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Enantioselective determination of trantinterol in rat plasma by ultra performance liquid chromatography–electrospray ionization mass spectrometry after derivatization

Jing Yang[a], Yanjuan Wang[a], Li Pan[b], Ning Li[a], Xiumei Lu[a], Jin Guan[c], Maosheng Cheng[b], Famei Li[a,*]

[a] Department of Analytical Chemistry, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[b] School of Pharmaceutical Engineering, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[c] School of Applied Chemistry, Shenyang Institute of Chemical Technology, Shenyang 110142, PR China

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ABSTRACT

An ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) method was developed and validated for the determination of trantinterol enantiomers in rat plasma. Diphenhydramine was employed as the internal standard. The plasma samples were prepared using liquid–liquid extraction with n-hexane–dichloromethane–isopropanol (20:10:1, v/v/v) as the extractant. Trantinterol enantiomers after pre-column derivatization using diacetyl-L-tartaric anhydride (DATAAN) were separated on a C18 column using a gradient solvent programme. The mobile phase was composed of 3 mM ammonium acetate and acetonitrile. The detection was performed on a triple-quadrupole tandem mass spectrometer by multiple reaction monitoring (MRM) mode via electrospray ionization (ESI). Linear calibration curve for each enantiomer was obtained in the concentration range of 1–80 ng/mL, with limit of quantification (LOQ) of 1 ng/mL. The intra- and inter- precision (R.S.D.) values were below 9.6% and accuracy (R.E.) was from −2.4 to 6.2% at all quality control (QC) levels. The developed method was applied to the enantioselective pharmacokinetic study of trantinterol in rats.

1. Introduction

Trantinterol, 2-(4-amino-3-chloro-5-trifluoromethyl-phenyl)-2-tert-butylamino-ethanol hydrochloride, is a novel β_2-adrenoceptor agonist which exhibited both potent trachea relaxing activity and high β_2 selectivity with low cardiac side effect [1]. It is currently in phase II clinical trials in China for the treatment of asthma. Chemically trantinterol is a chiral molecule, and the structure is given in Fig. 1. As shown by stereoselective pharmacological studies of trantinterol enantiomers, (−)-trantinterol exhibited more potent efficacy, higher affinity and better selectivity for β_2-adrenoceptor than (±)- and (+)-trantinterol [2]. Therefore, it is required to study the possibly different profiles of trantinterol enantiomers in pharmacokinetics and metabolic pathways. A sensitive analytical method for quantitative analysis of each enantiomer in biological samples is the prerequisite to the investigation of stereospecificity in pharmacokinetics, metabolism and disposition of trantinterol. The most powerful tools available for the enantioseparation and determination of chiral drugs are HPLC and CE [3–7].

An HPLC–MS/MS method for the determination of trantinterol in human plasma has been reported [8]. However, no analytical method was reported for the enantioselective determination of trantinterol enantiomers in biological samples. There were only one HPLC-CSP method using hexane–ethanol as mobile phase [9] and one CE method using cyclodextrins as chiral selector [10] reported by our group. Both of them with UV detection were unsuitable for the analysis of trace amount of enantiomers in biological samples because of the lack of sensitivity, which drove us to pursue the use of MS detector as an alternative. However, the reported normal phase CSP method [9] cannot be directly used with LC–MS due to the compatibility of hexane with MS detection is questionable in the aspects of flammability and low flashpoint [11]. Therefore, we decided to develop a pre-column chiral derivatization method to separate the trantinterol enantiomers. The derivatized diastereomers could be then separated on an achiral column using common LC solvents such as methanol, acetonitrile and water as mobile phase which are easily interfaced with high sensitive MS detection.

In this study, a method of pre-column derivatization ultra performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) with multiple reaction monitoring (MRM) was developed for determining trantinterol enantiomers in rat

* Corresponding author at: Department of Analytical Chemistry, Shenyang Pharmaceutical University, P.O. Box 39, 103 Wenhua Road, Shenyang 110016, PR China. Tel.: +86 24 2398 6289; fax: +86 24 2398 6289.
E-mail address: lifamei@syphu.edu.cn (F. Li).

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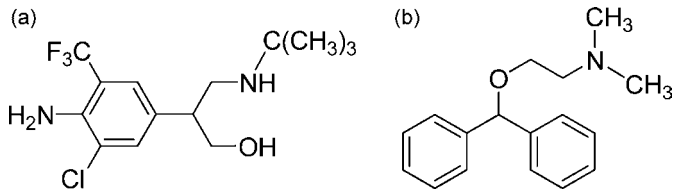

Fig. 1. Structures of trantinterol (a) and diphenhydramine (I.S.) (b).

plasma. The limit of quantification (LOQ) of the UPLC–MS/MS method is 1 ng/mL for each enantiomer. The method is reproducible and accurate and has been applied to the study of stereoselective pharmacokinetics of trantinterol enantiomers in rats after administration of trantinterol racemate.

2. Experimental

2.1. Reagents and chemicals

Racemic trantinterol and (−)-trantinterol hydrochlorides were synthesized in our laboratory. The purities of these compounds were 99.3 and 99.1%, respectively, verified using HPLC. Diphenhydramine hydrochloride (internal standard, I.S., 99.4% of purity) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Diacetyl-L-tartaric anhydride (DATAAN) was purchased from Fluka (Ronkonkoma, NY, USA). Acetonitrile and ammonium acetate were of HPLC grade and purchased from Dikma Company (Richmond Hill, NY, USA). Other chemicals were all of analytical grade. Water was purified by redistillation and filtered through 0.22 μm membrane filter before use.

2.2. Apparatus and operation conditions

2.2.1. Liquid chromatography

Liquid chromatography was performed on ACQUITY UPLC system (Waters, Milford, MA, USA) with autosampler and column oven. An ACQUITY UPLC® BEH C18 column (50 mm × 2.1 mm, 1.7 μm; Waters Corp., Milford, MA, USA) was employed. The column temperature was maintained at 40 °C. Chromatographic separation was achieved with gradient elution using a mobile phase composed of 3 mM ammonium acetate (A) and acetonitrile (B). The gradient elution started at 80% A and changed linearly to 70% A in 4 min, after being maintained at 70% A for 2.5 min it then returned to the initial condition. The flow rate was set at 0.2 mL/min. The autosampler was conditioned at 4 °C. The injection volume was 5 μL using partial loop mode for sample injection.

2.2.2. Mass spectrometry

Mass spectrometric detection was carried out on a Micromass Quattro micro API mass spectrometer (Waters) with an electrospray ionization (ESI) interface. The ESI source was set in positive ionization mode. Quantification was performed using multiple reaction monitoring (MRM) mode of the transitions of m/z 527 → m/z 454 for trantinterol derivative and m/z 256 → m/z 167 for I.S. with scan time of 0.05 s per transition. The optimized ionization conditions were as follows: capillary voltage 2.9 kV, cone voltage 20 kV, source temperature 110 °C and desolvation temperature 400 °C. Nitrogen was used as desolvation and cone gas with the flow rate at 700 and 70 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 2.53×10^{-3} mbar. The optimized collision energies for trantinterol derivative and diphenhydramine were 14 and 10 eV, respectively. All data collected in centroid mode were acquired and processed using MassLynx NT 4.1 software with QuanLynx program (Waters).

2.3. Preparation of stock solutions, calibration standards and quality control samples

Standard stock solutions of racemic trantinterol and I.S. were both prepared in methanol at the concentration of 100 and 50 μg/mL, respectively. All the stock solutions were stored at 4 °C and brought to room temperature before use. Working solutions were obtained by serial dilution of stock solution with methanol.

Calibration standards for trantinterol enantiomers were prepared by spiking blank rat plasma at 1.00, 2.00, 5.00, 10.0, 20.0, 40.0 and 80.0 ng/mL for each enantiomer. The quality control (QC) samples were prepared with blank plasma at low, middle and high concentrations of 2.50, 20.0, 70.0 ng/mL. The concentrations of trantinterol and I.S. were calculated as free base.

2.4. Plasma sample preparation and derivatization

I.S. solution (20 ng/mL) was prepared by dilution of I.S. stock solution, 100 μL was pipetted into 10 mL clean glass tube and evaporated to dryness, aliquot of plasma sample (100 μL) was then added. After alkalifying with 100 μL of 1 M NaOH, the analytes were extracted into 3 mL n-hexane–dichloromethane–isopropanol (20:10:1, v/v/v) by vortex-mixing for 30 s and shaking on a mechanical shaker for 10 min. After centrifugation at 3500 × g for 10 min, the upper organic layer was then transferred into another clean glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was derivatized by treating with 100 μL of DATAAN solution, 0.1 mg/mL in acetic acid–dichloromethane (1:4, v/v) solution (freshly prepared before use), and heated at 45 °C for 1 h. The reaction solution was cooled to room temperature, then 200 μL of methanol was added to react with excess reagent, and the solvent was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was dissolved in 100 μL of acetonitrile–water (70:30, v/v), and an aliquot of 10 μL was injected into the UPLC–MS/MS system for analysis.

2.5. Method validation

2.5.1. Selectivity

Selectivity was investigated by comparing chromatograms of blank plasma obtained from rats prior to dosing with those of blank plasma spiked with trantinterol and I.S. and plasma sample after administration of 3 mg/kg trantinterol racemate.

2.5.2. Linearity and LOQ

To evaluate linearity, calibration standards in plasma at seven concentration levels ranged 1.0–80.0 ng/mL for each trantinterol enantiomer were prepared and assayed on 3 consecutive days. Calibration curves for trantinterol enantiomers in plasma were generated by plotting the peak area ratio (y) of enantiomer derivatives to I.S. versus nominal concentrations (x) of trantinterol enantiomers by $1/x^2$ weighted least square linear regression. The LOQ is defined as the lowest concentration of trantinterol enantiomer for which an acceptable accuracy within ±20% and a precision below 20% were obtained.

2.5.3. Precision and accuracy

The accuracy and precision were assessed to determine QC samples at three concentration levels of trantinterol enantiomer (2.50, 20.0 and 70.0 ng/mL of each enantiomer) on 3 consecutive days. Precision was expressed as relative standard deviation (R.S.D.) and accuracy as relative error (R.E.). Intra-day precision and accuracy were determined by six replicate analysis of QC samples on 1 day, while inter-day precision and accuracy were determined by six replicate analysis on 3 consecutive days, using standard curve prepared on the same day.

2.5.4. Extraction recovery and matrix effect

Extraction recoveries of (−)- and (+)-trantinterol were determined by comparing the peak areas obtained from blank plasma samples spiked with analytes before extraction with those from blank plasma samples to which analytes were added after extraction. This procedure was performed at three QC levels. The recovery of I.S. was determined in a similar way.

In order to evaluate the matrix effect on the ionization of analytes, i.e. the potential ion suppression or enhancement due to the matrix components, three concentration levels of trantinterol enantiomers were added to the extract of 100 μL of blank plasma, derivatized as descried in Section 2.4, the corresponding peak areas (A) were compared with those of the trantinterol standard solutions derivatized directly (B). The ratio (A/B × 100) % was used to evaluate the matrix effect. The matrix effect of internal standard was also evaluated using the same method.

2.5.5. Stability

The stability tests were designed to cover the anticipated conditions that real samples may experience. The stability of trantinterol and I.S. stock solutions was evaluated after storage at room temperature for 4 h and at 4 °C for 30 days. QC plasma samples of three concentration levels were subjected to the conditions below. The stability of QC plasma samples kept at room temperature for 4 h was evaluated. This time exceeds the routine preparation time of samples. In order to estimate the stability of derivatized trantinterol enantiomers in processed extracts of rat plasma samples, the pretreated QC samples were kept in an autosampler maintained at 4 °C for 12 h.

2.6. Application of the assay

All animal procedures described in this report were approved by the Ethics Committee of Shenyang Pharmaceutical University. Adult Wistar rats (200 ± 20 g) obtained from the Animal Center of Shenyang Pharmaceutical University (Shenyang, China) were utilized for all the experiments. Each rat (n = 6) was given a single dose of 3 mg/kg (trantinterol racemate in aqueous solution) by i.g. route. The rats were fasted for 12 h before drug administration. Blood samples (0.3 mL) were collected into heparinized tubes from each rat by puncture of retroorbital sinus. This was performed at 0 h (predose), and 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0, 8.0, 12.0 and 24.0 h after i.g. administration. Blood was immediately processed for plasma by centrifugation at 3000 × g for 10 min. The plasma samples were stored at −20 °C until analysis.

3. Results and discussion

3.1. Chiral separation of trantinterol enantiomers

The indirect separation of trantinterol enantiomers was achieved by using the chiral derivatization reagent to yield diastereomeric derivatives that differ in their chemical and physical behavior and, therefore, can be separated on achiral stationary phase. To ensure a quantitative determination of each enantiomer, a baseline resolution of diastereomers is desired. Various parameters (buffer type, pH, organic modifier, etc.) of mobile phase for the separation of derivatized trantinterol enantiomers were studied. The separation was performed by using ammonium acetate and formic acid as the buffer finally. Although formic acid could improve the sensitivity, the enantioseparation was unsatisfactory. Ammonium acetate buffer could supply a better resolution than formic acid. However, we also found higher concentration of ammonium acetate in the mobile phase up to 10 mM resulted in a lower resolution with a shorter retention time. The best resolution was achieved with 3 mM ammonium acetate as the buffer. The effect of pH was examined ranging from 4 to 7 on the resolution of trantinterol enantiomers. It was found that the chromatographic separation of the two diastereomers strongly depended on the buffer pH. The increase in pH value leads to a general increase in both retention times and resolution. Ammonium acetate buffer (pH 7) without pH adjustment was selected at last for the best resolution. Mixtures of 3 mM ammonium acetate and acetonitrile using isocratic and different gradient elution programs were evaluated. Finally, the gradient elution program as listed in Section 2.2.1 was selected which resulted in a good peak shape and chromatographic resolution in a relative short analysis time. The use of gradient elution could extend column life, especially in the use of small particles of stationary phase.

The elution order of derivatized trantinterol enantiomers by this method is (+)-trantinterol followed by (−)-trantinterol. The peak assignment was performed by comparing the retention times of enantiomerically pure (−)-trantinterol standard with those of racemate after derivatization.

3.2. Sample preparation

Liquid–liquid extraction method was chosen for sample pretreatment because this technique could produce not only purified but also concentrated samples. Some solvents were attempted, and n-hexane–dichloromethane–isopropanol (20:10:1, v/v/v) was finally adopted because of its high extraction efficiency and good repeatability.

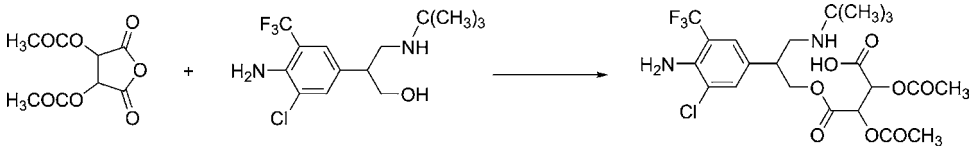

Fig. 2. The derivatization reaction between trantinterol and DATAAN.

Table 1
Precision and accuracy for the determination of trantinterol enantiomers in rat plasma (intra-day: n = 6; inter-day: n = 6 series per day, 3 days).

	(−)Trantinterol (ng/mL)				(+)-trantinterol (ng/mL)			
	1.00 (LLOQ)[a]	2.50 (low)[a]	20.0 (middle)[a]	70.0 (high)[a]	1.00 (LLOQ)[a]	2.50 (low)[a]	20.0 (middle)[a]	70.0 (high)[a]
Found C (ng/mL)	0.93 ± 0.07	2.63 ± 0.11	19.5 ± 1.2	69.4 ± 2.1	1.02 ± 0.084	2.66 ± 0.089	19.8 ± 1.4	69.7 ± 2.1
Intra-day R.S.D. (%)	6.4	8.6	6.1	2.7	7.9	8.7	6.8	2.8
Inter-day R.S.D. (%)	14	8.7	8.4	4.4	14	9.6	9.2	4.5
Accuracy R.E. (%)	−7.3	5.2	−2.4	−0.9	−4.6	6.2	−1.0	−0.5

[a]Values are added C (ng/mL).

Sodium hydroxide solution (100 μL × 1 M) was added into the plasma samples in order to improve the extraction efficiency of the enantiomers which exist in unionized state under alkaline conditions, and reduce interference since most endogenous materials were of acidic nature.

3.3. Derivatization of trantinterol with DATAAN

Derivatives of tartaric acid anhydride, developed by Lindner et al. [12], have shown their usefulness as chiral derivatizing agents for several β-blockers and other compounds possessing an aminoalcohol structure [13–18]. In our study, DATAAN was used as chiral derivative reagent, and reacted with the hydroxyl group of trantinterol enantiomers to form diastereomeric ester derivatives. The reaction scheme for the formation of the DATAAN derivatives of racemic trantinterol is shown in Fig. 2. During the method development, the effects of the parameters on the derivatization were evaluated on the peak area of the resulting derivatives. In the experiment, we found the derivatization reaction could proceed without the addition of any catalysts, that simplified the method process. The derivatization was performed at three reaction temperatures (45, 60, 75 °C) and monitored at four time points (20, 30, 60, 90 min). The results showed that the derivatization could be completed at 45 °C in 60 min. A higher temperature was not useful to accelerate the reaction. The molar excess of DATAAN to trantinterol was used for the derivatization to proceed completely. And a linear calibration function was achieved over the whole calibration range of each trantinterol enantiomer. No chiral inversion occurred during the tagging reaction and separation by UPLC, judging from the chromatogram of single enantiomer after derivatization. Residual water should been avoided in the solvent during the derivatization reaction.

3.4. Method validation

3.4.1. Selectivity

Selectivity was accessed by comparing the chromatograms of blank plasma with the corresponding spiked plasma. Typical chromatograms of blank rat plasma (a), a blank plasma sample spiked with trantinterol (20 ng/mL per enantiomer) and I.S. (b) and a rat plasma sample at 15 min after oral administration of racemic trantinterol (c) are shown in Fig. 3. No interferences were observed at the retention times of the analytes.

3.4.2. Linearity and LOQ

The calibration curves for trantinterol enantiomers were linear over the range of 1–80 ng/mL ($r > 0.99$) by using weighted ($1/x^2$) least squares linear regression. Typical equations for the calibration curves of (+)-and (−)-trantinterol were: $y = 5.696 \times 10^{-2}x + 4.939 \times 10^{-3}$ ($r = 0.9986$) and $y = 5.531 \times 10^{-2}x + 5.197 \times 10^{-3}$ ($r = 0.9981$), respectively.

The limit of quantification for each enantiomer was 1 ng/mL with acceptable precision and accuracy presented in Table 1.

3.4.3. Precision and accuracy

The data of intra- and inter-day precision and accuracy for trantinterol enantiomers are listed in Table 1. The intra- and inter-day precisions were less than 8.7 and 9.6%, while the corresponding accuracy was from −2.4 to 6.2%, indicating an acceptable accuracy and precision of the method.

3.4.4. Extraction recovery and matrix effect

The extraction recovery values from rat plasma at concentration levels of 2.5, 20 and 70 ng/mL were 78.5 ± 5.0%, 74.2 ± 4.5%, 76.1 ± 3.7% for (+)-trantinterol and 77.5 ± 4.3%, 73.8 ± 4.6%, 75.0 ± 3.4% for (−)-trantinterol, respectively. While the recovery of the I.S. was 78.2 ± 3.5%. These results indicated that the recovery of trantinterol enantiomers and the I.S. was consistent and not concentration dependent.

In terms of matrix effect, all the ratios (A/B × 100) % defined as in Section 2.5.4 were between 85 and 115%, which means no significant matrix effect in this method.

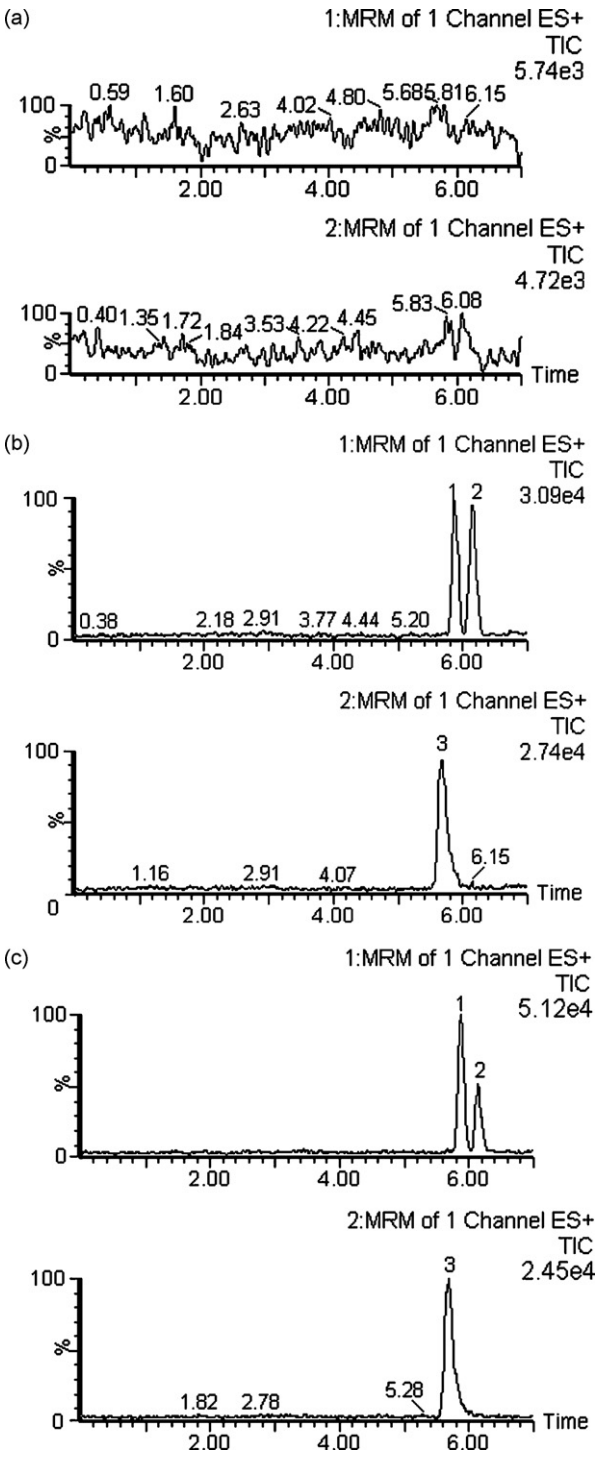

Fig. 3. Typical chromatograms of trantinterol derivatives and I.S. (a) Blank plasma, (b) blank plasma spiked with trantinterol and diphenhydramine and (c) plasma sample at 15 min after oral administration of racemic trantinterol. The retention times for trantinterol enantiomers and I.S. were 5.87, 6.13 and 5.70 min, respectively.

Table 2
Stability of (−)-trantinterol and (+)-trantinterol in rat plasma at three QC levels (n = 5).

	Concentration (ng/mL)	Stability (R.E. %, mean ± S.D.)	
		Short-term stability	Post-preparative stability
(−)-Trantinterol	2.50 (low)	95.1 ± 5.6	103.8 ± 4.3
	20.0 (middle)	93.8 ± 4.1	98.4 ± 8.6
	70.0 (high)	102.4 ± 5.2	107.4 ± 6.4
(+)-Trantinterol	2.50 (low)	94.0 ± 4.7	108.3 ± 5.3
	20.0 (middle)	96.3 ± 6.8	101.1 ± 5.1
	70.0 (high)	106.4 ± 3.8	103.6 ± 6.2

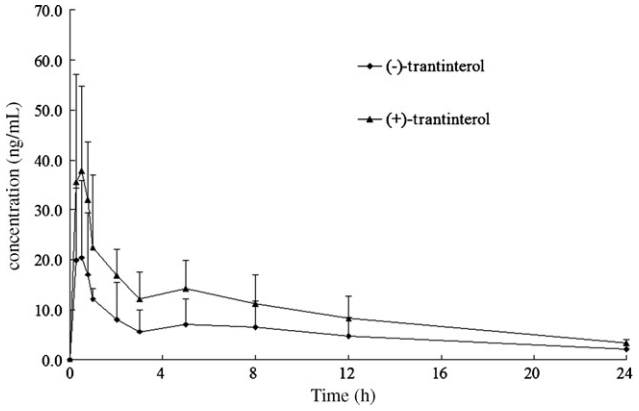

Fig. 4. Mean concentration–time profiles of trantinterol enantiomers after single oral administration of trantinterol (3 mg/kg).

3.4.5. Stability

The stock solutions of trantinterol and I.S. were found to be stable at room temperature for 4 h and 4 °C for one month. Table 2 summarizes the results for stability, and all the results well met the criterion for stability measurements.

3.5. Application of the analytical method to pharmacokinetic study

The validated analytical method was applied to the determination of trantinterol enantiomers in rat plasma after an oral administration of 3 mg/kg dose of racemic trantinterol. Fig. 4 shows the mean plasma concentration–time profiles of each enantiomers in six rats. The C_{max} (maximum drug concentration in plasma) for (−)- and (+)-trantinterol was found to be 22.2 ± 11 and 41.0 ± 18 ng/mL, respectively. Both (−)- and (+)-trantinterol reached their maximum concentration in plasma at 0.46 h (t_{max}). The AUC_{0-t} was 83.5 ± 31 and 155 ± 53 ng h/mL for (−) and (+) enantiomers, respectively. The AUC_{0-t} (+)/(−) and C_{max} (+)/(−) ratios of trantinterol were 1.85 and 1.84, respectively.

4. Conclusions

This paper reports, for the first time, the enantioselective analysis of trantinterol in plasma by UPLC–MS/MS with derivatization. The method is simple, rapid and provides baseline separation of derivatized trantinterol enantiomers. It met all requirements of specificity, sensitivity, linearity, precision, accuracy and stability generally accepted in bioanalytical chemistry. The method has been successfully applied to enantioselective pharmacokinetic study of trantinterol racemate in rats.

References

[1] L.L. Gan, M.W. Wang, M.S. Cheng, L. Pan, Biol. Pharm. Bull. 26 (2003) 323.
[2] Z.H. Hao, Y.Y. Zhang, L. Pan, X. Su, M.S. Cheng, M.W. Wang, H.D. Zhao, Y.L. Wu, Biol. Pharm. Bull. 31 (2008) 802.
[3] H.X. Chen, Q.P. Deng, L.W. Zhang, X.X. Zhang, Talanta 78 (2009) 464.
[4] G. Gübitz, M.G. Schmid, J. Chromatogr. A 1204 (2008) 140.
[5] A.A. Elbashir, F.E. Suliman, B. Saad, H.Y. Aboul-Enein, Talanta 77 (2009) 1388.
[6] G.L. Erny, A. Cifuentes, J. Pharm. Biomed. Anal. 40 (2006) 509.
[7] I. Ali, V.D. Gaitonde, H.Y. Aboul-Enein, A. Hussain, Talanta 78 (2009) 458.
[8] Y. Sun, M. Liu, J. Zhang, Y. Jiang, W. Yu, J.P. Fawcett, J. Gu, J. Pharm. Biomed. Anal. (2009), doi:10.1016/j.jpba.2009.01.012.
[9] J. Yang, J. Guan, L. Pan, K. Jiang, M. Cheng, F. Li, Anal. Chim. Acta 610 (2008) 263.
[10] J. Yang, X. Lu, L. Pan, K. Jiang, M. Cheng, F. Li, J. Sep. Sci. 31 (2008) 3749.
[11] K.V. Penmetsa, C.D. Reddick, S.W. Fink, B.L. Kleintop, G.C. DiDonato, K.J. Volk, S.E. Klohr, J. Liquid Chromatogr. Relat. Technol. 23 (2000) 831.
[12] W. Lindner, C. Leitner, G. Uray, J. Chromatogr. A 316 (1984) 605.
[13] D.R. Brocks, F.M. Pasutto, F. Jamali, J. Chromatogr. B 581 (1992) 83.
[14] W. Lindner, M. Rath, K. Stoschitzky, G. Uray, J. Chromatogr. B 487 (1989) 375.
[15] D.R. Brocks, M.J. Dennis, W.H. Schaefer, J. Pharm. Biomed. Anal. 13 (1995) 911.
[16] E.A. Struys, E.E.W. Jansen, N.M. Verhoeven, C. Jakobs, Clin. Chem. 50 (2004) 1391.
[17] M. Nicklasson, S. Bjorkman, B. Roth, M. Jonsson, P. Hoglund, Chirality 14 (2002) 643.
[18] D.R. Brocks, J. Pharm. Pharm. Sci. 4 (2001) 24.