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Determination of lansoprazole and its metabolites in plasma by high-performance liquid chromatography using a loop column

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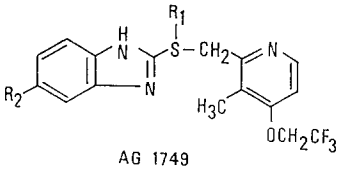
ABSTRACT

A high-performance liquid chromatographic method for the simultaneous determination of lansoprazole, a new proton pump inhibitor, and its metabolites in human plasma is described. Lansoprazole, its metabolites and an internal standard were extracted with *tert.*-butyl methyl ether. Samples were injected using an automatic injector via a loop column, and separation was obtained using a reversed-phase column under isocratic conditions. The absorbance was monitored at 285 and 303 nm. The quantification limit was 2 ng/ml for lansoprazole and 3 or 5 ng/ml for the metabolites. No endogenous compounds were found to interfere. The mean overall recovery was between 75 and 95% for lansoprazole and its metabolites. This method is suitable for pharmacokinetic studies.

INTRODUCTION

Lansoprazole (L) is a new substituted benzimidazole with a potent inhibitory effect on acid secretion. It exerts its effect on the gastric proton pump by antagonism of the enzyme H^+/K^+ ATPase, slowing the secretion of gastric acid from parietal cells [1–3]. L is acid-labile and is thus administered in the form of enteric-coated granules in capsules containing 30 mg of drug. L (AG 1749) is extensively biotransformed in humans [4,5]; urinary elimination, as hydroxylated metabolites and unchanged drug, represents *ca.* 15 and 1%, respectively, of the administered dose. L is found in the plasma, together with three metabolites, L sulphide (I), L sulphone (II) and a hydroxylated metabolite (III). The sulphone and sulphide metabolites can be oxidized to the corresponding 5-hydroxylated metabolites (IV and V, respectively) which are eliminated in the urine (Fig. 1).

This paper describes a simple and selective high-performance liquid chromatographic (HPLC) method that separates L and its metabolites. The use of a loop column permits good conservation of acid- and thermo-labile L and its metabolites until injection. This method has been used in several L pharmacokinetic studies [4,5].



AG 1749

	R1	R2
AG 1749 (L)	O	H
II	O2	H
III	O	OH
I	–	H
IV	O2	OH
V	–	OH

Fig. 1. Structures of lansoprazole (AG 1749) and its metabolites.

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EXPERIMENTAL

Equipment

The HPLC system consisted of a Shimadzu Model LC6A solvent-delivery system (Touzart et Matignon, Vitry, France), a Gilson 231 automatic injector (Villiers le bel, France) and a Waters 490 multiple-wavelength detector (Milford, MA, USA). The detector output was fed into a Shimadzu Model CR4A recorder-calculator. A Genie 2 vortex (Scientific Industries), a 401 dilutor (Gilson), a Jouan Model GR 4.11 refrigerated centrifuge (St. Nazaire, France) and a Sybron-Thermolyne dry bain-marie (Bioblock, Paris, France) were used to extract the analytes from plasma.

Reagents

L, L sulphide (I), L sulphone (II) and their hydroxylated products (III, IV and V, respectively) were kindly provided by Houdé and Takeda Labs. (Paris, France).

Triethylamine, acetonitrile, acetic acid and orthophosphoric acid were purchased from Prolabo (Paris, France), methanol from Carlo-Erba (Milan, Italy) and anhydrous potassium dihydrogenphosphate from Merck (Darmstadt, Germany). All solvents were HPLC-grade. *p*-Hydroxybenzoic acid N-butyl ester (IBPHA) was supplied by Sigma (St. Louis, MO, USA) and *tert.*-butyl methyl ether by Burdick and Jackson, USA (distributed by Fluka, Buchs, Switzerland). Deionized water was obtained using a Milli Q water system (18 MΩ, Millipore, Milford, MA, USA).

Extraction procedure

The internal standard (IBPHA, 20 μg/ml, 100 μl) (I.S.) and methanol (100 μl) were added to 1 ml of plasma in 16-ml glass-stoppered tubes. Vortex-mixing for a few seconds was followed by addition of 7 ml of *tert.*-butyl methyl ether. After vortex-mixing for 45 s and centrifugation at 4°C at 1500 *g* for 10 min, the organic phase was recovered, transferred to 8-ml tubes with screw-tops, and evaporated to dryness at 30°C under a gently stream of nitrogen. The walls of the tubes were rinsed with 1 ml of *tert.*-butyl methyl ether, and the solution was evaporated again at 30°C under a stream of nitrogen. The residue was reconstituted in 100 μl of methanol, vortex-mixed for 1 min, and transferred to 1.2-ml conical glass injection flasks.

Chromatographic conditions

The mobile phase consisted of 360 ml of acetonitrile, 105 ml of phosphoric acid, 40 ml of potassium dihydrogenphosphate (0.25 *M*), 1.5 ml of acetic acid, 1.5 ml of triethylamine and 492 ml of water. Before use, the solvent was degassed by vacuum filtration through a 0.45-μm Millipore membrane. The mobile phase was delivered at a flow-rate of 2.0 ml/min at room temperature through a Nucleosil C_{18} column (250 mm × 4.6 mm I.D., 5 μm particle size equipped with a 1-cm Nucleosil C_{18} precolumn (5 μm particle size (SFCC, Eragny, France). The absorbance was measured simultaneously at 285 and 303 nm, which provided two chromatograms per injection.

For the injection, a 1 cm Nucleosil CN 10 μm particle size column (SFCC) was mounted in place of the injection loop on the Rheodyne valve of the 231 automatic injector. The column was rinsed with 1 ml of water using the 231 injector, and 0.1 ml of methanolic extract was diluted tenfold with water immediately before being introduced into the loop column. The valve was washed with 1 ml of water, then turned to the inject position to allow the elution solvent to flow through.

Drug standards

Stock solutions of each compound were prepared in methanol and kept at 4°C for three months. Standards were prepared immediately prior to use by spiking 1 ml of drug-free pooled human plasma with 100 μl of the appropriate dilution of stock solutions in methanol to provide plasma concentrations ranging from 5 to 2000 ng/ml for L and from 2.5 to 400 ng/ml for the metabolites. These standards were extracted as described above. Peak heights were determined for each standard and sample at 285 nm for L, the unhydroxylated metabolites and the I.S., and

at 303 nm for the hydroxylated metabolites because the detector response (Σ) is higher. Least-squares regression analysis of peak-height ratios (product/I.S.) of the standards *versus* their concentrations was performed using a Wang 2200 computer. All compounds were quantified by comparison of the peak-height ratio of the compounds at 285 nm (L, I, II) or 303 nm (III, IV, V) with that of the I.S. at 285 nm, using a calibration curve.

Analytical variables

The extraction recoveries of L and its metabolites were calculated by comparing the signal obtained following the injection of the dry residues of plasma samples spiked with 200 ng/ml L and its metabolites and extracted as described above, with the signal obtained following the direct injection of the same amounts of L and its metabolites. The within-day precision was evaluated by determining coefficients of variation (C.V.) for L and its metabolites at plasma concentrations of 5, 50 and 100 ng/ml by replicate analyses of ten plasma samples on the same day. The between-day precision was evaluated by analysis of spiked control plasma samples stored at −20°C in 1.2-ml aliquots and tested each day. Quantification limits were defined as three times the S.D. of the mean intercept of the calibration curves.

Application

Concentrations of L and its metabolites were measured in plasma samples obtained from eighteen healthy subjects and twelve elderly patients treated with a single 30-mg oral dose in fasting. Blood samples were drawn during the 24 h following the administration.

RESULTS AND DISCUSSION

To determine the feasibility of solid-phase extraction of L and its metabolites, 1-ml samples were mixed with 0.1 ml of a 20 μg/ml IBPHA (I.S.) solution. The mixture was added to the top of a CN extraction column (Bond Elut 100 mg), which had been prewashed by successively passing 1 ml of methanol and two 1-ml volumes of water; the elution was then perfomed with 1 ml of methanol. The chromatogram of plasma blanks showed no interference. The recovery of L in aqueous solution was *ca.* 100%, but this fell to below 10% for plasma. This difference may be explained by the extensive protein binding of L (98%, unpublished data). This liquid extraction procedure was used because none of the organic or alkaline deproteinization methods and proteinase K treatment were able to release L from protein, and acid treatment was excluded.

The injection method with a loop column was chosen because L and its 5-hydroxy metabolite (III) are not stable in slightly acid medium at room temperature, whereas they are stable for three months in methanol and dimethylformamide. The lengthy analysis time does not permit the dry extract to be reconstituted with an acidic mobile phase, and it is thus necessary to redissolve it in methanol. Recovery was good with 100 μl of methanol, but the half-peak width increased with the injected volume of methanol [6]. The efficiency was maintained on the analytical column only when the injected volume was less than 20 μl. With such a small volume, it is not reasonable to expect a total and reproducible dissolution of the dry extract, and the injection of an aliquot involves a loss of sensitivity. In order to solve these problems, we used an injection technique via a CN loop column, using a procedure based on the solid-phase extraction described above.

The separation of L, five of its metabolites and the I.S. is shown in the chromatogram of an extracted standard (Fig. 2). The capacity factors (k') for the extracted plasmas are also shown, and it can be seem that these was no interference with L, its metabolites or the I.S. in the chromatograms of the blank plasmas. Furthermore, the absorbance ratio between 303 and 285 nm allows the identity of the products to be verified. No interfering peaks were observed with the other medication taken by the patients included in our studies (phenobarbital, sotalol, atenolol, pindolol, acebutolol, allopurinol, quinidine, prazosine, amiloride and ranitidine). Fig. 3 shows chromatograms of extracted plasma samples obtained 2 h after the administration of 30 mg of lansoprazole orally to an elderly patient.

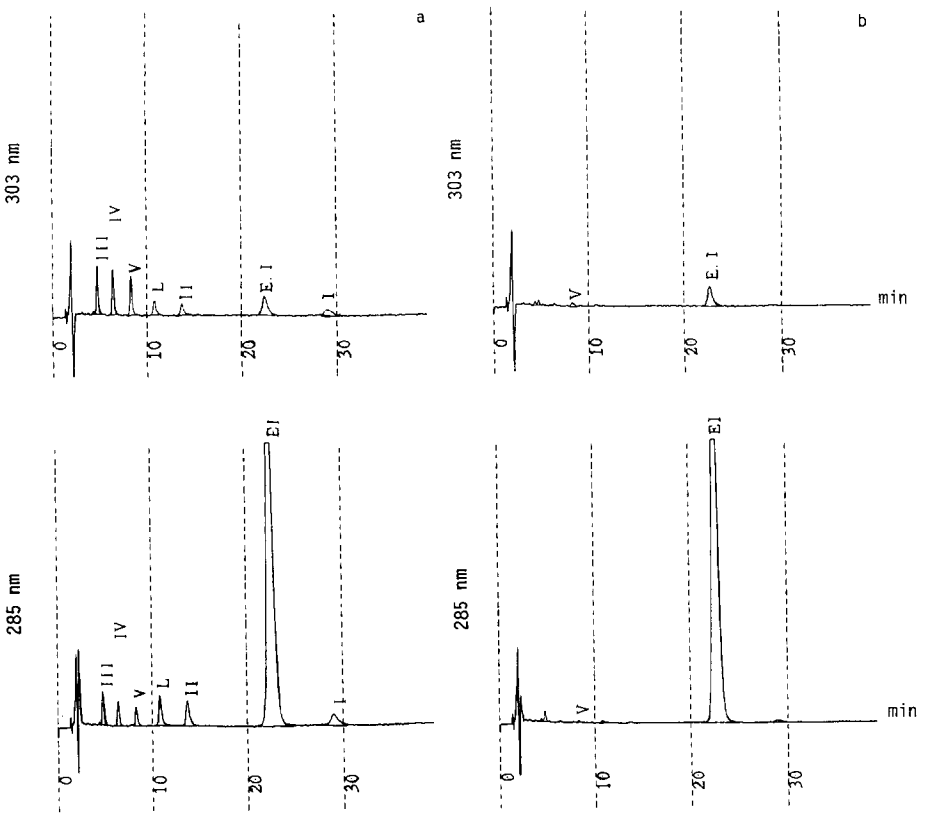

Fig. 2 (a) Chromatograms of extracted blank plasma spiked with 50 ng/ml I (15.8), II (7.9), III (2.7), IV (3.8), V (4.8) and 100 ng/ml L (6.0) at 285 and 303 nm. (The capacity factors are indicated in parentheses). (b) Chromatograms of blank plasma at 285 and 303 nm.

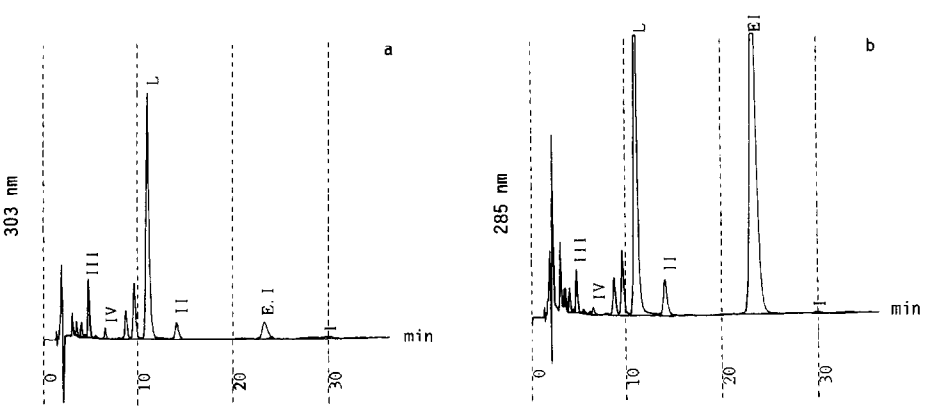

Fig. 3. Chromatograms of extracted plasma form a sample, taken 2 h after the administration of a single oral dose of 30 mg of lansoprazole to an elderly patient, and containing III (80 ng/ml), IV (15 ng/ml), L (965 ng/ml), II (92 ng/ml) and I (9.8 ng/ml). Detection wavelength: (a) 285 nm; (b) 303 nm.

TABLE I

EXTRACTION RECOVERIES FROM PLASMA

Compound	Recovery (%)
III	78
IV	82
V	87
Lansoprazole	74
II	83
I	94

The extraction recoveries are presented in Table I. The response was linear from 0 to 2000 ng/ml for L, from 0 to 1000 ng/ml for II and from 0 to 500 ng/ml for I, III, IV and V. Although the standard curve for L was apparently linear, the use of linear regression analysis for the entire range of standard values resulted in large predictor errors at low concentrations. The average percentage deviation value for the 5 ng/ml standard was 8.3%. Two standard curves were therefore used: a low range of 0–200 ng/ml (0, 5, 10,

TABLE II

ESTIMATION OF INTRA-ASSAY PRECISION

Spiked plasma samples were used. Values in parentheses are C.V.s (%).

Compound	Product/I.S. peak-height ratio (mean, n = 10)			
	5 ng/ml	50 ng/ml	100 ng/ml	1000 ng/ml
III	0.028 (8.1)		0.591 (1.3)	5.77 (1.2)
IV	0.054 (4.3)	0.417 (1.1)	0.818 (1.4)	
V	0.084 (6.9)	0.495 (1.6)	0.963 (1.0)	
Lansoprazole			0.443 (1.5)	4.18 (1.2)
II	0.026 (12)	0.252 (2.3)	0.509 (0.54)	
I	0.015 (13)	0.149 (3.2)	0.301 (1.5)	

TABLE III

ESTIMATION OF ACCURACY AND BETWEEN-DAY PRECISION

Spiked plasma samples were used. The concentrations of the quality controls were chosen according to the concentrations likely to be observed. Values in parentheses are C.V.s (%).

Compound	Accuracy (mean ± S.D., n = 30) (%)			
	8 ng/ml	80 ng/ml	400 ng/ml	2000 ng/ml
III	7.4 ± 1.01 (13.6)	75.1 ± 5.2 (6.9)		
IV	8.3 ± 0.58 (6.9)	82.0 ± 5.8 (7.0)		
V	8.5 ± 1.08 (12.8)	81.8 ± 4.4 (5.4)		
Lansoprazole	7.9 ± 1.4 (18.1)	78.5 ± 4.8 (6.1)	401 ± 20 (5.0)	1955 ± 75 (3.8)
II	7.8 ± 1.2 (15.1)	75.3 ± 4.1 (5.5)	365 ± 18 (4.9)	
I	8.5 ± 1.8 (21.3)	81.1 ± 3.1 (3.8)		

20, 50, 100, 150 and 200 ng/ml) and a high range of 200–2000 ng/ml (200, 400, 800, 1200, 1600 and 2000 ng/ml). This resulted in less than 4% deviation of calculated values from known values for all standards.

The method was sensitive, with quantification limits of 2 ng/ml for the hydroxylated metabolites, 3 ng/ml for L and L sulphone and 5 ng/ml for L sulphide.

The precision data for the concentrations L and its metabolites are presented in Tables II and III. The identity curve between the added and calculated values for the quality control standards, made up by another person from stock solutions different from those used for the calibration curve, was $y = 0.978x + 3.03$. The slope was not significantly from 1 and the intercept from zero, confirming the accuracy of the method.

Fig. 4 shows the levels of L and its metabolites in plasma from healthy subjects and elderly patients. There were three metabolites, *i.e.* L sulphone, L sulphide and 5-hydroxylansoprazole. The sulphone was the major metabolite in both populations. Measurable amounts of the hydroxylated sulphone metabolite were only present following the administration of the 30-mg daily dose to the elderly subjects.

Our results indicate that the proposed assay method has the sensitivity, selectivity and reproducibility necessary for use in pharmacokinetic studies.

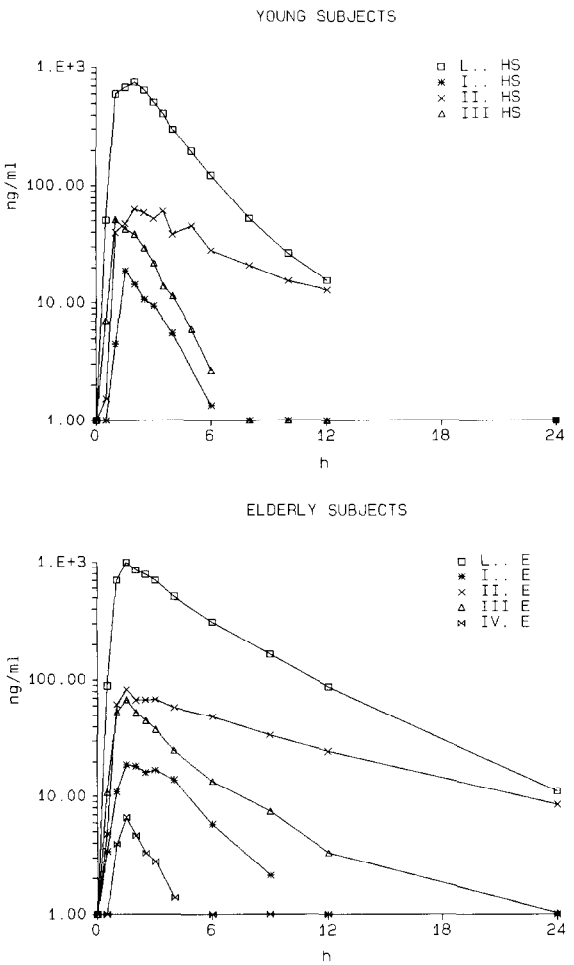

Fig. 4. Mean plasma levels of lansoprazole and its metabolites in young (n = 18) and elderly (n = 12) subjects.

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