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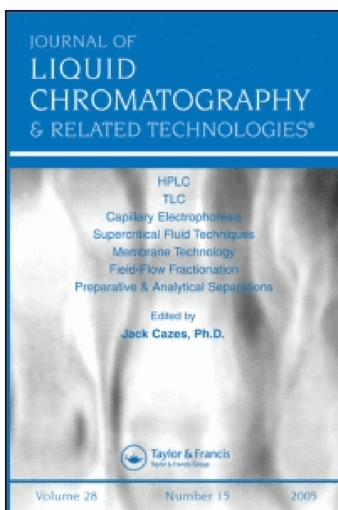

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LIQUID CHROMATOGRAPHY-ELECTROSPRAY TANDEM MASS SPECTROMETRY (LC-MS/MS) DETERMINATION OF LANSOPRAZOLE IN HUMAN PLASMA

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☐ *A rapid, selective, and sensitive LC-MS/MS method has been developed and validated for quantification of lansoprazole (LZ) in human plasma using esomeprazole as an internal standard (IS). The analyte and IS were extracted by single solvent liquid-liquid extraction using tert-Butyl methyl ether and separated by isocratic elution on C18 analytical column with 90:10 v/v acetonitrile, and 20 mM ammonium acetate (flow rate of 1 mL/min) as the mobile phase in the positive ion mode. Selected Reaction Monitoring transitions for LZ, internal standard and their daughter ion were observed at 369, 346, 252, and 198 m/z, respectively. The lower limit of quantification was 3.997 ng/mL and total run time for the analysis was 1.2 minutes. Linear calibration was observed in the range of 3.997 ng/mL to 2002.5 ng/mL. The method was validated for its selectivity, stability, accuracy, precision, and recovery.*

Keywords esomeprazole, lansoprazole, LC-MS/MS, SRM-Mode

INTRODUCTION

Lansoprazole (LZ) and esomeprazole (EZ) are well known proton pump inhibitors that are chemically known as 2-[[[3-methyl-4(2,2,2-trifluro-ethoxy)-2-pyridinyl]methyl]sulfinyl]-1H-benzimidazole and

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6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl]-1 H-benzimidazole, respectively (Figure 1). They inhibit the gastric acid secretion through interaction with the (H^+/K^+)-ATP-ase in gasstric parietal cells.[1–4] LZ is widely used in combination with antibiotic like clarithromycin and amoxicillin or with levofloaxacin for the eradication of *Helicobacter pylori* which causes duodenal ulcers.[5,6]

Several methods have been reported for the estimation of LZ in biological matrices and in pharmaceuticals.[7–30] In many studies, LZ was used as the main analyte or as internal standard (IS), in that HPLC by UV detection and UV spectroscopic methods were used, which were not suitable for clinical trials because for their low sensitivity.[22,23,28,29] Recent LC-MS/MS and Rapid Resolution-MS/MS methods for LZ also have demerits, like higher LLOQ and longer run time (RT), complex processing, double extraction, binary solvents for extraction, and instability of the analyte of interest in the mobile phase.[12,18,26] Recently, Hishinuma et al. reported estimation of LZ using lansoprazole-d4 as the IS by LC-MS/MS, which also has a longer run time of about 3.5 min.[25] Considering this, we developed a method with little sample volume, the shortest sample clean up procedure, the utilization of a simple liquid-liquid extraction using a single solvent, a new choice of IS, and the shortest run time.

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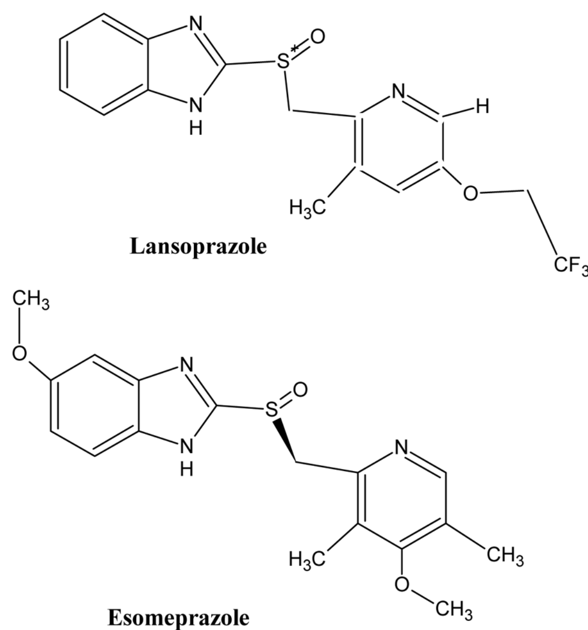

FIGURE 1 Structures of LZ and EZ.

EXPERIMENTAL

Chemicals and Reagents

LZ and Esomeprazole (EZ) received as gift samples from Micro Labs Pharmaceutical Co., Ltd. (Hossur, India). Acetonitrile (LC/MS grade) was purchased from J.T. Baker (New Jersey, USA). Milli-Q water from Millipore water system (Billerica, USA) was used throughout the experiment. Analytical grade ammonium acetate and tert-Butyl methyl ether (TBME) were obtained from Sigma Aldrich (Bangalore, India). Blank plasma was purchased from Prathma labs (Ahmadabad, India). Stock solutions of lansoprazole and IS (each 1 mg/mL) were prepared in water and methanol (HPLC grade) obtained from J.T. Baker (New Jersey, USA) and kept in glass tubes at −20°C.

LC-MS/MS Conditions

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Analysis were performed with API-3000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex, Canada) by the use of an electro spray source using a positive ion mode with selected reaction monitoring (SRM). 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 450°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 sample delivery to the ESI was done by HPLC autosampler at 4°C with SIL-HTC pump LC-200 series (Schimadzu, Japan) on C18 (50 × 4.6 mm, 5μ) analytical column (Thermo Hypurity, Thermo Fischer Scientific Inc. USA), system was operated at a temperature of 25°C. Acetonitrile and 20 mM ammonium acetate in the ratio of 90:10 v/v was used as mobile phase with a flow rate 1 mL/min, 50% split and with a total run time of 1.2 min.

Data Collection and Integration

The data were gathered and processed with Sciex analyst version 1.4.1 data collection and integration software on an IBM compatible computer.

Preparation of Standards and QC Solutions

A stock solution of LZ was prepared by dissolving an accurately weighed quantity of standard compound in methanol to give final concentrations of 1000 μg/mL in the volumetric flask. The solution was further diluted to achieve standard working solutions of desired concentrations. The internal

TABLE 1 Tandem Mass Working Parameters

Parameter	Value
Collision gas (CAD)	6.000
Curtain gas (CUR)	9.000
Nebulizer gas (NEB)	10.000
Ion source gas 1 (GS1)	40.000
Ion source gas 2 (GS2)	60.000
Ion spray voltage (ISV)	4500.000
Probe temperature °C (TEM)	450.000
Interface heater (ihe)	On
D well time per transition (MS)	300.000
Declustering Potential (DP)	
For LZ	35
For EZ	38
Focusing Potential (FP)	
For LZ	130
For EZ	120
Entrance Potential (EP)	
For LZ	9
For EZ	7
Collision Energy	
For LZ	16
For EZ	14
Cell Exit Potential (CXP)	
For LZ	10
For EZ	11

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standard working solution (1 μg/mL) was similarly prepared by diluting a stock standard solution of EZ in methanol. All the working solutions were refrigerated (4°C) and brought to room temperature before use.

Preparation of CC Standard and QC Samples

The standard solutions were used to spike 100 μl blank plasma samples either for CC standards of LZ or for QC's in a prestudy. During the validation, calibration standards were prepared at plasma concentration of 3.997, 7.994, 60.105, 400.699, 801.099, 1201.500, 1602.000, and 2002.500 ng/mL for LZ while QC's were prepared with blank plasma at LLOQ, Low, Medium, and high concentrations of 4.162, 11.404, 814.570, and 1522.561 ng/mL for LZ.

Plasma Sample Preparation

Aliquots of frozen plasma samples (100 μL) were thawed at room temperature and thoroughly vortexed prior to extraction. The calibration curve consists of a blank sample (matrix sample processed without IS), a zero sample (matrix sample processed with IS), and eight non-zero samples

covering the expected range, including LLOQ. To each calibration sample and quality control sample, 25 μL of IS was added followed by 2.5 mL of TBME as a solvent for extraction in an Eppendorf tube. They were vortexed well, and centrifuged at 4000 rpm for 5 min at 4°C. The supernatant layer was transferred to a polyethylene tube and evaporated in an nitrogen evaporator at 40°C for 6 min. Then, the residue, which was reconstituted with 500 μL mobile phase and vortexed for 1 min from this 5 μL, was injected into LC-MS/MS.

Method Validation

ICH guidelines and USFDA guidelines were followed for method validation.[31,32] The method was validated for its selectivity, stability, linearity, accuracy, precision, and recovery.

Linearity

Linear calibration curves were plotted by using the partial least square method in which least square regression of quantities versus peak area ratio to EZ with a weighted index (1/x) was utilized. Peak height of the LZ and the IS calibration points were evaluated; their ratio were linear in a range of 3.997 to 2002.5 ng/mL with a coefficient of determination greater than 0.99 (Table 2).

Stability

Stability of LZ concentration was carried out by comparison with freshly prepared samples. Herein, the short-term stability for 4.30 hr at room temperature, dry extract bench top stability for 24 hr, and three freeze thaw

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TABLE 2 Results of Linearity

S. No.	Conc. of LZ (ng/mL)				Mean	Standard deviation (%)	Accuracy (%)
	STD	Run 1	Run 2	Run 3			
1	3.997	3.931	3.858	3.893	3.894	0.030	97.400
2	7.994	8.259	8.585	8.382	8.400	0.160	105.200
3	60.105	60.341	58.248	61.874	60.150	1.800	100.700
4	400.699	382.447	398.242	404.767	395.150	11.470	98.600
5	801.399	818.407	786.390	773.802	792.860	22.990	98.900
6	1201.500	1144.257	1165.006	1216.323	1175.190	37.090	97.800
7	1602.000	1644.285	1659.735	1595.422	1633.140	33.570	101.900
8	2002.500	2052.394	2024.315	1930.809	2002.500	63.650	100.000
Correlation coefficient. (r)		0.999	0.999	0.998	–	–	–

TABLE 3 Accuracy and Precision of the Determination of LZ in Human Plasma

	Intra day (n = 3)					Inter-day (n = 3)			
Drug	Added (ng/mL)	Mean found	SD	CV (%)	Accuracy (%)	Mean found	SD	CV (%)	Accuracy (%)
LZ	4.162	4.614	0.198	4.300	110.900	4.120	0.189	4.500	98.990
	11.404	10.680	0.630	5.900	93.700	10.680	0.548	5.850	93.700
	814.570	777.190	0.876	2.900	95.400	789.90	0.876	3.200	96.970
	1522.561	1483.000	0.545	3.000	97.400	1483.500	0.675	2.850	97.400
	4.162	4.140	0.540	6.800	99.470	4.160	0.660	7.000	99.900
	11.404	9.970	0.530	5.300	87.420	10.080	0.970	5.700	88.400
	814.570	740.000	0.587	3.200	90.840	745.000	1.450	2.900	91.500
	1522.561	1411.000	0.876	1.900	92.700	1423.000	0.880	2.180	93.500
	4.162	4.142	0.240	5.500	106.400	4.380	0.360	4.980	105.600
	11.404	10.660	0.380	3.600	93.500	10.690	0.530	3.600	93.700
	814.570	789.060	1.060	3.300	96.900	792.040	0.950	3.890	97.230
	1522.561	1440.870	0.990	6.600	94.600	1442.80	0.860	6.820	94.800

cycles were carried out in intervals of 24 hr each with three successive cycles. Autosampler stability was carried out at 4°C.

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Precision and Accuracy

To evaluate the inter-day precision and accuracy, 5 quality control samples once a day for 3 days were examined while intra-day precision and accuracy were evaluated by analyzing the quality control sample spiked in the mobile phase three times a day at regular intervals. Coefficient of variation was determined for each concentration by calculating the standard deviation. The accuracy of the methods was calculated for each spiked concentrations with the comparison of nominal concentration and assayed concentration (Table 3).

In order to prove the extraction efficiency, recovery was studied by evaluating the concentration of extracted sample. Recovery was evaluated by calculating the mean response of extracted sample after the liquid-liquid extraction, together with the evaluation of concentration of the unextracted sample at LQC, MQC, and HQC level. Recovery of the sample was calculated by using these results by dividing mean of extracted sample by mean of unextracted sample of the corresponding concentration.

RESULTS AND DISCUSSION

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 LZ

suitable for pharmacokinetic studies. To achieve the goal during method development, different options were evaluated to optimize sample extraction, detection parameters, and chromatography. LZ was extracted by liquid-liquid extraction by using the tert Butyl methyl ether (TBME). TBME 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 the positive ion electrospray (ESI) mode. Mobile phase with 20 mM ammonium acetate buffer and acetonitrile (90:10%v/v) resulted in an improved signal. With this optimized mobile phase, the m/z value of the parent ions of LZ and EZ are 369 and 346, whereas daughter ions of LZ and EZ were observed at 252 and 198 (Figure 2a, 2b, 2c, and 2d). Use of short Thermo Hypurity C18 (50mX4.6IDX5μ) column resulted in reduced flow rate and run time (1.20 min) with column oven temperature kept at 40°C.

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Selectivity

Representative chromatogram obtained from blank plasma and plasma spiked with LOQ standard for LZ is represented in Figure 3 and Figure 4a, respectively. Similarly, a representative chromatogram obtained in its nominal concentration for EZ (IS) is shown in Figure 4b. No interfering peak of endogenous compounds was observed at the retention time of analyte or the IS in blank human plasma containing sodium EDTA as an anti-coagulant from six different lots.

Linearity

The peak area ratios for calibration standards were proportional to the concentration of analyte in each assay over the nominal concentration range of 3.997–2002.500 ng/mL for LZ. The calibration curves appeared linear and were well described by least square lines. A weighting factor on $1/\text{concentration}^2$ was chosen to achieve homogenicity of variance. Linear correlation co-efficient was $r = 0.9994$ and the regression equation was $y = 0.0064 \times -2.51e^{-006}$. Across the eight points taken as calibration standards, the % C.V obtained over five batches was between 0.77 and 3.18 LZ (Table 2).

Lower Limits of Quantitation and of Detection

The LLOQ for LZ was 4.162 ng/mL; it was possible to detect concentrations from real samples up to 3.997 ng/mL for Lansoprazole.

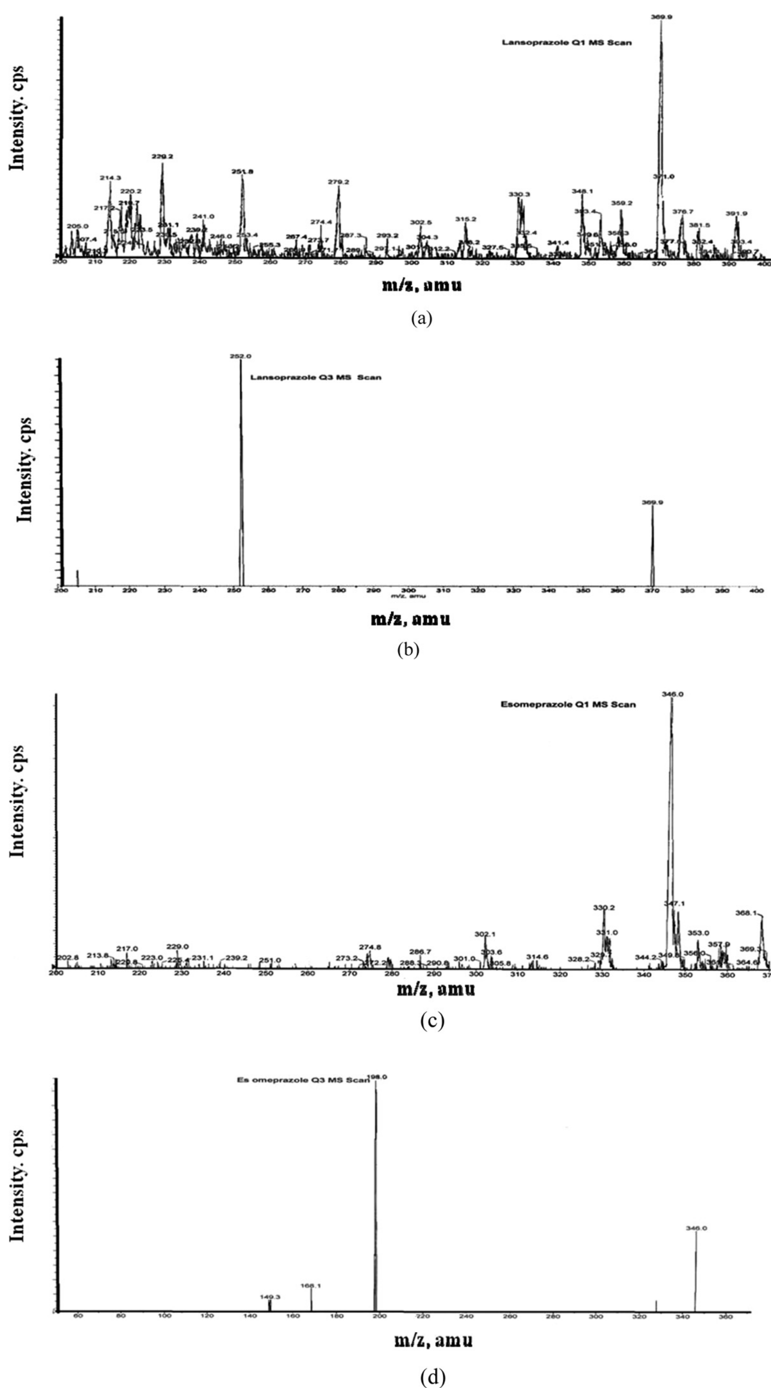

FIGURE 2 Full scan mass spectra of (a) LZ parent ion (m/z 369.9), (b) LZ Q3 product ion (m/z 252.0), (c) EZ (IS) parent ion (m/z 346.0), and (d) EZ Q3 product ion (m/z 198.0).

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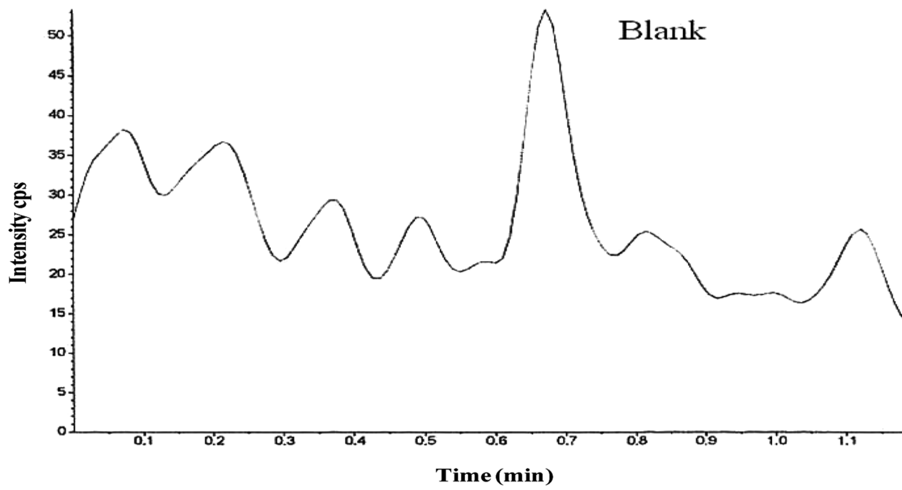

FIGURE 3 Chromatogram of blank plasma without drug, which shows the absence of endogenous peak. (The signal shown is more than 10 times shorter than that of the respective LLOQ peak).

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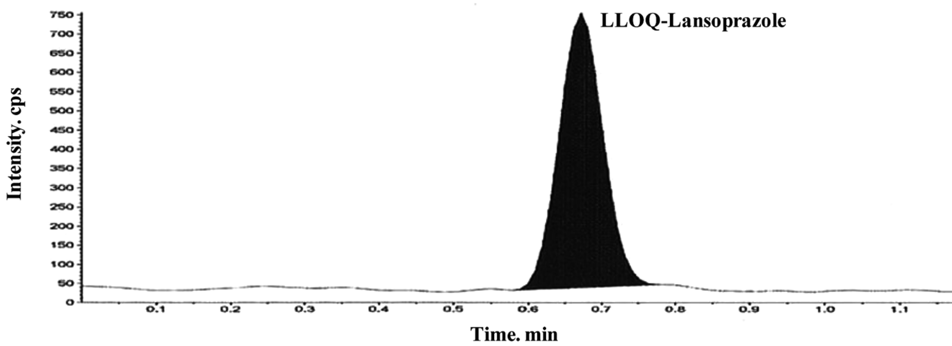

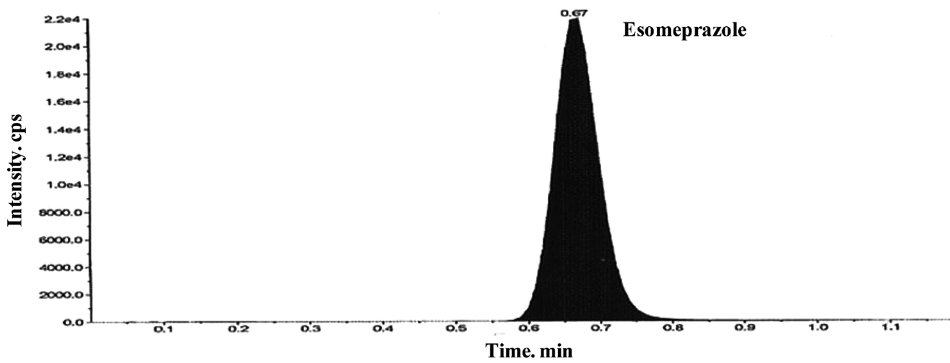

FIGURE 4 Chromatogram of LLOQ of (a) LZ and (b) EZ (IS).

Precision and Accuracy

The intra run precision was between 1.90 to 6.80% for LZ; whereas, at the LOQ levels, it was found to be 4.30 to 6.80% for LZ.

The intra run accuracy was within the range of 87.42% to 110.90% for LZ across all the five levels tested (Table 3). The inter run precision and accuracy were determined by pooling all individual assay results of quality control samples over the five separate batch runs. The inter run precision of LZ was found to be 2.18% to 7.00%, and LOQ levels, were 4.50% to 7.00%. The inter run accuracy was within the range of 88.40% to 105.60% for Lansoprazole (Table 3).

Recovery

Six replicates at low, medium, and high quality control concentration for Lansoprazole were prepared for recovery determination. The mean recovery for LZ was 58.6% with a % C.V value of 4.65, while for EZ mean recovery was 54.2% with a % C.V of 6.48%.

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Stability

Bench top, dry extract, and process stabilities for LZ were investigated at LQC and HQC levels. The results revealed that LZ was stable in plasma for 6 hrs at room temperature (25°C) and 24 hrs in the auto sampler at 4°C. It was confirmed that repeated freeze thawing (three cycles) of plasma spiked with LZ at LQC and HQC level did not affect the stability of LZ. Dry extract stability was also evaluated for a period of 10 hr at LQC and HQC levels. The long term stability results also indicated that the analyte Lansoprazole was stable in matrix up to 42 days at a storage temperature of −70°C. This long term stability resulted in data generated that was sufficient to cover the entire study period from the collection of blood samples to the final date of analysis. The stability of the main stock solutions of LZ and Esomeprazole were also proved by comparing their stored aliquots at a refrigerated temperature of 4°C versus freshly prepared stocks. This was done by comparing the areas obtained from aqueous samples prepared at the MQC level from both the stabilized and freshly prepared stock solutions of LZ and EZ, which were stable until 4.3 hr at room temperature (25°C) and under refrigeration at 4°C for up to 14 days. The mean ratios found were 104.4 and 92.2% for LZ and EZ, respectively, which is in agreement with the acceptance limit (±15%). The results obtained after three freeze and thaw cycles were within acceptance limits for LZ (mean ratio of 98.16%).

RESULT AND DISCUSSION

Method Development and Optimization

To optimize chromatographic conditions several trials were carried out to achieve good separation, peak symmetry, runtime, solvent for extraction, and suitable IS. Appropriate mobile phase was optimized by various combinations of acetic acid and/or ammonium acetate with acetonitrile, from which 20 mM ammonium acetate with acetonitrile in the ratio of 10:90 v/v gave better resolution. With this optimized mobile phase, the m/z value of the parent ions of LZ and EZ are 369 and 346, whereas daughter ions of LZ and EZ were observed at 252 and 198 (Figure 2a, 2b, 2c, and 2d). With the set instrumental parameters, the analyte of interest and IS produced higher peak intensity in positive ion modes; hence, positive ion mode was chosen for the entire study. The chromatograms of the sample revealed that the retention time for LZ and EZ (internal standard) was only 0.67 min, which is a shorter time than that of previously reported methods. The results also showed the absence of peak for endogenous substances in the blank plasma (Figure 3), and the signal observed was more than 10 times shorter than the respective LLOQ of LZ (Figure 3a).

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Linearity

The calibration curve was constructed using concentration vs peak response, which was found to be linear from 3.997 ng/mL to 2002.5 ng/mL. Linear correlation co-efficient was $r = 0.9994$ and the regression equation was $y = 0.0064 \times -2.51e^{-006}$; peak area ratios of the analyte to the IS was above 0.9986 in triplicate experiments (Table 2).

LLOQ

LLOQ of the method was fixed as 3.997 ng/mL, from the lowest concentration in the calibration curve standard.

Specificity

Analyte specificity in the method was studied by analyzing six plasma extracts. It was found that there were no interference or suppression observed at the RT of drug due to endogenous substance in the drug free plasma extract (Figure 3). Figure 4a and Figure 4b were the chromatogram of analyte and IS spiked in the plasma.

Precision and Accuracy

For the global precision and accuracy, three batches were performed; each batch consisted of one calibration curve and 4 QC samples including LLOQ QC. The inter- and intra-day precision and accuracy percentage were in the range of 87.42–110.90.

Stability

Short-term stock solution stability performance was studied for 4.5 hr. The mean ratios found were 104.4 and 92.2% for LZ and EZ, which is in agreement with the acceptance limit (±15%). The long-term stock solution stability was evaluated under refrigerated conditions (2 to 8°C) for 2 weeks. The results obtained after three freeze and thaw cycles were within acceptance limits for LZ (mean ratio of 98.16%). For testing the autosampler stability, three replicates of LQC and HQC samples were extracted and were analyzed after 24 hr with a fresh set of calibration curve standards. The mean values were found to be 95.2 and 97.8% for LQC and HQC, which was within the acceptance limit for stability.

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CONCLUSIONS

A rapid and highly sensitive LC-MS/MS method for quantifying LZ using EZ as a new choice of IS was developed and validated. This method was found to be advantageous over previously reported methods in terms of its small sample volume, fast elution of analyte and IS (0.67 min), simple single solvent extraction, sample cleanup procedure, accuracy, and precision. The LLOQ of the method is very low of 3.997 ng/mL for LZ, hence this method can be easily adapted for pharmacokinetic studies. The study of matrix effects proves that the method is sufficiently free from interference due to endogenous substances. The validation results confirm that the method can be considered suitable for bioequivalence and pharmacokinetic study of LZ in human plasma.

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