428 ± 316	1474 ± 440
V/F (L)	5922 ± 2198	5891 ± 1946	6592 ± 2871

4. Conclusions

A LC–MS/MS method was developed and validated for the determination of dauricine in human plasma. The method is rapid, sensitive and specific with a LLOQ of 1 ng/mL using 200 μL human plasma. The method was successfully applied to the pharmacokinetic study of dauricine in human.

References

[1] C.J. Hu, F.D. Zeng, Chin. Native Med. Pharmacol. Clin. Res. Prog. 1 (1992) 26.
[2] H. Li, G. Chen, F.K. Chen, Chin. Trad. Patent Med. (2001) 839.
[3] Y. Chen, H.X. Chen, Chin. J. Anal. Chem. 34 (2006) 675.
[4] S.J. Chen, Y.M. Yang, Y.M. Yang, Y.M. Liu, B. Zhang, X.B. Pang, F.D. Zeng, Chin. Pharmacol. Bull. 17 (2001) 225.
[5] S.X. Hou, Z.S. Dai, F.J. Wang, L.J. Guo, C.J. Hu, Acta Pharm. Sin. 17 (1982) 863.
[6] S.J. Chen, Y.M. Yang, Z.S. Dai, F.D. Zeng, J. Tongji Med. Univ. 20 (2000) 253.
[7] F.M. Han, Z.H. Peng, W. Song, H.M. Zhang, M.M. Zhu, Y. Chen, J. Chromatogr. B 854 (2007) 1.
[8] R.A. Yates, M. Tateno, K. Nairn, A. Ikegami, A. Dane, J. Kemp, Eur. J. Clin. Pharmacol. 58 (2002) 247.
[9] E. Seaber, N. On, S. Phillips, R. Churchus, J. Posner, P. Rolan, Eur. J. Clin. Pharmacol. 41 (1996) 141.
[10] E.J. Seaber, N. On, R.M. Dixon, M. Gibbens, W.J. Leavens, J. Liptrot, G. Chittick, J. Posner, P.E. Rolan, R.W. Pack, Br. J. Clin. Pharmacol. 43 (1997) 579.
[11] S.J. Chen, B. Yang, Y.M. Yang, Z.S. Dai, F.D. Dai, Chin. Pharmacol. Bull. 5 (2000) 213.