Journal of Chromatography B, 867 (2008) 144–148

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

Determination of ritodrine in human plasma by high-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry

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ARTICLE INFO

Article history:
Received 3 November 2007
Accepted 6 March 2008
Available online 14 March 2008

Keywords:
Ritodrine
Electrospray ionization tandem mass spectrometry
Determination
Human plasma

ABSTRACT

A simple and sensitive HPLC/MS/MS method was developed and evaluated to determine the concentration of ritodrine (RTD) in human plasma. Liquid–liquid extraction with ethyl acetate was employed as the sample preparation method. The structural analogue salbutamol was selected as the internal standard (IS). The liquid chromatography was performed on a Hanbon Sci. & Tech. Lichrospher CN (150 mm × 4.6 mm, i.d., 5 μm) column (Hanbon, China) at 20 °C. A mixture of 0.03% acetic acid and methanol (50:50, v/v) was used as isocratic mobile phase to give the retention time 3.60 min for ritodrine and 2.94 min for salbutamol. Selected reaction monitoring (SRM) in positive ionization mode was employed for mass detection. The calibration functions were linear over the concentration range 0.39–100 ng mL^{-1}. The intra- and inter-day precision of the method were less than 15%. The lower limit of quantification was 0.39 ng mL^{-1}. The method had been found to be suitable for application to a pharmacokinetic study after oral administration of 20 mg ritodrine hydrochloride tablet to 18 healthy female volunteers. The half-life is 2.54 ± 0.67 h.

1. Introduction

Ritodrine hydrochloride, 4-[2-[2-hydroxy-2-(4-hydroxyphenyl) -1-methyl-ethyl] aminoethyl]phenol hydrochloride, is the β_2 adrenergic receptor agonist which is effective in inhibiting uterine contraction. Clinically, it is widely used in obstetrics to stop premature labor and foetal asphyxia during labor [1,2].

Due to its efficacy in clinic, a rapid and sensitive quantification of this drug is imperative. To our best knowledge, some methods have been developed for the determination of ritodrine. These methods included fluorimetry [3], spectrophotometry [4–12], and high-performance liquid chromatography (HPLC) with spectrophotometric detection [13–17] and fluorescence detection [18]. Although some of these methods were sensitive, they could not be directly applied to the assay of ritodrine in human plasma. Recently, Nakamura and coworkers [19] have reported a HILIC–MS/MS method to determine the amount of ritodrine in human serum. The analytes were extracted by SPE with Waters Oasis MCX cartridges. But until now, there is no report about determination of ritodrine by HPLC–MS/MS method on RP column. The reason may be that the stricted retention of ritodrine on RP column makes it difficult to separate from matrix reference.

In this paper, we tried to analyze ritodrine in human plasma on a RP column by HPLC–MS/MS method. Considering economic aspect, liquid–liquid extraction was used to prepare the samples.

2. Experimental

2.1. Chemicals and reagents

Ritodrine hydrochloride was obtained from Hainan Chuntch pharmaceutical Co. Ltd. (Hainan, China) and Salbutamol from National Institute for The Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of HPLC-grade was obtained from Merck (Darmstadt, Germany). Acetic acid of HPLC-grade was purchased from Tedia (Fairfield, USA). Distilled water, prepared from demineralized water, was used throughout the study. Blank plasma was provided by The First Affiliated Hospital of Anhui Medical University (Hefei, China).

2.2. Instrumentation

A TSQ Quantum Ultra AM triple-quadrupole mass spectrometer (Thermo Finnigan), coupled with an electrospray ionization (ESI) source, a Finnigan Surveyor LC pump and Finnigan Surveyor autosampler, was used for HPLC/MS/MS analysis. Data acquisition was performed with Xcalibur 1.4 software (Thermo Finnigan).

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doi:10.1016/j.jchromb.2008.03.007

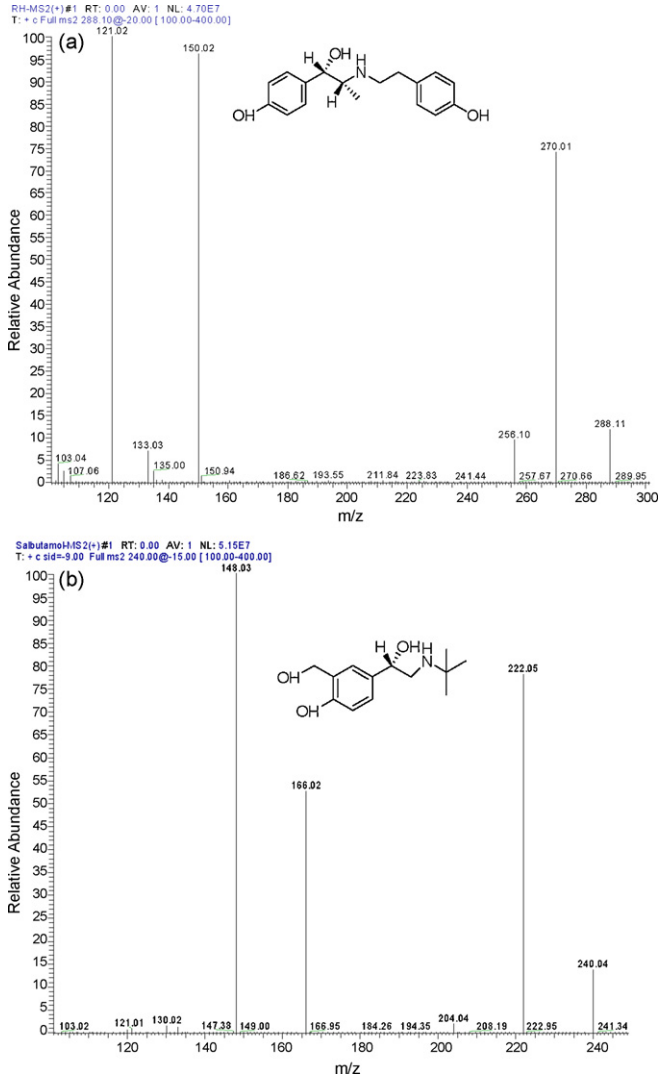

Fig. 1. Full scan product ion mass spectra of the $[M+H]^+$ ions of RTD (a) and IS (b). Capillary temperature 330 °C, electrospray voltage 4500 V, sheath gas 28, auxiliary gas 8, source collision-induced dissociation (CID) was at 9 eV and collision pressure of 1.4 mTorr. For ritodrine and sabutamol, collision energy was set at 20 and 15 eV, respectively.

2.3. LC/MS/MS conditions

Chromatographic analysis was performed on a Hanbon Sci. & Tech. Lichrospher CN column (150 mm × 4.6 mm, i.d., 5 μm, Huaiyin, China) with column oven at 20 °C. The mobile phase consisted of 0.03% acetic acid and methanol (50:50, v/v), which was pumped at a flow rate of 1.0 mL min^{-1} and 20% of the eluent was split into the inlet of the mass spectrometer.

Mass spectrometric detection was performed by means of selected reaction monitoring method with ESI source in positive ionization mode. The precursor ion for ritodrine was *m*/*z* 288.11 ($[M+H]^+$), and for salbutamol was *m*/*z* 240.04 ($[M+H]^+$). The product ions 121.02 (RTD, $[M+H]^+$) and 148.03 (IS, $[M+H]^+$) were chosen to quantification (Fig. 1).

2.4. Stock solutions

The stock solutions were prepared by dissolving the weighed standards into methanol at the concentration 120 μg mL^{-1} for RTD and 136 μg mL^{-1} for IS. The stock solution of RTD was diluted with methanol to get a series of working standard solutions at 1000, 500, 250, 125, 62.5, 31.3, 15.6, 7.8 and 3.9 ng mL^{-1} for calibration curve and 850, 62.5 and 7.8 ng mL^{-1} concentrations for QC samples. The internal standard, salbutamol was diluted to the working concentration 50 ng mL^{-1} from the stock solution 136 μg mL^{-1} with methanol. All stock solutions were stored at −20 °C.

2.5. Sample preparation

Liquid–liquid extraction with ethyl acetate was chosen as the sample preparation method. Internal standard working solution (10 μL, 50 ng mL^{-1}) was spiked into 100 μL unknown human plasma in a 2 mL centrifuge tube, and vortex-mixed for 30 s. Then 1 mL ethyl acetate was added into the tube. The sample mixture was mixed thoroughly for 2 min and centrifuged at 13,400 rpm for 10 min. The upper organic layer 800 μL was collected and evaporated to dryness under the gentle stream of nitrogen at the temperature of 50 °C. The residue was redissolved in 800 μL mobile phase. A 10 μL aliquot of the resulting solution was injected into the HPLC/MS/MS system for analysis.

2.6. Calibration curve and quality control samples

The calibration curve samples were prepared by spiking 10 μL IS (50 ng mL^{-1}) and 10 μL of one of the above-mentioned working solution into 100 μL blank human plasma at concentrations of 5 ng mL^{-1} for IS and 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 ng mL^{-1} for RTD. Quality control (QC) samples were generated with the same process to yield final concentrations of 85, 6.25, and 0.78 ng mL^{-1}. All spiked samples were then extracted by ethyl acetate using the method of sample preparation to get the samples that can be injected directly.

2.7. Method development

The specificity was examined by analyzing blank human plasma from six different sources. The samples were prepared by the process of sample preparation without spiking IS solution into the blank plasma. The interference was detected at the LLOQ. "Cross-talk" was evaluated by IS (5 ng mL^{-1}) and RTD (100 ng mL^{-1}).

The matrix effect was assessed by comparing peak areas of RTD obtained from the spiked-after-extraction samples with those from the unextracted pure standard solutions at the same concentration level. Three different concentration levels of RTD (85, 6.25 and 0.78 ng mL^{-1}) had been evaluated. The matrix effect of IS was also tested using the same method.

The recovery was evaluated by comparing the quality control sample mean peak areas to mean peak areas of spiked-after-extraction samples of corresponding concentration. All were performed in triplicate for every concentration.

The calibration curve was generated by plotting the peak area ratios (the peak area of RTD to that of IS) against RTD plasma concentrations, and calculated using a least-squares regression with a weighting factor of $1/x^2$. Linearity was determined to assess the performance of the method. Plasma calibration curves were prepared and analyzed in triplicate on three separate days. For quality control sample, it was used to evaluate the accuracy and precision of intra- and inter-day. Just like the calibration curves, QC samples were also prepared on three separate days, using quintuplicate ($n = 5$) preparations of plasma samples at each of three concentration levels, i.e., 15 determinations on each of the 3 days. The accuracy, i.e., percentage concentration deviation, was expressed by relative error (R.E.%), and the precision by relative standard deviation (R.S.D.%).

Ritodrine stability in human plasma was assessed by analyzing samples at concentrations of 0.78, 6.25 and 85 ng mL^{-1}, representing low, medium and high concentration QC samples, respectively, after exposure to different time and conditions. The results were

Table 1
Intra- and inter-day precision and accuracy data for the analysis of RTD in human plasma ($n = 5$)

	LLOQ (0.39 ng mL^{-1})	LQC (0.78 ng mL^{-1})	MQC (6.25 ng mL^{-1})	HQC (85 ng mL^{-1})
Day 1				
Mean ± S.D. (ng mL^{-1})	0.39 ± 0.05	0.83 ± 0.08	6.67 ± 0.50	86.12 ± 1.74
R.S.D. (%)	12.82	9.64	7.50	2.02
R.E. (%)	0.00	6.41	6.72	1.31
Day 2				
Mean ± S.D. (ng mL^{-1})		0.79 ± 0.09	5.59 ± 0.11	89.07 ± 5.82
R.S.D. (%)		11.39	1.97	6.53
R.E. (%)		1.28	−10.56	4.79
Day 3				
Mean ± S.D. (ng mL^{-1})		0.77 ± 0.04	5.78 ± 0.38	89.68 ± 3.51
R.S.D. (%)		5.19	6.57	3.91
R.E. (%)		−1.28	−7.52	5.51
Inter-day				
Mean ± S.D. (ng mL^{-1})		0.80 ± 0.07	6.01 ± 0.59	88.29 ± 4.08
R.S.D. (%)		8.75	9.82	4.62
R.E. (%)		2.56	−3.84	3.87

evaluated by the values of R.E.% and R.S.D.%. The short-term stability was studied after exposure of the plasma samples to room temperature (20 °C) for 4 and 24 h. The long-term stability was tested after keeping the samples at low temperature (−20 °C) for 40 days. While the freeze and thaw stability was assayed after three freeze (−20 °C)-thaw (room temperature) cycles.

2.8. Pharmacokinetic study

The method was applied to determine the concentrations of RTD in human plasma from healthy volunteers who received oral dosage ritodrine hydrochloride two tablets (containing 20 mg RH). Eighteen healthy female volunteers were selected for the study. Blood (3 mL) was removed by venepuncture into heparinized evacuated glass tubes prior to dosage and at 0.17, 0.33, 0.5, 0.67, 0.83, 1, 1.5, 2, 3, 4, 6 and 8 h thereafter. Following standing for 30 min and centrifugation (3000 g) for 10 min, the plasma was removed and stored at −20 °C until analysis.

3. Results and discussion

3.1. MS/MS conditions

Ritodrine has both amino and hydroxyphenyl groups in its structure. Full-scan mass spectra of RTD were acquired in both positive and negative ion modes. Since RTD had higher ionized efficiency in positive mode than in negative mode, RTD was monitored with the positive ESI source. The full scan product ion mass spectrum of RTD is shown in Fig. 1a. The precursor ion for RTD at m/z 288.11 gave three main product ions at m/z 121.02, 150.02 and 270.01 under the optimum collision energy 20 eV. The fragment ion at m/z 121.02 has highest abundance, while m/z 150.02 and 270.01 showed relative intensity above 50% of the base peak. Considering the sensitivity and stability, fragment ion at m/z 121.02 was selected to identify RTD. Fig. 1b is the mass spectrum of IS. The most abundant fragment ion at m/z 148.03 was chosen for SRM acquisition of it.

3.2. Chromatographic conditions

To optimize chromatographic conditions, we tried to find the suitable column and composition of mobile phase which can give good resolution and symmetric peak shapes of analytes as well as appropriate retention time. Many columns had been tried including C18 (Luna C18, Hypersil C18, Synergi C18), CN (Luna CN, Lichrospher CN), and phenyl-hexyl columns. Lichrospher CN column (150 mm × 4.6 mm i.d., 5 μm) gave the good chromatogram and appropriate retention time. For the mobile phase, water/methanol system was compared with water/acetonitrile system, and water/methanol system gave more symmetric peak shapes and better separation. In addition, we found the acidity of water had a great effect on the resolution of the analytes. For formic and acetic acid, the acetic acid was better because of its lower acidity, while formic acid gave too short retention time to distinguish the interference. Different percentages of acetic acid were tested including 0.1, 0.05, 0.03, 0.02 and 0.01%. The retention time was changed in all cases, but 0.03% acetic acid gave the acceptable retention time (3.60 min) and signal intensity. The peak breadth was narrow. So 0.03% acetic acid was selected. Ammonium acetic was also tried, but the signal depression was produced rapidly. Finally, a mixture of 0.03% acetic acid and methanol (50:50, v/v) was selected as the mobile phase. For IS, an interference at the retention time 1.83 min was detected, but separated well from IS.

3.3. IS selection

Two compounds, pseudoephedrine and salbutamol had been tried as IS because of their similar structure with RTD, and both of them ionized well in positive ESI source under the MS conditions of RTD. Suitable retention times for pseudoephedrine and salbutamol were gained on a Lichrospher CN column (150 mm × 4.6 mm i.d., 5 μm) using 0.03% acetic acid and methanol (50:50, v/v) as mobile phase. The results showed that pseudoephedrine gave poor accuracy and precision because of its unstable response. Therefore salbutamol was selected as IS.

3.4. Sample preparation

At first, protein precipitation was attempted in view of its time saving and process simplifying. Many solvents (methanol, acetonitrile and acetone) had been used, but none could give good result. Then, the liquid–liquid extraction was tried with different extraction solvents including ethyl acetate, dichloromethane, acetone, and the mixtures of them. In the end, ethyl acetate gave the best recovery and the least matrix effect.

3.5. Method validation

3.5.1. Specificity and selectivity

Fig. 2a–c are the chromatograms of the extracted blank human plasma for specificity and spiked human plasma with RTD (100 ng mL^{-1}) or IS (5 ng mL^{-1}) for "cross-talk". No interferences were found at the retention times of the analyte and IS.

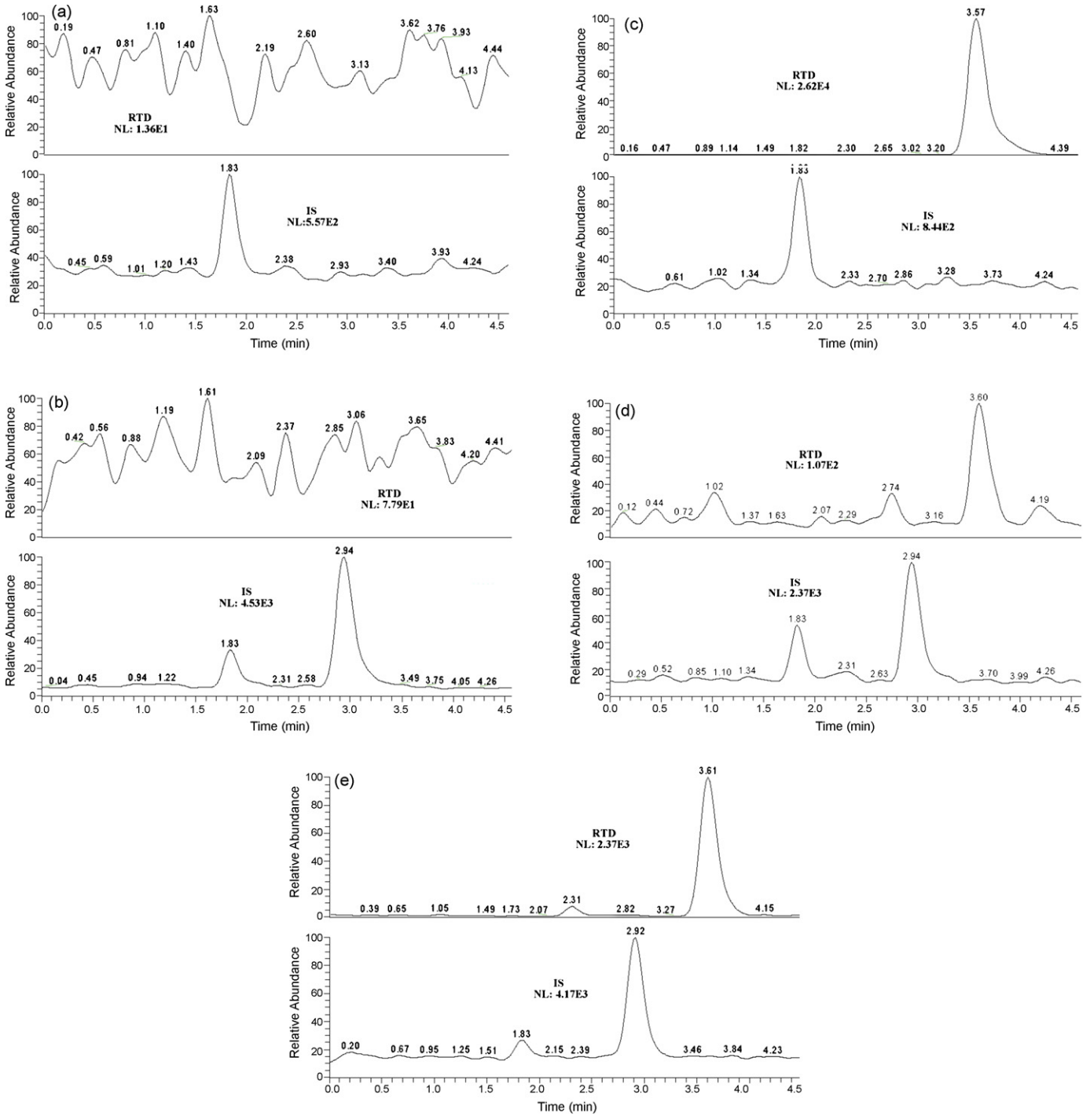

Fig. 2. SRM chromatograms for RTD and salbutamol (IS) in human plasma samples. (a) Blank plasma; (b) plasma sample with IS (5 ng mL^{-1}); (c) plasma sample with RTD (100 ng mL^{-1}); (d) plasma sample with RTD (0.39 ng mL^{-1}) and IS (5 ng mL^{-1}) (LLOQ); (e) a volunteer plasma sample 1.5 h after an oral dose of 20 mg ritodrine hydrochloride.

3.5.2. Linearity, precision and accuracy

Good linearity had been obtained at the concentrations ranged 0.39–100 ng mL^{-1}. The mean standard curves obtained on three separate days. The calibration coefficient fell between 0.998 and 0.999. Fifteen QC samples at three concentration levels were prepared every day following the standard curves, the concentrations of which were calculated by the equation of intra-day average calibration curve. The precision was assessed by the values of the relative standard deviation (R.S.D.%), while the accuracy by relative error (R.E.%). The acceptable criteria for the precision and accuracy are <15% [20]. Table 1 summarizes the results of quality control on 3 days which are all within the acceptable criteria.

3.5.3. Recovery and matrix effect

Matrix-induced signal suppression or enhancement is a well-known phenomenon in electrospray ionization mass spectrometry. To lessen the matrix effect, the compositions of the mobile phase and the extraction solvent had been studied carefully. The matrix effect ranged 94.40–99.86% for RTD and 96.77–98.66% for IS. The ion suppression nearly could be ignored. The recovery of RTD was 76.95–82.52% and that of IS was 49.41–51.60%. For IS, the recovery

Table 2
Stability for RTD in human plasma under various storage conditions ($n = 3$)

Storage condition	LQC (0.78 ng mL^{-1})		MQC (6.25 ng mL^{-1})		HQC (85 ng mL^{-1})	
	R.E. (%)	R.S.D. (%)	R.E. (%)	R.S.D. (%)	R.E. (%)	R.S.D. (%)
Short-term stability (4 h, room temperature)	9.31	0.44	9.62	1.98	2.05	1.77
24 h stability (room temperature)	−2.25	4.64	−8.98	3.71	1.19	5.95
Freeze-thaw stability (3 cycles, −20 °C)	6.66	2.81	2.40	10.10	−2.37	4.72
Long-term stability (40 days, −20 °C)	−9.58	6.96	1.66	7.90	8.95	3.57

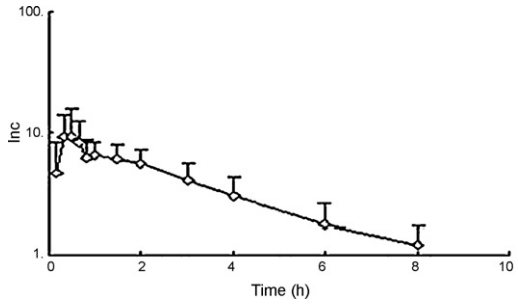

Fig. 3. Logarithm of mean plasma concentration–time profiles of IS healthy female volunteers after oral administration of 20 mg tablet of ritodrine hydrochloride. The error bars represent S.D.

was low, but it was consistent, precise and reproducible. With the low matrix effect and consistent recovery, the assay has proved to be reliable in bioanalysis.

3.5.4. Stability

The short-time, long-time, and three freeze-thaw cycles stabilities had been evaluated by R.S.D.% and R.E.%. The data of stabilities were shown in Table 2. No stability problems were found for the analyte and IS.

3.6. Application to pharmacokinetic study

The method was applied to determine the plasma concentrations of ritodrine after oral administration of 20 mg ritodrine hydrochloride to 18 volunteers. The logarithm of mean plasma concentration-time curve of RTD is shown in Fig. 3. Because of the relatively short chromatographic run time and simple sample preparation procedure, a throughput of 200 samples 1 day was achieved. In 24 h three sets of QC samples were regularly interspersed with the incurred samples, so that a set of QC samples (three different concentrations, each in quintuplicate) was analyzed every 8 h. This simple and selective method for the determination of RTD in human plasma was readily applicable to the clinical PK study for RTD. The PK parameters were received as follows: the maximum plasma concentration (C_{max}) 14.48 ± 11.26 ng mL^{-1}; area under the curve (AUC_{0-8}) 30.50 ± 11.40 ng h mL^{-1}; the time to maximum plasma concentration (T_{max}) 0.74 ± 0.51 h; half-life ($t_{1/2}$) 0.74 ± 0.51 h.

4. Conclusion

A simple and sensitive HPLC/MS/MS method on RP column for the quantification of ritodrine in human plasma was developed and validated. The method satisfied the requirements of high sensitivity, selectivity and throughput for pharmacokinetics.

Acknowledgement

The authors gratefully acknowledge University of Science and Technology of China for financial support.

References

[1] J.G. Hardman, L.E. Limbird, A.G. Gilman, Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed., McGraw-Hill, New York, 2001.
[2] D. S. Tatro, A-Z Drug Facts, Facts and Comparisons, Wolters Kluswer Health, St. Louis, 1999.
[3] O.A. Razak, J. Pharm. Biomed. Anal. 18 (1998) 359.
[4] C.S.P. Sastry, R. Chintalapati, Indian Drugs 38 (2001) 433.
[5] J.F. Van Staden, N.W. Beyene, R.I. Stefan, H.Y. Aboul-Enein, Talanta 68 (2005) 401.
[6] C.S.P. Sastry, R. Chintalapati, A.V.S.S. Prasad, B.S. Sastry, Talanta 53 (2001) 907.
[7] H.D. Revanasiddappa, B. Manju, Farmaco 56 (2001) 615.
[8] H.D. Revanasiddappa, B. Manju, J. AOAC Int. 83 (2000) 1440.
[9] H.D. Revanasiddappa, B. Manju, P.G. Ramappa, Anal. Sci. 15 (1999) 661.
[10] R.S. Bakry, A.F.M. El-Walily, S.F. Belal, Mikrochim. Acta 127 (1997) 89.
[11] R.S. Bakry, A.F.M. El-Walily, S.F. Belal, Anal. Lett. 29 (1996) 409.
[12] R.S. Bakry, A.F.M. El-Walily, S.F. Belal, Anal. Lett. 28 (1995) 2503.
[13] P. Kuhnert, P. Erhard, A. Dixon, B. Kuhnert, T. Gross, J. Liq. Chromatogr. 6 (1983) 2775.
[14] L.S. Lin, S.N. Caritis, L.K. Wong, J. Pharm. Sci. 73 (1984) 131.
[15] T.L. Gross, B.R. Kuhnert, P.M. Kuhnert, M.G. Rosen, N.J. Kazzi, Obstet. Gynecol. 65 (1985) 793.
[16] V.R. Bari, U.J. Dhorda, M. Sundaresan, Indian Drugs 39 (1999) 679.
[17] H.D. Revanasiddappa, B. Manju, J. Anal. Chem. 58 (2003) 775.
[18] A.S. Gross, K.F. Brown, J.A. Baird-Lambert, R.L. Nation, J. Chromatogr. 416 (1987) 400.
[19] T. Ohmori, M. Nakamura, S. Tada, T. Sugiyama, Y. Itoh, Y. Udagawa, K. Hirano, J. Chromatogr. B 861 (2008) 95.
[20] Guidance for industry bioanalytical method validation, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Veterinary Medicine (CVM), 2001, p. 19.