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RESEARCH ARTICLE

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STABILITY INDICATING ASSAY METHOD FOR LANSOPRAZOLE A COMPARATIVE STUDY BY UPLC AND HPLC

ABSTRACT

Lansoprazole is indicated for treatment of ulcers of the stomach and reflux Zollinger – Ellison's syndrome, treatment of H. pylori infections alongside antibiotics. Lansoprazole is belongs to the class of a proton pump inhibitors. A simple, Isocratic, rapid and selective RP-UPLC method was developed for the quantification of Lansoprazole in active pharmaceutical ingredients (API'S) using a simple mobile phase of Water: Acetonitrile: Triethyl amine in the raio of 50:50:1 and the pH of the mobile phase was adjusted to 6.8 with ortho phosphoric acid. Water: Acetonitrile: Triethyl amine in the ratio of 50:50:1 and the pH of the mobile phase was adjusted to 10.5 with ortho phosphoric acid was used as a sample diluent at a flow rate of 0.70 mL/min. The chromatographic separation was achieved on a Acquity uplc HSS T3 column of dimension 2.1 X 100 mm, 1.8 µm. The column and sample compartment temperatures were maintained at ambient. UV detector was monitored at 285 nm. The retention time (RT) of Lansoprazole was about 2 min at a overall runtime of 6 min. The developed method was validated as per the requirements of ICH guidelines. Finally a comparison of the method was done against conventional HPLC. The developed method was superior with respect to analysis time, efficiency and sensitivity. The runtime of Lansoprazole in conventional hplc method was about 15 min. Compare to HPLC method the developed method is cost effective and more rugged. The method can be used for routine analysis of assay in Quality Control laboratory on regular basis.

KEY WORDS Ultra performance liquid chromatography (UPLC), Lansoprazole assay, Stomach ulcers, method development and validation, comparison study.

INTRODUCTION

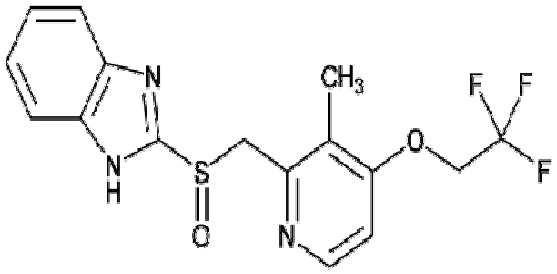

Figure-1: Structure of Lansoprazole

Lansoprazole is chemically 2- ([3-mehyl-4-(2, 2, 2 tri fluoroethoxy) pyridine-2-yl] methyl sulfinyl)-1H- benzo (D) imidazole, it is a class of proton pump inhibitors. It is an official drug listed in United States Pharmacopoeia (USP). Its active metabolite is absorbed with cytosine group in H+/K+ ATP ASE there by inhibiting the ability of parietal cells to produce gastric acid. Plasma elimination half life is 1.5 hours and is not proportional to the duration of drugs effect. It is indicated for treatment of ulcers of the stomach and reflux Zollinger – Ellison's syndrome, treatment of H. pylori infections alongside antibiotics [1]. The structures of Lansoprazole, Related compound A, Related compound B and Related compound C are shown in the figures-1, 2, 3 and 4 respectively.

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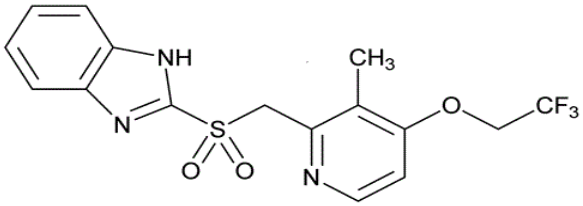

Figure-2: Structure of Lansoprazole Related compound A

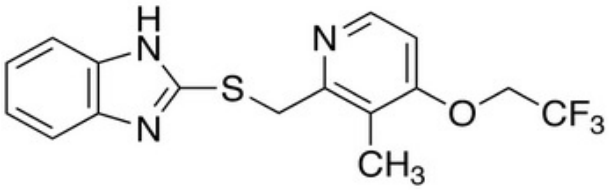

Figure-3: Structure of Lansoprazole Related compound B

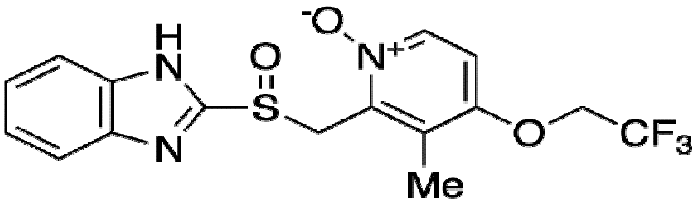

Figure-4: Structure of Lansoprazole Related compound C

Literature review reveals that some analytical methods have been reported for Lansoprazole by spectroscopic determination [3-7] and voltammetric [8].methods; in biological fluids Using LCMSMS and validated LC method [9-18] for the estimation of Lansoprazole in bulk and tablet dosage form.

MATERIALS

Lansoprazole API and its impurities and 4-Ethoxy Acetophenone were purchased from USP, Acetonitrile was obtained from rankem. Triethyl amine was purchased from Qualigens.

EQUIPMENTS

Waters Acquity UPLC system (Waters, USA), An Agilent HPLC system (Agilent, USA) equipped with binary gradient pump, auto sampler, column oven and photodiode array detector (PDA) was employed for analysis. Chromatographic data was acquired using Empower 2 software. Millipore Milli Q Plus water purification system were used for the analysis. Acquity UPLC HSS T3 column of dimension 2.1 X 100 mm, 1.8 µm and Metler balance were used for the study.

Chromatographic conditions

The Analysis was performed on Acquity UPLC HSS T3 column under reverse phase condition. The selected mobile phase composition was, Water: Acetonitrile: Triethyl amine in the raio of 50:50:1 and the pH of the mobile phase was adjusted to 6.8 with ortho phosphoric acid. The flow rate of the mobile phase was 0.7 mL/min. The column temperature and sample compartment

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temperature were maintained at ambient. The injection volume was 1.0 µL. Water: Acetonitrile: Triethyl amine in the raio of 50:50:1 and the pH of the mobile phase was adjusted to 10.5 with ortho phosphoric acid was used as sample diluent and detector was monitored at 285 nm. The retention time (RT) of Lansoprazole was about 2 min at a overall runtime of 6 minutes.

Preparation of standard and sample solutions

Internal Standard: 500 mg of 4-Ethoxy Acetophenone weighed and quantitatively transferred into 200 ml volumetric flask and diluted to volume with diluent.

50.0 mg of Lansoprazole reference standard and samples were separately weighed and transferred quantitatively into a 10 ml volumetric flask and diluted to volume with Internal Standard (IS) and further 1.0 ml this solution is diluted to 50 ml volumetric flask and make up to volume with diluent, to obtain a concentration of 0.1 mg/ml.

RESULTS AND DISCUSSIONS

Method Development

A simple isocratic method was selected with Acetonitrile and buffer composition in the ratio of 1:1. To ensure the specificity of the method specified impurities were individually injected and to prove the stability indicating nature of the method forced degradation studies were carried out. Blank and system suitability solution chromatograms are shown in figure-5 and 6. Blank solution chromatogram was free of interferences and system suitability chromatogram met as per USP system suitability criteria. Sample chromatogram acquired by uplc and hplc are given in figure-7 and figure-8 respectively. Chromatographic data of system suitability are shown in Table-I. A comparative chromatographic performance data of HPLC and UPLC are indicated in the Table-II. The elution of all the components in UPLC was observed to be three folds minimized to that of HPLC. The resolution and efficiency obtained for all the components selected in the study by UPLC showed comparatively better than HPLC. The tailing factors of all the components are less than 2.0.

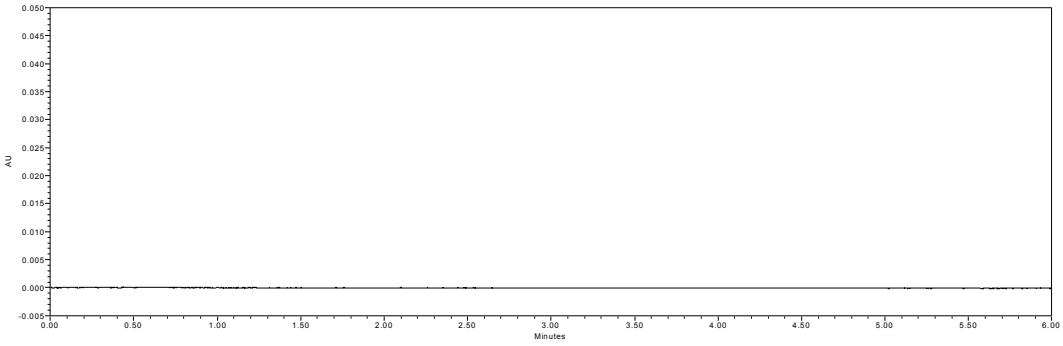

Figure-5: Blank chromatogram acquired by UPLC

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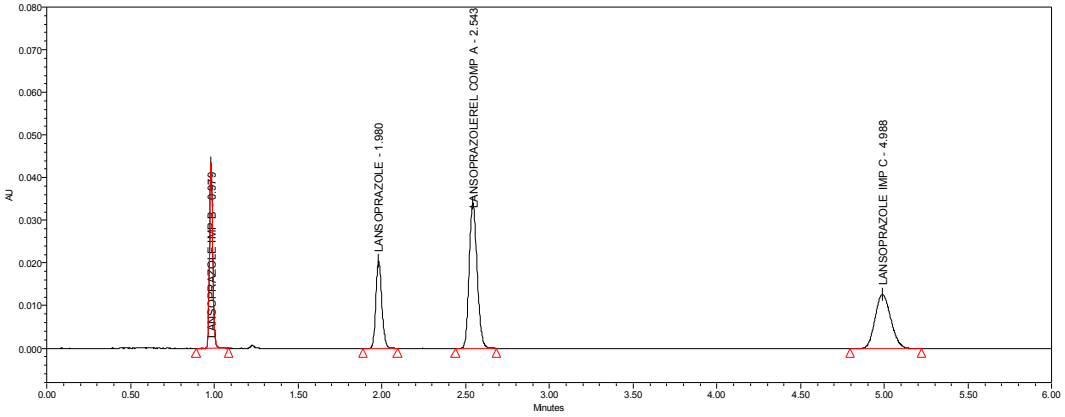

Figure-6: System suitability Chromatogram acquired by UPLC

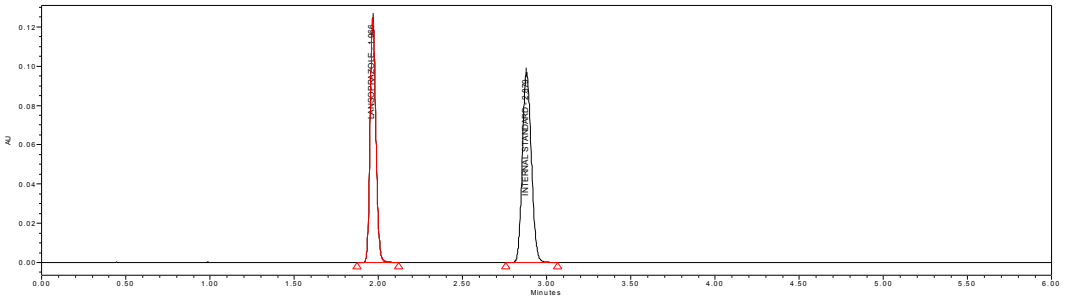

Figure-7: Assay sample chromatogram acquired by UPLC

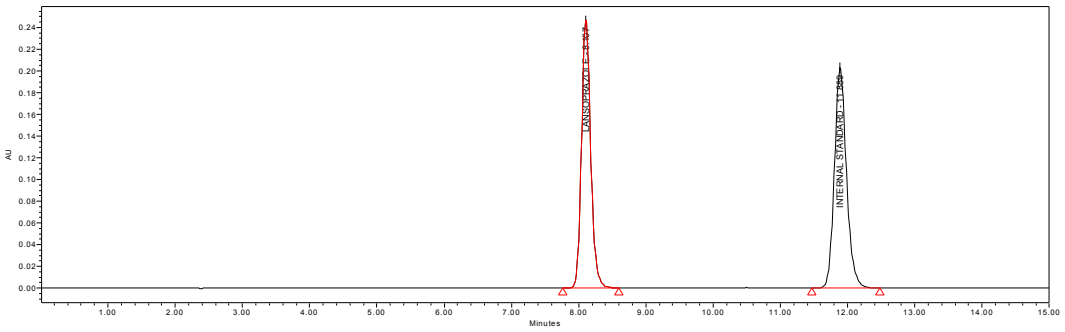

Figure-8: Assay sample chromatogram acquired by HPLC

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Table-I: system suitability results

S.No	Name of the compound	Retention Time(min)	USP Resolution	USP Tailing	USP Plate Count
1	Lansoprazole Rel. comp B	0.98	NA	1.2	12640
2	Lansoprazole	1.98	19.6	1.2	14762
3	Lansoprazole Rel. comp A	2.54	7.2	1.2	13350
4	Lansoprazole Rel. comp C	4.99	18.6	1.1	13399

Table-II: Comparison of system suitability data by UPLC and HPLC

	Name of drug component	Retention Time(min)		USP Resolution		USP Tailing		USP Plate Count	
		UPLC	HPLC	UPLC	HPLC	UPLC	HPLC	UPLC	HPLC
1	Rel. compound B	1.48	6.09	NA	NA	1.2	1.2	16034	10041
2	Lansoprazole	1.94	8.11	9.7	3.2	1.2	1.1	29170	17989
3	Rel. compound A	2.51	10.51	11.6	8.5	1.2	1.1	40017	18674
4	Rel. compound C	2.72	11.2	3.4	4.2	1.1	1.2	25299	35482

Figure-9: Spiked sample Chromatogram

Specificity

To conform the specificity of the method, sample was spiked with three impurities.Figure-9 represents spiked sample chromatogram.It was observed that the sample has no interference with the impurites and the peak purity angle is less than that of threshold which indicates the homogenity of the peak.

Precision

The precision of the assay method was evaluated by carrying out five replicate injections of Lansoprazole (0.1 mg mL−1) qualified reference standard. The percentage of RSD of five injections was calculated. The % RSD of areas was less than 0.5% which indicates the reproducibility of the system. Overlaid chromatogram of five replicate injections is given in figure-10. The tabulated data of precision are mentioned in Table-III.

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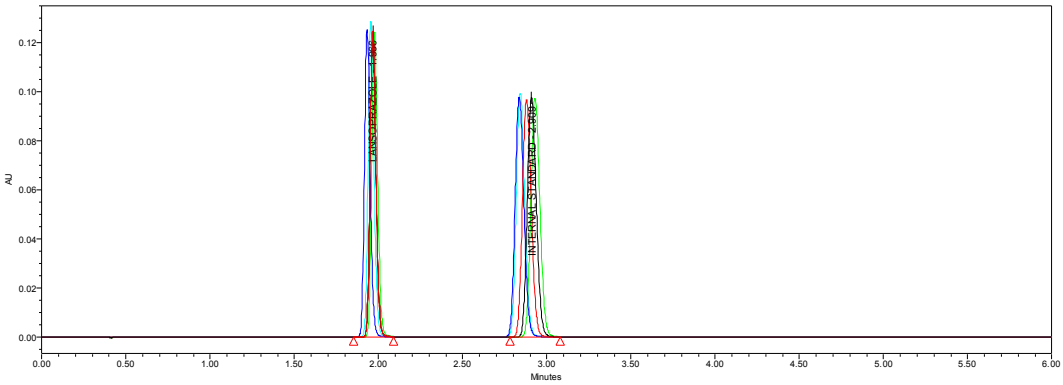

Figure-10: five replicate standard injection chromatogram

Table-III: precision results

S.No.	Retention Time		Area		Area ratio	
	Lansoprazole	IS*	Lansoprazole	IS*	Lansoprazole	IS*
1	1.97	2.88	310145	360248	0.8069	1.0000
2	1.95	2.84	310579	358894	0.8654	1.0000
3	1.98	2.88	311400	360013	0.8650	1.0000
4	1.96	2.84	315853	364290	0.8670	1.0000
5	1.97	2.89	316427	366342	0.8637	1.0000
MEAN	1.97	2.87	312881	361957	0.8644	1.0000
SD	0.01	0.024	3016.00	3191.9	0.0022	0.0
%RSD	0.6	0.8	1.0	0.9	0.26	0.0

*IS=Internal standard

Linearity

Linearity solutions were prepared from stock solution at five concentration levels from 40% to 160% of analyte concentrations (0.04 to 1.6mg mL−1). The linear regression analysis of Lansoprazole was constructed by plotting the peak area of the analyte (y) versus analyte concentration in (x) axis. The calibration curve was linear in the range of 0.04 to 1.6 mg/ml for Lansoprazole with a correlation coefficient of more than 0.999. The linearity plot is summarized in Figure -11 and the data are given in Table -IV.

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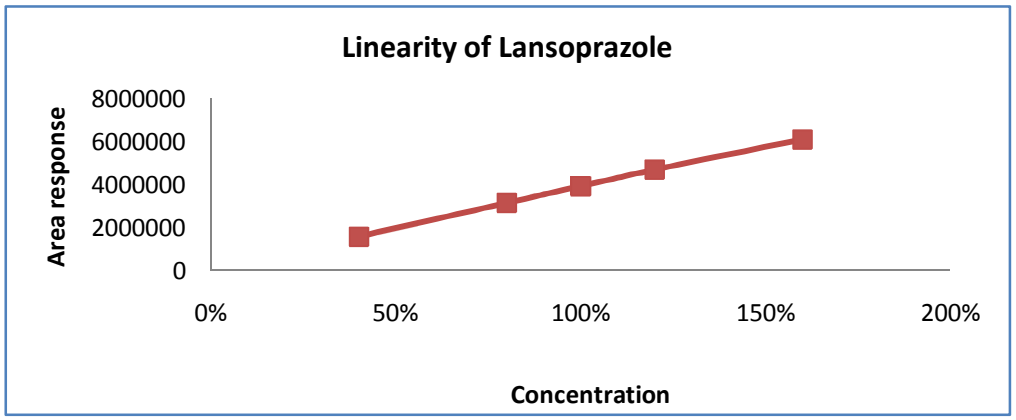

Figure-11: Linearity plot of Lansoprazole

Table-IV: Linearity results

S.No.	Name of the compound	Conc. of Lansoprazole (mg/ml)	Area		Area ratio	Standard Level
			Lansoprazole	Internal standard		
1	Lansoprazole	0.04	125657	149718	0.8393	40%
2	Lansoprazole	0.08	254917	297828	0.8559	80%
3	Lansoprazole	0.10	312614	366765	0.8524	100%
4	Lansoprazole	0.12	374448	438688	0.8536	120%
5	Lansoprazole	0.16	508733	601281	0.8461	160%
	Correlation coefficient		0.999			

Robustness

The robustness of the method was determined by making deliberate changes in the chromatographic conditions, such as change in mobile phase composition, flow rate and column temperature. In the all above conditions, the components of the mobile phase kept constant. It was observed that in all above conditions, there are no marked changes in the chromatograms, which demonstrates robustness of the method.

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Forced degradation studies

The parent drug stability test guideline Q1A (R2) issued by the International conference on Harmonization (ICH) suggests that stress studies should be carried out on a drug to establish its inherent stability characteristics, leading to identification of degradation products and hence supporting the suitability of the proposed analytical procedures. It also requires that analytical test procedures for stability samples should be stability-indicating and should be fully validated. Stress testing of a drug substance is to identify the likely degradation products, which can help to establish the degradation pathways [19] and the intrinsic stability of the molecule. Acidic, basic, peroxide, heat and humidity, dry heat and photo stability parameters were performed in order to prove the stability indicating nature of the method. In all the conditions the purity angle is less than that of the purity threshold. The results were tabulated in Table-V.

CONCLUSION

The developed method is specific, accurate and precise. The method is suitable for quantification of Lansoprazole. The shorter run time demonstrates that the method is cost and time effective aiming towards green chemistry. Thus developed RP-UPLC method was specific and stability indicating. This method can be effectively transferred to quality control labs and can be used for carry out the quantitative analysis Lansoprazole.

Table-V: Forced degradation results

Stress condition	Conc. µg/mL	Match angle	Match threshold	Purity angle	Purity Threshold	Mass balance
Non stressed	102	0.44	1.25	0.15	0.45	100
Acid hydrolysis	105	0.35	1.23	0.28	0.55	98.8
Base hydrolysis	104	0.75	1.42	0.32	0.53	98.1
Oxidation	106	0.69	1.20	0.31	0.55	100.1
Heat & Humidity	104	0.44	1.99	0.29	0.59	99.2
Photo stability	107	0.66	1.88	0.22	0.55	99.5
Dry heat	102	0.60	1.76	0.11	0.34	100.2

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