Journal of Chromatography, 571 (1991) 283–290
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 6018

Short Communication

High-performance liquid chromatographic determination of lansoprazole and its metabolites in human serum and urine

ISAMU AOKI*

Takeda Analytical Research Laboratories, Ltd., Juso-Honmachi, Yodogawa-ku, Osaka 532 (Japan)

MINORU OKUMURA

Pharmaceutical Production Research Laboratories, Takeda Chemical Industries, Ltd., Juso-Honmachi, Yodogawa-ku, Osaka 532 (Japan)

and

TAKATSUKA YASHIKI

Analytical Laboratories, Takeda Chemical Industries, Ltd., Juso-Honmachi, Yodogawa-ku, Osaka 532 (Japan)

(First received January 16th, 1991; revised manuscript received May 15th, 1991)

ABSTRACT

A simple and sensitive high-performance liquid chromatographic method with ultraviolet detection is described for the simultaneous determination of lansoprazole and its metabolites in human serum and urine. The analytes in serum or urine were extracted with diethyl ether–dichloromethane (7:3, v/v) followed by evaporation, dissolution and injection into a reversed-phase column. The recoveries of authentic analytes added to serum at 0.05–2 μg/ml or to urine at 1–20 μg/ml were greater than 88%, with the coefficients of variation less than 7.1%. The minimum determinable concentrations of all analytes were 5 ng/ml in serum and 50 ng/ml in urine. The method was successfully applied to a pharmacokinetic study of lansoprazole in human.

INTRODUCTION

Lansoprazole, (±)-2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl]-sulphinyl]benzimidazole (AG-1749), is a new substituted benzimidazole compound, which markedly inhibits the secretion of basal and stimulated gastric acid and is useful in the treatment of duodenal ulcers, gastric ulcers and reflux esophagitis [1–3]. The metabolism of lansoprazole was investigated with animals (rats, mice and dogs), and the main metabolites were identified [4,5] as shown in Fig. 1. The metabolites of lansoprazole include not only its sulphide (M-I) and sulphone (M-VII) but also 5-hydroxy metabolites (M-VI, -IV and -IX) of these.

0378-4347/91/$03.50

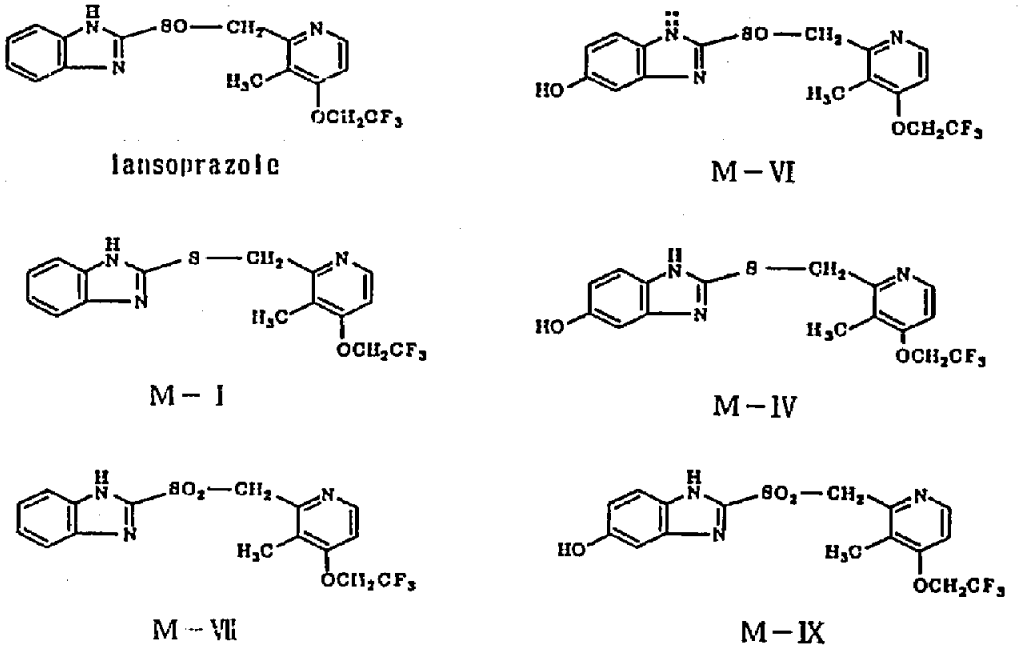

Fig. 1. Structures of lansoprazole and its possible metabolites in humans.

In the present paper, a simple and sensitive high-performance liquid chromatographic (HPLC) method for the simultaneous determination of lansoprazole and its main metabolites in human serum and urine is decribed.

EXPERIMENTAL

Reagents and materials

Test samples and reagents. Lansoprazole and its metabolites were synthesized in the Research and Development Division of Takeda Chemical Industries. Type VII *β*-glucuronidase was purchased from Sigma (St. Louis, MO, USA). Dichloromethane, acetonitrile and ethanol were of HPLC grade. All other reagents were of analytical-reagent grade.

Mobile phase I (MP-I) was water–acetonitrile–*n*-octylamine (620:380:1, v/v), adjusted to pH 7 with 85% phosphoric acid. Mobile phase II (MP-II) was 0.07 *M* phosphate buffer (pH 7.2)–ethanol–acetonitrile (20:10:3, v/v). The propylene glycol (PG) solution, propylene glycol–diethyl ether–dichloromethane (1:140:60, v/v), was used to depress the evaporation of the analytes. Internal standard solutions I (IS-I) and II (IS-II) consisted of 25 *μ*g/ml isobutyl *p*-hydoxybenzoate in dichloromethane and 6 *μ*g/ml isopropyl *p*-aminobenzoate in MP-II, respectively.

Serum and urine samples were collected from ten healthy male volunteers participating in the Phase I study, and the control samples were collected before administration of the test compound.

Standard samples. A serum standard sample was prepared to contain lan-

soprazole, M-I, M-IV, M-VI, M-VII and M-IX (each 0.5 μg/ml) in a control serum, and a urine standard sample was prepared to contain M-IV, M-VI and M-IX (each 5 μg/ml) in a mixture of control urine and 0.47 *M* sodium bicarbonate solution (1:1, v/v).

Extraction of the analytes from serum and urine samples

A 0.1-ml portion of IS-I was added to 0.5 ml of a serum sample and a serum standard sample, and the analytes were extracted twice with 3 ml of diethyl ether–dichloromethane (7:3, v/v). After centrifugation, 0.5 ml of the propylene glycol (PG) solution was added to the supernatant, and the solvent was evaporated under a nitrogen stream. The residue was dissolved in 0.5 ml of MP-I, and a 100-μl portion of the solution was subjected to HPLC. To 0.5 ml of a urine sample and a urine standard sample was added 0.5 ml of β-glucuronidase solution (1000 U/ml in 0.07 *M* phosphate buffer of pH 7), and the mixture was incubated at 37°C for 60 min. The analytes were extracted twice with 3 ml of diethyl ether–dichloromethane (7:3, v/v). After centrifugation, 0.5 ml of the PG solution was added to the supernatant, and the solvent was evaporated under a nitrogen stream. The residue was dissolved in 0.4 ml of IS-II, and a 20-μl portion of the solution was subjected to HPLC.

Instruments and conditions for determination

The HPLC system consisted of a Shimadzu LC-4A or LC-6A high-performance liquid chromatograph equipped with an autosampler with cooling device (Ishido, Chiba, Japan). A reversed-phase column of TSK gel ODS-120T (particle size 5 μm; 250 mm × 4.6 mm I.D., Tosoh, Tokyo, Japan) was used. The column temperature and the flow-rate were 40°C and 1.0 ml/min, respectively.

MP-I and MP-II were used for the determination of analytes in serum and urine, respectively. Lansoprazole, M-I, M-IV, M-VII and internal standard in serum were detected by UV absorption at 285 nm, and M-VI and M-IX were detected at 303 nm; M-IV, M-VI and M-IX in urine were detected at 303 nm.

Administration of lansoprazole and sample collection

A capsule containing 30 mg of lansoprazole as enteric-coated granules (lansoprazole capsule, 30 mg) was orally administered to each of ten fasted healthy male volunteers. The serum samples were collected before administration and 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h after administration. The samples were preserved at −20°C until analysis. The urine samples were collected before administration and at the intervals of 0–6, 6–12 and 12–24 h after administration. To stabilize the analytes, the same volume of 0.47 *M* sodium bicarbonate solution was added immediately after the collection of a urine sample and the mixture was stored at −20°C until analysis.

RESULTS AND DISCUSSION

From the results of metabolism of lansoprazole in animals, the sulphide (M-I) and sulphone (M-VII) metabolites and three corresponding 5-hydroxy metabolites (M-VI, M-IV, M-IX) were expected as metabolites in humans. Thus, we investigated a simultanous determination method for lansoprazole and its five possible metabolites to develop a reversed-phase HPLC technique with UV detection.

Lansoprazole and its metabolites were unstable in an acid solution, as is omeprazole [6], although they were rather stable in an alkaline solution; the half-lives of lansoprazole at 25°C were *ca.* 30 min at pH 5, *ca.* 18 h at pH 7 and *ca.* 37 h at pH 8. Accordingly, the analytes were stable in a serum sample (pH *ca.* 8), but unstable in a urine sample depending on the pH. Therefore, we stabilized the analytes by adding an equal volume of 0.47 *M* sodium bicarbonate solution to each urine sample to keep the pH *ca.* 9. The chromatograms of serum samples are shown in Fig. 2. The calibration curves of the peak-height ratios (analyte to

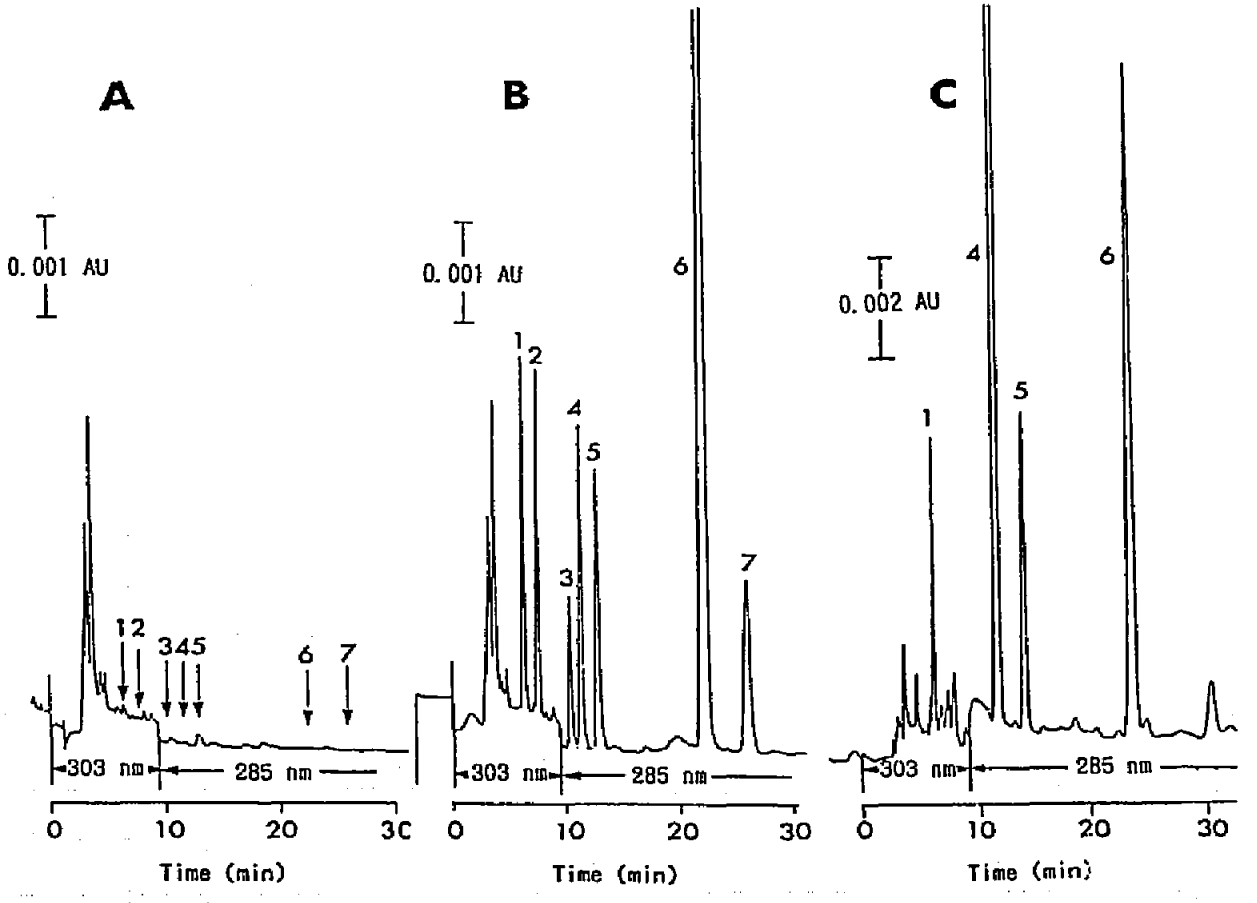

Fig. 2. Chromatograms of extracts from (A) analyte-free serum, (B) serum spiked with analytes (each concentration *ca.* 0.1 μg/ml) and (C) the serum sample of a volunteer (M-VI, 162 ng/ml; lansoprazole, 1748 ng/ml; M-VII, 268 ng/ml). Peaks: 1 = M-VI; 2 = M-IX; 3 = M-IV; 4 = lansoprazole; 5 = M-VII; 6 = internal standard; 7 = M-I.

internal standard) *versus* the concentration of the analytes show straight lines passing through the origin (correlation coefficient >0.999). The average recoveries of the analytes in the concentration range 0.05–2 μg/ml were more than 88%, with coefficients of variation (C.V) of less than 7.1% (Table I). The minimum determinable concentrations (signal-to-noise ratio of 3) for lansoprazole and its metabolites were all 5 ng/ml when 0.5 ml of a serum sample was used.

Preliminary investigation on the metabolites of lansoprazole in human urine revealed that only 5-hydroxy metabolites (M-IV, M-VI, M-IX) were excreted, mainly as the glucuronides. As the authentic samples of the glucuronides were not available, the conditions for the hydrolysis of glucuronides were investigated using β-glucuronidase. As a result, incubation at 37°C for 1 h was found enough for the hydrolysis.

The chromatograms of urine samples (after hydrolysis) are shown in Fig. 3. The calibration curves of the peak-height ratios (analyte to internal standard) *versus* the concentration of the analytes show straight lines passing through the

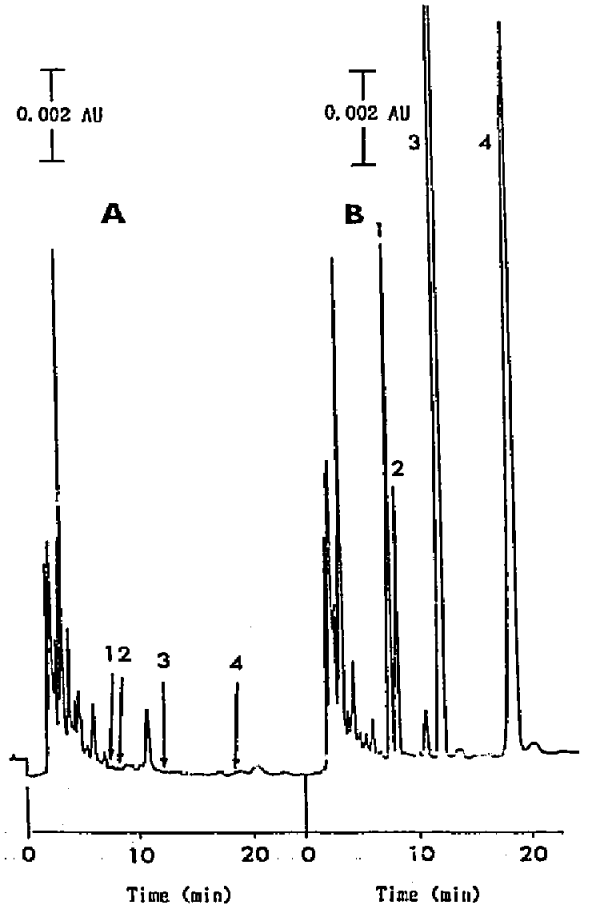

Fig. 3. Chromatograms of extracts from (A) analyte-free urine (after hydrolysis) and (B) the urine sample of a volunteer (after hydrolysis: M-IX, 5.75 μg/ml; M-VI, 4.35 μg/ml; M-IV, 17.09 μg/ml); detection wavelength, 303 nm. Peaks: 1 = M-IX; 2 = M-VI; 3 = internal standard; 4 = M-IV.

TABLE I

RECOVERY AND REPRODUCIBILITY FOR LANSOPRAZOLE AND ITS METABOLITES ADDED TO CONTROL HUMAN SERUM

Concentration of analyte in serum (μg/ml)	Recovery (mean ± R.S.D.) (%)					
	M-VI	M-IX	M-IV	Lansoprazole	M-VII	M-I
0.05 (n = 5)	88.6 (±5.7)	93.4 (±2.6)	97.8 (±3.2)	98.6 (±1.4)	94.1 (±4.6)	98.0 (±0.0)
0.1 (n = 5)	96.9 (±4.1)	101.2 (±4.6)	104.4 (±3.0)	98.7 (±2.2)	95.8 (±1.8)	95.9 (±1.5)
0.2 (n = 5)	109.0 (±1.2)	105.0 (±2.8)	104.9 (±5.1)	98.4 (±2.5)	97.8 (±2.1)	96.9 (±3.4)
0.5 (n = 5)	97.5 (±2.0)	97.8 (±1.1)	94.1 (±3.2)	94.2 (±1.2)	96.8 (±0.5)	95.8 (±1.8)
1.0 (n = 5)	95.8 (±2.0)	97.5 (±1.2)	93.4 (±3.0)	97.5 (±0.8)	98.5 (±1.4)	97.9 (±1.1)
2.0 (n = 5)	96.9 (±1.0)	97.5 (±0.7)	98.0 (±2.3)	99.1 (±0.4)	98.6 (±0.9)	99.4 (±0.3)
Overall (n = 30)	97.8 (±7.1)	98.7 (±4.4)	98.9 (±5.6)	97.8 (±2.3)	97.3 (±2.0)	100.6 (±1.29)
Correlation coefficient (r)	0.99975	0.99998	0.99932	0.99992	0.99999	0.99985

TABLE II

RECOVERY AND REPRODUCIBILITY FOR LANSOPRAZOLE METABOLITES ADDED TO CONTROL HUMAN URINE

Concentration of analytes in urine (μg/ml)	Recovery (mean ± R.S.D.) (%)		
	M-IX	M-VI	M-IV
1 (n = 5)	92.9 (±0.8)	89.6 (±1.8)	90.2 (±1.3)
2 (n = 5)	96.1 (±0.7)	96.5 (±1.5)	94.8 (±0.8)
4 (n = 5)	95.8 (±1.3)	93.1 (±1.0)	94.5 (±1.2)
10 (n = 5)	96.5 (±0.6)	94.6 (±0.9)	96.9 (±0.8)
20 (n = 5)	96.9 (±0.9)	94.1 (±1.0)	97.4 (±1.0)
Overall (n = 25)	95.7 (±1.8)	93.6 (±2.8)	94.7 (±2.9)
Correlation coefficient (r)	0.99995	0.99998	0.99997

origin (correlation coefficient >0.9999). The average recoveries of the analytes in the concentration range 1–20 μg/ml were more than 90%, with coefficients of variation less than 2.9% (Table II). The minimum determinable concentrations

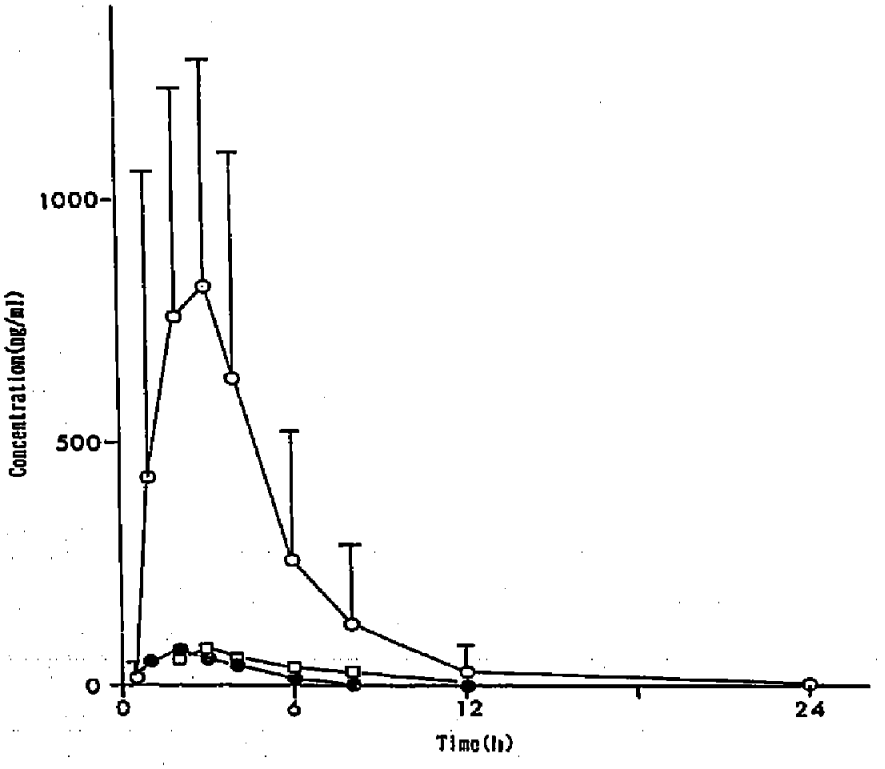

Fig. 4. Serum levels of lansoprazole and its metabolites. Data show the mean values and the standard deviations obtained from ten healthy volunteers (single oral dose of 30-mg lansoprazole capsule in the fasted state). (○) Lansoprazole; (●) M-VI; (□) M-VII.

(signal-to-noise ratio of 3) for lansoprazole and its metabolites were all 50 ng/ml when 0.5 ml of a urine samples was used.

By using the established method, the determination of lansoprazole and its main metabolites in serum and urine was performed after oral administration of the lansoprazole capsule (30 mg) to ten fasted, healthy male volunteers. The average time-courses of the serum concentrations are shown in Fig. 4. In serum, lansoprazole showed a higher concentration than M-VI or M-VII. The serum concentration of lansoprazole reached the maximum 2–3 h after administration and disappeared after 24 h.

M-IV, M-VI and M-IX were excreted mainly as glucuronides in the urine samples, and the total excretion ratios of the metabolites (glucuronides plus free forms) in 24 h were 8.55, 3.90 and 1.54%, respectively.

ACKNOWLEDGEMENTS

The authors thank Mr. T. Shima of Takeda Service Co. Ltd. and Mr. T. Amano of Takeda Analytical Research Laboratories for their cooperation.

REFERENCES

1 H. Satoh, N. Inatomi, H. Nagaya, I. Inada, A. Nohara and Y. Maki, *Jpn. J. Pharmacol.*, 40 (Suppl.) (1986) 226.
2 H. Satoh, N. Inatomi, H. Nagaya, I. Inada, A. Nohara, N. Nakamura and Y. Maki, *J. Pharmacol. Exp. Ther.*, 248 (1989) 806.
3 H. Nagaya, H. Satoh, K. Kubo and Y. Maki, *J. Pharmacol. Exp. Ther.*, 248 (1989) 799.
4 K. Miwa, M. Mitani, T. Tsukamoto, K. Yoshida, T. Kobayashi, T. Kimura, H. Shimomura and S. Tanayama, *Jpn. Pharmacol. Ther.*, 18 (1990) 3413.
5 T. Kobayashi, M. Mitani, K. Miwa, K. Yoshida, I. Naeshiro, M. Ikeda, T. Kimura, H. Shimomura and S. Tanayama, *Jpn. Pharmacol. Ther.*, 18 (1990) 3437.
6 A. Pilbrant and C. Cederberg, *Scand. J. Gastroenterol.*, 20 (Suppl.) (1985) 113.