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Simple quantification of lansoprazole and rabeprazole concentrations in human serum by liquid chromatography/tandem mass spectrometry

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ABSTRACT

A rapid, simple and highly sensitive method was developed for the quantitative determination of lansoprazole and rabeprazole concentrations in 20 μL of human serum using high-performance liquid chromatography/tandem mass spectrometry (LC/MS/MS). Analytes, along with an internal standard (lansoprazole deuterium derivatives), were separated using a mobile phase of acetonitrile/1 mM ammonium formate (140/60, v/v) on a C18 analytical column and analyzed in the selected reaction-monitoring (SRM) mode. The lower limit of quantification was 0.25 ng/mL. A good linear response was observed for each analyte (from 0.25 ng to 2.5 μg/mL). This method was useful for therapeutic drug monitoring and pharmacokinetic studies.

1. Introduction

Lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoro-ethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimid-azole and rabeprazole, 2-[[[4-(3-methoxypropoxy)-3-methyl-2-pyridil]-methyl]sulfinyl]-1H-benzimidazole are proton pump inhibitors (PPIs) that inhibit gastric acid secretion via interaction with the (H^+/K^+)-ATPase in gastric parietal cells [1,2]. Like most compounds of this class, these compounds (particularly rabeprazole) are acid labile and are reversibly transformed to sulfenamides in acidic media [3,4].

Lansoprazole concentrations have previously been determined in solutions and serum by spectroscopy and high-performance liquid chromatography (HPLC) [5,6]. Many studies using HPLC with UV detection have had higher ranges for their limits of quantification (LOQ) (between 5.0 and 20 ng/mL) [7–11] and longer retention times (RT) 11 min [7,8]. Landes et al. [12] observed an LOQ of 2.0 ng/mL using an HPLC method with a loop column, but their RT was approximately 11 min and their method required double extraction and two evaporation steps with nitrogen. Oliveira et al. [13] had an LOQ of 2.5 ng/mL using HPLC coupled to tandem mass spectrometry (LC/MS/MS) and a double extraction method, but this study did not use isotopic labeling derivatives as an internal standard.

Several methods have also been employed for quantification of rabeprazole in plasma [14–20]. Nakai et al. [14] 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 double extraction complicated the sample preparation. Nakai et al. [14] and Mano et al. [16] described gradient HPLC methods for determining rabeprazole in plasma, which required a long run time (>25 min) and had a higher LOQ (30 ng/mL). Moreover, the stability of rabeprazole was not determined. Recently, Zhong et al. [17] and Huang et al. [18] reported an LC/MS/MS method for quantification of rabeprazole in human plasma. Zhang et al. [17] 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). This process had an LOQ of 2.0 ng/mL and used 0.5 mL of plasma. Huang et al. [18] 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. Although this method was simple and sensitive, its background was not as clear as the LLE method, which may have affected its sensitivity and might cause column damage with long-term usage. El-Gindy et al. [19] used spectrophotometric and chromatographic methods to investigate

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Table 1
SRM conditions for the analytes analyzed in positive ion mode

Compound	SRM transition (*m*/*z*)	Retention time (min)	DP	EP	CE	CXP
Lansoprazole-d_4	374 > 252	2.92	81	10	19	24
Lansoprazole	370 > 252	2.95	116	10	19	8
Rabeprazole	360 > 242	2.53	16	10	19	22

DP: declustering potential, EP: entrance potential, CE: collision energy, CXP: collision cell exit potential

Table 2
Tandem mass spectrometer main working parameters

Parameters	Value
Collision gas (CAD)	4.5
Curtain gas (CUR)	40
Ion source gas 1 (GS1)	40
Ion source gas 2 (GS2)	60
Ionspray voltage (IS) (V)	5500
Probe temperature (TEM) (°C)	550
Interface heater (ihe)	on
Dwell time per transition (ms)	330

the stability of rabeprazole in solution under acidic and oxidative conditions and during photodegradation. They found that rabeprazole was rapidly degraded in acidic media, but was more stable in alkaline solutions. Thus, the mobile phases used by Huang et al. [18] may have adversely affected the stability of rabeprazole. Feng et al. [20] 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.

Recently, the use of LC/MS/MS in the quantification of drug concentrations in blood from patients has become widespread in the clinical field. Moreover, the practice of personalized medicine is now regarded as important in the prescription of medication. Even if the PPI in particular has the same effect, the structure and formulation of the compound is characteristic. Therefore, it is necessary to understand the individual differences of the curative effect of these drugs in order to promote individualized medical care. Through development of highly sensitive methods of quantification using a very small amount of sample, it is possible to avoid high-volume operations and reduce foreign elements. Furthermore, simple and easy operations in quantification of drug concentrations will be beneficial for pharmacists in clinical practice in the future.

In this work we describe a simple, rapid, sensitive and selective method for the quantitation of lansoprazole and rabeprazole using LC/MS/MS. The current method offers a number of advantages over existing methods, including shorter analysis time, smaller required sample volumes (20 μL serum), a less extensive sample clean up procedure, and the inclusion of lansoprazole-d_4 as an internal standard. This method was applied to a pharmacokinetic study of serum lansoprazole and rabeprazole concentrations, after administration of 30 mg of lansoprazole and 10 mg of rabeprazole to 14 and 8 healthy volunteers, respectively.

2. Experimental methods

2.1. Chemicals and reagents

Lansoprazole and lansoprazole deuterium derivatives were provided by Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). Rabeprazole was obtained from Eisai Co., Ltd. (Tokyo, Japan). Acetonitrile (LC/MS grade) was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Ultra-pure water was purified using a Purelab-Ultra-Analytic (Organo Corp., Tokyo, Japan). Analytical grade ammonium formate was obtained from Wako Pure Chemical Industries (Osaka, Japan). Blank serum was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Stock solution of lansoprazole, rabeprazole and internal standard (each 1 mg/mL) were prepared in acetonitrile and kept in glass tubes at −20 °C. Stock solutions were used for the preparation of calibration standards and quality control samples.

2.2. Sample preparation

Aliquots of frozen human serum samples (20 μL) were thawed at room temperature prior to use, then 40 μL of internal standard solution (25 pg/μL lansoprazole-d_4 in acetonitrile) was added to each sample. The tubes were briefly vortexed, sonicated for 60 s and centrifuged at 10,000 rpm for 5 min at 4 °C to precipitate solids. The supernatant then was filtrated and transferred to an autosampler vial, where 15 μL was injected for LC/MS/MS.

2.3. Chromatographic conditions

A Nanospace SI-2 HPLC system (Shiseido, Tokyo, Japan) was used. Chromatography was performed on a SunfireTM C18 3.5 μm (2.1 mm i.d. × 150 mm) analytical column (Waters, Milford, MA, USA) and a C18 Capcell Pak MGII 5 μm (2.0 mm i.d. × 10 mm) guard column (Shiseido, Tokyo, Japan) operated at 40 °C. The mobile phase was an isocratic elution with acetonitrile/1 mM ammonium formate (70/30, v/v) at a flow-rate of 200 μL/min. Under these conditions, RTs were typically 2.92 min for lansoprazole-d_4, 2.95 min for lansoprazole and 2.53 min for rabeprazole. Column effluent was introduced into the mass spectrometer using a fused silica capil-

Table 3
Accuracy and precision of the determination of lansoprazole and rabeprazole in human serum

Compounds	Added	Intra-day (*n* = 3)			Inter-day (*n* = 4)		
		Found mean ± S.D.	C.V. (%)	Accuracy (%)	Found mean ± S.D.	C.V. (%)	Accuracy (%)
	0.5 pg	0.52 ± 0.041	7.8	105	0.50 ± 0.028	5.6	101
	5 pg	5.5 ± 0.13	2.5	109	5.5 ± 0.10	1.8	111
	50 pg	51 ± 0.41	0.81	101	51 ± 1.3	2.6	102
Lansoprazole	0.5 ng	0.51 ± 0.0057	1.1	102	0.51 ± 0.0076	1.5	102
	5 ng	5.1 ± 0.022	0.43	101	5.1 ± 0.040	0.78	101
	25 ng	26 ± 0.14	0.55	102	26 ± 0.15	0.58	103
	50 ng	51 ± 0.19	0.37	101	51 ± 0.49	1.0	102
	0.5 pg	0.49 ± 0.010	2.0	98.7	0.51 ± 0.018	3.5	102
	5 pg	5.2 ± 026	5.0	105	5.4 ± 0.13	2.4	107
	50 pg	51 ± 0.99	1.9	103	51 ± 1.3	2.6	102
Rabeprazole	0.5 ng	0.50 ± 0.0085	1.7	101	0.50 ± 0.011	2.2	99.5
	5 ng	5.1 ± 0.0094	0.19	101	5.0 ± 0.052	1.0	99.7
	25 ng	25 ± 0.14	0.57	100	25 ± 0.33	1.3	101
	50 ng	49 ± 0.66	1.3	97.9	50 ± 0.69	1.4	100