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Research Article

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Development and validation of a rapid liquid chromatographic method for the analysis of Lansoprazole and its related production impurities

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ABSTRACT

A high performance liquid chromatographic (HPLC) method for the analysis of Lansoprazole and its associated impurities was examined with the aim of economic analysis, while maintaining good efficiency. The separation was carried out using a Chromatopak Peerless -C18 analytical column with a mobile phase composed of acetonitrile: buffer (500:500v/v) (buffer pH 10.0, adjusted with orthophosphoric acid) and was isocratically eluted at a flow rate of 1.0 mL min^{-1}. Column oven temperature was 30ºC. A small sample volume of 20 µL was used for each sample run, being injected into the HPLC system. The chromatogram was monitored with UV detection at a wavelength of 254 nm and the total run time was 30 min. The method was validated according to ICH (international conference on harmonization) guidelines with respect to precision, accuracy, linearity, specificity, robustness and limits of detection and quantification. All parameters examined were found to be well within the stated guidelines.

Keywords: Lansoprazole, Active pharmaceutical ingredient, Method development, Validation.

INTRODUCTION

Lansoprazole, 2-[(RS)-[[3-Methyl-4-(2, 2, 2-trifluoroethoxy) pyridin-2-yl] methyl] sulphinyl]-1H-benzimidazole. (API) (British Pharmacopoeia 2009) [Fig.1] is a proton-pump inhibitor (PPIs) which inhibits the stomach's production of gastric acidsProton-pump inhibitors (PPIs) are a group of drugswhose main action is a pronounced and long-lasting reduction of gastric acid production. They are the most potent inhibitors of acid secretion available. The group followed and has largely superseded another group of pharmaceuticals with similar effects, but a different mode of action, called H_2-receptor antagonists. These drugs are among the most widely sold drugs in the world, and are generally considered effective. The vast majority of these drugs are benzimidazole derivatives. [1][14][15]

Its main impurities are 2, 3-dimethyl-5-nitropyridine-N-Oxide (Impurity A) [refer Fig.2], 1H-benzimidazole-2-thiol, (Impurity B)[refer Fig. 3]. 2-[3-Methyl-4-(2,2,2-trifluoroethoxy) -2-pyridinyl] methylthio-1H-benzimidazole (Impurity C) [refer Fig 4]**.**[12]

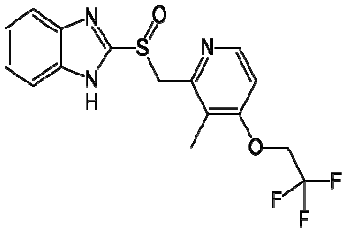

Fig 1. Lansoprazole

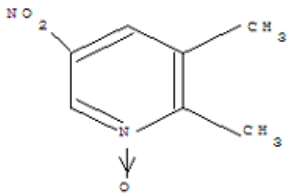

Fig 2. 2,3-dimethyl-5-nitropyridine-N-Oxide [Impurity A]

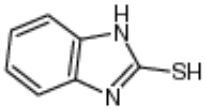

Fig 3. 1H-benzimidazole-2-thiol, [Impurity B]

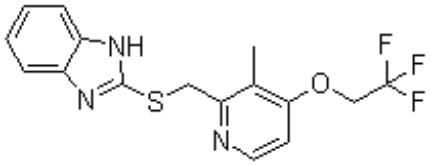

Fig 4. 2-[3-Methyl-4-(2,2,2-trifluoroethoxy) -2-pyridinyl]methylthio-1H-benzimidazole [Impurity C]

There are a number of methods described in the literature for the analysis of Lansoprazole by HPLC. TLC is also used for analysis of Lansoprazole, but are often complicated and time-consuming methods and also cannot be used for the simultaneous determination of the API and its impurities.[13]

High performance liquid chromatography (HPLC) is widely known to be one of the most important analytical techniques used in the pharmaceutical industry. [9][10]

The aim of this research was to achieve an economical, simple, faster separation of Lansoprazole and three main impurities in the bulk substance. An isocratic method was developed and validated according to ICH guidelines.

EXPERIMENTAL SECTION

Materials and reagents

Samples Lansoprazole and three impurities were received from Ultratech India Ltd. Mumbai, India. HPLC-grade acetonitrile (HPLC grade) and water (HPLC grade) were purchased from Merck (Mumbai, India).

Instrumentation

The HPLC system (Thermo) consisted of a U.V. Visible detector, column used was octadecylsilyl silica gel for chromatography R (5 µm) with a pore size of 10 nm, column size: l = 0.25 m, Ø = 4.6 mm of (Peerless, Chromatopak), at column temperature:30°C,pH meter of Lab India make.

Chromatographic conditions

An isocratic separation was carried out using a mobile phase consisting of acetonitrile- triethylamine (pH 10.0) (500:500 v/v) was used at a flow rate of 1.0ml/min with UV detection at 254nm. The column was heated to 30°C and an injection volume of 20µL was used. The mobile phase was filtered through 0.45µm nylon filters and degassed in an ultrasonic bath prior to use.

Preparation of Buffer Solution

Buffer solution was prepared by dissolving 10 mL of Triethylamine in 500 mL standard volumetric flask, dissolved with HPLC grade water pH adjusted to 10.0, with orthophosphoric acid.

Standard solution Preparation-

1. About 100 mg of Lansoprazole **Reference standard** was accurately weighed and transferred in 100 mL volumetric flask, dissolved in diluent up to the mark. (1000 ppm)
2. About 10 mg of **Impurity A** was dissolved with diluent upto the mark in 100 ml volumetric flask (100 ppm).

3. About 10 mg of **Impurity B** was dissolved with diluent up to the mark in 100 ml volumetric flask (100 ppm).
4. About 10 mg of **Impurity C** was dissolved with diluent up to the mark in 100 ml volumetric flask (100 ppm).
This solution was further diluted with diluent to obtain required ppm solutions [8].

Method development and optimization
The proposed method for estimation of related substances of Lansoprazole is validated as per the British Pharmacopoeia and ICH guidelines.Impurities determination is an integral part of pharmaceutical analysis. Here a specific, accurate, precise and cost effective method for estimation of Lansoprazole in the presence of its impurities was developed which fulfilled all parameters of validation as per given in the ICH guidelines.[6][7]

Specificity Graphs
For method optimization, a systematic examination of the mobile phase composition and flow rate was conducted. The flow rate and temperature were increased in increments taking retention times, as well as the resolution between the API and three impurities. An isocratic method using acetonitrile- triethylamine (pH10 0.) (500:500 v/v), with a flow rateof 1.0mL/min at a temperature of 30°C was found to give a retention time of between Lansoprazole and its three impurities.[3][5]

1. Selectivity
Samples of each of the separate impurities were prepared and injected 6 times. A Lansoprazole sample was also injected. All samples had different retention times. The test solution was also injected. All peaks were sufficiently separated and no interference was noted. [Refer fig 5]

2. Linearity
Each of the impurities and the API gave a linear response over the concentration ranges tested. The mean values of the slope, intercept and correlation co-efficient are given in Fig. 7-10. The impurities were run at a low concentration range 8.0 to12.0 µg/mL while the active ingredient Lansoprazole was run at a high standard range 80 to 120 µg/mL. The ICH guidelines state that a correlation co-efficient of 0.99 or over is desirable for linearity studies. All curves had values within this range showing there is a linear relationship across the range for the analytical procedure.

3. Accuracy
The percent recovery of the Impurity samples were calculated and are shown in Table 7. Good recoveries were obtained ranging from 98.83 to 99.77% for the API. The percent relative error was also calculated for each concentration giving RSD values of 0.13 and 0.57%. [Refer table no 7]

4. LOQ and LOD
The LOQ and LOD were determined based on signal-to noise ratios, where the analytical responses of approximately 10 and 3, respectively, were used. The concentrations found are seen in Table 8.

Limit of Detection (LOD) The detection limit of an individual analytical procedure
is the lowest amount of analyte in a sample, which can be detected but not necessarily quantities as an exact value. Based on the Standard Deviation of the Response and the Slope, The detection limit (DL) may be expressed as:

$$DL = \frac{3.3\,\sigma}{S}$$

Where, б = the standard deviation of the response for the lowest conc. in the range.
S = the slope of the calibration curve.

Limit of Quantification (LOQ)
The quantification limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be quantitatively determined with suitable precision and accuracy. Based on the Standard Deviation of the Response and the Slope, The quantitation limit (QL) may be expressed as:

$$QL = \frac{10\,\sigma}{S}$$

Where, ϭ = the standard deviation of the response for the lowest conc. in the range
S = the slope of the calibration curve.

5. Precision:

I. Repeatability:

Repeatability studies were performed by injecting 6 replicates of the Lansoprazole test solution (100 µg/mL). Repeatability studies on the impurities were performed by injecting 6 replicates of a 10µg/mL standard of the individual impurities. The %RSD values were found to range between 0.14% and 0.45% (Table 4). Results met with the test specifications for the API (1.5%) and the acceptable limit of 10% for the impurities.

II Intermediate:

Intermediate precision expresses within-laboratories variations:

i. Analysis on different day: was studied by injecting 100 µg/ ml of the drug, and process was repeated next day for three times each.
ii. Changing Chemist (3 chemists)

6. Robustness-

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage and done by observing - influence variations of buffer pH in a mobile phase, changing wavelength and flow rate.

RESULTS AND DISCUSSION

Main objective of this analytical method development was to separate Lansoprazole from Impurity A, Impurity B and Impurity C. Different Mobile phases and different stationary phases were tried but effective chromatographic separation was achieved with a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with octadecylsilyl silica gel. Flow rate of mobile phase was adjusted to 1.0 ml/min. Mobile phase composed of acetonitrile: buffer (500:500v/v). Buffer was prepared by dissolving 10mL of triethylamine in 500 mL standard volumetric flask, dissolved with HPLC grade water.Adjusted pH 10.0, with orthophosphoric acid. UV detector was set at 254 nm with column temperature 30°C. Peak shapes and separation of Lansoprazole and impurities were as follows:

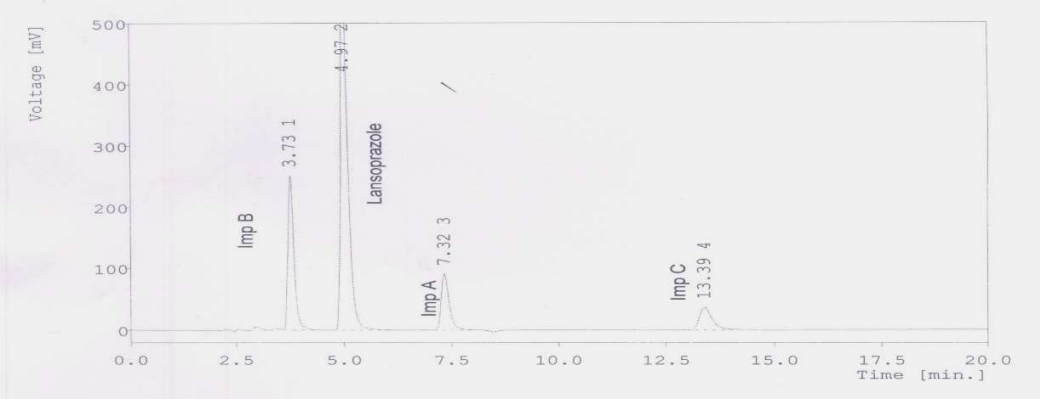

Fig 5 Typical chromatogram of specificity

Table 1 Retention Time

Sr.No	Name of API & its Impurity	Retention Time
1	Blank	--------
2	Lansoprazole (100 ppm)	4.97
3	Impurity A (10 ppm)	7.32
4	Impurity B (10 ppm)	3.73
5	Impurity C (10 ppm)	13.39

Specificity and selectivity studies results

Selectivity of the method was performed by separately injecting individual impurities and none of these impurities were seen to interfere with the Lansoprazole peak with minimum resolution of 1.24 between any two peaks. No interference of blank was observed (fig 6)

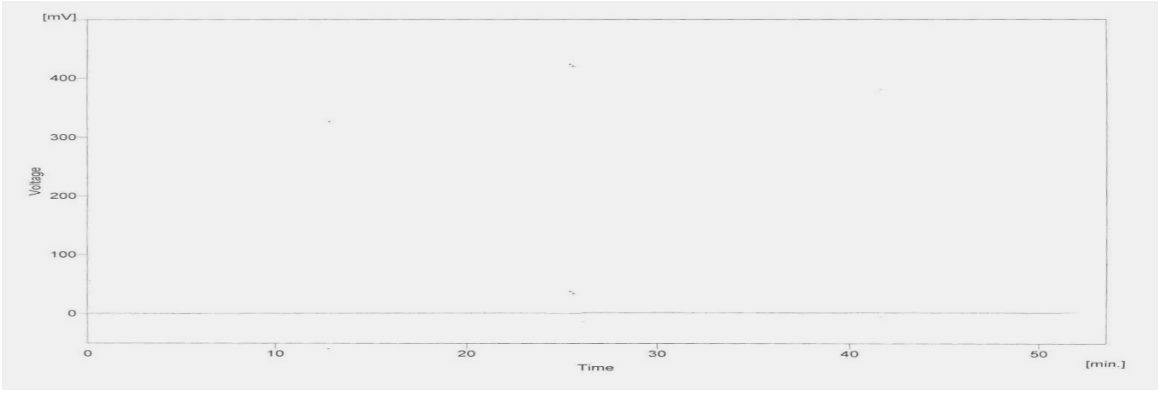

Fig 6 Typical chromatogram of Blank

Specificity Impurities were added to the stock solution and the mixture was subjected to chromatographic analysis and it was observed that impurity peaks were well resolved from peak of Lansoprazole (fig 5);system suitability parameters are shown in**(Table 1).**The method was considered to be specific since there was no interfering peak at the retention time of Lansoprazole and also the peak was well resolved from the peaks of all impurities.[2][4]

Linearity The data obtained in the linearity experiments was subjected to linear-regression analysis. A linear relationship between peak areas and concentrations was obtained in the range of 80- 120 µg ml-1with r = 0.999(Table 2) for Lansoprazole and for process impurity range was 8-12 µg ml-1 with r =0.998 Impurity A, r=0.997 Impurity B, r=0.998, Impurity C. [5] (Table 3).

Linear calibration plot for the method was obtained over the calibration ranges tested.
Stock solution: Lansoprazole (1000 ppm) andImpurity A,ImpurityB and Impurity C (100 ppm)

Table 2 Lansoprazole Linearity

Volume Of Stock Solution (ml)	**Final dilution (ml)**	**Final Conc. (µg/ml)**	**Area**			**Mean Area**	**Relative standard deviation (%)**
			1	2	3		
0.8	10	80	5389.76	5379.08	5297.63	5355.49	0.94
0.9	10	90	6065.27	6055.76	6076.33	6065.78	0.16
1.0	10	100	6634.47	6614.43	6594.90	6614.6	0.29
1.1	10	110	7363.77	7343.64	7381.83	7363.08	0.25
1.2	10	120	8021.53	8032.73	8049.07	8034.44	0.17
						Average	**0.368**
						Slope	**66.55**
						***Co-rel**	**0.999**

Table 3: Process Impurities

Volume Of Stock Solution (ml)	Final dilution (ml)	Conc in ppm	Mean Area of Imp A	Mean Area of Imp B	Mean Area of Imp C
0.8	10	8	843.91	1884.96	745.85
0.9	10	9	959.80	2043.14	818.69
1.0	10	10	1111.23	2299.81	910.49
1.1	10	11	1162.51	2638.03	1044.81
1.2	10	12	1293.23	2847.34	1134.20
*RSD			0.18	0.0964	0.514
Slope			110.13	251.96	100.28
*Co-rel			0.998	0.997	0.998

Co-rel : Correlation Coefficient
RSD : Relative standard Deviation

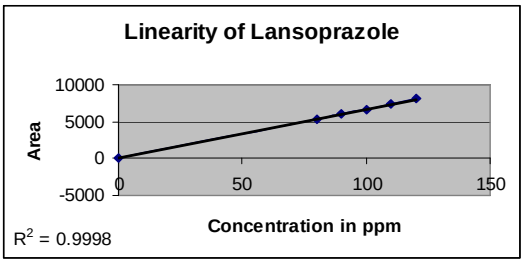

Fig 7. Linearity plot of Lansoprazole

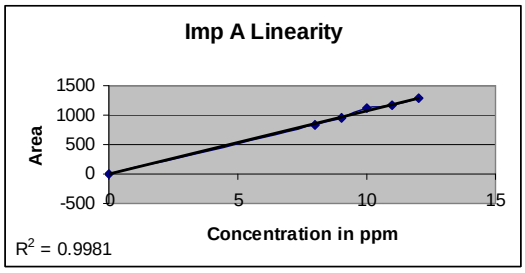

Fig 8. Linearity plot of Imp A

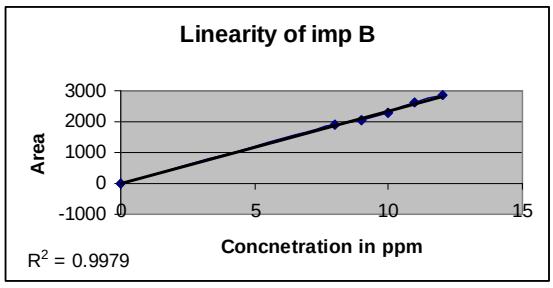

Fig 9. Linearity plot of Impurity B

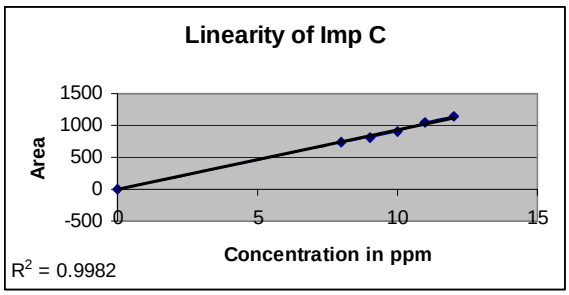

Fig 10. Linearity plot of Impurity C

Precision The developed method was found to be precise as the % RSD value for repeatability studies was less than 2.0%, where as the %RSD for inter-day precision was also less 2.0%. (Refer Table 4, 5 and 6).

Five replicate injections of Lansoprazole (100 ppm) and process Impurity A, Impurity B and Impurity C each of 10 ppm was made. The results for each impurity are summarized in the following table:

Table 4 Precision

Injection Details	Standard Deviation	Relative standard Deviation
Lansoprazole	9.86	0.145
Impurity A	4.566	0.439
Impurity B	8.22	0.45
Impurity C	2.65	0.29

Table 5- Intra day Precision

Injection Details	Standard Deviation	Relative standard Deviation
Lansoprazole	9.17	0.13
Impurity A	4.85	0.432
Impurity B	8.1	0.33
Impurity C	7.39	0.81

Table 6 - Change of chemist

Injection Details	Standard Deviation	Relative standard Deviation
Lansoprazole	16.26	0.23
Impurity A	9.73	0.92
Impurity B	8.81	0.34
Impurity C	8.46	0.93

Accuracy

The results of recovery studies for accuracy was calculated. Recovery observed was(98.83 -99.77%) for Lansoprazole process impurities (Refer table 7).

Table 7 - Accuracy (Mean recovery of all impurities at each level)

Sr.No	Percentage	Impurity A	Impurity B	Impurity C
1	**Level - 1 80%**	99.98	99.71	98.58
2		99.67	99.92	98.21
3		99.62	99.80	98.37
4	**Level 2 -100%**	99.57	99.57	98.92
5		99.72	99.72	98.19
6		99.77	99.77	98.71
7	**Level 3 -120%**	99.73	99.73	99.53
8		99.82	99.82	99.73
9		99.90	99.90	99.27
	Mean	**99.75**	**99.77**	**98.83**
	SD	**0.13**	**0.10**	**0.56**
	RSD (%)	**0.13**	**0.10**	**0.57**

Recovery limit - 90% -110% RSD NMT 2.0%

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The LOD and LOQ were found to be 1.02 ppm and 3.40 ppm for Lansoprazole and for corresponding process impurities LOD was 0.05 ppm, 0.02 ppm 0.13 ppm LOQ was 0.17, 0.1, and 0.44 respectively. (Refer Table 8)

The results of each impurity are summarized in the following table:

Table 8 LOD/LOQ

	Average Standard Deviation	Slope of Calibration Curve	Detection Limit in ppm	Quantitation Limit in ppm
Lansoprazole	22.68	66.55	1.022	3.40
Impurity A	1.97	110.13	0.053	0.17
Impurity B	2.30	251.96	0.02	0.10
Impurity C	4.49	100.28	0.134	0.44

Robustness:

The method was tested for capacity to remain unaffected by small variation in method parameters, such as change of flow rate, change of wavelength, change of pH. Sample of Lansoprazole and its process impurities were analyzed for the same. It was observed that the method is unaffected by small changes in the experimental conditions. Which confirms robustness of the method. Results are as follows. (Refer Table no 9, 10, 11)

Table 9: Change of Flow rate

Flow Rate	Lansoprazole (100ppm)		Impurity A (10 ppm)		Impurity B (10 ppm)		Impurity C (10 ppm)	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
0.8	5.81	8116.03	9.33	1235.82	4.67	1343.84	16.47	1108.97
	5.82	8120.05	9.32	1236.78	4.67	1345.16	16.47	1096.15
	5.81	8125.28	9.33	1238.32	4.68	1347.99	16.48	1103.92
1.0	4.98	7027.94	7.32	1126.18	3.74	2301.66	13.35	909.73
	4.98	7030.84	7.32	1130.93	3.75	2302.32	13.35	912.69
	4.98	7.32.88	7.34	1134.14	3.75	2301.44	13.35	314.70
1.2	3.96	5465.42	6.22	905.42	3.12	1831.03	11.04	760.65
	3.96	5479.55	6.21	906.18	3.12	1829.86	11.04	765.91
	3.96	5487.64	6.22	910.86	3.12	1828.12	11.05	771.37

Table 10 Change of Wavelength

Wave length	Lansoprazole (100ppm)		Impurity A (10 ppm)		Impurity B (10 ppm)		Impurity C (10 ppm)	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
252	4.87	6107.41	7.45	1094.81	3.73	3048.55	13.30	973.98
	4.87	6116.86	7.45	1097.10	3.73	3052.57	13.30	966.89
	4.87	6128.95	7.45	1095.74	3.73	3054.36	13.30	971.36
254	4.83	6635.36	7.50	1126.18	3.74	2301.66	13.35	909.73
	4.83	6636.62	7.50	1130.93	3.74	2302.32	13.35	912.69
	4.83	6638.04	7.50	1134.14	3.74	2301.44	13.35	914.70
256	4.82	7538.28	7.46	1200.33	3.73	2010.92	13.27	924.46
	4.82	7541.17	7.46	1203.92	3.73	2008.48	13.27	927.34
	4.82	7549.11	7.46	1206.61	3.73	2010.51	13.27	931.17

Table 11 Change of pH

pH	Lansoprazole (100ppm)		Impurity A (10 ppm)		Impurity B (10 ppm)		Impurity C (10 ppm)	
	RT (min)	Area	RT (min)	Area	RT (min)	Area	RT (min)	Area
9.8	5.21	4565.62	7.20	638.08	3.76	1946.99	12.95	547.37
	5.21	4560.73	7.20	640.13	3.76	1948.98	12.95	548.46
	5.21	4559.84	7.20	643.51	3.76	1950.22	12.95	549.01
10.0	4.83	6635.36	7.51	1126.18	3.74	2301.60	13.35	909.73
	4.83	6636.62	7.51	1130.93	3.74	2302.32	13.35	912.69
	4.83	6638.04	7.51	1134.14	3.74	2301.44	13.35	914.70
10.2	5.08	5794.22	7.42	690.23	3.77	2482.47	13.68	872.63
	5.08	5792.03	7.42	693.86	3.77	2486.43	13.68	862.06
	5.08	5794.22	7.42	698.10	3.77	2488.87	13.68	869.38

CONCLUSION

The analytical method was found to be specific as proved by injecting known components into the chromatographs when limit of detection and limit of quantitation for impurities has been established

The analytical method was found to be linear over a specified range, and to be precise, accurate, rugged and robust. The above mentioned isocratic method for the analysis of Lansoprazole and its related substance was found to be simple, rapid and sensitive. The method facilitated the separation of three of know impurities of drug with good resolution. Hence method was completely evaluated for its linearity precision, accuracy robustness, limit of quantitation and detection.

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