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VALIDATION AND STABILITY OF RP-HPLC FOR THE DETERMINATION OF LANSOPRAZOLE IN TABLET DOSAGE FORM AND HUMAN PLASMA

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ABSTRACT

A simple, selective, accurate high Performance Liquid Chromatographic (HPLC) method was developed and validated for the analysis of Lansoprazole. Chromatographic separation achieved isocratically on a C_{18} column [Use Inertsil C_{18}, 5µ , 150 mm x 4.6 mm] utilizing a mobile phase of acetonitrile/phosphate buffer (70:30, v/v, pH 7.0) at a flow rate of 0.8 ml/min with UV detection at 260 nm. The retention time of lansoprazole was 2.53 min. The method is accurate (99.15-101.85%), precise (intra-day variation 0.13-1.56% and inter-day variation 0.30-1.60%) and linear within range 0.1-30µg/ml (R^2=0.999) concentration and was successfully used in monitoring left over drug. The detection limit of lansoprazole at a signal-to-noise ratio of 3 was 1.80ng/ml in human plasma while quantification limit in human serum was 5.60 ng/ml. The proposed method is applicable to stability studies and routine analysis of lansoprazole in pharmaceutical formulations as well as in human plasma samples.

Keywords: RP-HPLC, Lansoprazole, Blood serum, Method validation, Tablet dosage form

INTRODUCTION

Lansoprazole[1] is chemically 2-({3-methyl-4-(2, 2, 2-trifluoroethoxy)-2-pyridyl) methyl} sulfinyl benzimidazole, is used as a gastric proton pump inhibitor. It has an empirical formula of $C_{16}H_{14}F_3N_3O_2S$ and a molecular weight of 369.36. Literature survey revealed HPTLC, spectrophotometric and spectrofluorometric methods for determination of lansoprazole in bulk, dosage forms, biological fluids and acid-induced degradation studies[2-6].

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MATERIALS AND METHODS

A High Performance Liquid Chromatograph system, with LC solutions data handling system (Shimadzu-LC 2010) with an auto sampler was used for the analysis. The data was recorded using LC 2010 solutions software. The purity determination performed on a stainless steel column 150 mm long, 4.6 mm internal diameter filled with Octadecyl silane chemically bonded to porous silica particles of 5 µm diameter (Inertsil C_{18}, 5µ , 150 mm x 4.6 mm, make: Shimadzu ltd, Japan) with the mobile phase containing acetonitrile and phosphate buffer in the ratio of 70:30 (v/v pH 7.0) at ambient temperature. Flow rate was kept at 0.8 ml/min, and the elution was monitored at 260 nm.

Lansoprazole working standard, used from Smilax Laboratories Limited. For the estimation of lansoprazole in bulk and commercial formulations of lansoprazole brand (LAN, Intas laboratories), 20 tablets were obtained from retail pharmacies. Each tablet was labeled contain 30 mg of Lansoprazole and had an expiry of not less than 365 days at the time of study. HPLC grade Sodium dihydrogen phosphate (NaH_2PO_4) Disodium hydrogen phosphate (Na_2HPO_4), Acetonitrile- procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system.

PREPARATION OF MOBILE PHASE:

Mobile phase was prepared by mixing 700 ml of acetonitrile with 300 ml of phosphate buffer and its pH adjusted to 7.0. The mobile phase was sonicated for 15 min and then it was filtered through a 0.45 µ membrane filter paper

PREPARATION OF STOCK AND STANDARD SOLUTIONS:

Accurately weighed 25 mg of test sample into a clean dry 50 ml volumetric flask, dissolve and dilute to the mark with mobile phase. Mark this solution as sample solution. This solution contains 0.5 mg/ml of sample. Qualified working standard of lansoprazole is used to carry out validation exercise. The potency of working standard is 99.77 %. With the optimized chromatographic conditions, a steady baseline was recorded, the standard solution was injected and the chromatogram was recorded. This procedure were repeated to sample preparation.

METHOD VALIDATION:

The method was validated for the parameters like specificity, range and linearity, limit of detection (LOD), limit of quantitation (LOQ), accuracy, and precision. In addition, system suitability parameters were also calculated. To demonstrate specificity in the presence of excipients used in formulation, lansoprazole was spiked (at approximately 25 µg/ml) in drug product, chromatogram was observed and compared with that of raw material. To evaluate the linearity, the LOD and LOQ of the method in reference drug and in serum, different serial dilutions (0.0980, 0.190, 0.80, 1.50, 3.12, 6.30, 12.50 and 25 µg/ml) were prepared from the standard stock solutions in 25 ml volumetric flasks and volume made up with diluent which is mixture of 70:30 acetonitrile & methanol. The samples were injected (10 µl) and signals from the samples were recorded at 2.02 minute which were compared with those of blank. LOD and LOQ values were calculated as signal-to-noise ratio of 3:1 and 10:1 respectively. To determine accuracy of the method, working standard of lansoprazole was prepared in triplicate at three concentration levels (10, 20 and 25 µg/ml) and analyzed. Repeatability of the method was checked by analyzing six replicate samples of lansoprazole (at the 100% concentration level) and calculating relative standard deviation (%RSD). To determine intermediate precision, standard solutions of lansoprazole at eight concentration levels were analyzed three times within the same day (intra-day variation) and three other days (inter-day variation).

ASSAY IN FORMULATIONS:

In case of marketed formulations, five accurately weighed tablets were crushed to a fine powder and an amount equivalent to 10 mg of lansoprazole was added into different 100 ml volumetric flasks and volume was made up with acetonitrile and methanol mixture. The samples were filtered through a 0.45-µm-membrane filter; different serial dilutions (3.12, 6.20, 12.40, 25µg/ml) were made from this solution in 25 ml volumetric flask and were injected for HPLC analysis.

ASSAY IN SERUM:

One volume of plasma was de-proteinated by nine volume of acetonitrile and filtered through 0.45 µm Millipore filter paper that was used to make serial dilution of

lansoprazole (0.0970 µg/ml to 25 µg/ml). Three replicates of each dilution were injected to HPLC system and linearity was evaluated. Repeatability of the method was checked by analyzing six replicate samples of lansoprazole (at the 100% concentration level) and calculating relative standard deviation (%RSD).

RESULTS AND DISCUSSION:

For validation of analytical methods, the guidelines of the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use [ICH 1996] and [USP 2002] have recommended the accomplishment of accuracy tests, precision, specificity, linearity of the method

SYSTEM SUITABILITY:

The HPLC system was equilibrated with the initial mobile phase composition, followed by 10 injections of the same standard. These 10 consecutive injections were used to evaluate the system suitability on each day of method validation.

The system suitability parameters including capacity factor >2, resolution>3 and asymmetric factor<2. All parameters were satisfactory with good specificity for the stability assessment of lansoprazole. Theoretical plates of the column were >3000.

ACCURACY:

The accuracy of an analytical method is the closeness of test results obtained by that method to true value. In case of the assay of a drug in a formulated product, accuracy may be determined by application of the analytical method to synthetic mixtures of the drug product components to which known amount of analyte has been added within the range of method. If it is not possible to obtain samples of all drug product components, it may be acceptable to add known quantities of the analyte to the drug product (i.e.,"to spike") (USP 2004). In our studies, the later technique was adopted and lansoprazole was spiked in drug product. The result of accuracy given in (Table-1) revealed that the method was found accurate for all above purposes.

TABLE 1: ACCURACY/RECOVERY OF LANSOPRAZOLE

Parameters	Conc (µg/ml)	% Recovery	% RSD
Assay (Spiking method)	10	96.02	1.70
	20	101.54	4.80
	25	95.15	4.60
Assay	6.30	99.18	0.5
	12.40	100	1.5
	25	99.99	0.3
Assay (in serum)	12.40	100	0.5
	6.20	100	1.2
	3.12	100	0.8

PRECISION:

Precision is the degree of reproducibility or repeatability of the analytical method under normal operating conditions (USP 2004). The method passed the test for repeatability as determined by %RSD of the area of the peaks

of six replicate injections at 100% test concentration. The results of intra-and inter-day variation are shown in (Table 2).

TABLE 2: INTERMEDIATE PRECISION OF THE METHOD

Concentration (µg/ml)	Assay in formulation		Serum
	Intra-day variation (%RSD)	Inter-day variation (%RSD)	Intra-day variation (%RSD
0.0980	0.13	0.90	4.12
0.190	0.35	0.33	0.08
0.80	0.40	1.60	2.70
1.50	0.85	1.08	3.20
3.12	0.26	0.20	1.15
12.5	1.65	0.80	0.40
25	0.25	1.08	3.30

RANGE AND LINEARITY:

The linearity of an analytical method is its ability to elicit test results that are directly, or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range (USP 2004). The linearity of the method was observed with in the expected concentration range demonstrating its suitability for analysis. The correlation coefficient (r^2) was found to be 0.999 and value of intercept was less than 25 of the response of 100% of the test concentration in all the cases indicating functional linear relationship between the concentration of analyte and area under the peak.

LIMITS OF DETECTION AND QUANTITATION:

The detection limit (LOD) is the lowest amount of an analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions. It may be expressed as a concentration that gives a signal-to-noise ratio of 2:1 or 3:1 (USP 2004, ICH Q2B guidelines, 1996 1997, FDA, Guidance for Industry 2000)[7, 8]. The lower limit of detection for lansoprazole is 2.40ng/ml in reference material and formulation and 1.70ng/ml serum. Limit of Quantitation (LOQ) is the lowest amount analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. A signal-to-noise ratio of 10:1 can be taken as LOQ of the method (USP 2004). The LOQ values were found to be 8.15ng/ml for raw material, formulations and 5.70ng/ml for serum.

SPECIFICITY:

Specificity is the ability to assess unequivocally the analyte in the presence of components that may be expected to be present in the sample matrix (USP 2004). For demonstrating the specificity of the method for drug formulation the drug was spiked and the representative chromatogram (Figure-1).

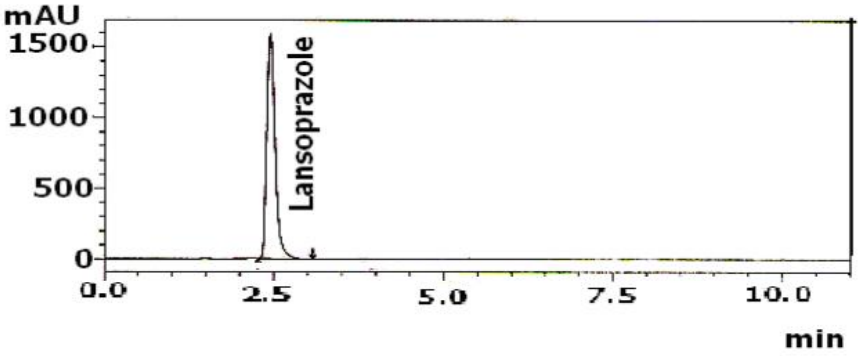

Figure 1 Chromatogram of Lansoprazole

The excipiants used in different formulation products did not interfere with the drug peak and thus, the method is specific for pantoprazole. To further confirm the specificity of the method, UV scans of spiked drug were taken in the range 200-400nm and no significant change was found by comparing the absorbance of pure drug and spiked drug at the analytical wavelength of drug.

STABILITY STUDIES

Stability of sample in Mobile phase:

The stability of sample solution (Lansoprazole) in mobile phase was demonstrated by injected the sample solution at different time intervals viz. 0, 3, 6, 9, 12 and 24 h of time intervals. Upto 9 h, no degradants were observed in the chromatogram. However, after 9 h the chromatographic peak area of lansoprazole decreased insignificantly. Hence, the sample solution was stable at least for 9 h after its preparation. Interestingly, after 9 h, when samples were stored at 20^0C under laboratory light conditions, significant rise in the peak areas were observed. Thus, it would be preferable that the sample solution is to be injected before 9 h of its preparation.

Hydrolysis:

Individually, 5 ml of the standard solution was transferred to a 10 ml distillation flask and boiled for 1 h at 80°C after adding : (a) 5 ml of water for neutral hydrolysis (b) 5 ml of 0.1N HCl for acid hydrolysis (c) 5 ml of 0.1 N NaOH for basic hydrolysis. Before the analysis, (b) and (c) solutions were neutralized. For chemical oxidation to 5 ml of the standard solution, 100 µL of 30 % H_2O_2 solution (v/v) were added and mixed. The solution was left at room temperature for 1 h in the dark.

Photochemical degradation:

The photochemical stability of the lansoprazole was studied by exposing the methanolic stock solution to direct sunlight for 8 h (from 9 AM to 5 PM, at 20°C).

Thermal Stress (test sample exposed to sunlight):

Transfer about 2 to 3 gm of sample into a clean dry watch glass and spread evenly. Expose to sunlight for 10 hours. After the sample got exposed to prescribed time, weigh accurately 25 mg of sample into a clean dry 50 ml volumetric flask, dissolve and dilute to the mark with mobile phase and exposed to sunlight.

Stability-indicating methods have received considerable attention for the determination of a vast number of drugs[9-12]. The international Conference on Harmonization (ICH) guideline entitled "Stability Testing of New Drug Substances and Products" requires the stress testing to be carried out to elucidate the inherent stability

characteristics of the active substances[13]. Susceptibility to oxidation is one of the required tests. The hydrolytic and photolytic stabilities are also required. An ideal stability-indicating method is one that quantifies the drug present and also resolves its degradation products. This study was carried out by employing the following tests: hydrolysis (neutral, acidic and basic), chemical oxidation, photolysis and thermolysis.No decomposition was observed when the lansoprazole was exposed to sunlight, temperature, UV; whereas significant change i.e., decrease of assay about 25 to 30 % observed when sample was treated with 0.1N NaOH and 0.1N HCl. The sample treated with 3 % H_2O_2 was almost completely degraded.

References

1. Parfitt K, editor. Martindale, the complete drug referencePharmaceutical Press London; 1999.

2. Gerloff J, Mignot A, Barth H, Heintze K. Pharmacokinetics and absolute bioavailability of lansoprazole. Eur J Clin Pharmacol 1996; 50:293-7.

3. Masatomo M, Tada H, Suzuki T. Simultaneous determination of lansoprazole enantomers and their metabolites in plasma by liquid chromatography with solid-phase extraction. J Chromatogr B Analyt Technol Biomed Life Sci 2004; 804:389-95.

4. Pandya KK, Mody VD, Satia MC, Nodi RI, Chakravarthy BK, Gandhi TP. High Performance thin-layer chromatographic method for the detection and determination of lansoprazole in human plasma and its use in pharmacokinetic studies. J Chromatogr B Biomed Sci Appl 1997; 693: 199-204.

5. Susheel J.V., M. Lekha and T.K.Ravi. High Performance Thin layer Chromatographic Estimation of Lansoprazole and Domperidone in Tablets. Indian J. Pharm. Sci, 2007 69(5): 684-686.

6. Zeinab Abdelaziz Et-Sherif, Afaf Osman Mohamad, El-Tarras RP-HPLC method for the determination of lansoprazole, omeprazole and pantoprazole sodium sesquihydrate in presence of their acid-induced degradation products. 2006: 54(6) 814-818.

7. ICH Q2B: Validation of Analytical Procedures: Methodology, May (1997).

8. International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceutical for Human Use (ICH) Q2B (1996). Validation of Analytical Procedures, Methodology.

9 Avarez-Lueje, A., Pujol, S., Squella, J.A. and Nunez Vergara, L. J. A Selective HPLC method for determination of lercanidipine in tablets. J. Pharm. Biomed Anal 2003; 31: 1-9.

10 bdul-Fattah. A. M. and Bhargava, H.N. A new high performance liquid Chromatography (HPLC) method for the analysis of halofantrine (HF) in pharmaceuticals. J. Pharm. Biomed. Anal 2002; 29: 901-908.

11 Lambropouls, J., Spanos, G. A. and Lozaridis, V.N. Method development

and validation for the HPLC assay (potency and related substances) for 20 mg paroxetine tablets. J. Pharm. Biomed. Anal 1999; 19: 793-802.

12 Bebawy, L. I.Spectrophotometric and HPLC determination of linezolid in the presence of its alkaline-induced degradation products and in pharmaceutical tablets. Anal. Let 2003; 36: 1147-1161.

13 ICH, Q1A Stability Testing of New Drug Substances and Products. October 1993; International Conference on Harmonization. Geneva.

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