Journal of Chromatography
Separation Techniques

Gosavi and Tayade, J Chromatogr Sep Tech 2017, 8:2
DOI: 10.4172/2157-7064.1000360

Research Article Open Access

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

Gosavi SM* and Tayade MA

Department of Pharmaceutical Chemistry, M. G. V.'s Pharmacy College, Panchavati, Mumbai, Agra Road, Nashik- 422003, Maharashtra, India

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). Densiometric 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-3,5dimethyl-2-pyridinyl) methyl] sulfinyl]-1-Hbenzimidazole-1-yl) magnesium dihydrate (Figure 1) a, is a compound that inhibits gastric acid secretion. ESO is cost-effective in the treatment of gastric oesophageal reflux diseases. 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 win CATS software (V1.4.2.8121 Camag). Evaluation of chromatogram was done by using peak areas of drug.

Chemicals

Esomeprazole magnesium (A.S Bulk drugs, Hyderabad, India), was used as such by without checking their purity. The HPLC grade methanol and Analytical Reagent grade ethyl acetate, ammonia solution, chloroform was purchased from Modern sciences, Nashik, India. Human plasma used for research work was supplied by Arpan Blood Bank, Nashik, Maharashtra, India.

Chromatographic condition

Mobile phase was selected as mixture of Ethyl acetate: methanol: ammonia solution (32%) in the ratio of (9:1:0.5 v/v/v) for the development of plates. Time for chamber saturation was optimized to 20 min. The length of chromatographic development was 70 mm. The densitometric scanning was performed at 301 nm.

Preparation of stock solution and working standard solution

Stock solutions1 mg/ml each of Esomeprazole magnesium was prepared in methanol.

Preparation of plasma sample

To 1 ml of fresh human plasma in separate 15 ml of centrifuge tubes, spiked 1 ml of drug solution from 1000 mg/ml stock solution. Wait for C_{max} i.e up to T_{max} of drug (1 and ½ h) to reach equilibrium concentration. 5 ml extracting solvent chloroform was then added in

***Corresponding author:** Gosavi SM, Department of Pharmaceutical Chemistry, M. G. V.'s Pharmacy College, Panchavati, Mumbai, Agra Road, Nashik- 422003, Maharashtra, India, Tel: +91 253 2515620; E-mail: suvi.gosavi999@gmail.com

Received August 04, 2016; **Accepted** March 13, 2017; **Published** March 20, 2017

Citation: Gosavi SM, Tayade MA (2017) Development and Validation of High Performance Thin Layer Chromatography for Determination of Esomeprazole Magnesium in Human Plasma. J Chromatogr Sep Tech 8: 360. doi: 10.4172/2157-7064.1000360

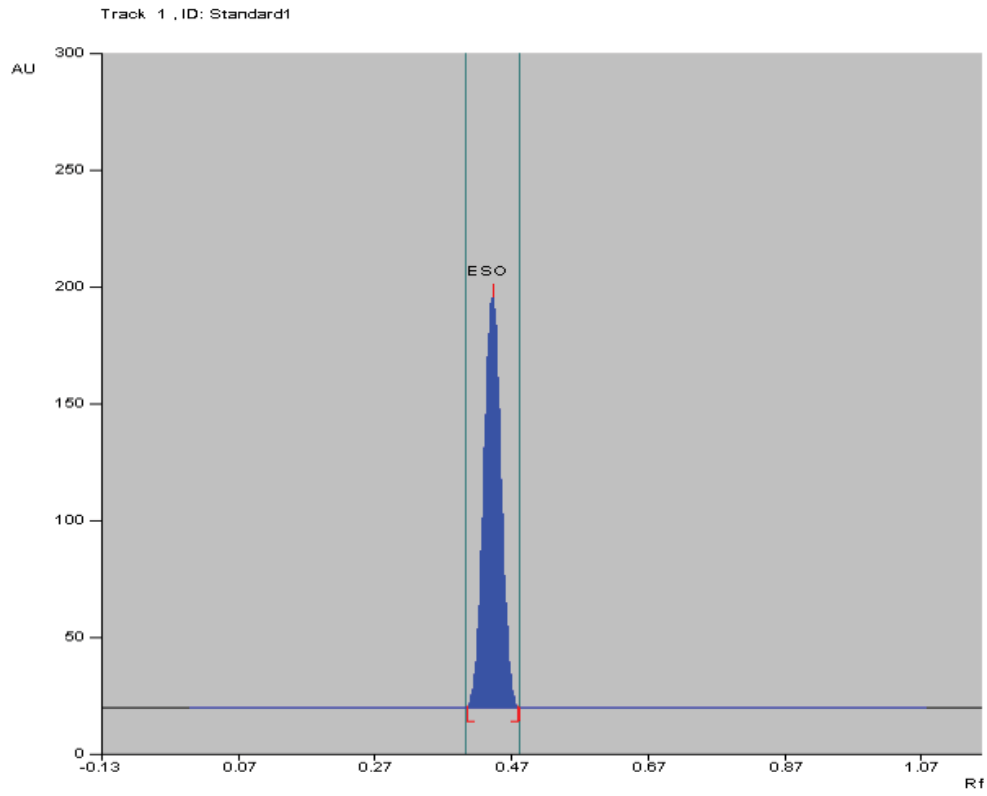

Figure 1: Typical densitogram of Esomeprazole magnesium in human plasma (300 ng/spot). Typical densitogram of Esomeprazole magnesium in human plasma (300 ng/spot).

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	Solvents used	Recovery
1	Acetonitrile	No recovery
2	Ethyl acetate	No recovery
3	Dimethyl ether	No recovery
4	Chloroform 1 ml	20%
5	Chloroform 2 ml	40%
6	Chloroform 4 ml	70-80%
7	Chloroform 5 ml	90%

Table 1: Extraction procedure optimization.

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.

Conc. in ng/spot	Mean	SD	% RSD	SE
200	3219.249	15.51388	0.48191	8.957203
400	5422.349	22.42588	0.413582	12.94797
600	7327.349	51.20786	0.698859	29.56574

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

Conc. in ng/spot	Mean	SD	% RSD	SE
200	3280.24	51.75421	1.57762	29.88118
400	5654.73	23.75512	0.420093	13.71543
600	7675.251	95.36232	1.242465	55.05908

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

Level of addition	Tablet conc. (ng/band)	API conc. (ng/band)	Total conc. In ng/spot	% recovery
80%	300	240	540	95.47
	300	240	540	99.19
	300	240	540	100.2
100%	300	300	600	99.90
	300	300	600	101.2
	300	300	600	100.25
120%	300	360	660	99.80
	300	360	660	100.01
	300	360	660	98.59

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

Sn no	Concentration (ng)	% Recovery
1	200	87.82
2	400	83.84
3	600	95.27

Table 6: Result of recovery of Esomeprazole magnesium in human plasma.

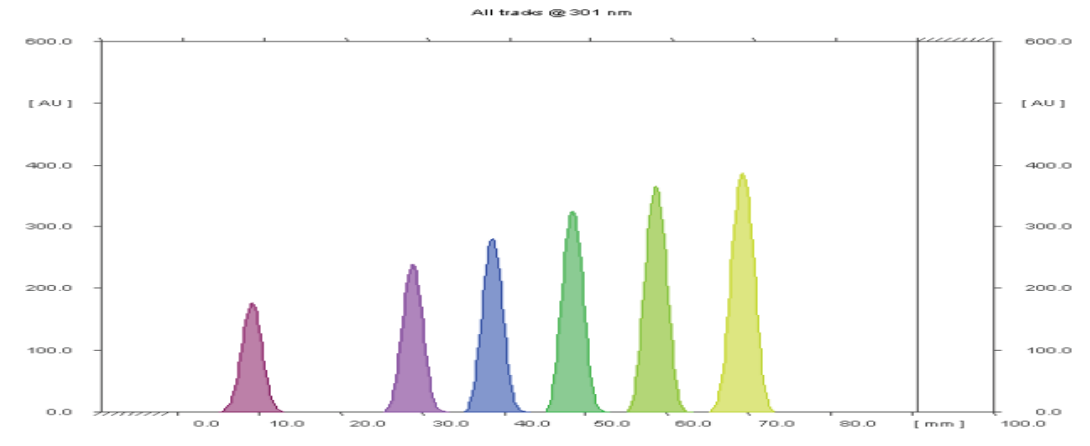

Figure 2: 3-D view of Esomeprazole magnesium 200-700 ng/band.

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.

Sn no	Conc. In ng/spot	Recovery (%) Thaw extract
1	200	94.23
2	400	100.69
3	600	100.2

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

Sn no	Conc. In ng/spot	Recovery (%) Thaw extract
1	200	93.18
2	400	95.74
3	600	97.36

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

Sn no	Conc. In ng/spot	Recovery (%) Thaw extract
1	**200**	**99.173**
2	**400**	**100.5**
3	**600**	**98.40**

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

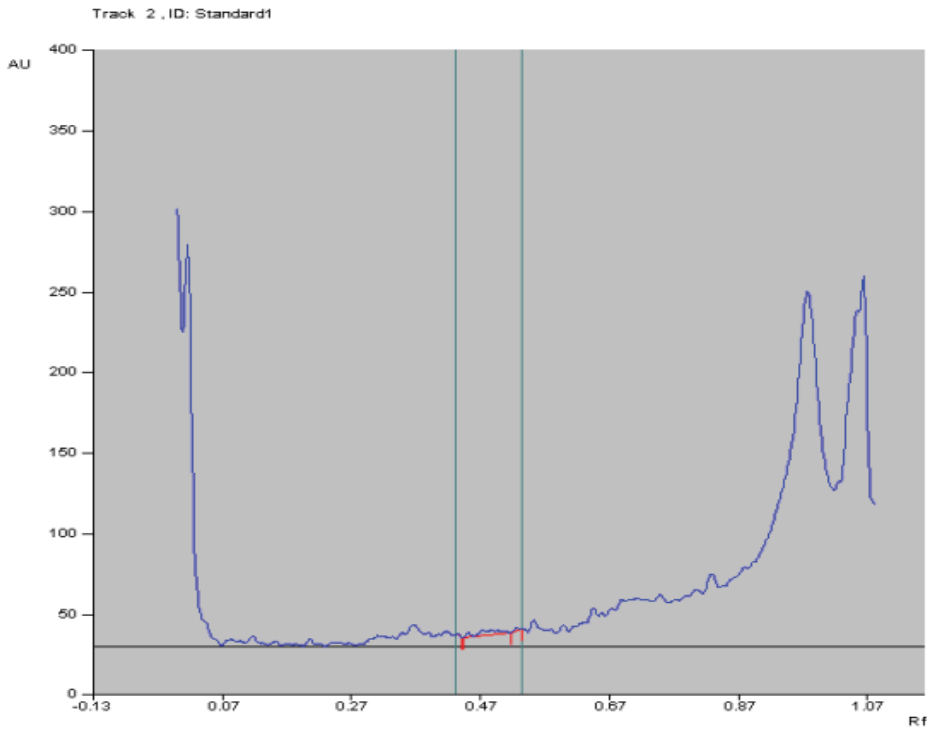

Figure 3: Chromatogram of Blank human plasma at 300 ng/spot.

References

1. Ashutosh KS, Manidipa D, Seshagiri R (2013) Stability indicating simultaneous estimation of assay method for esomeprazole and naproxen in bulk as well as in pharmaceutical formulation by using RP-HPLC. World J Pharm and Pharma Sci 4: 1897-1920.
2. Jain MS, Agrawal YS, Chavhan RB, Bari MM, Barhate SD (2012) UV Spectrophotometric Methods for simultaneous estimation of Levosulpiride and Esomeprazole in capsule dosage form. Asian J Pharm Ana 4: 106-109.
3. Putta RK, Somashekar S, Mallikarjuna GM, Shantakumar SM (2010) Physico-chemical characterization, UV spectrophotometric method development and validation studies of Esomeprazole Magnesium Trihydrate. J Chem Pharm Res 2: 484-490.
4. Lakshmana PS, Shirwaikar A, Annie S, Dineshkumar C, Joseph A, et al. (2008) Simultaneous estimation of Esomeprazole and Domperidon by UV spectrophotometric method. Indian J Pharm Sci 70: 128-131.
5. Singh S, Rai J, Inamullah, Nisha C, Hemendra G, et al. (2013) Stability Indicating Simultaneous Equation Method for Determination of Domperidone and (S)-Esomeprazole Magnesium in Capsule Dosage Form Using UV-Spectrophotometer. Br J Pharmaceut Res 3: 435-445.
6. Deepak KJ, Nitesh J, Rita C, Nilesh J (2011) The RP-HPLC method for simultaneous estimation of Esomeprazole and Naproxen in binary combination. Pharm Methods 2: 167-172.
7. Kumar SA (2016) Development and validation of a new bioanalytical method for simultaneous estimation of esomeprazole and naproxen in human plasma by using RP-HPLC. Br J Pharmaceut Res 4: 2312-2327.
8. Styadev TN, Sudhir KVR, Santosh T, Syam sundar B (2013) Development and validation of High performance liquid chromatographic technique for the determination of Esomeprazole in human plasma. Int J Res Pharma Chem pp: 552-559.
9. Palavai SR, Shakil S, Gururaj V, Badri V, Vure P, et al. (2011) Stability indicating simultaneous estimation of assay method for naproxen and esomeprazole in pharmaceutical formulations by RP-HPLC. Der Pharma Chemica 3: 553-564.
10. Santaji UN, Vangala RR, Dantu DR, Nagendrakumar M (2012) A validated stability indicating ultra performance liquid chromatographic method for determination of impurities in Esomeprazole magnesium gastro resistant tablets. J Pharm and Biom Anal 57: 109-114.
11. Mogili1 R (2011) Development and validation of Liquid Chromatography Tandem Mass Spectrometry for determination of Esomeprazole in Human plasma. Der Pharmacia Lettre 3: 138-145.
12. Sharma S, Sharma MC (2011) Densitometric method for the quantitative determination of Esomeprazole and Domperidon in dosage forms. Am Eur J Toxic Sci 3: 143-148.
13. Sharma S, Sharma MC (2011) Development and validation of densitometric method for quantitative determination of Esomeprazole and domperidone in dosage form. Am Eur J Toxic Sci 3: 143-148.
14. Shaikh NC, Bandgar SA, Kadam AM, Dhaneshwar SR (2012) Development and validation for simultaneous estimation of Esomeprazole and domperidone as bulk drug and in tablet dosage form by high performance thin layer chromatography. Int J Res Ayurveda Pharma 3: 421-424.
15. Validation of chromatographic methods (2001) (Center for drug evaluation and research CDER), pp: 2-26.
16. Lakshmana PS, Suriyaprakash TNK (2012) Extraction of Drug from the Biological Matrix: A Review. Biol Mat pp: 480-506.
17. Aryal B, Mamata SN, Kalpana D, Harish CN (2012) Pharmacokinetics of Esomeprazole in Oryctolagus Cuniculus Male Rabbit with Immobilized Stress. Int J Pharmaceut Biol Arch 3: 49-55.