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Short communication

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

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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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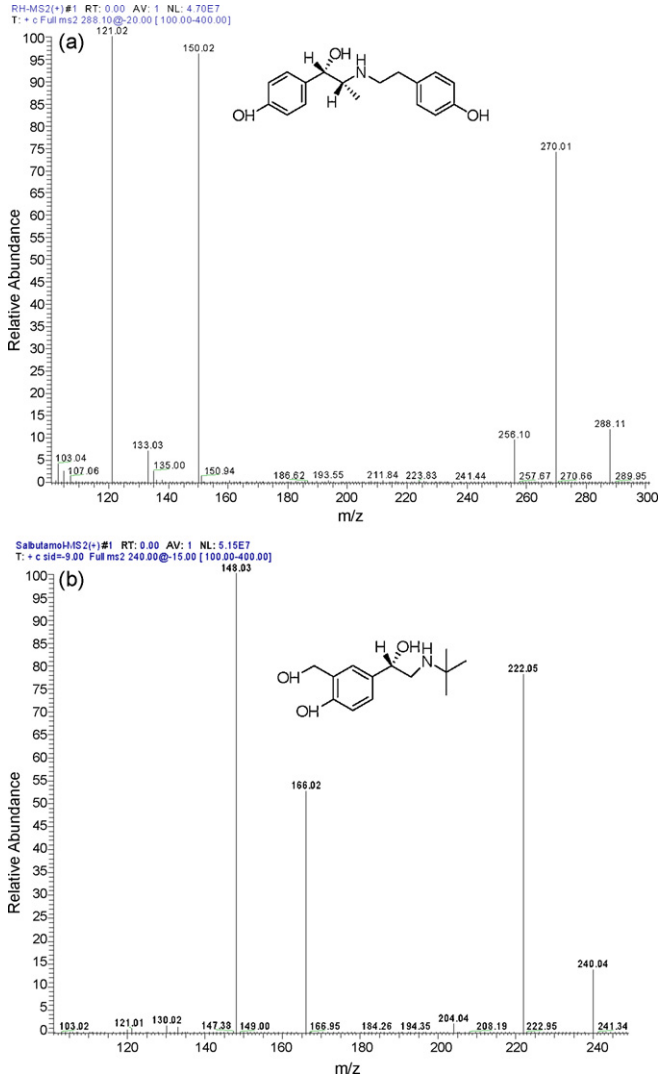

Fig. 1. Full scan product ion mass spectra of the $[M+H]^+$ ions of RTD (a) and IS (b). Capillary temperature 330 °C, electrospray voltage 4500 V, sheath gas 28, auxiliary gas 8, source collision-induced dissociation (CID) was at 9 eV and collision pressure of 1.4 mTorr. For ritodrine and sabutamol,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