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Determination of roxithromycin in rat lung tissue by liquid chromatography–mass spectrometry

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Abstract

A liquid chromatography–mass spectrometry (LC–MS) method for the determination of roxithromycin in rat lung tissue is described. Liquid–liquid extraction was adopted for sample preparation with recoveries from 72.5 to 76.9% at levels of 0.1, 5.0 and 20.0 μg/ml. Chromatographic separation was performed on a C_{18} column using a mixture of methanol, water and formic acid (80:20:1, v/v/v) as mobile phase delivered at a flow rate of 0.5 ml/min. Positive selected ion monitoring (SIM) mode was used for the quantification of roxithromycin at *m/z* 837.7 and clarithromycin (internal standard) at *m/z* 748.7. The linearity was obtained over the concentration range of 0.05–20.0 μg/ml and the lower limit of quantification was 0.05 μg/ml. For each QC level of roxithromycin, the intra- and inter-day precisions relative standard deviation (R.S.D.) were less than 4.1 and 7.5%, respectively, and accuracy (RE) was ±10.0%. The proposed LC–MS method has been successfully used for the determination of roxithromycin in rat lung tissue after oral administration of roxithromycin formulations to 44 SD rats. The present study demonstrates that the concentration of roxithromycin in rat lung tissues can be significantly increased by ambroxol when they are formulated in combination.

Keywords: Roxithromycin; Liquid chromatography–mass spectrometry; Rat lung tissue

1. Introduction

Roxithromycin (Fig. 1), a semi-synthetic 14-membered-ring macrolide antibiotic derived from erythromycin, is more stable than erythromycin under acidic conditions and exhibits improved clinical effects on respiratory infections [1]. It was found that ambroxol (Fig. 1), an expectorant, could increase the concentrations of such antibiotics as ampicillin, erythromycin and amoxycillin in rat lung tissues [2]. But no references are available concerning the effect of ambroxol on the concentration of roxithromycin in the lungs of rats when they are used in combination. Based on the synergic effect between erythromycin and ambroxol, it was supposed that ambroxol might have similar effect on roxithromycin. On the basis of the above supposition, a combination formulation of roxithromycin and ambroxol was developed in our laboratory.

In the preliminary phase of the development of this formulation, a biopharmaceutical test in rats was necessary in order to demonstrate if the concentration of roxithromycin in rat lung tissues can be increased by ambroxol when they are formulated in combination, which can provide the basis for the development of this combination formulation. To achieve this purpose, an analytical method of simplicity and sensitivity was required to determine the concentrations of roxithromycin in rat lung tissues. Among the analytical methods for biopharmaceutical analysis, liquid chromatography with mass spectrometry detection (LC–MS) was preferred because of its high sensitivity and selectivity. As a result of survey, several LC–MS methods related with roxithromycin were available, some of which were just used for some qual-

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itative purpose or for the determination of other drugs while using roxithromycin as internal standard, which did not provide details in terms of accuracy, precision, linearity and stability. In addition, there were several methods used for the determination of roxithromycin in biological samples [3–10] and most of them adopted tandem MS or ion-trap MS detection [3,5–7,9,10]. Our study focused on LC–single quadrupole MS because of its wider availability in ordinary laboratories as well as its sufficient sensitivity and selectivity for the present work.

The present paper describes a rapid and sensitive LC–single quadrupole MS method for the determination of roxithromycin in rat lung tissue. The developed method was validated in terms of selectivity, linearity, limit of quantification, precision and accuracy and has been successfully applied for the determination of roxithromycin in rat lung tissue after oral administration of roxithromycin formulations to 44 rats. The present study demonstrates that the concentration of roxithromycin in rat lung tissues can be significantly increased by ambroxol when they are formulated in combination.

2. Experimental

2.1. Chemicals and reagents

Roxithromycin reference standard was from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). Clarithromycin was a gift from Shenyang Pharmaceutical University and used as internal standard (IS) in present work. A mixture suspension of ambroxol hydrochloride and roxithromycin (1:5, w/w) in PEG 400 and a suspension of roxithromycin in PEG 400 were used as test formulation and reference formulation, respectively. HPLC-grade methanol was from Tianjin Concord Reagent Company (Tianjin, China). Sodium carbonate, formic acid, *n*-hexane, dichloromethane and isopropyl alcohol were from Shenyang Chemical Reagent Company and of analytical grade.

2.2. Instrument and LC–MS conditions

HP 1100 series LC/MSD G1946D (Agilent, USA) was used.

Chromatographic separation was performed on a Diamonsil^{TM} C_{18} column (150 mm × 4.6 mm i.d., 5 μm, Dikma, China) at ambient temperature. The mobile phase consisting of a mixture of methanol, water and formic acid (80:20:1, v/v/v) was delivered at a flow rate of 0.5 ml/min. The injection volume was 20 μl.

The mass spectrometer was operated in the positive electrospray ionization (ESI) mode. The optimized ionization conditions were: nitrogen flow rate, 8.0 ml/min; gas temperature, 325 °C; nitrogen pressure, 30 psig; capillary current, 24 nA; fragmentation voltage, 150 V for roxithromycin and 170 V for clarithromycin (IS). Selected ion monitoring (SIM) mode was used for the quantification of quasi-molecular ion $[M + H]^+$ at *m/z* 837.7 for roxithromycin and *m/z* 748.7 for clarithromycin.

2.3. Animals

Sprague–Dawley rats weighing 200 g on an average were from Laboratory Animal Center of Shengyang Pharmaceutical University (certificate no. 042).

2.4. Preparation of calibration standards and quality control samples

Stock solutions (1 mg/ml) of roxithromycin and clarithromycin were separately prepared in methanol. The stock solutions were further individually diluted with methanol to give diluted standard solutions (100 μg/ml). Calibration standards of roxithromycin (0.05, 0.1, 0.2, 1.0, 5.0, 10.0 and 20.0 μg/ml) were prepared by spiking appropriate amount of the standard solutions of roxithromycin in blank rat lung homogenates. Quality control (QC) samples were prepared in blank rat lung homogenates at concentrations of 0.1, 5.0 and 20.0 μg/ml for roxithromycin. The Calibration standards and QC samples were then treated following the sample preparation procedure, as indicated in Section 2.5.

2.5. Sample preparation

Two hundred microliter aliquot of rat lung homogenate, 100 μ1 of the IS solution in methanol (13 μg/ml) and 100 μ1of mobile phase were mixed well. And then 200 μ1 of sodium carbonate aqueous solution (0.1 mol/l) was added into the mixture and shaken well. Two milliliters of a mixture of *n*-hexane–dichloromethane–isopropyl alcohol (20:10:1, v/v/v) were added and the contents were vortexed for 1 min and centrifuged for 5 min to separate the phases. The supernatant was separated and dried under a stream of nitrogen at room temperature. The residue was reconstituted with 200 μl of mobile phase and 20 μl was injected onto the LC column.

2.6. Method validation

Validation runs were conducted on three separate days. Each validation run consisted of a set of the spiked calibration standards at seven concentrations over the concentration range (each in triplicate) and QC samples at three concentrations ($n = 6$ at each concentration). The linearity of each calibration curve was determined by plotting the peak-area ratio (y) of roxithromycin to internal standard versus the nominal concentration (x) of the analyte. The calibration curves were obtained by weighted linear regression analysis ($1/x^2$ weighing factor). Calibration standards and QC samples were