Journal of Chromatography B, 988 (2015) 75–80

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Application of a liquid chromatographic/tandem mass spectrometric method to a urinary excretion study of rabeprazole and two of its metabolites in healthy human urine

Chengtao Lu[a], Yanyan Jia[a,c,1], Ying Song[a], Xueqing Li[a], Yuan Sun[a], Jinyi Zhao[a], Shan Wang[a], Lei Shi[c], Aidong Wen[a,*], Li Ding[b,**]

[a] Department of Pharmacy, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, China
[b] Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing 210009, China
[c] Department of Pharmacy, General Hospital of Guangzhou Military Command of PLA, China

ARTICLE INFO

Article history:
Received 5 June 2014
Accepted 18 January 2015
Available online 23 January 2015

Keywords:
LC–MS/MS
Rabeprazole
Rabeprazole thioether
Desmethyl rabeprazole thioether
Pharmacokinetics

ABSTRACT

To study urinary excretion properties of rabeprazole and two of its metabolites, i.e. rabeprazole thioether and desmethyl rabeprazole thioether in human urine, a sensitive, selective, accurate and precise method for the quantification of rabeprazole and two of its metabolites using a liquid chromatographic/tandem mass spectrometric method has been developed and validated. Starting with a 200 μL urine aliquot, a general sample preparation was performed using protein precipitation with methanol. Analytes were separated on a Dikma Inspire™ C_{18} column (150 mm × 2.1 mm, 5 μm) using a mixture of methanol and aqueous 10 mM ammonium acetate buffer containing 0.05% formic acid (55:45, v/v) as mobile phase. Linearity was obtained over the concentration range of 0.1446–96.38 ng/mL, 0.3198–319.8 ng/mL and 0.05160–82.53 ng/mL for rabeprazole, rabeprazole thioether, desmethyl rabeprazole thioether in human urine, respectively. The fully validated method was applied to a urine excretion study of rabeprazole sodium administered as a 30 min intravenous infusion for the first time. The calculated cumulative urinary recovery just reached 0.04745‰, 1.272‰ and 0.1631‰ of dose within 24 h post-dose for rabeprazole, rabeprazole thioether, and desmethyl rabeprazole thioether, respectively, after intravenous infusion administration, indicating that rabeprazole and its two main metabolites undergo substantial non-renal elimination in healthy Chinese volunteers.

1. Introduction

Rabeprazole is a proton pump inhibitor (PPI) that inhibits gastric acid secretion via interaction with the (H^+/K^+)-ATPase in gastric parietal cells [1,2]. Rabeprazole is used for the treatment of acid-peptic disease, such as duodenal, gastric and oesophageal ulceration. Like most compounds of this class, these compounds (particularly rabeprazole) are labile under acid condition [3,4]. PPIs (e.g. omeprazole, lansoprazole and rabeprazole) are pro-drugs that are activated by conversion to sulphenamides in the acidic environment. The metabolism of rabeprazole, like omeprazole, is regulated by an enzyme of cytochrome P450 system in liver, CYP2C19. Rabeprazole is mainly metabolized via nonenzymatic reduction to its thioether metabolites, which is pharmacologically active, and it inhibits the motility of *H. Pylori*, which colonizes the gastric mucosa and is closely associated with gastritis and peptic ulcers [5].

There have been several papers focusing on the determination of rabeprazole and its metabolites in human plasma using HPLC with UV detection. Nakai et al. [6] established a method using HPLC with UV detection for the simultaneous determination of rabeprazole and its four metabolites in 1 mL human plasma, The LOQ was 5 ng/mL for rabeprazole and 20 ng/mL for each of its four metabolites, but the sample preparation was complex involving a double extraction, and the stability after sample preparation and during the analytical process was not reported. Uno et al. [7] established a column-switching HPLC method with UV detection for the simultaneous determination of rabeprazole and rabeprazole thioether in human plasma, the LOQ was 1 ng/mL for rabeprazole and 3 ng/mL for rabeprazole thioether. Miura et al. [8] described a HPLC method

* Corresponding author. Tel.: +86 29 84773636.
** Corresponding author. Tel.: +86 25 83271505.
E-mail addresses: ad_wen@163.com (A. Wen), dinglidl@sina.com (L. Ding).
[1] Co first author.

http://dx.doi.org/10.1016/j.jchromb.2015.01.023
1570-0232/

for the simultaneous determination of rabeprazole enantiomers and their metabolites, rabeprazole thioether and rabeprazole sulfone in human plasma obtained from renal transplant recipients. The method provided 5 ng/mL for rabeprazole and rabeprazole sulfone, 10 ng/mL for rabeprazole thioether.

Several methods have been employed for quantification of rabeprazole alone in plasma. Takakuwa et al. [9] and Mano et al. [10] described a gradient HPLC method for determination of rabeprazole in plasma which required a long chromatographic run time (>25 min) and the LLOQ (30 ng/mL) was high, and the stability of rabeprazole was not described. Zhang et al. [11] used methanol as a mobile phase, but the sample was extracted with a mixture of *n*-hexane/dichloromethane/isopropanol 20/10/1, v/v/v). The method had an LOQ of 2.0 ng/mL and used 0.5 mL of plasma. Ramakrishna et al. [12] developed a HPLC method with UV detection for quantification of rabeprazole in human plasma following solid-phase extraction with a LOQ of 20 ng/mL. Huang et al. [13] observed an LOQ of 0.2 ng/mL using methanol/water (50/50, v/v) containing 0.1% formic acid as a mobile phase and methanol for precipitation of the plasma proteins. Shao et al. [14] reported an LC/MS method for the quantification of rabeprazole in 0.1 mL of dog plasma, which had an LOQ of 1 ng/mL. However, their selected ion monitoring (SIM) method lacked selectivity and their gradient elution mode had unstable ionization. In addition, El-Gindy et al. [15] used spectrophotometric and chromatographic methods to investigate the stability of rabeprazole in solution under acidic, oxidative and photodegradation conditions. They found that rabeprazole was rapidly degraded in acidic media and was more stable in alkaline solutions. However, the above reported biological samples were obtained after oral administration of rabeprazole tablets to healthy volunteers, respectively.

So far as we know, no analytical method has been developed for the determination of rabeprazole and its two main metabolites in human urine. The aim of the present study was to develop a reliable and sensitive LC–MS/MS assay for the simultaneous determination of rabeprazole, rabeprazole thioether and desmethyl rabeprazole thioether in healthy human urine, which was applied to a urinary excretion study of urine rabeprazole and its two main metabolites concentrations, after a single dose of intravenous administration of 20 mg rabeprazole sodium for injection. The structures of analytes are presented in Fig. 1.

2. Experiment

2.1. Chemicals and reagents

Rabeprazole sodium sterile powder for injection (RSPI, 20 mg) was supplied by JinCheng Health Pharmaceutical Co., Ltd. (Shannxi, China). The reference substance of rabeprazole sodium was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The reference substances of rabeprazole thioether and desmethyl rabeprazole thioether were provided by epichem (Australia). HPLC-grade methanol was purchased from Tedia Company Inc. (Fairfield, USA). Purified water obtained through Milli-Q system (Millipore, USA) was used throughout the experiment.

2.2. Instrumentation

The LC–MS/MS system consisted of an Agilent 1100 series HPLC and an Agilent 6410 triple quadrupole mass spectrometer equipped with an electrospray ionization source (Agilent Technologies, USA). The chromatographic separation was performed on a Dikma inspire^{TM} C_{18} column (150 mm × 2.1 mm, 5 μm) column. All data were acquired employing Agilent 6410 quantitative analysis version analyst data processing software.

2.3. Calibration standards and quality control (QC) samples

The stock solutions of rabeprazole, rabeprazole thioether and desmethyl rabeprazole thioether were separately prepared in methanol containing 0.5‰ diethylamine (DEA). The concentration of the three analytes stock solutions was 1 mg/mL. Working solutions were prepared by diluting appropriate concentrations of rabeprazole, rabeprazole thioether and desmethyl rabeprazole thioether in methanol containing 0.5‰ DEA. The stock and working solutions of internal standard donepezil were separately prepared in methanol. All the working standard solutions were stored at −5 °C. Calibration standards and QC samples in urine were prepared by diluting 10 μL corresponding working solutions with 200 μL drug-free human urine, respectively. Resulting calibration concentrations ranged from 0.1446 to 96.38, 0.3189–426.4 and 0.05160–103.2 ng/mL for rabeprazole, rabeprazole thioether and desmethyl rabeprazole thioether, respectively.

The concentration of QC samples of the three analytes was as follows: 0.3855, 5.783, and 77.11 ng/mL for rabeprazole; 0.8428, 15.99, and 319.8 ng/mL for rabeprazole thioether; 0.1032, 3.095, and 82.53 ng/mL for desmethyl rabeprazole thioether.

2.4. Sample preparation

Internal standard solution 20 μL of 926.0 ng/mL and 20 μL of 10% ammonia solution were added to 200 μL of urine. The tubes were vortex-mixed for 10 s. Protein precipitation was performed by adding 600 μL methanol. Samples were then centrifuged at 25 °C and 16,000 rpm for 10 min, and then a 5 μL aliquot of the resulting supernatants was injected onto the column.

2.5. Chromatographic condition

The mobile phase was a mixture of methanol and aqueous ammonium acetate buffer (10 mM) containing 0.05% (v/v) formic acid (55:45, v/v), which was pumped at a flow rate of 0.35 mL/min and the total run time was 8.0 min. The chromatographic separation was performed at 35 °C with a small injection of 5.0 μL of urine sample.

2.6. MS spectrometric conditions

In order to optimize the MS parameters, a standard solution of analytes and IS was infused into the mass spectrometer using a syringe pump. The optimized parameters were: nebulizer pressure, 50 psi; drying gas temperature, 350 °C; dry gas flow, 11 L/min. Analytes were quantificated by multiple monitoring (MRM) employing the following precursor to product ion transitions and parameters: transitions of m/z 360.2 → 242.2 for rabeprazole with fragmentor voltage (FV) 100 V and collision energy (CE) 7 eV; transitions of m/z 344.2 → 226.2 for rabeprazole thioether with FV 130 V and CE 18 eV; transitions of m/z 330.2 → 212.1 for desmethyl rabeprazole thioether with FV 125 V and CE 18 eV; transitions of m/z 380.4 → 243.2 for donepezil with FV 120 V and CE 26 eV. The product ion mass spectra of analytes and the IS are shown in Fig. 2.

2.7. Method validation

2.7.1. Specificity

Specificity of the method was investigated by analyzing the chromatograms of six different individuals of blank human urine, as well as the corresponding spiked samples. Each blank sample was tested following the sample preparation procedures and under the