International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

"Development and validation ofanLC-MS/MSmethod for the determination ofLansoprazole in humanplasma"

Pavan kumar Gangavarapu,
PhD, Senior Director, Amneal Pharmaceuticals, LLC, USA

Date Of Submission: 20-03-2021 Date Of Acceptance: 05-04-2021

ABSTRACT: Background: For the determination of lansoprazole (LNZ) in human plasma, a fast and sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay system has been developed and thoroughly validated.
Objectives: To develop a liquid chromatographic method for the determination of Lansoprazole.
Methods: In order to develop a liquid chromatographic method for the determination of Lansoprazole using an isocratic Shimadzu HPLC equipment comprising of two LC10AT VP pumps, VP CTO-10AS VP column oven, a Hypurity advance C_{18} column (4.6 ID X 50 mm, 5µ) and anAPI 4000 (MDS Sciex) mass detector was used for chromatographic separation. The contents of the tubes were vortexed and transferred intoauto-samplervialsandthenanalyzedbyLC-MS/MS.Analiquotof10µLofthe sample was drawn each time from the vials in the auto sampler. Data acquisition was done by using Analyst 1.4.2 software.
Results: Within-batch accuracy for LLOQ QC ranged from 104.54 to 108.85%. Within-batch accuracy for LQC, MQC1, MQC2 and HQC ranged from 92.91 to 112.01%. Intra-day accuracy for LLOQ QC was 107.15%Intra-day accuracy for LQC, MQC1, MQC2 and HQC ranged from 94.72 to 101.33%. The outcomes were well within the acceptable limits of the intra day precision tests. The cumulative average analyte recovery was found to be 92.91 percent.
Conclusion: The analyte was shown to be stable in the stability analysis. The analysis technique developed and validated was found to be quick, easy, precise, responsive, reliable and cost-effective compared to other techniques published. In the analysis of a preclinical pharmacokinetic sample, the procedure has been effectively extended with the required specificity and precision together with high throughput.
KEYWORDS: Lansoprazole, Benzimidazole, Proton pump inhibitor, LC-MS

I. INTRODUCTION

Drugprofile: Lansoprazole, a benzimidazole derivative, is a proton pump inhibitor that acts on the membrane H+/K+-ATP (adenosine triphosphatase) in gastric parietal cells.[1]It is effective in the treatment of various peptic diseases, including gastric and duodenal ulcer, reflux esophagitis, Zollinger–Ellison syndrome, and other hyperacidic-related conditions.[2] Lansoprazole is metabolized in the liver and the main metabolites are 5- hydroxy lansoprazole and lansoprazole sulphone. Formation of the 5-hydroxy metabolite is mainly by cytochrome P4502C19 (CYP2C19), whereas CYP3A4 is involved in the formation of the sulphone.[3] It is clinically important to measure CYP2C19 activity using the hydroxylation and sulfoxidation indexes of lansoprazole, which reflects phenotype, and genotype of CYP2C19.[4] Lansoprazole is active against Helicobacter pylori.[5]The plasma elimination half-life of lansoprazole does not reflect its duration of suppression of gastric acid secretion. Thus, the plasma elimination half-life is less than two hours, while the acid inhibitory effect lasts more than 24hours.[6]

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

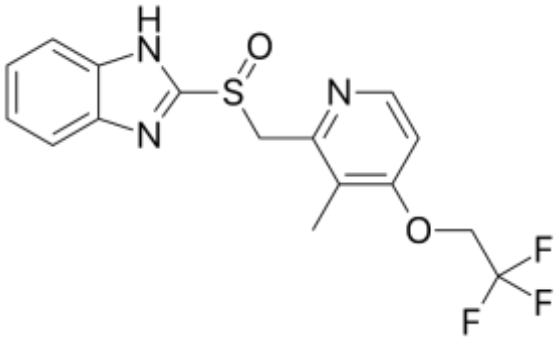

Figure 1: Molecular structure of Lansoprazole

Antisecretory activity: After oral administration, lansoprazole was shown to significantly decrease the basal acid output and significantly increase the mean gastric pH and percent of time the gastric pH was >3 and >4. Lansoprazole also significantly reduced meal- stimulated gastric acid output and secretion volume, as well as pentagastrin-stimulated acid output. In patients with hypersecretion of acid, lansoprazole significantly reduced basal and pentagastrin- stimulated gastric acid secretion. Lansoprazole inhibited the normal increases in secretion volume, acidity and acid output induced byinsulin.[7]

Drug interactions: The absorption of certain drugs may be affected by stomach acidity, and, as a result, lansoprazole and other PPIs that reduce stomach acid also reduce the absorption and concentration in blood of ketoconazole and increase the absorption and concentration in blood of digoxin. This may lead to reduced effectiveness of ketoconazole or increased digoxin toxicity,respectively.[8]

Side effects: Lansoprazole like other PPIs were well-tolerated. The most common side effects are diarrhea, nausea, vomiting, constipation, rash and headaches. Dizziness, nervousness, abnormal heartbeat, muscle pain, weakness, leg cramps and water retention rarely occur.[9]

High doses and long-term use (1 year or longer) may increase the risk of osteoporosis-related fractures of the hip, wrist, or spine. Therefore, it is important to use the lowest doses and shortest duration of treatment necessary for the condition being treated.[10]

II. MATERIALS AND METHODS

Instrumentation: The author had attempted to develop a liquid chromatographic method for the determination of Lansoprazole using an isocratic Shimadzu HPLC equipment comprising of two LC10AT VP pumps, VP CTO-10AS VP column oven, a Hypurity advance C_{18} column (4.6 ID X 50 mm, 5µ) and anAPI 4000 (MDS Sciex) mass detector was used for chromatographic separation. Data acquisition was done by using Analyst 1.4.2 software. The details of the instruments employed in the study are as follows.

HPLC System	**Shimadzu**
Mass Spectrometer	API 4000, MDS Sciex
Deep Freezer	Sanyo (-86°C) VIP Series
Refrigerated centrifuge	Heraeusmegafuge
Microbalance	Sartorius
Vibramax	Heidolph
Vacuum pump	Millipore
Refrigerator	Samsung
PH meter	Orion
Micropipettes, Multipette and Micro tips	Brand and Eppendorf
Vortexer	Spinix

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

Poly propylene tubes	Torson's
Water Purification System	Elix 10 / Milli-Q gradient
Ultra sonicator	Power Sonic510, (Hwashin Technology)
Nitrogen Evaporator	ZymarkTurbovap LV station, Caliper

Chemical and reagents: Lansoprazole reference standard, Pantoprazole reference standard Methanol (HPLC grade), Milli-Q water, Ammonium acetate (AR grade), Sodium hydrogen carbonate (AR grade), Methyl tert-butyl ether (TMBE) (HPLC grade), Human plasma 0.45µ Membrane filter.

Preparation ofsolutions

- **Lansoprazole StockSolution:** About 5 mg of Lansoprazole, working standard was weighed accurately, and transferred to a 5 mL clean glass volumetric flask, dissolved in HPLC grade methanol and made up the volume with the same to produce a solution of 1 mg/mL of Lansoprazole.The stock solutions were diluted to suitable concentrations using diluent for spiking in to plasma to obtain calibration curve (CC) standards, quality control (QC) samples and DIQC samples. All other final dilutions (system suitability dilutions, aqueous mixture, recovery etc.) of Lansoprazole were prepared in mobilephase.
- **Pantoprazole Stock Solution (InternalStandard):**About 10 mg of Pantoprazole Sodium Reference standard was weighed accurately and transferred to a 10 mL volumetric flask, dissolved in HPLC grade methanol and made up the volume with the same to produce a solution of 1 mg/mL of Pantoprazole. The stock solution was diluted to suitable concentration using diluent for internal standard dilution and mobile phase for system suitabilitydilution.
- **BiologicalMatrix:** Six K_2 EDTA human plasma lots were screened for selectivity test. All six human plasma lots were found free of any significant interference for Lansoprazole and internal standard. Selectivity, Matrix Effect and sensitivity tests were performed. After bulk spiking, aliquots (sample contained 0.5M Sodium Hydrogen Carbonate: plasma 1:10) of 600 µL for CCs and 600 µL for QCs of spiked plasma samples were pipetted out in prelabelled polypropylene RIA vials and then all the bulk spiked samples were transferred to deep freezer maintained at -70 ℃ ± 10 ℃, except twelve sets of LQC and HQC, which were transferred to deep freezer maintained at -20 °C ± 5 °C for generation of stability data at -20°C.
- **Calibration Curve Standards and Quality ControlSamples:**Calibration curve standards consisting of a set of nine non-zero concentrations ranging from 10.02 ng/mL to 1999.21ng/mL for Lansoprazole were prepared. Quality control samples consisted of Lansoprazole concentrations of 10.13 ng/mL (LLOQ QC), 30.24 ng/mL (LQC), 503.99 ng/mL (MQC1), 1007.98 ng/mL (MQC2)and1799.97 ng/mL (HQC) were prepared. These samples were stored below -70 °C until use. Twelve sets of LQC and HQC were transferred to the -20 °C deep freezer to check stability at -20°C.

Standard	**Concentration**	**Lansoprazole (ng/mL)**
Standard I	2 - 3 times of C_{max}	1999.21
Standard H	80% of I	1599.37
Standard G	60% of I	1199.53
Standard F	40 % of I	801.28
Standard E	20% of I	400.64
Standard D	10% of I	200.32

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

Standard C	5% of I	50.08
Standard B	40% of Cconc.	20.03
Standard A	50% of Bconc.	10.02
LLOQ QC	Conc equal toA	10.13
LQC	2.5-3 times ofLLOQ	30.24
MQC 1	50% of I	503.99
MQC 2	50% of I	1007.98
HQC	75- 90% ofI	1799.97

- **5mM Ammonium AcetateBuffer:** About 385.00 mg of ammonium Acetate was transferred to a 1000 mL reagent bottle and dissolved in 1000 mL of Milli Q water. The buffer was mixed well and sonicated in an ultrasonicator for 3 to 5 minutes. The amount weighed depends on total volume of Milli Q water to be used for preparation of 5mM ammonium Acetate buffer.Buffer was prepared as and when required and used within a period of 4 days of preparation.
- **MobilePhase:** 800 mL of HPLC Methanol was transferred to a 1000 mL reagent bottle and 200 mL of 5mM Ammonium Acetate Buffer was added it. It was mixed well, sonicated in an ultrasonic bath for 3 to 5 minutes. The mobile phase was prepared as and when required and used within a period of 7 days of preparation.
- **Diluent:** A mixture of HPLC grade Methanol and Milli Q water was prepared in the volume ratio of 60:40 (v/v) as diluent. It was then sonicated in an ultrasonicator for 3 to 5 minutes. The diluent was prepared as and when required and used within a period of 7 days ofpreparation.
- **0.5M Sodium HydrogenCarbonate:** About 2g of Sodium Hydrogen Carbonate was transferred to a 50 mL reagent bottle and dissolved in 50 mL of Milli Q water. The buffer was mixed well and sonicated in an ultrasonicator for 3 to 5 minutes. The amount weighed depends on total volume of Milli Q water to be used for preparation of 0.5M sodium hydrogen carbonate. Buffer was prepared as and when required and used within a period of 4 days of preparation.
- **RinsingSolution:** Diluent was used as rinsing solution
- **System SuitabilitySolution:** A mixture of drug and internal standard was prepared for system suitability test. The concentration of drug corresponds to mean concentration of 250.18ng/mL and that of internal standard corresponds to working concentration used for calibration range (251.66 ng/mL). The same solution was injected as an aqueous mixture. Area Ratio was considered for System Suitability.

Optimized Chromatographic Conditions

Parameter	**Value**
Column	Hypurity advance C_{18} (4.6 X 50 mm, 5µ)
Mobile phase	Methanol: 5mM ammonium acetate buffer (80: 20 v/v)
Buffer	5mM ammonium acetate buffer
Isocratic/gradient mode	Isocratic

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

Flow rate	0.70 mL/min
Run time	2.5 min
Column oven temperature	40 ± 2^0C
Auto sampler temperature	15^0C
Volume of injection	20 µL
Rinsing volume	500 µL

Extraction process of plasma samples and their drying: Four hundred micro liters of spiked plasma calibration curve standards and the quality control samples were transferred to a set of pre-labeled poly propylene tubes containing each 25 µL of pantoprazole dilution (internal standard; 10.06 µg/mL). The tubes were added with 5mL of TMBE solution and vortexed for ten seconds. To each of the tubes 2.5 mL of extraction solvent was added. The tubes were further vortexed for 20 minat 200rpmona vibramaxunit and thenwerecentrifugedat4000rpmfor 10mininarefrigeratedcentrifugeat4^{0}C.Fromthecentrifugedtubesapproximately 4.0 mL of the supernatant layer was transferred to each of a new set of pre-labeled poly propylene tubes. The contents of the tubes were evaporated in a stream of nitrogen at 40^{0}C for 10 min and the residues of the dried tubes were reconstituted with 1000µL of the mobile phase. The contents of the tubes were vortexed and transferred intoauto-samplervialsandthenanalyzedbyLC-MS/MS.Analiquotof10µLofthe sample was drawn each time from the vials in the auto sampler.

III. RESULTS AND DISCUSSION

ANALYTICAL METHODVALIDATION

- **Selectivity:** The selectivity of the present method was evaluated by checking the blank EDTA (Ethylene di-amine tetra acetic acid) plasma (without spiking with Lansoprazole) obtained. Six different lots of blank plasma were screened and all of them were found to have no significant endogenous interferences at the retention times of the analyte and the internal standard. The representative chromatogram of the extracted blank plasma sample. The same human EDTA plasma lots free of interfering substances were used to prepare the calibration curve standards and the quality control samples for the validationstudy.

S.No	Conc. (ng/mL)
	LLOQ
	10.02
1	10.47
2	9.97
3	9.92
4	10.50
5	10.38
6	10.26
AVERAGE	10.250
SD	0.2511
%CV	2.45
% NOMINAL	102.30

Table 1: Within Batch Precision and Accuracy for Sensitivity of Lansoprazol

- **Matrixeffect:**Matrix effect for Lansoprazole was evaluated by analyzing all the six batches of plasma at low (LQC) and high (HQC) concentrations. No significant matrix effect was observed in all the six batches (batch no.P220410-284, P050510-286, P050510-287,P050510-288,P050510-289,P050510-290) of plasma for Lansoprazole at low (LQC) and high (HQC) concentrations. The precision and accuracy for Lansoprazole at LQC

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

concentration was found to be 1.95% and 107.10% respectively and at HQC concentration was found to be 0.73% and 98.45% respectively. The results are within the acceptable limits

- **Precision andAccuracy:** The precision of the assay was measured by the percent coefficient of variation for QC samples of Lansoprazole. The accuracy of the assay was measured by computing the ratio of the calculated mean values of the QC samples to their respective nominal values, expressed as percentagenominal.

	Concentration (ng/mL)				
	LLOQ QC	LQC	MQC1	MQC2	HQC
QC#	10.13	30.24	503.99	1007.98	1799.97
1	10.66	29.98	504.65	990.79	1714.71
2	10.24	30.31	514.35	1005.27	1714.12
3	11.01	30.94	516.54	1025.05	1734.93
4	11.36	30.18	525.21	1029.88	1750.03
5	10.88	31.53	523.57	1042.3	1751.56
6	10.99	31.59	530.62	1044.67	1758.67
Mean	10.857	30.755	519.157	1022.993	1737.337
S.D.	0.3779	0.7016	9.2512	21.1918	19.3665
C.V.%	3.48	2.28	1.78	2.07	1.11
% Nominal	107.17	101.70	103.01	101.49	96.52
N	6	6	6	6	6
7	10.88	30.90	513.35	1006.75	1697.13
8	11.05	30.20	505.36	999.28	1676.98
9	10.74	29.99	509.55	986.33	1672.60
10	10.73	30.14	491.59	977.67	1639.99
11	10.73	29.71	492.32	977.58	1663.46
12	10.98	30.53	501.42	979.77	1684.49
Mean	10.852	30.245	502.265	987.897	1672.442
S.D.	0.1405	0.4182	8.9342	12.3656	19.5401
C.V.%	1.29	1.38	1.78	1.25	1.17
% Nominal	107.12	100.02	99.66	98.01	92.91
N	6	6	6	6	6
13	11.20	30.74	515.76	1012.14	1710.81
14	10.73	30.36	513.42	994.20	1692.78
15	10.99	30.47	513.32	1005.30	1710.39
16	11.04	30.89	522.71	1013.34	1718.93
17	11.38	31.14	520.72	1003.50	1728.20
18	10.82	31.22	523.89	1007.62	1712.95
Mean	11.027	30.803	518.303	1006.017	1712.343
S.D.	0.2396	0.3482	4.7248	6.9308	11.6968
C.V.%	2.17	1.13	0.91	0.69	0.68
% Nominal	108.85	101.86	102.84	99.81	95.13
N	6	6	6	6	6
19	10.41	30.36	513.28	1005.02	1677.72
20	10.76	29.55	522.99	994.90	1692.64
21	10.46	30.23	521.42	997.92	1725.45
22	11.03	29.96	522.78	1028.93	1701.96
23	10.47	29.70	509.83	999.74	1694.72
24	10.41	29.86	515.60	987.87	1691.47
Mean	10.590	29.943	517.650	1002.397	1697.327
S.D.	0.2524	0.3088	5.5407	14.1790	15.8737

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

C.V.%	2.38	1.03	1.07	1.41	0.94
% Nominal	104.54	99.02	102.71	99.45	94.30
N	6	6	6	6	6
31	10.71	32.87	551.90	1068.35	1895.00
32	10.80	33.66	552.54	1076.24	1892.64
33	10.47	34.22	558.44	1076.13	1906.83
34	11.10	33.70	550.32	1080.33	1908.45
35	10.89	34.30	558.44	1093.48	1903.87
36	10.91	34.49	560.51	1083.86	1930.99
Mean	10.813	33.873	555.358	1079.732	1906.297
S.D.	0.2127	0.5941	4.2619	8.4985	13.6725
C.V.%	1.97	1.75	0.77	0.79	0.72
% Nominal	106.75	112.01	110.19	107.12	105.91
N	6	6	6	6	6

Table 2: Within Batch Precision and Accuracy of Lansoprazole

Within-batch Precision for Lansoprazole (% CV)
Within-batch precision for LLOQ QC ranged from 1.29 to 3.48%
Within-batch precision for LQC, MQC1, MQC2 and HQC ranged from 0.68 to 2.28%

Intra-day Precision for Lansoprazole (% CV) Intra-day precision for LLOQ QC was 2.50%
Intra-day precision for LQC, MQC1, MQC2 and HQC ranged from 2.01 to 2.46%

	Concentration (ng/mL)				
	LLOQ QC	**LQC**	**MQC1**	**MQC2**	**HQC**
QC#	**10.13**	**30.24**	**503.99**	**1007.98**	**1799.97**
1	10.66	29.98	504.65	990.79	1714.71
2	10.24	30.31	514.35	1005.27	1714.12
3	11.01	30.94	516.54	1025.05	1734.93
4	11.36	30.18	525.21	1029.88	1750.03
5	10.88	31.53	523.57	1042.3	1751.56
6	10.99	31.59	530.62	1044.67	1758.67
7	10.88	30.90	513.35	1006.75	1697.13
8	11.05	30.20	505.36	999.28	1676.98
9	10.74	29.99	509.55	986.33	1672.60
10	10.73	30.14	491.59	977.67	1639.99
11	10.73	29.71	492.32	977.58	1663.46
12	10.98	30.53	501.42	979.77	1684.49
Mean	**10.854**	**30.500**	**510.711**	**1005.445**	**1704.889**
S.D.	**0.2718**	**0.6117**	**12.3693**	**24.6896**	**38.6340**
C.V.%	**2.50**	**2.01**	**2.42**	**2.46**	**2.27**
% Nominal	**107.15**	**100.86**	**101.33**	**99.75**	**94.72**
N	**12**	**12**	**12**	**12**	**12**

Table 3: Intra-day Precision and Accuracy of Lansoprazole

Between-batch Precision for Lansoprazole (% CV)
Between-batch precision for LLOQ QC was 2.56%

Between-batch precision for LQC, MQC1, MQC2 and HQC ranged from 3.42 to 4.93%

	Concentration (ng/mL)				
	LLOQ QC	**LQC**	**MQC1**	**MQC2**	**HQC**
QC#	**10.13**	**30.24**	**503.99**	**1007.98**	**1799.97**
1	10.66	29.98	504.65	990.79	1714.71
2	10.24	30.31	514.35	1005.27	1714.12
3	11.01	30.94	516.54	1025.05	1734.93

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

4	11.36	30.18	525.21	1029.88	1750.03
5	10.88	31.53	523.57	1042.3	1751.56
6	10.99	31.59	530.62	1044.67	1758.67
7	10.88	30.90	513.35	1006.75	1697.13
8	11.05	30.20	505.36	999.28	1676.98
9	10.74	29.99	509.55	986.33	1672.60
10	10.73	30.14	491.59	977.67	1639.99
11	10.73	29.71	492.32	977.58	1663.46
12	10.98	30.53	501.42	979.77	1684.49
13	11.20	30.74	515.76	1012.14	1710.81
14	10.73	30.36	513.42	994.20	1692.78
15	10.99	30.47	513.32	1005.30	1710.39
16	11.04	30.89	522.71	1013.34	1718.93
17	11.38	31.14	520.72	1003.50	1728.20
18	10.82	31.22	523.89	1007.62	1712.95
19	10.41	30.36	513.28	1005.02	1677.72
20	10.76	29.55	522.99	994.90	1692.64
21	10.46	30.23	521.42	997.92	1725.45
22	11.03	29.96	522.78	1028.93	1701.96
23	10.47	29.70	509.83	999.74	1694.72
24	10.41	29.86	515.60	987.87	1691.47
31	10.71	32.87	551.90	1068.35	1895.00
32	10.80	33.66	552.54	1076.24	1892.64
33	10.47	34.22	558.44	1076.13	1906.83
34	11.10	33.70	550.32	1080.33	1908.45
35	10.89	34.30	558.44	1093.48	1903.87
36	10.91	34.49	560.51	1083.86	1930.99
Mean	**10.828**	**31.124**	**522.547**	**1019.807**	**1745.149**
S.D.	**0.2774**	**1.5083**	**18.9660**	**34.8853**	**86.0509**
C.V.%	**2.56**	**4.85**	**3.63**	**3.42**	**4.93**
% Nominal	**106.89**	**102.92**	**103.68**	**101.17**	**96.95**
N	**30**	**30**	**30**	**30**	**30**

Table 4: Between Batch / Inter-day Precision and Accuracy of Lansoprazole.

Within-batch Accuracy for Lansoprazole (% Nominal)
Within-batch accuracy for LLOQ QC ranged from 104.54 to 108.85%
Within-batch accuracy for LQC, MQC1, MQC2 and HQC ranged from 92.91 to 112.01%

Intra-day Accuracy for Lansoprazole (% Nominal)
Intra-day accuracy for LLOQ QC was 107.15%
Intra-day accuracy for LQC, MQC1, MQC2 and HQC ranged from 94.72 to 101.33%

Between-batch Accuracy for Lansoprazole (% Nominal)
Between-batch accuracy for LLOQ QC was 106.89%

Between-batch accuracy for LQC, MQC1, MQC2 and HQC ranged from 96.95 to 103.68%

- **Stability**

a) Room Temperature Stock SolutionStability: Room temperature stock solution stability was carried out at 0 and 7 hours for Lansoprazole by injecting six replicates of prepared stock dilutions of Lansoprazole equivalent to the middle concentration (MQC2). Comparison of the mean area response of Lansoprazole at 7 hours was carried out against the zero hour value, the stability was found to be 103.20% for Lansoprazole. The results are within the acceptable limits.

S.No.	Area	
	0 hr	7 hr

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

1	4748537	4859540
2	4671954	4803355
3	4749115	4879012
4	4693151	4879191
5	4814485	4978978
6	4683376	4867933
Mean	4726769.7	4878001.5
S.D.	54194.27	56943.82
C.V.%	1.15	1.17
% Stability	103.20	

Table 5: Room Temperature Stock Solution Stability of Lansoprazole (0 and 7 Hours)

b) Room Temperature Spiking SolutionStability: Room Temperature Spiking Solution Stability was carried out at 0 and 7 hours for Lansoprazole by injecting six replicates of prepared Spiking Solution of Lansoprazole equivalent to the middle concentration (MQC2). Comparison of the mean area response of Lansoprazole at 7 hours was carried out against the zero hour value of Room temperature Spiking Solution, the stability was found to be 102.45% for Lansoprazole.

S.No.	**Area**	
	0 hr	**7 hr**
1	4748537	4818287
2	4671954	4826900
3	4749115	4782957
4	4693151	4902753
5	4814485	4815843
6	4683376	4908636
Mean	**4726769.7**	**4842562.7**
S.D.	**54194.27**	**51166.33**
C.V.%	**1.15**	**1.06**
% Stability	**102.45**	

Table 6: Room Temperature Spiking Solution Stability of Lansoprazole (0 and 7 Hours)

c) Refrigerated Stock Solution Stability (at 2-8°C): Refrigerated stock solution stability of Lansoprazole and Pantoprazole was carried out by injecting six replicates of stock dilutions. The stock solution was found to be stable for maximum of 6 days. The six days stock Solution stability of Lansoprazole was found to be 99.45%. Stability standard stock solution and comparison (fresh) standard stock solution of Lansoprazole were prepared equivalent to the middle concentration (MQC2) level during method validation. The response of comparison (fresh) sample was corrected by multiplying with correctionfactor.

Correction factor was calculated as follows:

Correction factor = Corrected concentration of

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

stability standard stock solution Corrected Solution concentration of comparison (fresh) standard stock

S.No.	Stability standard stock 1000731.0000 Area	Comparison standard stock 1000641.3600 Area	Corrected Response
1	3954331	4039479	4039117
2	4048669	4058941	4058577
3	3988335	4048605	4048242
4	4091253	4082588	4082222
5	4043378	4068955	4068591
6	4065024	4027806	4027445
Mean	4031831.7	4054395.7	4054032.5
SD	50887.63	19952.40	19950.61
%CV	1.26	0.49	0.49
N	6	6	6
Correcting factor	0.9999		
Mean response of Standard stock	4031831.7		
Mean corrected response	4054032.5		
% Response	99.45		

Table 7: Refrigerated Stock Solution Stability for Lansoprazole 2-8 °C (6 days)

d) Bench TopStability: In short-term room temperature stability, six sets each of LQC and HQC, were determined at 5 hours. The quality control samples were quantified against the freshly spiked calibration curve standards of concentration range equivalent to that used for calculation of precision and accuracy, Lansoprazole was found to be stable upto 5 hours as per the acceptance criteria. The percent mean nominal of LQC and HQC was 90.37% and 89.30% respectively and the precision of LQC and HQC was 2.86% and 0.43% respectively. The results are within the acceptance limits.

	Concentration (ng/mL)	
	LQC	**HQC**
QC#	**30.24**	**1799.97**
97	28.86	1596.18
98	27.37	1608.61
99	27.12	1611.18
100	26.74	1616.07
101	26.92	1608.63
102	26.95	1603.38
Mean	**27.327**	**1607.342**
S.D.	**0.7807**	**6.8478**

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

C.V.(%)	**2.86**	**0.43**
% Nominal	**90.37**	**89.30**
N	**6**	**6**

Table 8: Bench Top Stability of Lansoprazole (5 hours)

e) Freeze-thawStability: The stability of QC samples was determined after three freeze-thaw cycles. Six replicates of LQC and HQC were analyzed after three freeze-thaw cycles. The freeze-thaw quality control samples were quantified against the freshly spiked calibration curve standards of concentration range equivalent to that used for the calculation of precision and accuracy. The percent nominal for LQC & HQC was 92.11% and 90.07% respectively and the precision for LQC & HQC was 2.97% and 0.56% respectively for three freeze-thaw cycles. The results are within the acceptance limits.

	Concentration (ng/mL)	
	LQC	**HQC**
QC#	**30.24**	**1799.97**
61	26.72	1617.76
62	27.69	1614.42
63	27.92	1608.19
64	29.27	1627.98
65	27.95	1631.07
66	27.57	1628.08
Mean	**27.853**	**1621.250**
S.D.	**0.8261**	**9.1408**
C.V.(%)	**2.97**	**0.56**
% Nominal	**92.11**	**90.07**
N	**6**	**6**

Table 9: Freeze Thaw Stability (FT– III Cycle) of Lansoprazole

f) Plasma samples Stability at-20^{0}C: Plasma samples stability at -20^{0}C was determined for 3 days using six sets each of LQC and HQC. The quality control samples were quantified against the freshly spiked calibration curve standards of concentration range equivalent to that used for calculation of precision and accuracy. The mean concentration of stability QCs were compared against the mean of the of the Ist day QCs when injected for first time after the bulk spiking (first passing P& A Batch). Lansoprazole was found to be stable up to 3 days at –20^{0}C as per the acceptance criteria. The percent stability was 88.66% for LQC and 92.72% for HQC and the precision was 1.91% for LQC and 0.64% for HQC. The results are within the acceptable limits.

	Concentration (ng/mL)			
	Day 0		Day 3	
	LQC	HQC	LQC	HQC
QC#	30.24	1799.97	30.24	1799.97
1	29.98	1714.71	27.13	1604.27
2	30.31	1714.12	27.76	1602.93
3	30.94	1734.93	26.66	1627.19
4	30.18	1750.03	27.92	1603.59
5	31.53	1751.56	27.40	1620.48
6	31.59	1758.67	26.73	1606.88
Mean	30.755	1737.337	27.267	1610.890
S.D.	0.7016	19.3665	0.5221	10.3365
C.V.(%)	2.28	1.11	1.91	0.64

International Journal of Pharmaceutical Research and Applications
Volume 6, Issue 2 Mar-Apr 2021, pp: 76-87 www.ijprajournal.com ISSN: 2249-7781

% Nominal	101.70	96.52	90.17	89.50
N	6	6	6	6
% Stability			88.66	92.72

Table 10: Short Term Stability at -20 ˚C Lansoprazole Data for Day0 and Day 3

IV. CONCLUSION

The aim was to develop and validate an effective procedure for estimating the uncertain concentration of drugs in plasma. A highly precise, adaptive, reliable and reproducible LC-MS/MS system for quantifying Lansoprazole using commercially available small-volume human plasma IS with a clear solid phase extraction process has been developed and validated. The analytical method developed and validated was found to be easy, rapid, precise, sensitive, reliable and cost-effective than the other methods published. The approach was effectively extended to the analysis of a preclinical pharmacokinetic sample with the required precision and accuracy together with a strong throughput.

REFERENCES

[1]. Sethi, P.D. High Performance Liquid Chromatography; Quantitative Analysis of Pharmaceutical Formulations, 1st ed.; CBS Publishers and Distributors Pvt. Ltd. : New Delhi, 2010, Vol. I, pp. 59-63.
[2]. Sethi, P.D. High Performance Liquid Chromatography; Quantitative Analysis of Pharmaceutical Formulations, 1st ed.; CBS Publishers and Distributors Pvt. Ltd. : New Delhi, 2010, Vol. I, pp. 11-14, 101-103. [3]
[3]. Brahmankar, M. Biopharmaceutics and Pharmacokinetics, 1st ed.; Vallabh Prakashan: Delhi, 2005; pp. 282-283, 294. [4]
[4]. The Merck Index - An Encyclopaedia of Chemicals, Drugs, and Biological, 13th ed.; Merck Research Laboratories, Whitehouse Station. New Jersey, 2001. [5]
[5]. Joel, G. H.; Lee, E. L. The Pharmacological Basis of Therapeutics, 9th ed.; Mc Graw Hill Publication: New York, 1996. [6]
[6]. Barar, F. S. K. Essentials of Pharmacotherapeutics, 4th ed.; S. Chand & Company Ltd. : New Delhi, 2000. [7]
[7]. Venkateshwarlu, V. Biopharmaceutics and Pharmacokinetics, 1st ed.; Pharm Book Syndicate: Hyderabad, 2004, p. 331. [8]
[8]. Robert, E. N., Biopharmaceutics & Clinical Pharmacokinetics, 4th ed.; Marcel Dekker, New York, 2005, Vol. 3, p.352. [9]
[9]. Malcol, M., Thomas, N. Clinical Pharmacokinetics Concepts and Application, 3rd ed.; Waverly Pvt Ltd.: New Delhi, 1996, p. 57. [10]
[10]. Willard. Instrumental Methods of Analysis, CBS Publishers: New Delhi, 1982, 6th ed.; pp. 467-476.