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Short communication

Rapid and sensitive assay for trantinterol, a novel β_2-adrenoceptor agonist, in human plasma using liquid chromatography–tandem mass spectrometry

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ABSTRACT

A rapid and sensitive assay for trantinterol, a novel β_2-adrenoceptor agonist, in human plasma has been developed. Samples containing the analyte and internal standard, clenbuterol, were analyzed by liquid chromatography–tandem mass spectrometry after liquid–liquid extraction with diethyl ether:dichloromethane (60:40, v/v). Separation was performed on a Venusil MP C_{18} column (50 mm × 4.6 mm, 5 μm) using methanol:1% formic acid (50:50, v/v) as mobile phase and monitored by multiple reaction monitoring of the precursor-to-product ion transitions of trantinterol at *m/z* 311.2 → 238.1 and clenbuterol at *m/z* 277.2 → 203.1. The total run time was only 1.5 min and the method was linear over the concentration range 1–1000 pg/mL with a lower limit of quantitation of 1 pg/mL. Intra- and inter-day precisions (relative standard deviation) were below 7% and 12%, respectively, with accuracy (relative error) below 8%. The method was successfully applied to a pharmacokinetic study involving oral administration of a 50 μg trantinterol tablet to healthy volunteers.

1. Introduction

β_2-Adrenoceptor agonists such as salbutamol, terbutaline, etc. are effective bronchodilators widely used in the symptomatic treatment of asthma. They are generally used by the inhaled route but clenbuterol, a particularly potent and long acting bronchodilator, is also used by the oral route [1]. Trantinterol, 2-(4-amino-3-chloro-5-trifluomethylphenyl)-2-*t*-butylaminoethanol, is a novel phenylmethylamine β_2-adrenoceptor agonist currently undergoing clinical trials in China. Preclinical trials have revealed that trantinterol is a potent and highly selective β_2-adrenergic receptor agonist with long duration of action and low cardiac side effects [2].

A number of methods have been reported for the quantitation of β_2-adrenoceptor agonists in biological fluids. These methods include radioimmunoassay [3], ion chromatography [4], capillary gas chromatography [5,6], gas chromatography mass spectrometry [7] and liquid chromatography tandem mass spectrometry (LC–MS/MS) [8–11]. In this paper we report the first quantitative method to determine trantinterol in biological fluids using LC–MS/MS with clenbuterol as the internal standard (I.S.). The method has been fully validated and successfully applied to a pharmacokinetic study in healthy volunteers administered a single 50 μg oral dose.

2. Experimental

2.1. Chemicals and reagents

Trantinterol hydrochloride (>99.0% purity) was generously supplied by the Department of Pharmaceutical Chemistry, Shenyang Pharmaceutical University (Shenyang, P.R. China). Clenbuterol hydrochloride (purity >99.5%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, P.R. China). Formic acid and sodium hydroxide were obtained from Beijing Chemical Plant (Beijing, P.R. China) and HPLC grade methanol was from Fisher Scientific (Fair Lawn, NJ, USA). Distilled water, prepared from demineralized water, was used throughout the study. Trantinterol 50 μg tablets were prepared and supplied by Jiutai Pharmaceutical Co. Ltd. (Jinzhou, P.R. China).

2.2. Preparation of calibration standards and QC samples

All concentrations refer to trantinterol and clenbuterol as the free base. Stock solutions containing 1 mg/mL trantinterol and

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clenbuterol were prepared in methanol/water (50:50, v/v). Standard solutions of trantinterol (5, 15, 50, 150, 500, 1500, and 5000 pg/mL) were prepared by serial dilution of the stock solution with methanol/water (50:50, v/v). Low, medium and high concentration quality control (QC) solutions of trantinterol (15, 150 and 4000 pg/mL) were prepared independently in the same manner. A working 10 ng/mL I.S. solution was also prepared by dilution of the stock solution with methanol/water (50:50, v/v). All solutions were stored at 4 °C. Calibration standards were prepared by adding 100 μl standard solutions to 500 μl blank human plasma to generate concentrations in plasma in the range 1–1000 pg/mL. QC samples (3, 30, and 800 pg/mL) were prepared in the same way using QC solutions.

2.3. LC–MS/MS conditions

The HPLC system was an Agilent 1100 Series (Agilent Technologies, Palo Alto, CA, USA) fitted with a switching valve to minimize solvent entering the ion source. Chromatographic separation was achieved on a Venusil MP C_{18} column (50 mm × 4.6 mm, 5 μm) maintained at 30 °C. The mobile phase consisted of methanol/1% formic acid (50:50, v/v) at a flow rate of 0.8 mL/min. Mass spectrometric detection employed an Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ont., Canada) equipped with an electrospray ionization (ESI) source operated in the positive ion mode. The curtain, nebulizer and turbo gas were nitrogen set at 20, 50, and 45 psi, respectively. The ion spray voltage was adjusted to 5000 V and the source temperature set at 550 °C. The mass spectrometer was operated in the multiple reaction monitoring (MRM) mode at unit resolution for both Q1 and Q3 with a dwell time of 200 ms per MRM channel. Trantinterol and clenbuterol were monitored using the transitions of the protonated molecular ions at *m/z* 311.2–238.1 and *m/z* 277.2–203.1, respectively with declustering potentials of 32 and 40 eV, respectively and collision energies of 18 and 22 eV, respectively. MS/MS settings were tuned to maximize the response of the precursor/product ion combinations by introducing solutions into the ESI source via a syringe pump. Data acquisition and integration were controlled by Applied Biosystems Analyst version 1.3 software.

2.4. Sample preparation

To 500 μl human plasma in a glass tube, 100 μl I.S. working solution, 100 μl methanol/water (50:50, v/v) and 100 μl NaOH (1 mol/L) were added. The mixture was then subjected to liquid–liquid extraction (LLE) using 3 mL diethyl ether/dichloromethane (60:40, v/v). After centrifugation for 5 min at 3500 × *g*, the organic layer was transferred to another glass tube and evaporated to dryness at 40 °C under nitrogen. The residue was reconstituted in mobile phase (150 μl) and a 20 μl aliquot injected into the LC–MS/MS system.

2.5. Assay validation

Assay validation was performed according to FDA guidelines [12]. Linearity was assessed by weighted ($1/x^2$) least-squares linear regression of calibration curves based on peak area ratios and prepared in triplicate on three separate days. Precision (as relative standard deviation (R.S.D.)) and accuracy (as relative error (R.E.)) were evaluated based on assay of QC samples on three different days. The lower limit of quantitation (LLOQ) was defined as the lowest concentration with accuracy of ±15% and precision <15%. Selectivity was investigated by analyzing six different batches of drug-free human plasma spiked at the LLOQ. Recovery was calculated by comparing peak areas of QC samples with those prepared by spiking the upper organic layer of extracted blank plasma with the corresponding concentrations. Matrix effects were evaluated by comparing peak area responses of QC samples prepared using three different lots of human plasma with those of samples prepared by adding the same solutions into water. The stability of trantinterol was investigated by analysis in triplicate of QC samples after three successive freeze–thaw cycles and after storage at −20 °C for 1 month. Stability of processed samples stored at room temperature (18–20 °C) for 12 h was also assessed.

2.6. Application of the method

The method was used to investigate the plasma profile of trantinterol in 10 healthy volunteers (six males and four females, ranged from 30 to 40 years old) given a single oral dose of a trantinterol 50 μg tablet. The clinical protocol was approved by the Ethics Committee of Peking University First Hospital, China. All volunteers gave written informed consent before entering the study. Blood samples were collected before the dose and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8, 12, 24, and 36 h post-dose. Plasma was separated immediately by centrifugation at 3000 × *g* for 10 min and stored at −20 °C until analysis.

3. Results and discussion

3.1. LC–MS/MS conditions

Both analyte and I.S. responded best to positive ionization and gave protonated molecular ions $[M+H]^+$ as major peaks. The chemical structures of trantinterol and clenbuterol with proposed fragmentation patterns based on the full-scan product ion spectra of $[M+H]^+$ ions are shown in Fig. 1. The most abundant product ions of the analyte and I.S. were selected for MRM monitoring. Evaluation

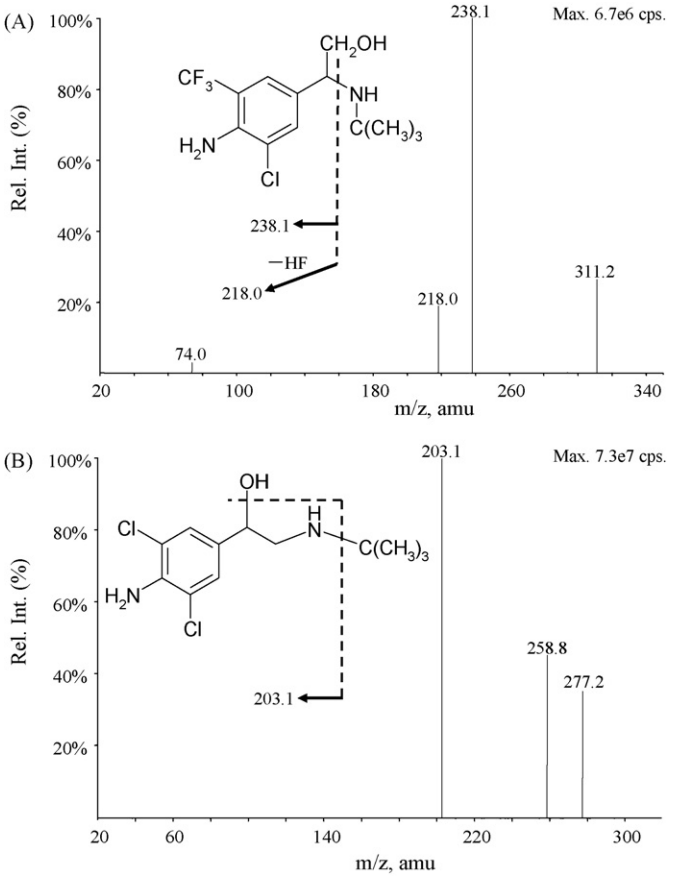

Fig. 1. Structures, proposed fragmentation patterns and full-scan product ion spectra of (A) trantinterol and (B) clenbuterol.

of mobile phases containing various combinations of acetonitrile, methanol, 10 mM ammonium acetate and water showed that inclusion of methanol produced strong signals with no solvent-clustered ions and inclusion of 1% formic acid improved peak shape. Several commercial reversed-phase HPLC columns (Nucleosil, Hypersil, Zorbax and Venusil) were investigated with the Venusil MP C_{18} column giving the most satisfactory chromatography. At a flow rate of 0.8 mL/min the analytical run time was only 1.5 min.

3.2. Sample preparation

Sample preparation by solid-phase extraction, simple protein precipitation and LLE were evaluated. LLE was adopted because pH adjustment and diethyl ether/dichloromethane gave efficient extraction of both trantinterol and clenbuterol with minimum interference.

3.3. Assay validation

As shown in Fig. 2A, no significant interference was observed from endogenous substances in plasma at the retention times of the analyte and I.S. Fig. 2B shows a representative ion chromatogram of a standard sample at the LLOQ (1 pg/mL) and Fig. 2C shows the chromatogram of a plasma sample from a healthy volunteer after oral administration of a trantinterol 50 μg tablet. The method was linear over the concentration range 1–1000 pg/mL with correlation coefficients in the range 0.9961–0.9984. A typical equation of a calibration curve was $y = 3.04x + 0.00266$, $r = 0.9984$. Precision and accuracy data are presented in Table 1. Intra- and inter-day precisions were below 7% and 12%, respectively, with accuracy (relative error) below 8%. Recovery of trantinterol for low, medium and high QC samples was 86.1 ± 7.8%, 84.2 ± 7.3%, 89.2 ± 5.9% (mean ± S.D.%), respectively. In terms of matrix effects, the ratios of the peak responses for low, medium and high QC samples were 78.0 ± 5.4%, 83.1 ± 4.8% and 87.8 ± 2.4%, respectively. The ratio for the I.S. was 79.8 ± 3.3%. It was concluded that matrix effects did not compromise the performance of the method. Results of stability tests are presented in Table 2. Trantinterol was stable under all conditions investigated.

Table 1
Accuracy and precision for the determination of trantinterol in human plasma (data are based on assay of six replicates on three different days).

Concentration (pg/mL)		R.S.D. (%)		R.E. (%)
Nominal	Mean found	Intra-day	Inter-day	
3.00	2.78	6.58	5.74	−7.30
30.0	28.1	6.01	4.81	−6.31
800	813	6.83	11.5	1.66

3.4. Application of the method

Fig. 3 shows concentration–time curves of trantinterol in plasma from healthy volunteers given a trantinterol 50 μg tablet. The maximum concentration (C_{max}) was 12.2 ± 4.2 pg/mL, the plasma elimination half-life ($t_{1/2}$) 16.4 ± 3.6 h and the mean area under the

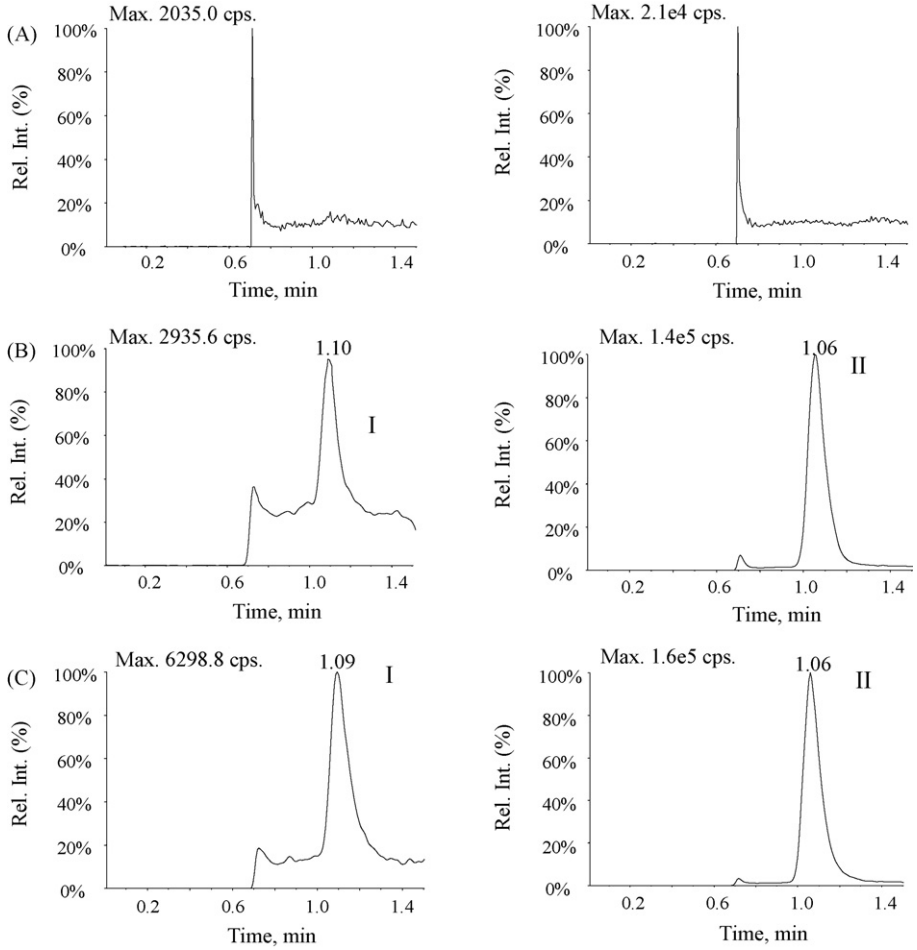

Fig. 2. Representative LC–MRM chromatograms for trantinterol (I) and clenbuterol (II) in human plasma samples (A) blank plasma, (B) blank plasma spiked with trantinterol at the LLOQ (1 pg/mL) and I.S. (10 ng/mL) and (C) plasma from a healthy volunteer 1.5 h after a single oral administration of a trantinterol 50 μg tablet.

Table 2
Stability data for trantinterol under various conditions (three samples each concentration).

Conditions	Concentration (pg/mL)		R.S.D. (%)	R.E. (%)
	Nominal	Mean found ± S.D.		
Long-term storage at −20 °C for 1 month	3.00	3.10 ± 0.15	4.87	3.37
	30.0	28.7 ± 0.9	3.17	−4.47
	800	861 ± 8	0.87	7.63
Three freeze–thaw cycles	3.00	2.86 ± 0.26	8.92	−4.67
	30.0	28.6 ± 2.3	8.14	−4.80
	800	853 ± 45	5.26	6.67
Storage of processed samples at room temperature for 12 h	3.00	3.06 ± 0.27	8.97	2.10
	30.0	30.8 ± 1.8	5.84	2.53
	800	848 ± 32	3.78	6.07

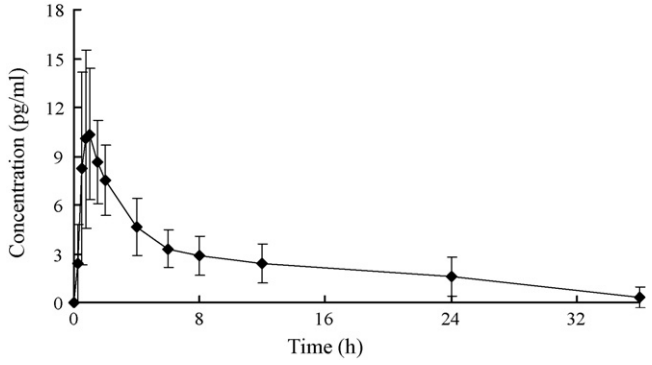

Fig. 3. Plasma concentration–time profile of trantinterol after a single oral dose of a trantinterol 50 μg tablet to healthy volunteers. (Data are mean ± S.D., $n = 10$.)

plasma concentration–time curve (AUC_{0-t}) 87.0 ± 40.7 (pg h)/mL. The results confirm that the assay has sufficient sensitivity to allow investigation of the pharmacokinetics of trantinterol given at therapeutic doses in the treatment of asthma. They also show that trantinterol has a long elimination half-life consistent with its reported long duration of action.

4. Conclusions

A rapid and sensitive LC–MS/MS method for the determination of trantinterol in human plasma at therapeutic doses has been developed. The relatively simple sample preparation procedure and short run time make it suitable for clinical pharmacokinetic studies of trantinterol.

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