Journal of Pharmaceutical and Biomedical Analysis 52 (2010) 227–231

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Simultaneous analysis of bambuterol and its active metabolite terbutaline enantiomers in rat plasma by chiral liquid chromatography–tandem mass spectrometry

Wenxia Luo [a], Lin Zhu [a], Jifeng Deng [a], Aiming Liu [a], Bin Guo [a], Wen Tan [b], Renke Dai [a,*]

[a] Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou 510663, PR China
[b] Key Pharma Inc., Song-Shang Lake, Dongguan 523808, PR China

A R T I C L E   I N F O

Article history:
Received 29 August 2009
Received in revised form 8 December 2009
Accepted 20 December 2009
Available online 28 December 2009

Keywords:
Stereoselective separation
Bambuterol
Terbutaline
LC–MS/MS
Plasma

A B S T R A C T

A chiral liquid chromatography–tandem mass spectrometry (LC–MS/MS) simultaneous stereoselective analysis of bambuterol and its active metabolite terbutaline enantiomers in Wistar rat plasma has been developed and validated. All analytes and the internal standard were extracted from rat plasma samples by liquid–liquid extraction, separated on macrocyclic glycopeptide teicoplanin column with mobile phase constituted of 20 mM ammonium acetate solution–methanol (10:90, v/v) at a flow-rate of 0.4 mL/min. Detection was performed on an API 3000 tandem mass spectrometer with positive electrospray ionization in multiple reaction monitoring mode. The calibration curves in the range 1–800 ng/mL were linear and the accuracy for each analyte was within 8.0%. The intra- and inter-day precision as determined from quality control samples was less than 10.1%. The validated assay was successfully used to determine the enantiomers of bambuterol and terbutaline in rat plasma samples in the pharmacokinetic studies of *rac*-bambuterol.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Bambuterol, an amphiphilic bis-*N*,*N*-dimethylcarbamate prodrug of the selective β_2-adrenoceptor agonist terbutaline [1], is an orally effective long-acting sympathomimetic bronchodilator. It was widely used for asthma and chronic obstructive pulmonary diseases in clinic [2–5]. Although bambuterol did not display direct affinity for the β_2-adrenoceptor *per se*, it is slowly converted to terbutaline, the active metabolite, mainly via hydrolysis by butyrylcholinesterase (BuChE) (EC 3.1.1.8) [6]. The structures of racemic bambuterol and terbutaline are shown in Fig. 1.

As most chiral drugs, for technical or economic reason, terbutaline and bambuterol are administrated as racemate although the pharmacological activity resides mainly in the *R*-enantiomer [3]. Recent studies have indicated that *S*-enantiomer (distomer) of β_2-adrenoceptor agonists is a less active or inactive bronchodilatorily, while *S*-terbutaline may cause adverse effects contributing to morbidity and even mortality in asthma patients [3,7]. Additionally, *R*-bambuterol displayed two-fold higher potency than *rac*-bambuterol in the treatment of asthma in guinea pigs [8] and five-fold higher potency for *in vitro* inhibition of BuChE than *S*-bambuterol in human, mouse, and horse BuChE [9]. Different pharmacokinetic profiles of the enantiomers may occur and subsequent formation of racemic metabolites would vary. Therefore, the racemic form of bambuterol would play different roles in the therapeutic or adverse effects in clinic. Furthermore, with the continued technical advances in chiral analysis, the US Food and Drug Administration (FDA) has defined more strict requirements for the pharmacological and toxicological evaluation of each single enantiomer of a developing stereoisomeric drug [10,11]. Thus it is a high need to scream for a rugged, sensitive and rapid quantitative method for analysis of individual enantiomer in biological samples.

There have been a body of assays reported for the determination of terbutaline enantiomers. The enantioseparation methods included capillary electrophoresis (CE) with cyclodextrins or their derivatives as chiral additives [12–15], CE with ionic liquids [16], partial filling method for non-aqueous CE technique [17], chiral column liquid chromatography with UV or MS detection [18–23], and high-field asymmetric waveform ion mobility spectrometry after ionization in tandem mass spectrometry [24]. While, few reports on enantioseparation of bambuterol were currently available [25,26]. And there is no reliable analytical method described in the literature for simultaneous enantiomeric quantification of prodrug bambuterol and its active metabolite terbutaline yet.

* Corresponding author at: Guangzhou Institute of Biomedicine and Health, Chinese Academy of Sciences, Guangzhou International Business Incubator, Guangzhou Science Park, Guangzhou 510663, PR China. Tel.: +86 13527857328; fax: +86 20 32290606.
E-mail address: renke_dai@yahoo.com (R. Dai).

0731-7085/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2009.12.020

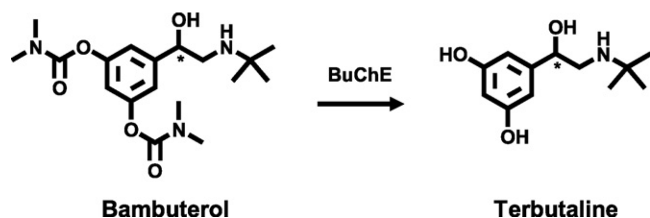

Fig. 1. The reaction of bambuterol to terbutaline catalyzed by BuChE. *Marks the chiral center.

In the present study, we describe a new enantioselective bioanalytical method using a teicoplanin-containing Chirobiotic T chiral column coupled to LC–MS/MS detection for simultaneous measurement of all enantiomers of bambuterol and its metabolite terbutaline in Wistar rat plasma. The developed analytical method was precise and accurate. It can be utilized as a valuable tool for stereoselective pharmacokinetic study of bambuterol and its metabolite terbutaline.

2. Experimental

2.1. Reagents and materials

R-Bambuterol hydrochloride (purity >99.5%) was from Key Pharma Inc. (Dongguan, P.R. China) and *rac*-bambuterol was from 3B Pharmachem International (Wuhan, P.R. China) Co., Ltd. Terbutaline sulfate was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China). *S*-Propranolol hydrochloride (internal standard, I.S.) was purchased from Sigma (St. Louis, USA). Methanol (HPLC grade) was from Burdick and Jackson (Muskegon, USA) and ammonium acetate (HPLC grade) from Dima Technology INC (Richmond Hill, Canada). Demineralized water was produced by Elix System (Millipore Corporation, USA). Other chemicals used were of analytical grade from commercial sources.

2.2. Stock solution

Standard solutions of racemic bambuterol and terbutaline (R/S = 1:1, w/w) were prepared in water–methanol (50:50, v/v) to give a final concentration of 1.0 mg/mL for each analyte. The solutions were successively diluted with water–methanol (50:50, v/v) to prepare a series of working solutions in the concentration range of 5–4000 ng/mL for each analyte. Calibration curves were then prepared by spiking 20 μL aliquots of the appropriate standard solution to 100 μL blank rat plasma containing 1 μg/mL neostigmine metilsulfate as BuChE inhibitor. The final concentrations for each analyte were prepared to be 1, 3, 8, 20, 60, 400, and 800 ng/mL. The quality control samples (QCs) were prepared in a similar way as the calibration standards to give three final concentrations of 3, 60, and 640 ng/mL for all analytes. I.S. working solution (3 μg/mL) was also prepared by diluting the 1 mg/mL *S*-propranolol stock solutions. All the solutions were stored at 4 °C and were brought to room temperature before use.

2.3. Sample preparation

To a 100 μL aliquot of plasma sample containing 1 μg/mL neostigmine metilsulfate, was added 20 μL of water–methanol (50:50, v/v) and 50 μL of I.S. (3 μg/mL). The mixed sample was then alkalinized by adding 40 μL 0.1 M sodium hydroxide. Sample was shaken for 1 min and 700 μL ethyl acetate was then added. The mixture was vortex-mixed for approximate 15 min, then centrifuged at 16,100 × *g* for 10 min, and the upper layer (750 μL) was carefully aspirated. The remainder was extracted once again with 700 μL ethyl acetate and the upper layer was combined with the former. The combined upper layer solution was evaporated to dryness at 45 °C under vacuum. The residue was reconstituted with 100 μL of the mobile phase, and vortex-mixed. 20 μL was injected into the LC–MS/MS system for analysis.

2.4. LC–MS/MS

Determination of all analytes was carried out on an LC–MS/MS system, API3000 mass spectrometer (Applied Biosystems, USA) coupled with HPLC (Shimadzu, Japan) and MPS3C autosampler (Gerstel, Germany).

An Astec Chirobiotic T column (250 mm × 4.6 mm I.D., 5 μm, Advanced Separation Technologies Inc., USA) was equipped with an ODS guard column (4 mm × 2.0 mm I.D., Phenomenex, USA). The mobile phase for chiral HPLC analysis consisted of water–methanol (10:90, v/v) containing 20 mM ammonium acetate (pH 6.4), which was degassed. The flow-rate was set at 0.4 mL/min and the column temperature was at 25 °C.

The mass spectrometer with electrospray ionization source was operated in the positive mode. Mass spectrometric conditions were optimized to obtain maximum sensitivity. The optimized heater temperature was 400 °C. The ion spray voltage was set at 4500 V. The nebulizer gas and the curtain gas (nitrogen) were both set at 8 psi. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 368 → 294 for bambuterol, m/z 226 → 152 for terbutalin and m/z 260 → 183 for *S*-propranolol with a scan time of 0.2 s per transition. The optimized collision energies chosen for bambuterol, terbutalin and I.S. were 27, 24 and 26 eV, respectively.

2.5. Method validation

Assay validation was performed according to FDA guideline [27]. During validation, the calibration curves were defined on three different days based upon assays of the spiked duplicate plasma samples, and QCs were determined in six replicates on the same day. Accuracy and precision were also assessed by determining QC samples at three concentration levels on three validation days. The accuracy was expressed by relative errors (RE) and precision by relative standard deviation (RSD).

The lower limits of detection, defined as the lowest concentration of an analyte were determined, which signal/noise ratio should be larger than 3. The lower limits of quantification (LLOQ), defined as the lowest concentration at which both precision and accuracy were less than or equal to a deviation of ±20%, were evaluated by analyzing samples which were prepared in six replicates.

The extraction recoveries of all analytes were determined by comparing the peak areas of QC samples to those of each analyte added post-extraction in blank plasma. To evaluate the matrix effect, a post-column infusion method was also utilized using an infusion pump (Harvard Apparatus, USA).

The robustness of the method was determined by according to the International Conference on Harmonization guidelines for validation of analytical procedures by typical variations, such as the extraction time, pH in the mobile phase, the mobile phase composition, and the different lots of columns [28].

The analytes stability in plasma samples under processing was evaluated at the concentrations of QCs. The short-term stability was determined after processed samples at room temperature for 2 h. The freeze–thaw stability was studied after three successive freeze–thaw cycles at −80 °C. The results were compared with those QC samples freshly prepared.

2.6. Pharmacokinetic study

The analytical method was applied to determine plasma concentrations of bambuterol and its active metabolite terbutaline enantiomers in Wistar rats after a single intravenous administration with 5 mg/kg *rac*-bambuterol. The Wistar rats (3 males, 3 females, weighing 190–210 g) were originally provided by the Experimental Animal Center, Southern Medical University, and maintained under specific pathogen-free conditions in the Animal Center of Guangzhou Institute of Biomedicine and Health (GIBH), Chinese Academy of Sciences. The animal protocols for experiments were reviewed and approved by the Institutional Laboratory Animal Care and Use Committee of GIBH. Approximately 0.3 mL blood of rats was collected during the time course of 0, 0.083, 0.167, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 8.0, 12.0, 24.0, 36.0 h for intravenous does of 5 mg/kg *rac*-bambuterol. Blood samples collected were centrifuged at 3000 × *g* for 10 min, and the supernatant was separated and stored at −80 °C until analysis. The pharmacokinetic parameters were calculated using the Drug and Statistics (DAS) version 2.0 software package (Anhui Provincial Center for Drug Clinical Evaluation, P.R. China).

3. Results and discussion

3.1. Optimization of chromatographic conditions

The enantioselective separations were achieved with the teicoplanin-based macrocyclic antibiotic-type chiral column using mobile phase of water–methanol (10:90, v/v). Baseline separation of both bambuterol and terbutaline enantiomers was obtained with their resolution factor >1.6. Other organic modifiers have also been studied for elution such as acetonitrile and 2-propanol. Acetonitrile and 2-propanol could not separate either enantiomers of bambuterol and terbutaline, while the column pressure increased greatly when 2-propanol was used.

For better peak shape and higher signal response, the buffer selected for this study was ammonium acetate because of its volatilization and compatibility. The buffer concentrations of 50, 20, 10, 5, 2 mM were investigated. No significant influence of these buffer concentrations was detected on the enantioselective separation, while the retention times of these enantiomers became shorter and the signal response of MS became lower when increasing the buffer concentrations. Therefore, 20 mM concentration of ammonium acetate was chosen as the optimized conditions. The effect of pH on the enantioselective separation of bambuterol and terbutaline was also studied in the range of pH 4.0–6.0 by adding acetic acid. The results displayed no notable difference on the separation, retention time and signal response in this pH range. Based upon these results, the mobile phase composition for the validation was set at ammonium acetate buffer–methanol (20 mM, pH 6.4) (10:90, v/v). When the mobile phase conditions were intentionally made small changes, no significant effect was observed in the chromatogram. Moreover, the results of enantioselective separation were reproduced in different lots of columns.

3.2. Method validation

3.2.1. Selectivity

Potential interference from endogenous compounds was investigated by analyzing rat plasma of six different subjects. Fig. 2 showed typical MRM chromatograms of (A) a blank plasma sample, (B) blank plasma spiked with 1 ng/mL (LLOQ) of both bambuterol and terbutaline, and (C) plasma sample 4 h after intravenous infusion of 5 mg/kg *rac*-bambuterol. No significant interference or ion suppression from endogenous substances was observed at the retention time of each analyte and I.S. The retention times were approximately 19.5, 21.6, 15.1, 16.9 and 20.1 min for *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, *S*-terbutaline, and *S*-propranolol, respectively.

3.2.2. Linearity of calibration curves and sensitivity

Good linear relationship was obtained over the concentration range of 1–800 ng/mL for all analytes in rat plasma. The correlation coefficients for the weighted ($1/x^2$) least-squares linear regression curves were over 0.991. The typical linear equations of the calibration curves regressed were: $y = 2.8 \times 10^{-2} + 3.56 \times 10^{-2}x$ for *R*-bambuterol, $y = 4.1 \times 10^{-2} + 3.58 \times 10^{-2}x$ for *S*-bambuterol, $y = 5.8 \times 10^{-5} + 1.48 \times 10^{-3}x$ for *R*-terbutaline, and $y = 3.86 \times 10^{-4} + 1.93 \times 10^{-3}x$ for *S*-terbutaline. Here y represents the ratios of bambuterol or terbutaline enantiomers peak-area to that of I.S. and x represents the plasma concentrations of analytes. The LLOD for *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline were 0.2, 0.2, 0.4 and 0.4 ng h/mL, respectively. The LLOQ were 1 ng/mL for each of these analytes, MRM chromatograms of LLOQs plasma samples are shown in Fig. 2.

3.2.3. Precision and accuracy

The intra- and inter-day precision and accuracy for *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline from the QCs were summarized in Table 1. In this assay, the intra- and inter-assay precision was 2.0–10%, 2.1–7.5%, 3.9–8.8%, and 2.3–6.8% for *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline, respectively. The accuracy was in the range of −8.0% to 7.6%, −9.2% to 5.6%, −1.6% to 7.2% and −3.7% to 4.4% for *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline, respectively.

3.2.4. Extraction recovery and matrix effect

The mean extraction recoveries (means ± SD) for *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline, are shown in Table 1, and the extraction recovery of I.S. was 71.8 ± 1.1%. The RSD was less than 10% for all recoveries throughout the entire standard concentration ranges, showing good consistency. The extraction time, which was altered, had not obviously effects for the recovery.

No significant ion suppression or enhancement from plasma matrix was shown for bambuterol and terbutaline in post-column infusion experiments when the liquid–liquid extraction (LLE) method was applied. In contrast, when a simple protein precipitation with methanol was used for sample preparation, significant ion enhancement was observed at the retention time of *R*-bambuterol and *S*-bambuterol. The variability in matrix factor for bambuterol was up to 60% in protein precipitations (data not shown). The matrix effect should be taken into account when applying limited standard preparations to all different subject measurements. According to the result of matrix effect, the LLE method was chosen rather than the protein precipitation.

3.2.5. Stability

The processing stability of bambuterol and terbutaline enantiomers after the exposure of the spiked samples at room temperature (25 °C) for 2 h and three freeze–thaw cycles at −80 °C was evaluated by comparing the concentration with fresh QC plasma samples. The REs were within 12% for all the analytes, indicating the bambuterol and terbutaline enantiomers were stable in 2 h at room temperature. The bambuterol and terbutaline enantiomers were also stable in plasma after three freeze–thaw cycles at −80 °C (REs ≤ 15%).

3.2.6. Method application

This validated analytical method was applied for pharmacokinetic studies of bambuterol enantiomers and its active metabolite terbutaline after intravenous administration of 5 mg/kg

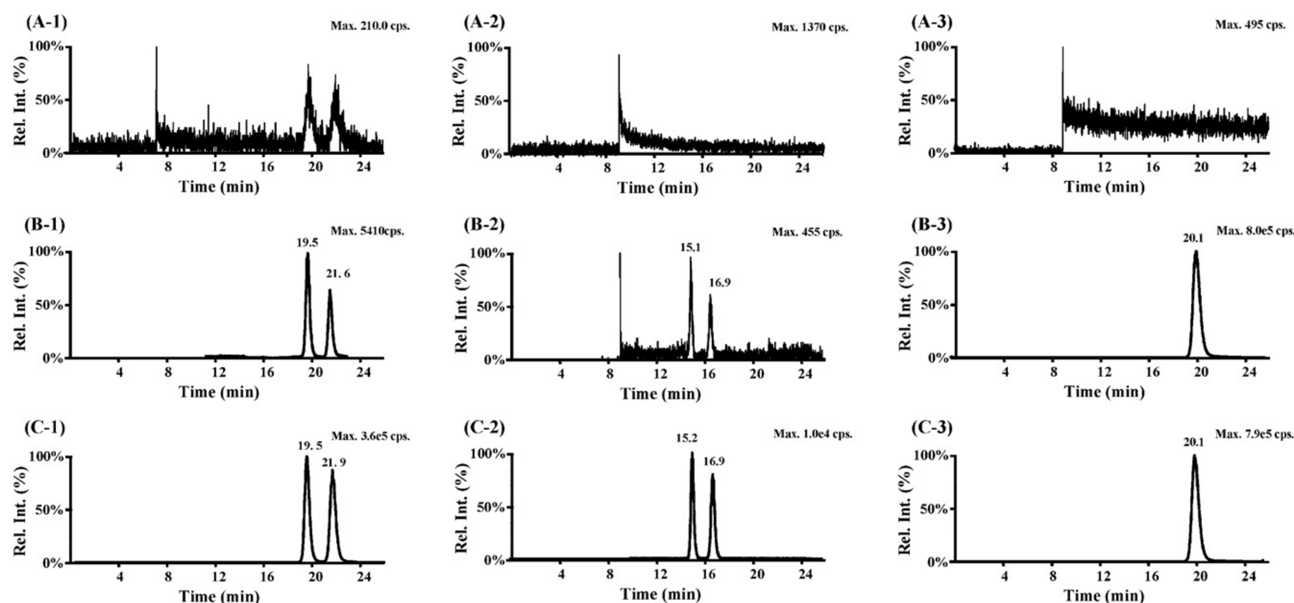

Fig. 2. Typical MRM chromatograms of *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, *S*-terbutaline, and *S*-propranolol (I.S.) in Wistar rat plasma. The retention times were approximately 19.5, 21.6, 15.1, 16.9, and 20.1 min, respectively. (A) Blank plasma sample; (B) plasma spiked with 1 ng/mL of bambuterol and terbutaline enantiomers and 1.5 μg/mL *S*-propranolol; (C) plasma sample 4 h after intravenous infusion of 5 mg/kg *rac*-bambuterol. Panels marked are (1) for bambuterol (*m*/*z* 368 → 294); (2) for terbutaline (226 → 152); and (3) for I.S. (260 → 183).

Table 1
Precision, accuracy and extraction recoveries of the method to determine *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline in rat plasma.

Analyte	Concentration added (ng/mL)	Within-batch (n = 6)			Between-batch (n = 6)			Extraction recoveries (n = 3) (mean ± SD) (%)
		Concentration found (ng/mL) (mean ± SD)	Precision RSD (%)	Accuracy RE (%)	Concentration found (ng/mL) (mean ± SD)	Precision RSD (%)	Accuracy RE (%)	
R-Bambuterol	1	1.01 ± 0.08	8.4	0.6	/	/	/	/
	3	2.89 ± 0.06	2.0	−3.6	2.88 ± 0.19	6.7	−4.0	74.9 ± 1.4
	60	64.1 ± 2.8	4.3	6.9	64.6 ± 3.0	4.7	7.6	86.8 ± 3.8
	640	619.2 ± 62.7	10.1	−3.3	588.7	7.9	−8.0	80.2 ± 0.5
S-Bambuterol	1	0.97 ± 0.03	2.7	−2.3	/	/	/	/
	3	3.09 ± 0.06	2.1	3.0	3.2 ± 0.16	5.0	5.6	80.8 ± 2.3
	60	61.5 ± 1.9	3.2	2.5	63.1 ± 2.3	3.7	4.7	89.7 ± 4.1
	640	606.8 ± 45.4	7.5	−5.2	581.3 ± 39.3	6.8	−9.2	79.7 ± 0.4
R-Terbutaline	1	1.01 ± 0.09	8.8	1.1	/	/	/	/
	3	2.98 ± 0.13	4.6	−0.4	2.95 ± 0.20	6.9	−1.6	41.8 ± 2.3
	60	60.1 ± 2.4	4.0	0.1	61.1 ± 2.8	4.5	1.9	41.5 ± 4.1
	640	686.1 ± 26.4	3.9	7.2	649.0 ± 44.6	6.9	1.4	39.5 ± 0.4
S-Terbutaline	1	1.00 ± 0.05	5.2	0.3	/	/	/	/
	3	2.89 ± 0.09	3.0	−3.7	2.98 ± 0.12	4.1	−0.6	50.3 ± 2.4
	60	57.7 ± 1.33	2.3	96.1	59.1 ± 1.9	3.3	−1.6	50.6 ± 3.6
	640	661.3 ± 36.2	5.5	3.3	668.4 ± 45.4	6.8	4.4	44.0 ± 1.4

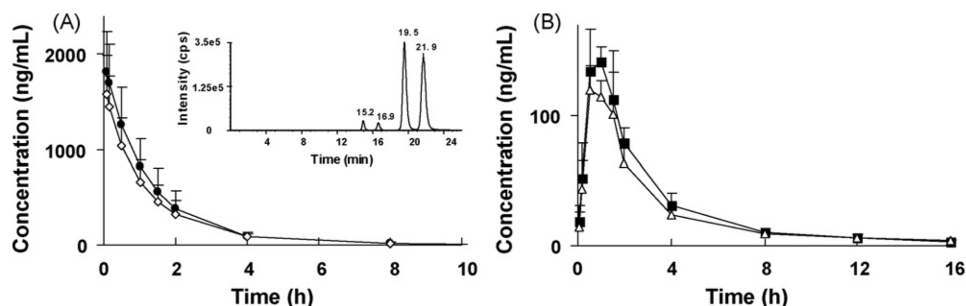

Fig. 3. Mean plasma concentration–time course of the enantiomers of bambuterol and terbutaline after an intravenous infusion of 5 mg/kg *rac*-bambuterol in Wistar rats (n = 6). (A) ●: *R*-bambuterol, ◊: *S*-bambuterol; (B) ■: *R*-terbutaline, □: *S*-terbutaline. The insert in panel A is the typical total chromatograms of plasma sample after intravenous infusion of 5 mg/kg *rac*-bambuterol. The retention times of *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline were 19.5, 21.9, 15.2, and 16.9 min, respectively.

rac-bambuterol in Wistar rats. Their plasma concentration–time courses were shown in Fig. 3. It confirms that the method was suitable for pharmacokinetic studies of bambuterol and terbutaline enantiomers in Wistar rat plasma. The mean area under the plasma concentration–time curve (AUC_{0-t}) for *R*-bambuterol, *S*-bambuterol, *R*-terbutaline, and *S*-terbutaline were 2735 ± 919, 2333 ± 646, 535 ± 82, and 474 ± 84 ng·h/mL, respectively. And the mean elimination half-life ($t_{1/2}$) was 6.43 ± 2.85, 7.16 ± 3.19, 7.74 ± 1.83, and 10.85 ± 1.42 h, respectively. The mean peak plasma concentration (*C*max) for *R*-terbutaline and *S*-terbutaline were 152 ± 20 and 138 ± 34 ng/mL, respectively, at a time (*T*max) of 0.92 ± 0.38 and 0.83 ± 0.41 h.

4. Conclusions

This method offers significant advantages over those previously reported, in terms of enantioselective and simultaneous determination of bambuterol and its active metabolite terbutaline. The assay was successfully employed for pharmacokinetic studies of the enantiomers of bambuterol and terbutaline in Wistar rat plasma with acceptable precision, adequate sensitivity and satisfied accuracy. It would be, furthermore, applicable for clinical studies of each enantiomer of bambuterol and terbutaline.

Acknowledgments

This work was supported in part by the National High-tech R&D Program [Grant 2006AA02Z339]; the Guangzhou Science and Technology Bureau [Grant 2006Z1-E4031, 2006P067]; and the Guangzhou Development District [Grant 2006Ss-P067].

References

[1] B. Testa, Prodrug research: futile or fertile? Biochem. Pharmacol. 68 (2004) 2097–2106.
[2] G. Persson, A. Baas, A. Knight, B. Larsen, H. Olsson, One month treatment with the once daily oral beta 2-agonist bambuterol in asthmatic patients, Eur. Respir. J. 8 (1995) 34–39.
[3] B. Waldeck, Beta-adrenoceptor agonists and asthma—100 years of development, Eur. J. Pharmacol. 445 (2002) 1–12.
[4] D.S. Sitar, C.P. Warren, F.Y. Aoki, Pharmacokinetics and pharmacodynamics of bambuterol, a long-acting bronchodilator pro-drug of terbutaline, in young and elderly patients with asthma, Clin. Pharmacol. Ther. 52 (1992) 297–306.
[5] M Cazzola, F. Calderaro, C. Califano, F. Di Pema, A. Vinciguerra, C.F. Donner, M.G. Matera, Oral bambuterol compared to inhaled salmeterol in patients with partially reversible chronic obstructive pulmonary disease, Eur. J. Clin. Pharmacol. 54 (1999) 829–833.
[6] A. Tunek, L.A. Svensson, Bambuterol, a carbamate ester prodrug of terbutaline, as inhibitor of cholinesterases in human blood, Drug Metab. Dispos. 16 (1988) 759–764.
[7] D.A Handley, J.R. McCullough, S.D. Crowther, J. Morley, Sympathomimetic enantiomers and asthma, Chirality 10 (1998) 262–272.
[8] W. Tan, J.L. Cheng, R-bambuterol, its preparation and therapeutic uses, US Patent No. 2005/0171197 (2005).
[9] A. Bosak, I. Gazic, V. Vinkovic, Z. Kovarik, Stereoselective inhibition of human, mouse, and horse cholinesterases by bambuterol enantiomers, Chem. Biol. Interact. 175 (2008) 192–195.
[10] N.M Maier, P. Franco, W. Lindner, Separation of enantiomers: needs, challenges, perspectives, J. Chromatogr. A. 906 (2001) 3–33.
[11] FDA, Policy Statement for the Development of New Stereoisomeric Drugs. (1992; Corrections made on 1997). http://www.fda.gov/Drugs/GuidanceCompliance Regulatory Information/Guidances/ucm122883.htm.
[12] R.L. Sheppard, X. Tong, J. Cai, J.D. Henion, Chiral separation and detection of terbutaline and ephedrine by capillary electrophoresis coupled with ion spray mass spectrometry, Anal. Chem. 67 (1995) 2054–2058.
[13] H.Y. Aboul-Enein, M.D. Efstatiade, G.E. Baiulescu, Cyclodextrins as chiral selectors in capillary electrophoresis: a comparative study for the enantiomeric separation of some beta-agonists, Electrophoresis 20 (1999) 2686–2690.
[14] K.H. Kim, S.H. Seo, H.J. Kim, E.Y. Jeun, J.S. Kang, W. Mar, J.R. Youm, Determination of terbutaline enantiomers in human urine by capillary electrophoresis using hydroxypropyl-beta-cyclodextrin as a chiral selector, Arch. Pharm. Res. 26 (2003) 120–123.
[15] J. Yang, X. Lu, L. Pan, K. Jiang, M. Cheng, F. Li, Simultaneous enantioseparation of four beta2-agonists by capillary electrophoresis with cyclodextrin additives. Study of the enantioselective mechanism, J. Sep. Sci. 31 (2008) 3749–3754.
[16] L. Huang, J.M. Lin, L. Yu, L. Xu, G. Chen, Improved simultaneous enantioseparation of beta-agonists in CE using beta-CD and ionic liquids, Electrophoresis 30 (2009) 1030–1036.
[17] H. Loden, Y. Hedeland, M. Hedeland, U. Bondesson, C. Pettersson, Development of a chiral non-aqueous capillary electrophoretic system using the partial filling technique with UV and mass spectrometric detection, J. Chromatogr. A 986 (2003) 143–152.
[18] A. Walhagen, L.E. Edholm, B.M. Kennedy, L.C. Xiao, Determination of terbutaline enantiomers in biological samples using liquid chromatography with coupled columns, Chirality 1 (1989) 20–26.
[19] K.H. Kim, D.S. Kim, S.P. Hong, O.S. Keon, Reversed-phase high performance liquid chromatographic separation of the enantiomers of terbutaline by derivatization with 2,3,4,6-tetra-O-acetyl-beta-D-glucopyranosyl isothiocyanate, Arch. Pharm. Res. 23 (2000) 26–30.
[20] R. Bakhtiar, F.L. Tse, High-throughput chiral liquid chromatography/tandem mass spectrometry, Rapid Commun. Mass Spectrom. 14 (2000) 1128–1135.
[21] K.H. Kim, H.J. Kim, J.H. Kim, S.D. Shin, Determination of terbutaline enantiomers in human urine by coupled achiral–chiral high-performance liquid chromatography with fluorescence detection, J. Chromatogr. B: Biomed. Sci. Appl. 751 (2001) 69–77.
[22] Y.Q. Xia, D.Q. Liu, R. Bakhtiar, Use of online-dual-column extraction in conjunction with chiral liquid chromatography tandem mass spectrometry for determination of terbutaline enantiomers in human plasma, Chirality 14 (2002) 742–749.
[23] O. Saleh, A. El-Azzouny, H. Aboul-Enein, A. Badawy, Validated HPLC method for separation and determination of terbutaline enantiomers, Anal. Lett. 41 (2008) 3221–3231.
[24] A. Mie, A. Ray, B.O. Axelsson, M. Jornten-Karlsson, C.T. Reimann, Terbutaline enantiomer separation and quantification by complexation and field asymmetric ion mobility spectrometry–tandem mass spectrometry, Anal. Chem. 80 (2008) 4133–4140.
[25] I. Gazic, A. Bosak, G. Sinko, V. Vinkovic, Z. Kovarik, Preparative HPLC separation of bambuterol enantiomers and stereoselective inhibition of human cholinesterases, Anal. Bioanal. Chem. 385 (2006) 1513–1519.
[26] A. Bartolincic, V. Druskovic, A. Sporec, V. Vinkovic, Development and validation of HPLC methods for the enantioselective analysis of bambuterol and albuterol, J. Pharm. Biomed. Anal. 36 (2005) 1003–1010.
[27] FDA, Guidance for Industry Bioanalytical Method Validation, 2001, http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf.
[28] ICH, International Conference on Harmonisation (ICH) of Technical Requirements for the Registration of Pharmaceutical for Human Use, Validation of Analytical Procedures: Text and Methodology Q2(R1), 2005, http://www.ich.org/LOB/media/MEDIA417.pdf.C-3.