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High-performance thin-layer chromatographic method for the detection and determination of lansoprazole in human plasma and its use in pharmacokinetic studies

K.K. Pandya[a], V.D. Mody[a], M.C. Satia[b], I.A. Modi[c], R.I. Modi[d], B.K. Chakravarthy[a], T.P. Gandhi[b,*]

[a]*Department of Phytochemistry, R & D, Cadila Pharmaceuticals Ltd., 244 Ghodasar, Ahmedabad 380 050, India*
[b]*Department of Pharmacology, R & D, Cadila Pharmaceuticals Ltd., 244 Ghodasar, Ahmedabad 380 050, India*
[c]*Chairman, Cadila Pharmaceuticals Ltd., 244 Ghodasar, Ahmedabad 380 050, India*
[d]*Managing Director, Cadila Pharmaceuticals Ltd., 244 Ghodasar, Ahmedabad 380 050, India*

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Abstract

A rapid and sensitive high-performance thin-layer chromatography (HPTLC) method has been developed for the measurement of lansoprazole in human plasma and its use for pharmacokinetic study has been evaluated. Detection and quantitation were performed without using an internal standard. A single stage extraction procedure was followed for extracting lansoprazole from plasma and a known amount of the extract was spotted on precoated silica gel 60 F_{254} plates using a Camag Linomat IV autosampler. Lansoprazole was quantified using a Camag TLC Scanner 3. The recovery study of authentic analytes added to plasma at 0.05 to 0.25 μg/ml was 95.37±2.15% and the lowest amount of lansoprazole that could be detected was 20 ng/ml plasma. The method provides a direct estimate of the amount of lansoprazole present in plasma. The method was used for the determination of plasma levels as well as pharmacokinetic parameters of lansoprazole after oral administration of two marketed preparations to healthy volunteers.

Keywords: Lansoprazole

1. Introduction

Lansoprazole, 2-[[[3-methyl-4-(2,2,2-trifluoroethoxy)-2-pyridyl]methyl] sulfinyl]benzimidazole (AG-1749), is a new substituted benzimidazole compound, containing a novel trifluoroethoxy group (Fig. 1), is structurally related to omeprazole and differs both structurally and pharmacologically from the H_2-receptor antagonists [1]. Lansoprazole has potent antisecretory effects [2,3] and it inhibits both the activity of K^+-ATPase and ATP-dependent H^+ accumulation in canine gastric microsomes [4].

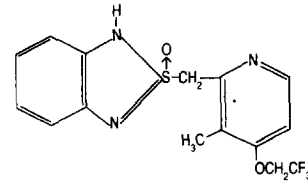

Fig. 1. Chemical structure of lansoprazole.

*Corresponding author.

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Lansoprazole was found to inhibit acutely induced gastroduodenal lesions and reflux esophagitis and promote ulcer healing [5]. The effect of lansoprazole (30 mg once daily) was evaluated in patients with gastric ulcers and duodenal ulcers [6,7].

Data on the pharmacokinetic profile of lansoprazole are limited, as most studies monitor the effect of lansoprazole via inhibition of gastric acid secretion rather than by assessing serum or plasma drug concentrations. The antisecretory effect of lansoprazole persists for approximately 24 h, despite an elimination half-life of less than 2 h, after treatment with a single oral dose (30 mg) to healthy volunteers, however, its antisecretory effect does not appear to correlate with the concentration of lansoprazole in serum [1]. The compound was mostly converted into metabolites and excreted in the feces more than in urine within 72 h in rats and dogs [8].

A previously reported method for the estimation of lansoprazole and its metabolites by high-performance liquid chromatography (HPLC) [9–11] used internal standards. This paper describes the development of an easy, economical and sensitive high-performance thin-layer chromatographic (HPTLC) method for the estimation of lansoprazole without the use of internal standards. The method was used to detect lansoprazole in plasma samples obtained from a bioequivalence study carried out in normal volunteers after administration of a single 30-mg dose p.o. As lansoprazole is acid labile, formulations used for these experiments were enteric coated granules of the drug in capsule form to prevent gastric decomposition and improve its systemic bioavailability, as described elsewhere [1].

2. Experimental

2.1. Reagents

A reference standard of lansoprazole was obtained from Dr. Reddy's Laboratories, Hyderabad, India. Two marketed formulations (A and B) of 30-mg lansoprazole capsules were used for comparative pharmacokinetic studies. Alkaline borate buffer (pH 10) and dichloromethane (analytical-grade) were used for extractions. Chloroform and methanol (analytical-grade) were used for developing TLC plates (Silica gel 60 F_{254}, Art 5554, DC-Alufolien, Kieselgel 60 F_{254}, E. Merck, Darmstadt, Germany).

2.2. Preparation of standards

A stock solution of lansoprazole was prepared in methanol at a concentration of 0.1 mg/ml. Standard solutions were obtained by diluting the stock solutions to concentrations ranging from 1 to 25 μg/ml.

2.3. Preparation of plasma samples

In a 15-ml graduated glass centrifuge tube, lansoprazole working standard (2 μg/ml) was added in volumes of 0, 25, 50, 75, 100 and 125 μl to 1 ml of drug-free plasma to provide calibration standards of 0 (no lansoprazole added), 50, 100, 150, 200 and 250 ng. Each test sample was mixed with 100 μl of alkaline borate buffer (pH 10) by vigorous vortex-mixing for 2 min and was extracted with 2×3 ml of dichloromethane on a vortex-mixer for 2 min and centrifuged at 700 *g* for 10 min. The combined dichloromethane extract was evaporated to dryness, in the dark, at 45°C in a water bath. Unknown plasma samples were prepared in an identical manner except for the addition of lansoprazole.

2.4. Instrumentation and chromatographic conditions

All residues were redissolved in 100 μl of methanol by vigorous vortex-mixing and 30-μl aliquots of the samples were spotted onto TLC plates with the help of a Camag Linomat IV autosampler. Lansoprazole (100 ng, 200 ng) reference standards were separately spotted on each TLC plate, as the external standard. The TLC plates were developed (10 cm) in a Camag Twin Trough glass chamber with a solvent system consisting of chloroform–methanol (15:1, v/v), in which the drug had an R_F value of 0.36 and was separated from other components in plasma. The TLC plates were dried completely, using a hot air drier, after development. The spots of lansoprazole were observed by fluorescence quenching under UV illumination (wavelength, 286 nm) and the total area of each spot was determined with the help of a Camag TLC scanner 3.

2.5. Quantitation

The quantitation of the chromatograms was performed using the ratio of the peak area of the unknown to that of a standard. A representative standard curve of lansoprazole was obtained by plotting the area under the peak of lansoprazole against the concentration over the range 10–250 ng. The minimum quantifiable concentration of lansoprazole in human plasma samples was 20 ng/ml.

2.6. Method validation

The recovery of lansprazole from plasma was determined by comparing peak areas obtained from plasma to which lansoprazole (50, 100, 150, 200 and 250 ng) had been added with that of the peak areas obtained from standards. The intra-day precision (random analytical variation) was evaluated by analyzing drug-free plasma samples, to which lansoprazole had been added at concentrations of 50, 150 and 200 ng, in triplicate. The inter-day precision was determined by analyzing 100, 150 and 250 ng standards simultaneously with the plasma from subjects daily for five days. The linearity of the detector response was tested by spotting standards (in triplicate) for each concentration over the range 10–250 ng.

2.7. Pharmacokinetic studies

Six healthy male human volunteers were selected for the study after giving written informed consent and having normal biochemical parameters. Subjects were aged 26±3.0 years (mean±S.D., range 20 to 34), with a mean body weight of 54.4±4.8 kg (range 46.0 to 62.5). The study protocol was approved by the institutional review board and the Ethics Committee of Cadila Pharmaceuticals Ltd., Research and Development Unit. Two formulations of lansoprazole capsules (30 mg) (product A and product B) were selected for the study. One capsule of either brand was administered to each volunteer with 200 ml of water, such that half of the number of volunteers received product A and the rest received product B in the first part of a single blind cross-over study. The second part of the study was carried out after seven days. Identical conditions were maintained on both occasions. Blood samples, obtained from an antecubetal vein prior to dosing and at 1.0, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0 and 12.0 h after dosing, were placed in heparinised tubes. The samples were immediately centrifuged at 1000 g for 20 min, and the plasma samples were separated and frozen at −20°C until analysis.

Pharmacokinetic parameters were calculated using a model independent method [12]. The peak level (C_{max}) and the time taken to reach peak level (t_{max}) were observed data. The elimination rate constant (K_{el}) and the terminal elimination half-life ($t_{1/2}$) were estimated by linear regression of the terminal part of the log concentration–time curve. The area under the plasma concentration–time curve (AUC) was determined by the linear trapezoidal rule, and extrapolated to infinity ($AUC_{0\to\infty}$) by dividing the last measurable concentration by the elimination rate constant.

The pharmacokinetic parameters for C_{max}, $AUC_{0\to\infty}$ and $t_{1/2}$ were compared using analysis of variance and the results are given as the mean value±S.E.M. The Wilcoxon test was used to compare t_{max} values. Statistical significance was defined at the $P<0.05$ level and 95% confidence intervals for the mean values of C_{max}, and $AUC_{0\to\infty}$ for product A vs. product B was calculated [13].

3. Results

The peak area was observed to be dependent on the amount of the standard, lansoprazole, and a linear relationship ($r=0.999$) was found between the peak areas of lansoprazole at various concentrations over the range 10–250 ng. The solvent system used for development of the plates produced no interference peaks in the area under the curve, and all other compounds were distinctly separated. The R_F value of lansoprazole under the conditions used was found to be 0.36±0.05 and spots were quantified at a wavelength of 286 nm. The accuracy, precision and reliability of the procedure were ascertained by adding known concentrations of drug to drug-free plasma and analyzing five samples of each concentration by the method described for extraction (Table 1). The recovery of lansoprazole in the extraction procedure from 1 ml of plasma was found

Table 1
Accuracy and precision of a HPTLC method for the determination of lansoprazole in plasma

Concentration added (ng/ml)	Concentration detected (mean±S.D., n=5) (ng/ml)	C.V.[a] (%)	Accuracy[b] (%)
50	47.89±0.49	1.03	100.43
100	91.74±0.98	1.06	96.19
150	145.12±1.61	1.11	101.44
200	188.09±2.15	1.14	99.14
250	245.08±3.05	1.25	102.79

[a]Coefficient of variation.
[b]After correction for recovery.

to be 95.37±2.15% (n=5). The intra-day and inter-day precisions are given in Table 2.

In order to verify the applicability of this method, the bioequivalence study with two marketed lansoprazole capsule preparations was conducted in healthy volunteers at a dose of 30 mg. The mean plasma concentration of lansoprazole at various time points after administration of drug is shown in Fig. 2. The mean maximum concentration (C_{max}) and the mean area under the plasma concentration curve ($AUC_{0\rightarrow\infty}$) for both preparations were comparable and were not significantly different (Table 3). The t_{max} for both preparations ranged between 2.1 and 2.2 h. The pharmacokinetic parameters were subject to two-way analysis of variance. The result indicates that the pharmacokinetic profiles of the two formulations are identical and the bioavailability is not significantly different at $P<0.05$, indicating the bioequivalence of the two products. The spot of lansoprazole in plasma is distinctly separated and, in volunteer plasma, is clearly identified in comparison with blank plasma (Fig. 3). The marketed products were analyzed for lansoprazole content using the proposed technique. The R_F values were found to be the same for capsules and standard lansoprazole, and there was no interference from the excipients. The contents of lansoprazole were found to be 97.9±2.3 and 98.2±2.1%, respectively, for products A and B.

Table 2
Precision data of the HPTLC assay for lansoprazole

Concentration added (ng)	Peak area[a] (mean±S.D.)	C.V. (%)
Inter-day		
100	2309.39±24.08	1.04
150	3647.34±39.24	1.08
250	6211.20±77.40	1.25
Intra-day		
50	1213.69±12.53	1.03
150	3664.14±40.09	1.10
200	4792.25±54.50	1.14

[a]Calculated for total concentration (integrated value).

4. Discussion

The proposed HPTLC method can measure the total concentration of plasma lansoprazole at a dose of 30 mg per oral administration. By this established method, the plasma concentration of lansoprazole reached a maximum 2–3 h after administration and disappeared after 24 h (Fig. 2). This trend is very much in agreement with the HPLC method reported earlier [9].

The applicability of the extraction process employed in the reported HPLC method [9] to HPTLC

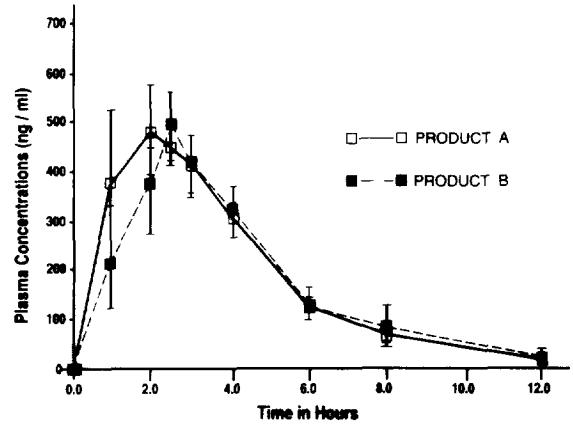

Fig. 2. Plasma concentration after oral administration of 30 mg of lansoprazole from two marketed products determined by HPTLC. Each point represents the mean±S.E. (n=6, crossover design).

Table 3
Pharmacokinetic parameters of lansoprazole (30 mg) in human volunteers

Parameter	Product A (n=6)	Product B (n=6)	p Value
C_{max} (ng ml^{-1})	614.23±50.73	568.47±47.39	NS
t_{max} (h)	2.10±0.33	2.20±0.34	NS
AUC (0→12 h) (ng h ml^{-1})	2261.68±227.47	2050.80±315.25	NS
AUC (0→∞) (ng h ml^{-1})	2364.41±264.73	2138.94±224.94	NS
K_{el} (h^{-1})	0.315±0.028	0.450±0.118	NS
$t_{1/2}$B (h)	2.312±0.252	2.24±0.572	NS

Data obtained by cross-over design and presented as mean±S.E.M.
n=Number of volunteers.
NS=not significant (p<0.05).

and vice-versa was verified using plasma samples. Two methods were adopted. First, the sample was processed by the HPLC method and dissolved in the reported mobile phase (water–acetonitrile–n-octylamine; 680:320:1, v/v/v). This was subsequently spotted (100 μl) onto the TLC plate and developed in the proposed TLC solvent system. However, the high water content in the HPLC mobile phase resulted in tailing of the spot, precluding the possibility of scanning. In the second method, the sample was processed by the proposed HPTLC method, then a 30-μl volume was injected onto the HPLC column and eluted with the reported mobile phase. This yielded a large number of interfering peaks and poor separation. However, by processing with the proposed HPTLC method, it is possible to quantify the levels of lansoprazole in plasma by spotting and detecting the samples without using an internal standard. By comparing the key analytical data of the proposed HPTLC method with that of previously reported HPLC analysis [9], it seems that the limit of detection is indeed lower in HPLC analysis (5 ng/ml), using isopropyl p-aminobenzoate as an internal standard, whereas, in the proposed HPTLC analysis, the limit of detection is 20 ng/ml, without using any internal standard. However, the recovery and coefficient of variation are improved in the proposed HPTLC analysis, compared to the reported HPLC method. The recovery of lansoprazole by the proposed HPTLC analysis was found to be 95.37±2.15%, compared to more than 88%, as reported for HPLC analysis. The coefficient of variation was found to be much less using the proposed HPTLC analysis (less than 1.25%), com-

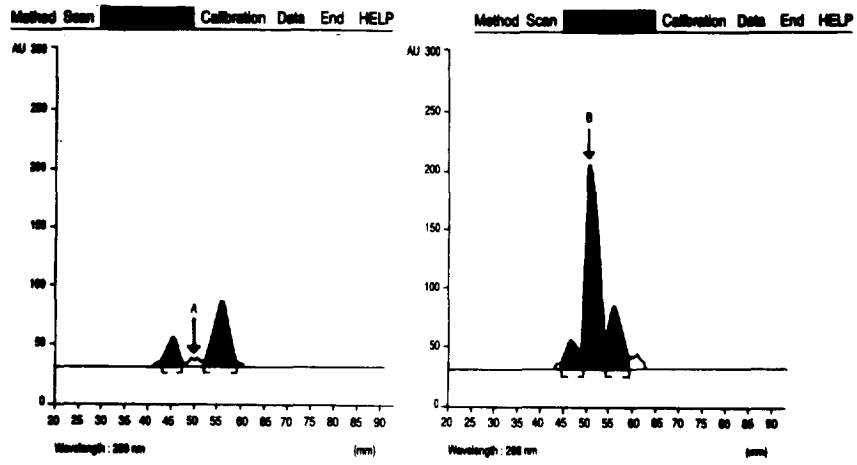

Fig. 3. Scanning profile of a volunteer's sample taken at 0 h (A) and at 2.5 h (B) post dose of 30-mg lansoprazole administration.

pared to that reported using HPLC analysis (less than 7.1%). The proposed method can also be used to accurately determine lansoprazole in capsules without interference from the excipients. Like the HPLC method for the detection of metabolites of lansoprazole [9], the HPTLC method can also be used for detection of those metabolites by using the respective metabolite reference standards.

5. Conclusions

The proposed HPTLC method for the estimation of lansoprazole in plasma has certain advantages over other reported methods. For example, (1) it gives a clear picture of the total drug present after absorption and thus has direct clinical relevance; (2) it is economical and faster than previously published methods: On a single plate, at least 10–12 samples can be analyzed in 4–5 h; (3) unlike earlier methods, this method does not require an internal standard and quantification can be done using reference drug as the external standard; (4) the recovery of the drug is improved compared with the HPLC method (95.37±2.15%); (5) the method described is a sensitive and specific assay for lansoprazole in plasma and is suitable for pharmacokinetic studies after therapeutic doses.

References

[1] L.B. Barradell, D. Faulds and D. McTavish, Drugs, 44 (1992) 225.
[2] H. Satoh, N. Inatomi, H. Nagaya, I. Inada, A. Nohara and Y. Mahi, Jpn. J. Pharmacol., 40 (Suppl.) (1986) 226.
[3] H. Satoh, N. Inatomi, H. Nagaya, I. Inada, A. Nohara, N. Nakamura and Y. Mahi, J. Pharmacol. Exp. Ther., 248 (1989) 806.
[4] H. Nagaya, H. Satoh, K. Kubo and Y. Maki, J. Pharmacol. Exp. Ther., 248 (1989) 799.
[5] N. Inatomi, H. Nagaya, K. Takami, A. Shino and H. Satoh, Jpn. J. Pharmacol., 55 (Suppl. 1) (1991) P-556.
[6] T. Kodama, Y. Fuse, Y. Ohkawara, Y. Maruyam and H. Tsuji, Jpn. Pharmacol. Ther., 18 (1990) 137.
[7] K. Makiyama, Y. Umene, I. Murata, M. Izuno and K. Hara, Jpn. Pharmacol. Ther., 19 (1991) 327.
[8] K. Miwa, M. Mitsuya and T. Tsukamoto, Jpn. Pharmacol. Ther., 18 (1990) 165.
[9] I. Aoki, M. Okumura and T. Yashiki, J. Chromatogr., 571 (1991) 283.
[10] B. Delhotal, G. Miscoria and B. Flouvat, J. Chromatogr., 577 (1992) 117.
[11] M. Toteno and N. Nakamura, Rinsho Iyaku, 7 (1991) 51.
[12] M. Gibaldi and D. Perrier, Pharmacokinetics, Marcel Dekker, New York and Basel, 2nd ed., 1982.
[13] W. Westlake, Biometrics, 32 (1976) 741.