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ANALYTICAL METHOD DEVELOPMENT, VALIDATION AND FORCED DEGRADATION STUDIES OF LANSOPRAZOLE BY RP-HPLC

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ABSTRACT

The objective of this work is to develop a rapid, precise, accurate and sensitive revere phase liquid chromatographic method and Forced degradation studies for the estimation of Lansoprazole. The chromatographic method was standardized for Lansoprazole using Shimadzu HPLC model reverse phase analytical Inspire grace C18 column (250 mm x 4.5 mm, 5mm particle size) with PC-3000-M Reciprocating Pump (40 Mpa) and UV-3000-M Detector, at 285nm and flow rate of 0.8 ml/min. The mobile phase consists of 80:20 Methanol: water. The linearity of proposed method was investigated in the range of 10-50 µm/ml (R2 = 0.999) of Lansoprazole. The limit of detection (LOD) was found to be 0.12 mm/ml. The limit of quantification (LOQ) was found to be 0.36 mg/ml. The retention time of Lansoprazole found to be 5.4 min. The method was statistically validated and % RSD was found to be less than 2 indicating high degree of accuracy and precision. Hence proposed method can be successfully applied for the estimation of Lansoprazole in further studies.

Keywords: Lansoprazole, RP-HPLC, Chromatogram, validation, estimation.

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INTRODUCTION

Selection of analytical method[1]

First stage in the selection or development of method is to establish what is to be measured and how accurately it should be measured.

The following analytical techniques are usually employed for estimations of different components in formulations:

Titrimetric and Gravimetric
Ultraviolet and Visible Spectrophotometry
Thin layer Chromatography
High Performance Liquid Chromatography

Gas Chromatography (GC)
Atomic Absorption Spectrometry (AAS)
Infra Red Absorption Spectrometry.

CHROMATOGRAPHY[3,5,6,7]

The term chromatography was first used by the Russian chemist and botanist Michael Tswett in 1906, to describe his work on the separation of colored plant pigments into bands on a column of chalk and other materials such as polysaccharides, sucrose and indulin. The term chromatography is derived from the Greek words : Chroma for color and Graphein to write. Tswett defined Chromatography as a method in which the components of a mixture are separated on an adsorbent column in a flowing system. As chromatography has progressed considerably from Tswett's time, standard organizations in some countries such as the British Standards Institute (BSI) in the UK and the American society for Testing Materials (ASTM) has produced their own definitions. In order to bring some order to the language of Chromatography IUPAC, the International Union of Pure and Applied Chemistry, in 1993 published their updated nomenclature and defination for chromatography, Unified Nomenclature for Chromatography. The IUPAC defination of Chromatography states that 'Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary while the other moves in a definite direction.

Table. No.1 Terms Associated with Chromatography[7]

Analyte	The Substance to be separated during chromatography
Bonded phase	Stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing
Chromatogram	The visual output of the chromatograph
Chromatograph	Equipment that enables a sophisticated separation eg. gas chromatography or liquid chromatographic separation
Elute	The mobile phase leave the column
Eluent	The solvent that will carry the analyte
Immobilized Phase	A stationary phase which is immobilized on the support particles or on the inner wall of the column tubing
Mobile Phase	The phase which moves in a definite direction. It may be a liquid (LC and CEC), a gas (GC), or a supercritical fluid (SFC)
Retention Time	The characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions
Sample	The matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components
Solute	Sample components in partition chromatography
Solvent	Any substance capable of solubilizing other substance
Stationary Phase	Substance which is fixed in place for the chromatography procedure

Table. No.2 Classification of Chromatographic Methods[7]

Stationary Phase	**Mobile Phase**	**Name**
Solid	Liquid	Plane Chromatography Paper Chromatography Thin Layer Chromatography Adsorption Column Chromatography High Performance Liquid Chromatography
Solid (Ion Exchange Resin)	Liquid	Ion Exchange Chromatography
Solid	Gas	Gas solid Chromatography

Instrumentation[9]

High performance liquid chromatography (HPLC) is basically a advanced form of column liquid chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That

makes it much faster. All chromatographic separations, including HPLC, operate under the same basic principle; separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules(polar and non-polar affinities) for the mobile phase and the stationary phase used in the separation.

There are few basic components in HPLC that plays a major role in the separation of the different molecules based on their affinities:

Solvent Reservoir
Pump
Sample Injector
Column
Detector
Data collection device

Similarly few important parameters are

Theoretical plates
Internal Diameter of a column
Particle and pore size of Stationary phase
Pump pressure
Detector
Auto sampler

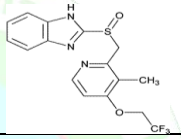
Analytical Method Development and Validation[10]

Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance. According to the International Conference on Harmonization (ICH), the most common types of analytic procedures are: (i) identification tests, (ii) quantitative tests of the active moiety in samples of API or drug product or other selected component(s) in the drug product,(iii) quantitative tests for impurities' content, (iv) limits tests for the control of impurities.

The most important parameters of validation are as follows:

Accuracy, precision, specificity, Limit of Detection, Limit of Quantitation, Linearity and range, Robustness and Ruggedness.

Table No. 3 Drug Profile[11]

S.No.	Name of Drug:	Lansoprazole
1	Structure:	
2	Molecular formula	C16H14F3N3O2.
3	Molecular weight	369.363 g/mol.
4	Chemical name	(RS)-2-([3-methyl-4-(2,2,2-trifluoroethoxy)pyridin-2-yl]methylsulfinyl)-1H benzimidazole.
5	Description	White to brownish-white crystalline powder
6	Melting point	178-182 ˚C
7	Solubility	Freely soluble in dimethylformamide; soluble in ethyl acetate, dichloromethane and acetonitrile; very slightly soluble in ether, and practically insoluble in hexane and water.
8	Mode of action	Lansoprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production.Lansoprazole is a selective and irreversible proton pump inhibitor.
9	Adverse effect	Headache, dizziness, diarrhoea, constipation, nausea, vomiting, stomach, dry mouth or throat, fatigue
10	Storge	Store in a well closed container protected from light
11	Dose	The usual dose is 15 and 30 mg daily
12	Category	Anti- ulcers

Mechansim of Action:

Pharmacokinetics:

Absorption:

The absorption of lansoprazole is rapid, with mean C_{max} occurring approximately 1.7 hours after oral dosing, and relatively complete with absolute bioavailability over 80%.Since lansoprazole is acid-labile, it is administered as a capsule containing enter-coated granules to prevent gastric decomposition and to increase bioavailability. Once lansoprazole has left the stomach, absorption is rapid and relatively complete; with absolute bioavailability over 80%.Bioavailability

may be decreased if lansoprazole is administered within 30 minutes of food intake as compared to that of a fasting state. Absorption may be delayed in patients with hepatic cirrhosis.

Distribution:

Distributed in tissue, particularly gastric parietal cells. Apparent oral volume of distribution following administration of 30mg of lansoprazole is about 0.5 L/kg.

Elimination:

Renal: Approximately 14 to 25% of a dose of lansoprazole is excreted in the urine, as conjugated and un conjugated hydroxylated metabolites. Less than 1% of unchanged lansoprazole is detectable in the urine. Biliary/fecal: Approximately two-thirds of a dose of lansoprazole is detected as metabolites in the feces. In dialysis: Lansoprazole and its metabolites are not significantly dialyzed; no appreciable fraction is removed by hemodialysis. Note: Elimination is prolonged in healthy elderly subjects, in adult and elderly patient with mild renal impairment, and in patients with severe liver disease.

Pharmacodynamics:

Lansoprazole is extensively metabolized in the liver to two main excretory metabolites that are inactive. In the acidic environment of the gastric parietal cell, lansoprazole is converted to two active compounds that inhibit acid secretion by (H+,K+)-ATPase within the parietal cell canaliculus, but that are not present in the systemic circulation.

Two metabolites have been identified in measurable quantities in plasma (the hydroxylated sulfinyl and sulfone derivatives of lansoprazole). These metabolites have very little or no anti secretory activity. Lansoprazole is thought to be transformed into two active species which inhibit acid secretion by (H^+,K^+)-ATPase within the parietal cell canaliculus, but are not present in the systemic circulation.

Standard bulk drug of Lansoprazole was provided by Swapnaroop Agency, Aurangabad. Tablets of Lansoprazole were procured from the local market. All other reagents used were of HPLC grade. HPLC (Shimadzu LC-20AT) method was developed using HPLC model reverse phase analytical Inspire grace C18 column (250 mm x 4.5 mm, 5mm particle size) with PC-3000-MReciprocating Pump (40 Mpa) Mobile phase selected for this method contained 80 parts of Methanol. Flow rate employed was 0.8 ml/min. Detection of eluent was carried out at 285 nm using UV detector. Method was developed. Standard stock solutions of pure drug were made separately in mobile phase containing 10-50µg /ml of Lansoprazole. Each solution was injected and a chromatogram was recorded. Mean retention time of Lansoprazole was found to be 5.4 min.

MATERIALS AND INSTRUMENTS:

MATERIALS AND REAGENTS:

Lansoprazole was arranged by Swapnaroop agency Aurangabad. Methanol, Water solvents were used for UV and RP-HPLC Studies of UV and HPLC grade.

Table No. 4 Name of Instruments

Sr.No.	Name of Instruments	Model	Company
1	HPLC	HPLC 3000 series	Analytical Technology Ltd
2	UV-VIS SPECTROPHOTOMETER	UV 2012	Analytical technology ltd
3	Balance	PGB 100	Wenser High Precision Balance
4	Sonicator	WUC-4L	Wenser Ultra
5	Digital pH meter	DPH-500	Global Electronics

Method:

Solubility Studies:

These studies were out to find an ideal solvent in which drugs were completely soluble and stable. It was concluded that the drug is soluble in distilled water and methanol so these were selected as solvent for further analysis.

.

Selection of Analytical Wavelength:

A stock solution of drug was prepared in distilled water and UV spectrum of 25 µg/ml solutions of Lansoprazole was taken. For RP-HLC analysis, spectra of drug were taken. Wavelength selected for analysis was such that the drug exhibited maximum absorbance. After taking spectra 285 nm was selected, as drug shows good Absorbance

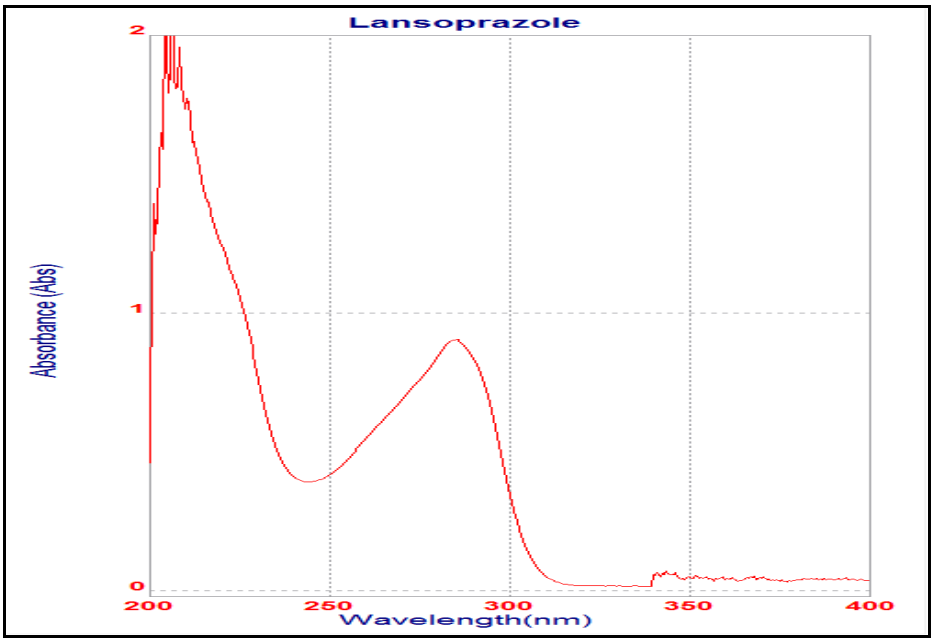


Fig. No 1. UV Absorbance of Lansoprazole

HPLC METHOD DEVELOPMENT:

RP-HPLC Analysis:

Optimization of RP-HPLC Method:

Lansopraozle was injected into the RP-HPLC system and run in different solvent systems. Mixture of different solvents were tried in order to determine optimum chromatographic conditions for effective separation.

After several permutation and combination, it was found that mixture of Methanol and water gives satisfactory results as compared to other mobile phases. Finally the optimal composition of the mobile phase 80 volume of Methanol and 20 volume of water was selected as it gave high resolution of Lansoprazole with minimal tailing. The summary of the method development is given in the table.

. **Table No. 5 Summary of Method Optimization:**

Column Used	Mobile Phase	Flow Rate	Wavelength	Observation	Result
Grace C18 (250mm x 4.6ID, Particle size:5 micron	Methanol 60ml and water 40ml	0.8 ml/min	285 nm	Peak Tailing	Method Rejected
Grace C18 (250mm x 4.6ID, Particle size: 5 micron	Methanol 70ml and water 30ml	0.8 ml/min	285 nm	Quite broad peak	Method Rejected
Grace C18 (250mm x 4.6ID, Particle size: 5 micron	Methanol 80ml and water 20ml	0.8 ml/min	285 nm	Peak with proper shape	Method Accepted

Standard solution and calibration graph:

Standard stock solution was prepared by dissolving 0.01 g of Lansoprazole in 10 ml of methanol:water (80:20) in volumetric flask to give a stock solution of 1000 µg/ml. The standard solutions were prepared by dilution of the stock solution with mobile phase Methanol: Water (80:20% v/v) to reach a concentration range 10-50 µg/ml. 20 µl injections were made for each concentration and chromatographed under the optimized conditions described above. The peak areas were plotted against the corresponding concentrations to obtain the calibration curves.

Sample preparation for Assay:

Ten Tablets containing label claim of 30 mg of Lansoprazole were weighed and finely powdered. Powdered tablets were accurately weighed, 30 mg drug transferred into a 10 ml volumetric flask, dissolved in 10 ml methanol:water (80:20) .The clear solution obtained was diluted to get appropriate concentration in linearity ranges and absorbance were measured.

Analysis of formulation:

Ten tablets of the formulation were weighed and the average weight per tablet wascalculated. Ten tablets were crushed and ground to a fine powder. Powder equivalent to 30mg of Lansoprazole was weighed and transferred to a 10 ml volumetric flask. The tablet powder was dissolved in the mobile phase .Fromthis 0.3 ml filtered solution pipette out into 10 ml volumetric flask. Then 10 ml of mobile phase isadded, and then the volume is made up to mark with mobile phase. The sample solution was ready for the analysis. After setting the chromatographic conditions and stabilizing the instrument to obtain a steady baseline, the tablet sample solution was loaded in the 10 µl fixed - sample loop of the injection port. The solution was injected and a chromatogram was recorded. The injections were repeated six times and the peak areas were recorded. A

representative chromatogram has been given inFigure-1. The peak area ratio of the drug was calculated and the amount of drug present per tablet was estimated from the respective calibration curves. The result of analysis reported in table-1.

Assay of marketed formulations:

Sample Name : Lansoprazole 30ppm of capsules
Wavelength : 285nm
Mobile Phase : Methanol:Water (80:20)
Sample volume : 20µl
Flow rate : 0.8 ml/min
Pressure :9-10MPa
Run time : 8.36min

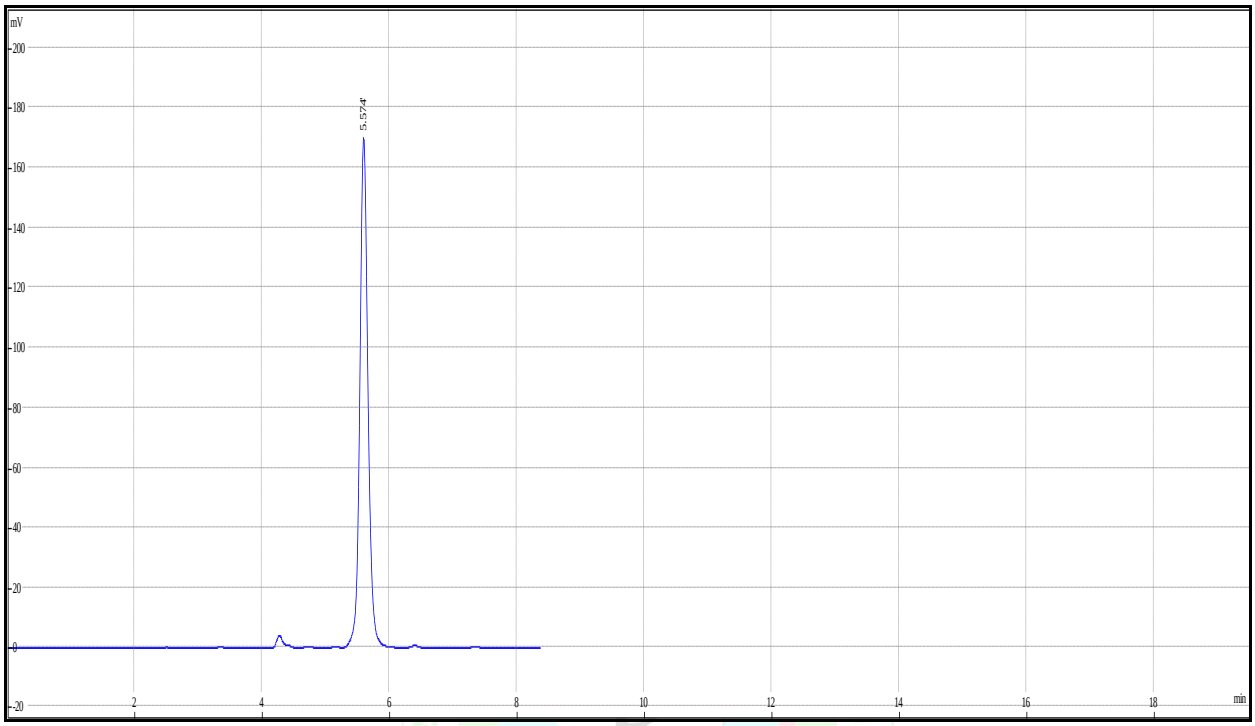


Fig. No.2 Assay of marketed formulations:

Tablet No 6: Chromatogram Assay of marketed formulations of Lansoprazole

Time	Conc	Area	Resolution	T. Plate	Asymmetry
5.574	30 ppm	1625895	00	9001	1.09

Tablet No 7: RP-HPLC Assay of Tablet Formulation

Sr.No.	Name of drug	Label Claim (mg)	Peak Area	Amount of Drug found*	% Drug content	%RSD
1	Lansoprazole	30	1621516	100.27	100.27	0.109

I Method Validation:

Linearity and range:

A calibration curve was constructed from five samples covering the total range of 10-50 µg/ml. The peak area was plotted versus the concentration. The resultant calibration curvewith a linear regression coefficient of 0.99.

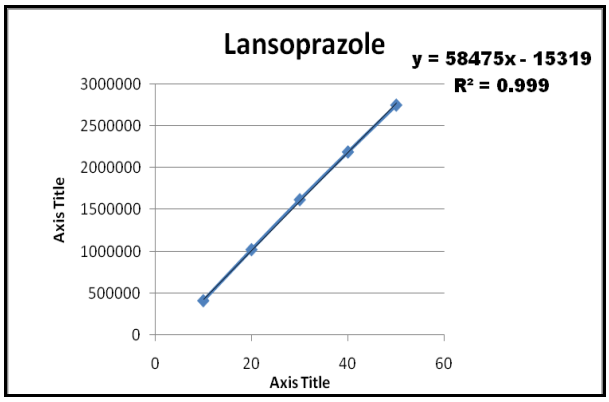
II Method Validation:

A. Linearity and Range:

Linearity was evaluated by determining five standard working solutions of drug triplicate for RP-HPLC.

Table -8 Linearity Data of Lansoprazole for RP-HPLC

Sr.No.	Conc.(µg/ml)	Peak Area
1	10	413467
2	20	1024494
3	30	1621516
4	40	2192673
5	50	2753128



.**Fig. No.3** Calibration curve of Lansoprazole by RP-HPLC

Table -9 Linear regression data of Lansoprazole for RP-HPLC.

Parameters	Lansoprazole
Linearity range (µg/ml)	10-50 µg/ml
r^2	0.999
Slope	58475
Intercept	15319

Discussion: Calibration curves showed good correlation coefficient in concentration range of 10-50 µg/ml for Lansoprazole

Precision:

Intra-assay precision (repeatability) and inter-day (intermediate) precision were determined. The analysis were performed using concentration at only one level ,i.e 30µg/ml. The concentration was analyzed in triplicate (n = 3) and intra-assay precision was found to be lessthan 2 % relative standard deviation (RSD) for all samples on all days. Inter-day precision % RSD for analyses conducted on two separate days was found to be less than 2% RSD for thesaid concentration studied.

Accuracy:

Accuracy was determined at three concentrations, similar to those used to assess the precision of the method. Each of the solutions was analyzed and the percentage standard deviation was determined. The method showed percentage RSD of less than 2 for all solutions tested which indicated good accuracy of the method.

Robustness:

Robustness was determined by varying the pH of the mobile phase by ±0.4 units and wavelength of the mobile phase by ± 2 units. The deliberate changes in the pH and wave length, did not affect the recovery of the drug which indicated the robustness of the method.

Degradation :

Sample Name : Lansoprazole 50ppm Thermally at RT for 24hrs
Wavelength : 285nm
Mobile Phase : Methanol: Water (80:20)
Sample volume : 20µl
Flow rate : 0.8 ml/min
Pressure :9-10MPa
Run time : 8.09min

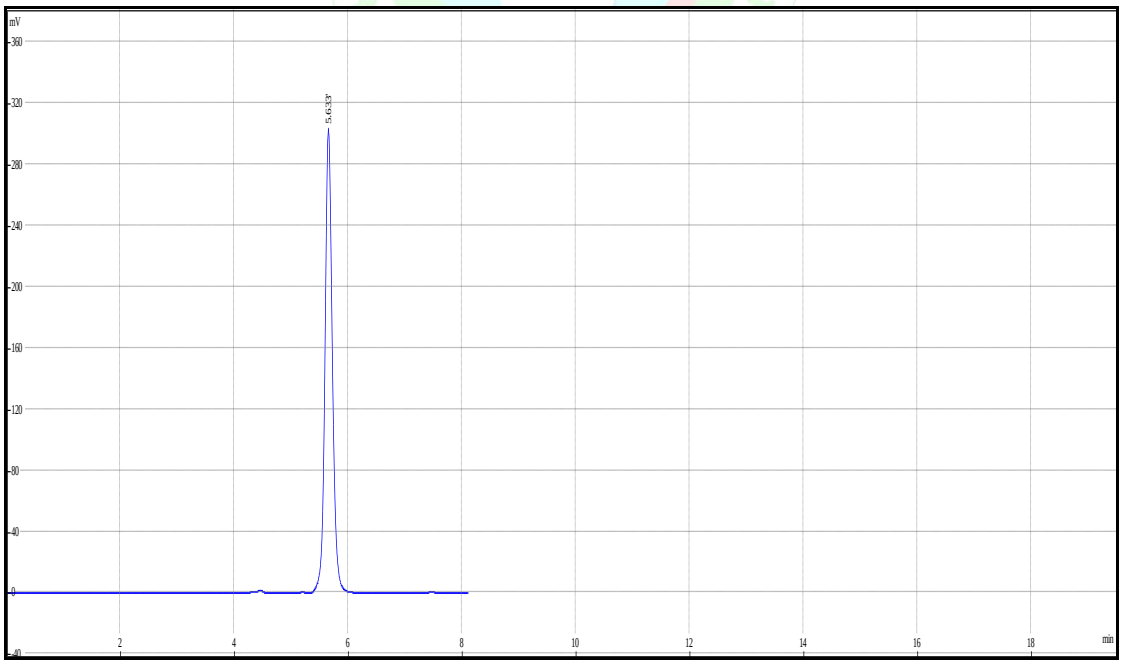


Fig. No.3 Assay of Lansoprazole formulations

Tablet No 10: Chromatogram of Assay of Lansoprazole Formulation

Time	Conc	Area	Resolution	T. Plate	Asymmetry
5.633	50 ppm	2742094	0.00	8950	1.09

Table 11: Degradation parameter of Lansoprazole formulations

Sr. NO.	Degradation	Area of	Area of degraded	Degraded	Actual %
1	0.1N Hcl at Room Temperature	2753128	659725	23.9627435	0.7603726
2	0.1N NaoH at Room Temperature	2753128	2768164	100.546142	0.9945386
3	3% H_2O_2 for at Room Temperature	2753128	361705	13.137953	0.8686203
4	Photolytic Degradation	2753128	2742143	99.6009993	0.0399
5	Thermal Degradation	2753128	2742094	99.59921951	0.400780494

Table: 12: Validation Parameters

	Validation Parameters	Lansoprazole
System suitability	Theoretical Plates	9001
Tailing Factor	Tailing Factor	1.09
Linearity	Coefficient of variation	0.999
Precision	Intraday: % RSD	0.13%
	Inter day: % RSD	0.11%
Accuracy	% Recovery	99.94
Robustness	pH ±0.4 units % SD	0.43
	Wavelength ±2 units % SD	0.22
Degradation	Degraded upto %	99.59921951
	Actual % degradation	0.400780494

Table 13: Summary of Method:

Parameters	Results
Linearity range [µg/mL]	10- 50
Regression Equation	Y= 58475 X - 15319
Correlation coefficient	0.999
LOD [µg]	0.12
LOQ [µg]	0.36
Accuracy [%RSD]	0.04 -0.24
Precision [%RSD]	
Intra-day	0.13
Inter-day	0.11
Ruggedness [% RSD]	
Analyst I	0.999
Analyst II	0.999
Robustness	Robust

RESULTS AND DISCUSSION

The developed RP-HPLC method for estimation of Lansoprazole from C_{18} column and methanol and water in the ratio of 80:20 as mobile phase. Detection was carried out using UV detector at 285nm. The method was developed. The run time per sample is just 8.29 min. Mean retention time of Lansoprazole was found to be 5.4 min. The method was validated as per ICH guidelines in terms of linearity, accuracy, specificity, intraday and interday precision, repeatability of measurement of peak area as well as repeatability of sample application and degradation studies and the results are shown in [Table –7]. Since none of the methods is reported for estimation of Lansoprazole in dosage form, this developed method can be used for routine analysis in formulation.

CONCLUSION:

The developed RP-HPLC method for estimation of Lansoprazole is accurate, precise, robust and specific. The method has been found to be better than previously reported method because of its less retention time, use of an economical and readily available mobile phase, UV detection and better resolution of peaks. The run time is relatively short, which will enable rapid quantification of many samples in routine and quality-control analysis of various formulations containing Lansoprazole. All the factors make this method suitable for quantification of Lansoprazole in bulk drugs and in pharmaceutical dosage forms without any interference. The results undertaken according to the International conference on Harmonization (ICH) guidelines reveal that the method is selective and specific.

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