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Short communication

HPLC–electrospray mass spectrometric assay for the determination of (R,R)-fenoterol in rat plasma

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ABSTRACT

A fast and specific liquid chromatography–mass spectrometry method for the determination of (R,R)-fenoterol ((R,R)-Fen) in rat plasma has been developed and validated. (R,R)-Fen was extracted from 125 μl of plasma using solid phase extraction and analyzed on Atlantis HILIC Silica 3 μm column. The mobile phase was composed of acetonitrile:ammonium acetate (pH 4.1; 20 mM) (85:15, v/v), at a flow rate of 0.2 ml/min. The lower limit of detection (LLOD) was 2 ng/ml . The procedure was validated and applied to the analysis of plasma samples from rats previously administered (R,R)-Fen in an intravenous bolus.

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1. Introduction

Fenoterol (Fen, Fig. 1) is a β_2-adrenoceptor agonist that may have clinical value in the treatment of congestive heart failure [1,2]. Fen possesses two chiral centers and the drug is supplied as a racemic mixture of (R,R)-Fen and (S,S)-Fen. Previous studies using cardiomyocytes contractility have demonstrated that (R,R)-Fen is the active component and this compound is currently under development as a therapeutic agent [1,2].

As part of the preclinical studies, the pharmacokinetics of (R,R)-Fen in rats was investigated, and an assay was developed to quantify (R,R)-Fen concentrations in rat plasma. The determination of Fen in plasma requires a sensitive assay as the plasma concentrations of the drug are commonly <10 ng/ml due to the compound's poor bioavailability and extensive metabolism via phase II pathways [3]. Plasma concentrations of Fen have been measured using a radioimmunoassay [4] and enzyme immunoassay [5] techniques as well as gas chromatography–MS [6,7], LC–APCI–MS [8] and HPLC with fluorescence detection [9]. However, these methods require either expensive antibodies, derivatization procedures or are not sensitive enough for the determination of low Fen plasma concentrations that are required for pharmacokinetic studies. Here, we present a fast, sensitive and specific assay for the determination of (R,R)-Fen in rat plasma which can be also adapted to other matrixes.

2. Experimental

2.1. Materials

(R,R)-Fenoterol ((R,R)-Fen) was prepared as previously described [2]. Ritodrine (Rit) hydrochloride, metaproterenol hemisulfate, ractopamine hydrochloride and formic acid were purchased from Sigma–Aldrich (St. Louis, MO). HPLC-grade acetonitrile, methanol and ethyl acetate were supplied by Fisher Scientific (Pittsburgh, PA). Purified water was prepared using a Milli-Q system (Millipore, Milford, MA). Control rat plasma collected from whole blood onto sodium EDTA was purchased from Innovative Research (Novi, MI).

2.2. Chromatographic conditions

The chromatography was carried out using an Agilent Technologies (Palo Alto, CA) 1100 LC/MSD Series (liquid chromatography-mass selective detector) composed of a vacuum degasser (G1379 A), a quarternary pump (1311A) a thermostated autosampler (G1329 A) and a thermostated column compartment (G1316A). The mass selective detector (MSD Quad SL, G1956B) was used with

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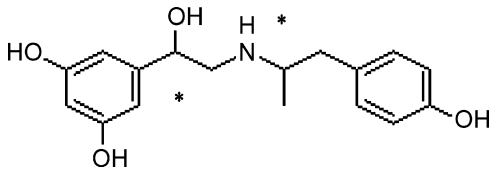

Fig. 1. Molecular structure of fenoterol. Asterisk "*" indicates position of a chiral carbon.

electrospray ionization interface (ESI) and on-line nitrogen generation system (Parker, Haverhill, MA, USA). The data was acquired by ChemStation software, Rev.A.10.02 [1757] (Agilent Technologies, Palo Alto, CA). The analysis was achieved with the use of an Atlantis HILIC Silica 3 μm (2.1 mm × 150 mm) column connected to an Atlantis HILIC Silica 3 μm guard column (2.1 mm × 10 mm) (Waters, Milford, MA).

The mobile phase was composed of acetonitrile:ammonium acetate (pH 4.1; 20 mM) (85:15, v/v) and was used at a flow rate ranging from 0.2 ml/min at time 0–10.0 min and from 15.1 to 17.0 min while flow rate of 0.3 ml/min was used between 10.01 and 15.0 min. To avoid excessive entry of complex plasma components into MS detector the flow was directed into the instrument only during the window time for the target compounds analysis, between 4.5 and 10 min of each chromatographic run.

2.3. Optimization of the mass selective detector (MSD) parameters

An ESI was used with the MSD operating in the positive ion mode. The optimized conditions for (R,R)-Fen and Rit were as follows: fragmentor voltage 70 and 100 V, respectively, gain 10, drying gas flow 10 l/min, nebulizer pressure 20 psig, drying gas temperature at 350 °C, capillary voltage 4000 V. Target compounds were quantified in a single ion-monitoring (SIM) mode. (R,R)-Fen was monitored at m/z 304.2 while Rit at m/z 288.2.

2.4. Extraction procedure

Rat plasma samples, ~0.150 ml, were thawed and centrifuged at 2643 × g for 5 min, a 0.125-ml aliquot was transferred to a polypropylene Eppendorf tube, acidified with 0.250 ml of 2% formic acid and vortex-mixed for 30 s. An aliquot of 20 μl of internal standard (Rit hydrochloride, working solution concentration 0.2 μg/ml) was added and the solution was vortex-mixed again for 30 s. The analytes were extracted using solid-phase extraction (SPE) with Bond Elut Plexa 1 ml cartridges (Varian, Palo Alto, CA). Cartridges were conditioned with 1 ml of MeOH and equilibrated with 1 ml of 2% formic acid. The cartridges were then loaded with samples and washed with 1 ml of water. The analytes were eluted with 2 ml of a mixture of ethyl acetate:methanol (1:1, v/v). The solution was evaporated to dryness in a Speed-Vac (Thermo Savant, NY) for a 1.5 h at a temperature of 80 °C. The residue was reconstituted in 20 μl of methanol, vortex-mixed for 20 s followed by the addition of 80 μl of acetonitrile and vortex-mixed for 10 s. The reconstituted samples were centrifuged for 6 min at 2643 × g and a 15-μl aliquot of the supernatant was injected onto HPLC system.

2.5. Preparation of standard solutions

A stock solution of (R,R)-Fen was prepared in methanol at a concentration of 1 mg/ml. Working solutions were prepared in methanol in amounts corresponding to concentrations of standard curve points, 2.0, 4.0, 8.0, 40.0, 160.0, 400.0, 1600.0 ng/ml (final concentrations). All solutions were kept at −20 °C.

2.6. Calibration curves, quality controls

Calibration and quality controls were prepared daily by spiking 115 μl of plasma with 10 μl of a corresponding working solution. (R,R)-Fen calibration curves were prepared in the following concentrations: 2.0, 4.0, 8.0, 40.0, 160.0, 400.0, 1600.0 ng/ml using Rit (28 ng/ml) as internal standard (final concentrations). One standard curve along with two sets of quality control samples were prepared each day of analysis. The quality control concentrations were as follows: 4.0, 40.0 and 400.0 ng/ml for low quality control (LQC), middle quality control (MQC) and high quality control (HQC), respectively.

2.7. Matrix effect (ME), recovery (RE) and process efficiency (PE)

Matrix effect studies were performed according to Matuszewski et al. [10]. They were studied at three quality control levels, 4.0, 40.0, and 400.0 ng/ml. The detailed description of quantification has been previously published [11].

2.8. Precision and accuracy

Both precision and accuracy were studied on three different days by analyzing QC samples with $n = 5$. Precision and accuracy were also determined for lower limit of quantification (LLOQ) with $n = 5$. The acceptance criteria were taken from the FDA Guidance [12] where the mean accuracy value for QCs should be within 15% of the actual value except for LLOQ, 20%. Precision determined for each QC level also should not exceed 15% of CVs and 20% for LLOQ.

2.9. Stability studies

(R,R)-Fen stability tests investigated were freeze–thaw and post-preparative studies for plasma extracts kept in the auto-sampler up to 12 h. Freeze–thaw tests were performed for three levels of QCs over 3 days while post-preparative tests were done for low and high QC.

2.10. Animal study

The pharmacokinetic study of (R,R)-Fen was performed by SRI International (Menlo Park, CA). The study was approved by Institutional Animal Care and Use Committee (study nb. B246-07). The drug was administered to male Sprague–Dawley rats at a dose of 5 mg/kg by IV administration. The blood samples (0.250 ml) were collected and processed to plasma at nine timepoints; predose, 5, 15, and 30 min; 1, 2, 4, 5 and 6 h post-dose. Samples were shipped on dry ice and stored at −80 to −75 °C for 5 months before analysis.

3. Results and discussion

3.1. Chromatographic conditions

Since reversed-phase C_{18} columns are highly compatible with mass spectrometer, initial LC/MS studies of (R,R)-Fen were carried out by using a C_{18} column (Phenomenex Luna, 150 mm × 2.1 mm i.d.) with mobile phases containing varying mixtures of ammonium acetate (50 mM, pH 6.0) and methanol. Retention of (R,R)-Fen on the Phenomenex Luna C_{18} column was low ($k \leq 3$) even at high composition of aqueous mobile phase (95% of ammonium acetate). At a mobile phase composition of ammonium acetate (pH 6.0; 50 mM):methanol (95:5, v/v)) ion suppression was observed due