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Development and Validation of a LC-MS/MS Method to Determine Lansoprazole in Human Plasma

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Abstract: A rapid and sensitive liquid chromatography-tandem mass spectrometric (LC-MS/MS) assay method has been developed and fully validated for the determination of lansoprazole (LNZ) in human plasma. Pantaprazole was used as an internal standard (IS). Analytes and the internal standard were extracted from human plasma by solid-phase extraction technique using Oasis HLB, Oasis Max, Varian Bond Elute Plexa, Orochem cartridges. The reconstituted samples were chromatographed on a thermo hypurity Advance, 50 X 4.6mm, 5 µ by using acetonitrile and 2 mM ammonium acetate solution(80:20 v/v) as the mobile phase at a flow rate of 1.0 mL/min. Detection was carried out LC-MS/MS (API 3000) in negative ion mode. The calibration curves obtained were linear (R^2-0.999) over the concentration range of 4.50- 2800.00 ng/ml for lansaprazole. The results of the intra- and inter-day precision studies were well within the acceptable limits. The overall average recoveries of analyte and IS were found to be 92.10-99.11%. The analyte were found to be stable of stability study. Developed and validated analytical method was found to be simple, rapid, specific, sensitive, precise and cost effective than reported methods. The method has been successfully applied to the investigation of a preclinical pharmacokinetic study with desired precision and accuracy along with high throughput.

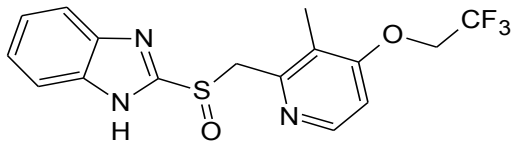
Keywords: Lansoprazole, liquid chromatography, MS/MS, pantaprazole.

1. INTRODUCTION

Lansoprazol (Fig. **1A**) and Pantaprazole (Fig. **1B**) belongs to a class of anti-secretory compounds, the substituted benzimidazoles, that do not exhibit anticholinergic or histamine H_2 -receptor antagonist properties, but rather suppress gastric acid secretion by specific inhibition of the (H^+, K^+)-ATPase enzyme system at the secretory surface of the gastric parietal cell. This enzyme system is regarded as the acid (proton) pump within the parietal cell, lansoprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. This effect is dose-related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus. Lansoprazol does not exhibit anticholinergic or histamine type-2 antagonist activity [3-9].

As per the literature, several LC-MS/MS methods have been reported for the determination of Lansoprazole individually or with other drugs in biological samples. In many studies, LNZ was used as the main analyte or as internal standard (IS), in that HPLC [1, 2] by UV detection and UV spectroscopic methods were used, which were not suitable for clinical trials because of their low sensitivity [14-26]. Thus, the objective of the project was to develop and validate suitable method for estimation of unknown concentration of drug in plasma. A highly accurate, sensitive, specific and reproducible LC-MS/MS method for the quantification of Lansoprazole using commercially available IS from small volumes of human plasma with a simple Solid Phase Extraction process was developed and validated [10-13]. Developed and validated analytical method was found to be simple, rapid, specific, sensitive, precise and cost effective than the reported methods. The method has been successfully applied to the investigation of a preclinical pharmacokinetic study with desired precision and accuracy along with high throughput [27-29].

(**A**)



(**B**)

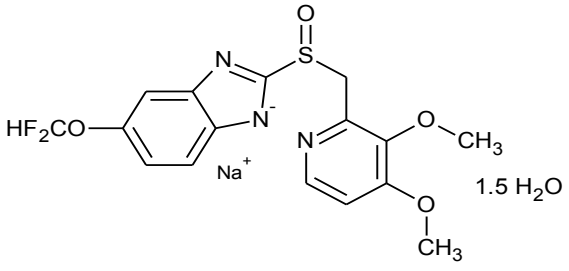


Fig. (1). Chemical structures of (**A**) Lansoprazole (**B**) Pantaprazole.

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2. EXPERIMENTAL

2.1. Chemicals and Reagents

Acetonitrile (HPLC grade, Merck), Ammonium acetate (HPLC grade, Merck), Human plasma, Milli-Q/HPLC water (In House), Methanol (HPLC grade, Merck), pantaprazole working standard (IS), Lansoprazole reference standard were provided by Wockhardt Research Centre, Aurangabad.

2.2. Instrumentation and Chromatographic Conditions

An HPLC system (Shimadzu, Kyoto, Japan)consisting of a Thermo Hypurity Advance, 50 X 4.6mm, 5µ, binary LC-20AD prominence pump, an auto sampler (SIL-HTc) and a solvent degasser (DGU-20A3) was used for the study. Aliquots of the processed samples (10 µL) were injected into the column, which was kept at 40 ±1 ^{0} C. The isocratic mobile phase, a mixture of Acetonitrile: 2 mM Ammonium Acetate (80:20 % v/v) was delivered at 1.0 mL/min LC-MS/MS (API 3000) in negative ion mode. Lansoprazole m/z 370.20/252.10 Pantaprazole (IS) m/z 384.20/200.00 in Table **2**. The parameters set were curtain gas, gas 1, and gas 2 (nitrogen) as 40, 40, and 60 units, respectively, while Dwell time was 300 s and source temperature was 500 ^{0} C. Ion spray voltage 4500 V unit mass resolution was set in an Q1 and Q3 analyzer and the main working parameters are summarized in Table **1**. The developed and optimized analytical method has been validated for quality control samples. The linearity range was found to be 4.50 ng/ml - 2800.00 ng/ml with regression value (r^2) 0.999.

2.3. Preparation of Standard Solutions

Lansoprazole stock solution: Approximately 25 mg of LNZ was weighed and transferred to 50 mL volumetric flask containing 10µl Liquor ammonia (25%). Then Methanol was dissolved so that the volume reaches the mark to make approximately 500000.00ng/ml stock solution. This stock solution was transferred in a reagent bottle with appropriate label and stored at 2-8 °C. Further dilutions of LNZ were prepared in dilution solution for spiking in plasma. The stock solution must be used within 28 days from the date of preparation. However if stock solution stability generated later with maximum generated stability period.

Pantaprazole (Internal standard) stock solution: Approximately 25 mg of PNZ was weighed and transferred to 25 mL volumetric containing 10µl of Liquor Ammonia to get 1000000 ng/mL stocks with methanol. Stock solution was transferred in a reagent bottle with appropriate label and stored at 2-8 °C

Table 1. Tandem mass working parameters.

Parameter	Value
CAD gas	2
Nebulizer Gas	14
Curtain gas	12
Ion Spray Voltage	4500 volts
Temperature	500 ^{0} C

2.4. Preparation of Standard Solutions for CC and QC of Drug LNZ

The selected range for validation was from 4.5ng/mL to 2800.00ng/mL concentrations for LNZ. The quality control samples for LNZ were prepared at concentrations of 4.61ng/ml (LLOQ), 11.54ng/mL (LQC), 1153.49ng/mL (MQC) and 2306.98ng/mL (HQC).

Table 2. Chromatographic conditions.

Column	Thermo Hypurity Advance, 50 X 4.6mm, 5 µ
Mobile phase	Acetonitrile and 2 mM Ammonium Acetate solution(80:20 v/v)
Rinsing solution	Acetonitrile: Milli Q Water (80:20v/v)
Flow rate	1.000 mL/minute with splitter (70% to Waste)
Column oven temperature	40 ± 1° C
Detection	LC-MS/MS (API 3000) in negative ion mode. Lansoprazole m/z 370.20/252.10 Pantaprazole (IS) m/z 384.20/200.00
Sample Cooler Temperature	5 °C ± 1 °C
Injection volume	µL

Table 3. Precision and accuracy of the method for determining LNZ in plasma samples.

Analyte		Conc. Added (ng/mL)	Intra-day Precision and Accuracy			Inter-Day Precision and Accuracy		
			Mean Found ng/mL)	±S.D.	% CV	Mean Found ng/mL)	±S.D.	% CV
LNZ	LLOQ QC	4.61	4.320	0.2069	4.79	4.246	0.3997	9.41
	LQC	11.54	11.044	0.2848	2.58	11.166	0.6575	4.89
	MQC	1153.49	1124.715	34.315	3.14	1143.251	53.0277	4.64
	HQC	2306.98	2179.283	34.81	1.64	2190.382	47.823	2.18

Table 4. Ruggedness of lansoprazole.

Sr. No.	LLOQ QC	LQC	MQC	HQC
	4.61	11.54	1153.49	2306.98
1	3.81	11.24	1104.70	2104.40
2	4.28	10.30	1098.16	2218.24
3	3.69	10.38	1121.38	2134.99
4	4.43	11.28	1077.09	2117.92
5	4.33	10.71	1094.76	2111.75
6	4.20	10.42	1134.69	2077.85
Mean	4.123	10.722	1104.463	2127.858
±S.D.	0.3010	0.4396	20.2984	48.1631
% CV	7.30	4.10	1.84	2.26
% Nominal	89.44	92.91	94.84	92.24

3. RESULT AND DISCUSSION

3.1. Method Development

The goal of this work was to develop and validate a simple, rapid, selective, and sensitive assay method for the extraction and quantitation of LNZ. To achieve the goal during method development, different options were evaluated to optimize sample extraction, detection parameters, and chromatography. LNZ was extracted by Solid Phase extraction by using the Varian Bond Elute Plexa. It was found to be the most reproducible and gave less batch variation when compared with other organic solvents. It was found that the best signal was achieved with (API 3000) in negative ion mode the isocratic mobile phase, a mixture of Acetonitrile: 2 mM Ammonium Acetate (80:20 % v/v) was delivered at 1.0 mL/min. With this optimized mobile phase, the m/z value of the parent ions of Lansoprazole 370.20/252.10 Pantaprazole (IS) 384.20/200.00. The spiked plasma was retrieved from the deep freezer and thawed in a water bath at room temperature. The thawed samples were vortexed to ensure complete mixing of the contents.50 µl of internal standard (10000.0 ng/ml) was added in prelabelled RIA vials except in blank samples where 50 µl of dilution solution was added. A 250 µl of the sample was added to it and the RIA vials. 500 µl 10Mm Sodium Dihydrogen Orthophosphate was added and vortex again. The samples was extracted using SPE technique. Varian Bond Elute Plexa 30 mg/cc was conditioned on a Speed-disk pressure processor using 1 ml Methanol followed by 1ml milli-Q water/HPLC grade water at a constant positive pressure. Prepared plasma samples were loaded and then washed with 1ml of Milli-Q water/ HPLC grade water then again washed with 1 ml of 5 %(v/v) Methanol in water. The cartridges were dried under positive pressure and eluted with 2 ml (1 ml × 2) of Methanol. The elute was dried at 50^0C under stream of nitrogen and reconstituted with 500 µl of reconstitution solution and filled into vials. The samples were analyzed on LC/MS/MS.

3.2. Validation

ICH guidelines and USFDA guidelines were followed for method validation [27-29] the method was validated for its selectivity, stability, linearity, accuracy, precision, and recovery.

3.2.1. Selectivity

The selectivity of the method was assessed by comparing chromatogram of blank plasma and spiked plasma. The retention times were 1.48 min for analyte and 1.47 for internal standard 1.47 represented in Figs. (**2**, **3**). There were no significant endogenous peaks. That could not interfere with retention time of Analyte and Internal standard. The results indicate that the method exhibited good selectivity.

$$\%\ \text{Interferance} = \frac{\text{Area at RT of Analyte (or IS) in the blank matrix}}{\text{Mean of Area at RT of Analyte (or IS)in hte LLOQ}} \text{X100}$$

3.2.2. Matrix Effect

The ion suppression/enhancement in the signal were found % CV 4.41 at low QC level and %CV 2.48 at high QC level for analyte, indicating that the matrix effect of analyte is not obvious under these conditions.

$$\text{Accuracy (\% Nominal)} = \frac{\text{Mean of Each QC concentration level}}{\text{Nominal Value}} \text{X100}$$

$$\text{Precision (\% CV)} = \frac{\text{S. D. at QC concentration level}}{\text{Mean of Each QC concentration Level}} \text{X100}$$

3.2.3. Sensitivity

The LLOQ signal-to-noise (S/N) values found for six injections of analyte at LLOQ concentration was 89.85. The Limit of Detection (LOD) was 4.00ng/ml and Limit of Quantitation (LOQ) was 4.60ng/ml. It can be concluded that the sensitivity is more for this method.

3.2.4. Linearity

Calibration curves were plotted as the peak area ratio *versus* analyte concentration. Calibration was found to be linear over the concentration range of 4.50 ng/ml to 2800.00 ng/ml. The linearity was represented by a linear regression equation as follows.

$Y = 269.8\ x + 5.146\ (R^2 = 0.999)$

3.2.5. Precision and Accuracy

The precision was less than 2.25% and the accuracy of the mean of measured concentrations ranged from 96.45 to 103.20%. Precision and accuracy for this method were controlled by calculating the intra and inter-batch variations of QC samples in six replicates. The intra-batch precision and accuracy were between 1.64 to 4.79 and 93.71 to 97.59%. Similarly, the inter-batch precision and accuracy were between 2.18 to 9.41 and 92.10 to 99.11% are

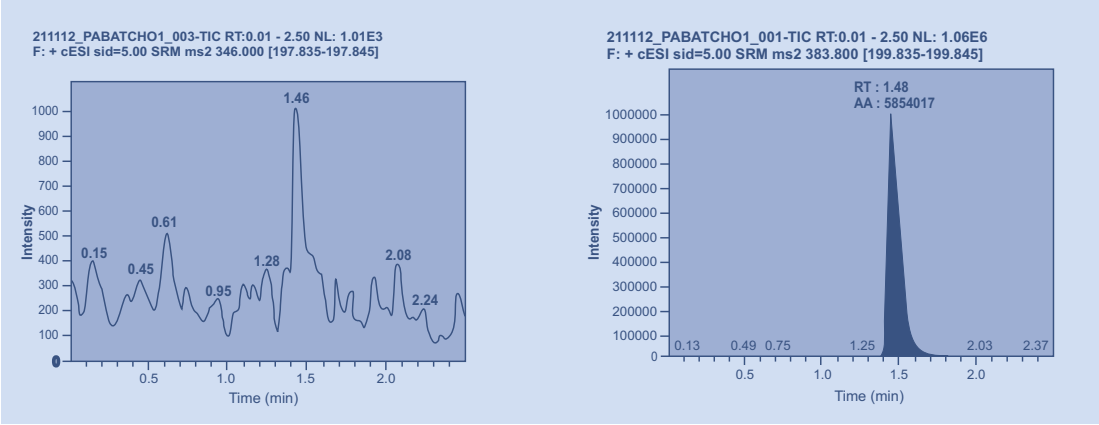

Fig. (2). Blank + Internal slandered.

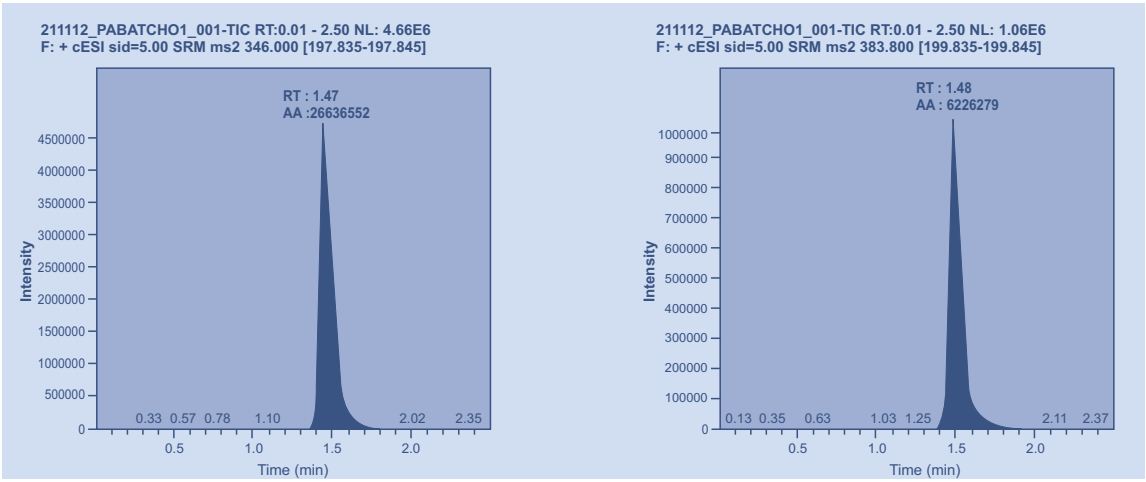

Fig. (3). Representative chromatograms for standard solution of LNZ and (IS) PNZ.

summarized in Table **3**. These results indicate the adequate reliability and reproducibility of this method within the analytical range.

3.2.6. Recovery

The extraction recoveries was determined at three different concentrations, and were found to be 11.54ng/mL,1153.49ng/mL and 2306.98ng/mL respectively. The overall average recoveries of analyte and IS were found to be 92.10-99.11%. Recoveries of the analyte and IS were high and consistent, precise and reproducible.

3.2.7. Stability

Analyte in plasma was subjected to three freeze/thaw cycles. The obtained accuracy was between 96.45% and 99.42% of the theoretical values. No significant degradation of the analyte was observed even after 48 h storage period in Autosampler tray and the final concentrations of analyte was found in between 96.42% and 92.08% of the theoretical values. In addition, the long-term stability of QC samples after 90 days of storage was at -20^0c, -50^0c. The concentrations ranged from 92.24 to 95.13% for long term stability and room temperature stability for 48 h was also evaluated for Analyte and IS. The % comparison response 101.81 to 99.64% for Room Temperature and Refrigeration stock solution stability studies. These results confirmed the stability of analyte human plasma for at least 90 Days at -20^0c, -50^0c.

3.2.8. Reinjection Reproducibility

Calibration was found to be linear over the concentration range of 4.50 ng/ml to 2800.00 ng/ml. The precision was less than 3.09 to 2.62 and the accuracy of the mean of measured concentrations ranged from 98.24 to 99.94%. This produced almost reproducible result.

3.2.9. Dilution Integrity

The dilution of samples should not affect the accuracy and precision (two times and four times as per dilution factor). Accuracy of 92.30 to 94.45% was observed.

3.2.10. Ruggedness

By method transfer, the Precision and Accuracy 1.84 to 7.30 and 89.44 to 92.08% was found showing reproducible results with Accuracy and Precision in Table **4**.

CONCLUSION

Thus, the objective was to develop and validate suitable method for estimation of unknown concentration of drug in plasma. A highly accurate, sensitive, specific and reproducible LC-MS/MS method for the quantification of

Lansoprazole using commercially available IS from small volumes of human plasma with a simple Solid Phase Extraction process was developed and validated. Developed and validated analytical method was found to be simple, rapid, specific, sensitive, precise and cost effective than the other reported methods. The method has been successfully applied to the investigation of a preclinical pharmacokinetic study with desired precision and accuracy along with high throughput.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest

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