Development and Validation of High Performance Thin Layer Chromatography for Determination of Esomeprazole Magnesium in Human Plasma

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Abstract

A simple, sensitive, rapid and economic high performance thin layer chromatographic method has been developed for determination of Esomeprazole magnesium in human plasma by liquid-liquid extraction. The plasma sample was extracted using chloroform. A concentration range from 200-700 ng/spot of Esomeprazole magnesium was used for calibration curve. The percent recovery of Esomeprazole magnesium was found 101.61 percent. The mobile phase constitute of ethyl acetate: methanol: ammonia (32%) (9: 1: 0.5 v/v). Densitometric analysis was carried out at wavelength 301 nm. The Rf value for Esomeprazole magnesium was found 0.54 ± 0.05. The stability of Esomeprazole magnesium in human plasma was confirmed during freeze thaw cycles at -30°C, on bench top during 24 h at room temperature and post preparative for 48 h. The proposed method was validated statistically by performing recovery study for determination of Esomeprazole magnesium in human plasma by liquid-liquid extraction.

Keywords: HPTLC; Esomeprazole magnesium; Human plasma; Liquid-liquid extraction

Introduction

Esomeprazole magnesium dihydrate1 (ESO), bis[5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1-Hbenzimidazol-1-yl] magnesium dihydrate (Figure 1) a, is a compound that inhibits gastric acid secretion. ESO is the S-isomer of omeprazole, the first single optical isomer proton pump inhibitor, generally provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to omeprazole. Several methods have been employed for the estimation of ESO alone and combination with other drugs such as UV and RP-HPLC methods. Literature survey reveals that many analytical methods such as UV spectrophotometric [1-4], HPLC methods [5-10], LCMS [11], HPTLC [12-14] methods are reported for determination of Esomeprazole magnesium individually as well in combination. Specific and sensitive methods based on mass spectrometry methods were reported earlier. Earlier reports on HPLC based bioanalytical estimation of esomeprazole resulted in lesser sensitivity, and high noise in the base line indicating a need to develop a more efficient, sensitive, simple and rapid method in human plasma. To access the reproducibility and wide applicability of the developed method, it was validated as per FDA guidelines [15].

Materials and Methods

Instrumentation

HPTLC Camag with precoated silica gel Plate 60F254 (20 cm × 10 cm) 250 µm thicknesses (E. Merck, Darmstadt, Germany) was used as stationary phase. Sample application was done by using Camag 100 µl syringe and Camag Linomat V applicator. The sample was sprayed in the form of narrow bands of 8 mm length at a constant rate 2 µl/s. Linear ascending development was carried out in 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland). The densitometric scanning was performed by using Camag TLC scanner III supported by winCATS software (V1.4.2.8121 Camag). Evaluation of chromatogram was done by using peak areas of drug.

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 centrifuge tube. Tube was cycomix for 5 min and then centrifuged at 3000 for 20 min. After centrifugation process, the supernatant liquid was collected in another tube and volume make up to 10 ml with methanol. The analysis was carried on HPTLC.

Method validation

Calibration plot: The calibration plot for the HPTLC method was constructed by analysis of six solutions containing different concentrations of Esomeprazole magnesium (200-700 ng/ml). The Esomeprazole magnesium can be determined at LLOQ 2 µl/ml. In the range 200-700 ng/ml the data were best fitted by a linear equation y=mx+b, the coefficient of determination (R2) was 0.9958.

Selectivity: For selectivity, analyses of blank sample of the appropriate biological matrix (plasma) were done. Each blank sample was tested for interference, and selectivity was ensured at the lower limit of quantification (LLOQ) i.e., 200 ng/ml [16,17].

Precision: Precision was measured using a minimum of three determinations per concentration (200, 400, 600 ng/spot). Intraday precision (Repeatability) was performed by taking three different concentrations (200, 00, 600 ng/spot) covering specified range in the triplicates and were analyzed three times within a day with same operator and with same equipment. Inter day precision was determined by analyzing three different concentrations (200, 400, 600 ng/spot) in triplicates on three different days within same laboratory conditions.

Accuracy: Accuracy was determined by replicate analysis of samples containing known amounts of the analyte (200, 400, 600 ng/spot). The study was determined by spiking known amount of standard stock to the test solution prepared from tablet formulation at three different spiking level 80%, 100%, 120% of target concentration.

Recovery: Recovery experiments was performed by comparing the analytical result for extracted samples at three concentrations (200, 400, 600 ng/spot) with unextracted standards that represent 100% recovery.

Limit of detection (LOD) and limit of quantitation (LOQ): The parameters LOD and LOQ were determined using the signal-to-noise ratio by comparing results of the test of samples with known concentrations of analyte to blank samples. The analyte concentration that produced a signal-to noise ratio of 3:1 was accepted as the LOD. The LOQ was identified as the lowest plasma concentration of the standard curve that could be quantified with acceptable accuracy, precision and variability.

Stability

Bench top stability: A Stock solution of Esomeprazole magnesium was kept at room temperature for 24 hours.

Post preparative stability: A Stock solution of Esomeprazole magnesium was kept at room temperature for 48 days.

Freeze thaw stability: The stability of low and high quality control samples were determined after three freeze thaw cycles.

Results and Discussion

Extraction Procedure Optimization One of the most difficult task during the method development was to achieve a high and reproducible recovery from the solvent, which is used for extraction of the drug.
(Table 1). Different solvents were tried for the extraction of from human plasma. Densitogram obtained shows the Rf at 0.52 and is shown in Figure 1. HPTLC densitogram of 200-700 ng/band was analyzed and observed 3-D view was shown in Figure 2.

**Linearity**

The linear plot was observed in the concentration range of 200-700 ng/spot. Results obtained are shown in Table 2 and calibration plot obtained was shown in Figure 2.

**Precision**

Intraday and interday precision of Esomeprazole magnesium in human plasma assures the repeatability of test results. The % RSD found was below 2. Results of intraday and interday precision were shown in Table 3 and Table 4, respectively.

**Accuracy**

Accuracy was studied by standard addition method and % recovery found was within acceptable limit. Results of recovery study are shown in Table 2 and Table 4 and statistical validation is shown in Table 5.

**Recovery**

The recovery of Esomeprazole magnesium for HPTLC recovery at the three concentrations 200, 400, 600 ng/spot were found to 87.82%, 83.84% and 95.27%, respectively (Table 6).

**Selectivity**

Analyses of blank sample of the appropriate biological matrix (plasma) were done. Each blank sample was tested for interference, Sn no | Conc. in ng/spot | Area
--- | --- | ---
1 | 200 | 3197.20
2 | 300 | 4317.58
3 | 400 | 5101.51
4 | 500 | 6038.19
5 | 600 | 7014.55
6 | 700 | 7795.89

Table 2: Data of calibration curve of Esomeprazole magnesium in human plasma.

<table>
<thead>
<tr>
<th>Conc. in ng/spot</th>
<th>Mean</th>
<th>SD</th>
<th>% RSD</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>3219.249</td>
<td>15.51388</td>
<td>0.48191</td>
<td>8.957203</td>
</tr>
<tr>
<td>400</td>
<td>5422.349</td>
<td>22.42588</td>
<td>0.413582</td>
<td>12.94797</td>
</tr>
<tr>
<td>600</td>
<td>7327.349</td>
<td>51.20786</td>
<td>0.698859</td>
<td>29.56574</td>
</tr>
</tbody>
</table>

Table 3: Data for intraday precision of Esomeprazole magnesium in human plasma by HPTLC method.

<table>
<thead>
<tr>
<th>Level of addition</th>
<th>Tablet conc. (ng/band)</th>
<th>API conc. (ng/band)</th>
<th>Total conc. In ng/spot</th>
<th>% recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>80%</td>
<td>300 240 540</td>
<td>95.47</td>
<td></td>
<td></td>
</tr>
<tr>
<td>100%</td>
<td>300 240 540</td>
<td>99.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120%</td>
<td>300 240 540</td>
<td>100.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4: Data for interday precision of Esomeprazole magnesium in human plasma by HPTLC method.

<table>
<thead>
<tr>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>95.47</td>
</tr>
<tr>
<td>99.19</td>
</tr>
<tr>
<td>100.2</td>
</tr>
</tbody>
</table>

Table 5: Data for recovery study of Esomeprazole magnesium in human plasma by HPTLC method.

<table>
<thead>
<tr>
<th>Sn no</th>
<th>Concentration (ng)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>87.82</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>83.84</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>95.27</td>
</tr>
</tbody>
</table>

Table 6: Result of recovery of Esomeprazole magnesium in human plasma.
and selectivity was ensured at the lower limit quantification and chromatogram was shown in Figure 3.

**Stability**

**Freeze thaw stability:** The stability of low and high quality control samples were determined after three freeze thaw cycles (Table 7).

**Short term stock stability:** A Stock solution of Esomeprazole magnesium was kept at room temperature for 24 hours (Table 8).

**Post preparative stability:** A Stock solution of Esomeprazole magnesium was kept at room temperature for 48 days (Table 9).

### Conclusion

The proposed HPTLC method for the estimation of Esomeprazole magnesium in human plasma is selective and sensitive. Sensitivity of the method is suitable for handling various plasma levels of the drug. The method is economical and faster than earlier published methods. In future, we can use this method for bioequivalence study.

### Acknowledgement

The authors are thankful to the Management and Principal, Dr. Rajendra S. Bhambar of M G V’s Pharmacy College, Nashik for providing necessary facilities for the research work. The authors are also thankful to Arpan Blood Bank, Nashik for providing human plasma, (A.S. Bulk Drugs, Hyderabad, India) for providing Esomeprazole Magnesium, as a gift sample for the research work.

<table>
<thead>
<tr>
<th>Sn no</th>
<th>Conc. In ng/spot</th>
<th>Recovery (%) Thaw extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>94.23</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>100.69</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>100.2</td>
</tr>
</tbody>
</table>

Table 7: Result of freeze thaw stability of Esomeprazole in human plasma.

<table>
<thead>
<tr>
<th>Sn no</th>
<th>Conc. In ng/spot</th>
<th>Recovery (%) Thaw extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>93.18</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>95.74</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>97.36</td>
</tr>
</tbody>
</table>

Table 8: Results of Bench top stability of ESO in human plasma.

<table>
<thead>
<tr>
<th>Sn no</th>
<th>Conc. In ng/spot</th>
<th>Recovery (%) Thaw extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>99.173</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>100.5</td>
</tr>
<tr>
<td>3</td>
<td>600</td>
<td>98.40</td>
</tr>
</tbody>
</table>

Table 9: Results for post preparative stability of Esomeprazole in human plasma.

![Figure 3: Chromatogram of Blank human plasma at 300 ng/spot.](image)
References


