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Short communication

Determination of lansoprazole and five metabolites in plasma by high-performance liquid chromatography

M.D. Karol*, G.R. Granneman, K. Alexander

Drug Metabolism Department, Abbott Laboratories, D4PK, AP13A, 100 Abbott Park Road, Abbott Park, IL 60064-3500, USA

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Abstract

A high-performance liquid chromatographic method for the determination of lansoprazole, a new proton-pump inhibitor, and five of its metabolites in human plasma is described. Lansoprazole, its metabolites, and internal standard (omeprazole) were extracted into diethyl ether–methylene chloride and separation was obtained using a reversed-phase column under isocratic conditions. The method features monochromatic ultraviolet detection at 285 nm, and single extraction, single evaporation sample handling. The lower limit of quantitation, based on standards with acceptable coefficients of variation, was 10 ng/ml for all compounds. No endogenous compounds were found to interfere. This method has been demonstrated to be suitable for pharmacokinetic studies in humans.

1. Introduction

Lansoprazole is a new (H^+, K^+)-ATPase ("proton pump") inhibitor which has been demonstrated to be effective in the treatment of duodenal and gastric ulcers, reflux esophagitis, and Zollinger–Ellison syndrome [1]. This paper describes a simple and selective high-performance liquid chromatographic (HPLC) method for lansoprazole (ABT-006, AG-1749) and five of its metabolites [M-I (AG-1777), M-IV (AG- 1907), M-VI (AG-1908), M-VII (AG-1813), and M-IX (AG-1909)] in human plasma. This method has been used in several pharmacokinetic studies (data on file, Abbott Laboratories, Abbott Park, IL, USA).

Two HPLC methods for the determination of lansoprazole and lansoprazole metabolite concentrations have been previously published [2,3]. In contrast to those methods, the method reported here uses an internal standard much more structurally similar to the analytes. Also, the sample preparation is simpler in the method we present. The previous methods require either dual extraction [2] or two evaporation steps [3]. In contrast, this method uses a single extraction and a single evaporation step. Furthermore, the previous methods utilize dual wavelength detectors. The method reported here is monochromatic permitting analysis using less expensive and more readily available instruments.

* Corresponding author.

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2. Experimental

2.1. Equipment

The HPLC system consisted of a Model 8100 refrigerated chromatograph/autosampler system (Spectra-Physics, San Jose, CA, USA), Model 4272 integrator (Spectra-Physics), and Kratos Spectroflow 783 variable wavelength ultraviolet detector (Applied Biosystems, Ramsey, NJ, USA).

2.2. Reagents

Lansoprazole, internal standard (omeprazole), and lansoprazole metabolites (M-I, M-IV, M-VI, M-VII, and M-IX) (Fig. 1) were provided by Takeda Chemical Industries (Osaka, Japan). Acetonitrile, diethyl ether and methylene chloride were HPLC grade (Fisher Scientific, Fairlawn, NJ, USA). Phosphoric acid (85%) was also purchased from Fisher Scientific. N-octylamine, N-acetohydroxamic acid (NAHA), and N,N-dimethylformamide (DMF) were purchased from Aldrich Chemical (Milwaukee, WI, USA).

2.3. Chromatographic conditions

The mobile phase consisted of 35% aqueous acetonitrile to which 1 ml/l *n*-octylamine and NAHA (0.005 *M*) were added. The pH of the mobile phase was adjusted to 7.0 with 85% phosphoric acid. The mobile phase was delivered at flow-rates of 1.0 ml/min (initial 15 min of each run) and 2.5 ml/min (remainder of run, total run duration = 20 or 30 min). Separation was accomplished at 40–43°C on an octyldecylsilane stainless-steel column [5 μm, 150 or 250 mm × 4.6 mm I.D., Regis Hi-Chrom Reversible (Regis Chemical, Morton Grove, IL, USA) or Ultrasphere, Beckman (Allendale, NJ, USA)]. The ultraviolet detector was operated at 285 nm.

2.4. Extraction procedure

The internal standard (150 μl of a 4 μg/ml aqueous stock solution) was added to 0.5 ml plasma. After addition of 5 ml of diethyl ether–methylene chloride (7:3, v/v), contents were vortexed for 5 min and centrifuged at 2000 rpm (ca. 300–850 *g*) at 6°C for 10 min. Aliquots of 4 ml of the organic extracts were then removed and evaporated to dryness under vacuum while being centrifuged (Speedvac, Savant) at ambient temperature. Residues were then reconstituted in 500 μl of mobile phase (adjusted to pH 7.5 with 85% phosphoric acid) and refrigerated until injection of 100 μl onto the column.

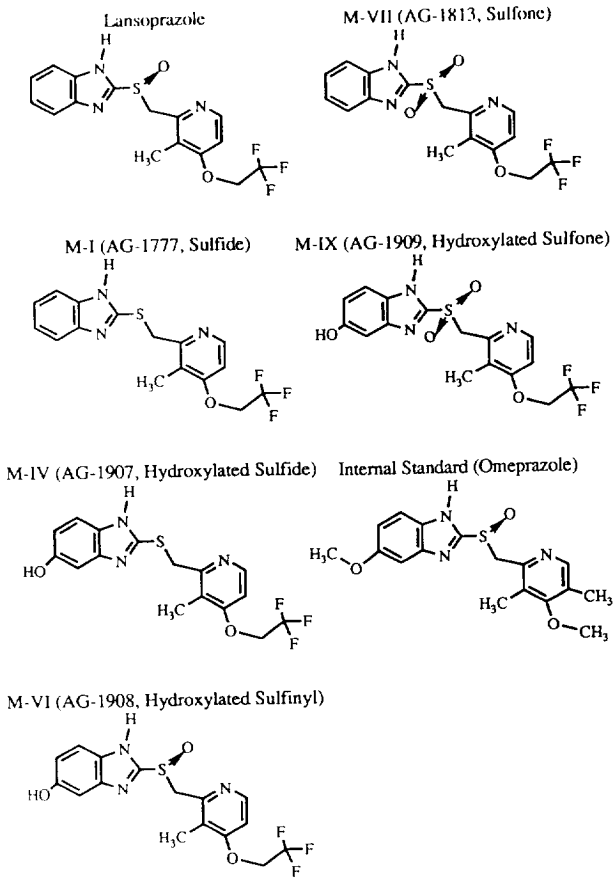

Fig. 1. Structures of lansoprazole, lansoprazole metabolites M-I, M-IV, M-VI, M-VII, and M-IX, and internal standard (omeprazole).

2.5. Drug standards

Working stock solutions for each compound were prepared in DMF at a concentration of 4

mg/ml. From this stock solution, plasma stock solutions of 100 and 4 μg/ml were prepared using drug-free human plasma. Standards for calibration curves in plasma (10, 20, 50, 100, 200, 500, 1000, 2000, and 4000 ng/ml) were prepared by serial dilution of the 4 μg/ml plasma stock solution. Analyte/internal standard ratios of observed peak heights from the calibration standards were subjected to linear regression to derive daily calibration curves.

2.6. Analytical variables

Absolute extraction recoveries of lansoprazole and its metabolites from human plasma were estimated using standard samples at concentrations ranging from 0.01 to 4.2 μg/ml by comparing the peak heights from processed plasma standard samples to those from a calibration curve prepared from analytes in the mobile phase. Plasma standard samples (10, 20, 50, 100, 200, 500, 1000, 2000, and 4000 ng/ml concentrations) were analyzed in quadruplicate on three separate days. Intra-assay precision was determined from these data. Inter-assay precision and accuracy were assessed using quality control samples (80 and 800 ng/ml) assayed on 18 separate occasions. Using quality control samples (80 and 800 ng/ml), the stability of lansoprazole in the frozen state over a period of 62 days was assessed. The impact of pH on the stability of lansoprazole was examined by preparing a 400 ng/ml solution in mobile phase at a variety of pH values (5.2, 6.0, 6.3, 7.1, 7.5, and 7.9) and storing these solutions under refrigeration for 24 h and at room temperature for up to 24 h. Each solution was assayed in triplicate.

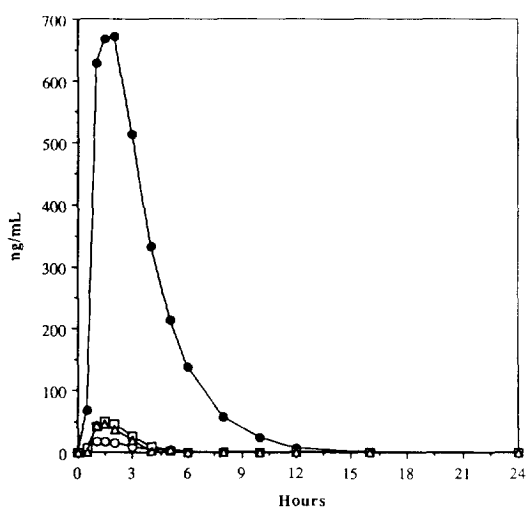

Fig. 2. Mean plasma lansoprazole and metabolite concentration versus time profiles in eight healthy volunteers after administration of a single 30-mg oral dose. ● = Lansoprazole; ○ = M-IV; □ = M-VI; △ = M-VII. Mean M-I and M-IX concentrations were below the limit of quantitation.

2.7. Application

The assay has been applied in several pharmacokinetic studies. Concentrations of lansoprazole and its metabolites measured in plasma samples obtained from eight healthy subjects given a single oral 30 mg dose of lansoprazole are shown in Fig. 2. Lansoprazole concentrations return to baseline by monoexponential pharmacokinetics with an arithmetic mean half-life of 1.5 h. Peak concentrations are seen at about 1.7 h after dosing. Blood samples were obtained over a 24-h period following dose administration.

3. Results and discussion

Fig. 3 illustrates a representative chromatogram of blank plasma and calibration plasma containing 0.5 μg/ml each of lansoprazole and its metabolites, and a subject's sample. Drug-free pooled human plasma yielded relatively clean chromatograms with no significant interfering peaks. Retention times of M-VI, omeprazole, M-IX, M-IV, lansoprazole, M-VII, and M-I were 6.5, 7.6, 8.7, 12.0, 13.2, 16.4, and 22.4 min, respectively (total run time = 30 min). When only lansoprazole quantitation is desired, the run time may be abbreviated to 20 min without interfering with the lansoprazole or internal standard peaks.

The calibration curves for lansoprazole and its metabolites were linear over a concentration range of 10 to 4000 ng/ml. Mean ± standard deviation for the coefficients of determination

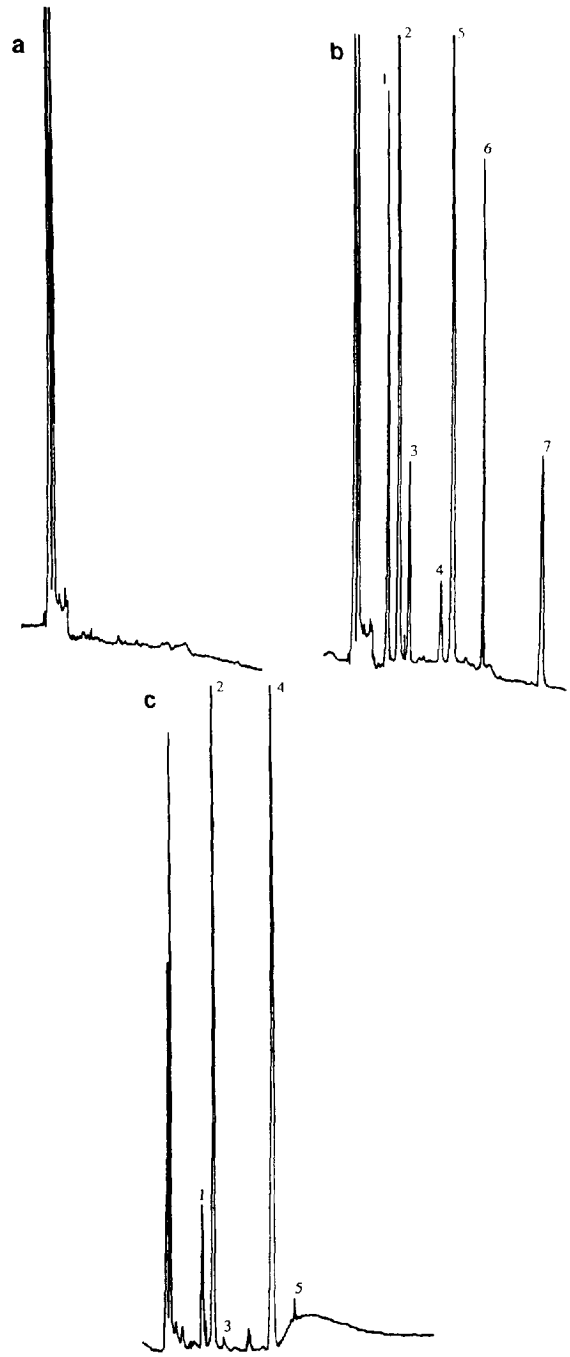

Fig. 3. (a) Chromatogram of blank pooled human plasma. (b) Chromatogram of pooled human plasma spiked with lansoprazole and metabolite concentrations of 500 ng/ml (peaks: 1 = M-VI; 2 = omeprazole internal standard; 3 = M-IX; 4 = M-IV; 5 = lansoprazole; 6 = M-VII; 7 = M-I). (c) Chromatogram of subject's plasma sample (peaks: 1 = M-VI; 2 = omeprazole internal standard; 3 = M-IX; 4 = lansoprazole; 5 = M-VII).

Table 1
Calibration curve coefficients of determination

Compound	Coefficient of determination (mean ± S.D., $n = 12$)
Lansoprazole	0.9989 ± 0.0007
M-I	0.9989 ± 0.0014
M-IV	0.9970 ± 0.0028
M-VI	0.9984 ± 0.0018
M-VII	0.9974 ± 0.0019
M-IX	0.9991 ± 0.0011

are shown in Table 1. The lower limit of quantitation for each compound was 10 ng/ml, the concentration of the lowest calibrator. The mean percentage absolute recoveries of lansoprazole, M-I, M-IV, M-VI, M-VII, and M-IX over a concentration range of 0.01 to 4.2 μg/ml were 99.67%, 78.36%, 105.55%, 99.18%, 90.62%, and 94.95%, respectively. Means were based on 7 to 9 replicates.

The intra-assay precision is illustrated in Table 2. For lansoprazole, the intra-assay coefficients of variation (C.V.) were relatively low, being less than 5.9% over the 10 to 4000 ng/ml concentration range. For the metabolites, the intra-assay C.V. values were generally comparable to or only slightly higher than those of lansoprazole until near the limit of quantitation, wherein the values rose to the 9 to 16% range. The inter-assay C.V. values for lansoprazole, M-VI, and M-VII at plasma concentrations of 80 and 800 ng/ml were low as well, ranging from 4.5 to 11.0%. For lansoprazole, M-VI, and M-II, they were 4.9 and 4.5, 11.0 and 7.3, 8.7 and 7.3%, respectively. The percentage differences between the mean assayed concentrations and the target concentrations (accuracy) for lansoprazole, M-VI, and M-VII at plasma concentrations of 80 and 800 ng/ml ranged from 0.31 to 2.38%. The specific values were 2.03 and 1.90, 2.38 and 1.85, and 0.31 and 0.44%, respectively.

Lansoprazole was stable in plasma in the frozen state for at least two months at concentrations of 80 and 800 ng/ml. Fig. 4 illustrates the peak height ratio for lansoprazole versus the internal standard as a function of pH at various temperatures. These results suggest instability of

Table 2
Estimation of intra-assay precision (%C.V.)

Conc. (ng/ml)	Lansoprazole		M-I		M-IV		M-VI		M-VII		M-IX	
	Overall precision	SEM	Overall precision	SEM	Overall precision	SEM	Overall precision	SEM	Overall precision	SEM	Overall precision	SEM
10	4.93	1.09			12.44	3.88	15.60	1.96	10.08	2.93	9.21	3.01
20	4.25	0.49	14.48	0.81	8.52	2.12	7.63	2.57	6.76	1.52	9.09	0.98
40					6.74	1.71						
50	2.19	0.67	4.27	0.89			5.13	1.46	3.70	1.01	4.53	0.98
100	2.74	0.47	4.27	1.34	2.63	0.64	3.52	1.20	4.01	0.09	2.02	0.66
200	4.90	1.78	4.92	2.14	3.07	0.70	2.26	0.44	5.72	2.06	2.35	0.23
400					5.24	1.15						
500	1.83	0.67	3.66	1.66			2.32	1.04	2.76	0.82	1.40	0.41
800					5.31	0.74						
1000	3.40	0.98	4.44	1.74			2.58	0.38	3.90	0.95	1.82	0.80
2000	4.97	0.62	9.29	1.02			3.21	0.23	6.71	1.24	0.77	0.25
4000	5.85	0.85	9.45	0.98			3.13	0.35	7.22	0.76	1.13	0.54

%C.V. based on analyte/internal standard peak height ratio. SEM = standard error of the mean computed as the standard error of the intra-assay percent coefficients of variability.

this compound in acidic media and sensitivity to temperature. To minimize degradation, especially during automated analyses, solution were maintained refrigerated at a pH value of 7.5. The slight pH adjustment to 7.5 provides added protection from degradation during sample handling at room temperature.

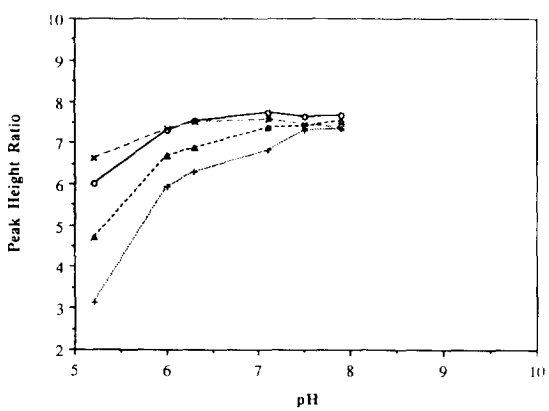

Fig. 4. pH and temperature dependence of lansoprazole stability in mobile phase at a concentration of 400 ng/ml. ○ = Room temperature, 0 h incubation; △ = room temperature, 7 h incubation; + = room temperature, 24 h incubation; × = refrigerated, 24 h incubation.

This analytical method has been applied to clinical samples in pharmacokinetic studies. Mean plasma lansoprazole and metabolite concentration versus time profiles from dosing eight healthy volunteers are illustrated in Fig. 2.

This paper describes a sensitive, specific, rapid, and robust reversed-phase HPLC method with ultraviolet detection for the measurement of lansoprazole and five of its metabolites in human plasma. This method has been demonstrated to be suitable for use in pharmacokinetic studies of lansoprazole.

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