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Analysis of Esomeprazole Magnesium Trihydrate in Pharmaceutical Formulation by Hptlc

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ABSTRACT:
A simple, precise, specific and accurate HPTLC method has been developed and validated for the analysis of Esomeprazole Magnesium Trihydrate in pharmaceutical dosage form by using internal standard . The separation was carried out on HPTLC Aluminium plates precoated with silica-gel 60 F254(10 ×10 cm) using Ethyl Acetate: n-Hexane : methanol [8:1:1v/v] as mobile phase. HPTLC separation of the two drugs followed by chromatographic measurement was carried out in the absorbance mode at 294 nm. The drugs were resolved satisfactorily with RF values of 0.436 ± 0.01 and 0.556 ± 0.01 for and Esomeprazole Magnesium Trihydrate and Lansoprazole(IS) respectively. The linear regression analysis data for the calibration plots showed good linear relationship with R2= 0.998 for Lansoprazole(IS)and Esomeprazole Magnesium Trihydrate, respectively in the concentration range of 0.016-0.8 µg/spot. The method was validated for accuracy, precision, specificity and robustness. The RSD values for intra- and inter-day precision were 0.008921 and 0.002886 for Esomeprazole Magnesium Trihydrate. The mean recovery for ES from the tablets is 99.42%.The proposed developed HPTLC method can be applied for identification and quantitative determination of Esomeprazole Magnesium Trihydrate in bulk drug and drug formulation.

KEYWORDS: Esomeprazole Magnesium Trihydrate, Lansoprazole, internal standard , HPTLC, validation.

I. INTRODUCTION [1,2,3,4]

Quality assurance is a wide ranging concept covering all matters that individually or collectively influence the quality of the product. It plays a central role in determining the safety and efficiency of medicines. Highly specific and sensitive analytical techniques hold the key to the design, development, standard and quality control of medicinal products. High Performance Thin layer Chromatography (HPTLC) is simplest of all the chromatographic techniques. Separation is based on migration of the sample spotted on a coated(stationary phase) plate with one edge dipped in a mixture of solvents (mobile phase). The whole system is contained in an enclosed tank. Detection techniques include fluorescence, UV and sprays(universal and specific) for compounds that are not naturally colored. The location of the analyte on the TLC plate is described by the Rf value which is the ratio of the migration distance of the compound of interest to the mobile phase front.

The internal standard [7,8] is a compound that matches as closely, but not completely with analyte.An internal standard is a known amount of a compound, different from analyte that is added to the unknown. Signal from analyte is compared with signal from the internal standard to find out how much analyte is present. Internal standards are especially useful for analysis in which the quantity of sample analyzed or the instrument response varies slightly from run to run for reasons that are difficult to control. Internal standards are widely used in chromatography because the small quantity of sample solution injected into the chromatograph is not very reproducible in some experiments.

As per FDA Validation [9,10,11]is "An Established documentary evidence that provides high degree of assurance that a specific process will consistently produce a product meeting its predetermined specifications and quality characteristics". Typical analytical characteristics used in method validation are Accuracy, Precision, Specificity, Robustness, Limit of detection, Limit of quantitation , Linearity and range, Ruggedness.

DRUG PROFILE:
esomeprazole magnesium trihydrate[12, 13,14,15]
Structure:

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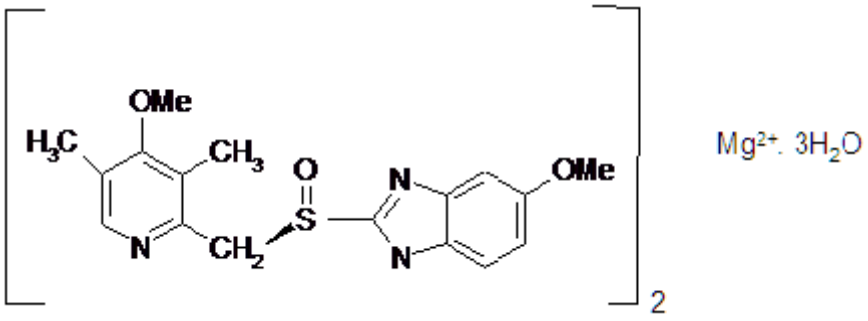
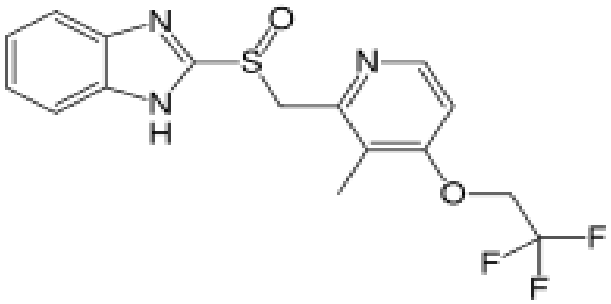


LANSOPRAZOLE (Internal Standard)[16,17]

Structure:



II. MATERIAL AND METHODS:

A. Estimation of Esomeprazole Magnesium Trihydrate in Tablet Dosage Form by HPTLC.

1. **Determination of wavelength for detection of Esomeprazole Magnesium Trihydrate (ESO) and Lansoprazole (LAN) (IS).**

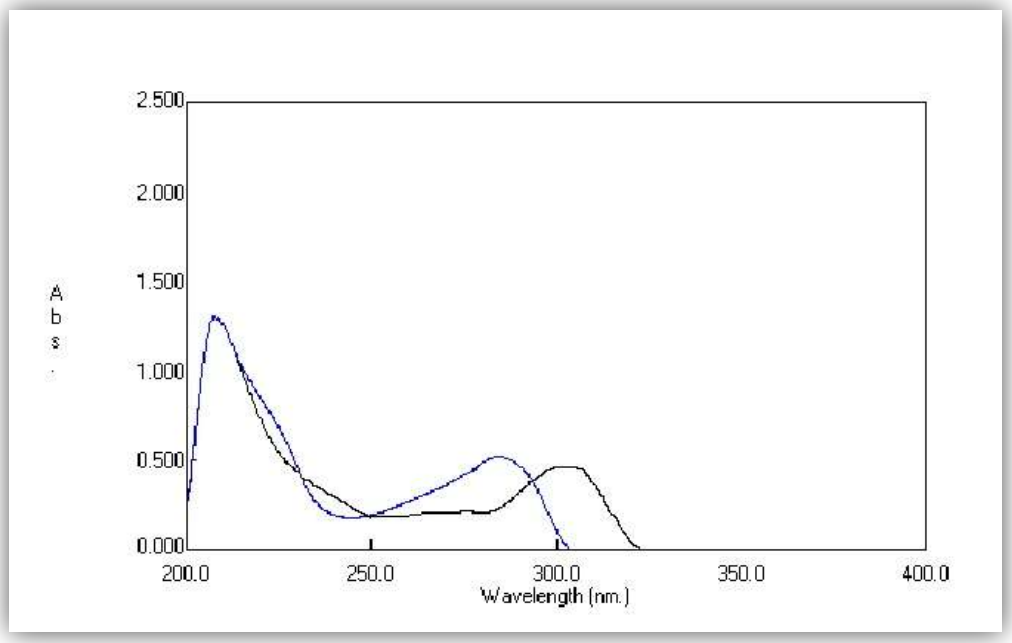


Fig No.2: Overlain Spectrum of Esomeprazole Magnesium Trihydrate (ESO) and Lansoprazole (LAN) (IS). The suitable wavelength selected for detection of ESO and LAN (IS) from the overlain spectrum was 294nm

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2. **Selection of Mobile Phase**

Based on sample solubility, stability and suitability, various mobile phase compositions were tried to get a good separation. The standard solution containing mixture of ESO and LAN as well as individual drugs were run in different mobile phases.

Aliquot portion of standard solutions of containing mixture of ESO and LAN (IS) were applied on 10 x10 cm Silica Gel 60 F_{254} TLC plate in the form of 6 mm band with 21.2 mm interval using Linomat V semiautomatic sample applicator and migration distance allowed was 80 mm. The plates were allowed to develop using different mobile phases in Twin trough glass chamber. After development plates were withdrawn out of chamber dried in hot air and spots were located under UV/ Fluorescence Lamp [254 nm/366 nm]. The Rf values were determined after scanning with CAMAG TLC Scanner III using Win CATS software.

From the various mobile phases tried, mobile phase containing Ethyl Acetate: n-Hexane: Methanol [8:1:1 v/v] with 30 min time of saturation with filter paper with plate equilibrium was selected and it was used throughout the further experimentation.

3. Chromatographic conditions:

The following chromatographic conditions were maintained throughout the method development.
Plates : Silica Gel 60 F_{254} HPTLC Aluminum sheets
Particle size : 7 µm
Thickness: 200 µm
Developing Chamber: Twin trough glass chamber
Mobile Phase : Ethyl Acetate: n-Hexane: Methanol [8:1:1 v/v] with 30 min time of saturation with filter paper with plate equilibrium
Sample application : As a band with Linomat V
Application position : 8 mm
Migration Distance : 80 mm
Detection : U.V Densitometric scanning
Scanning mode Absorbance
Detection wavelength : 294 nm
Temperature: Room temperature
Separation Technique : Ascending

4. **Preparation of standard calibration curve**

Standard stock solution:

ESO standard stock solution:

An accurately weighed quantity of ESO [= 20mg] was dissolved in methanol and volume was made up to 25 ml with methanol [0.8 µg/µl].

LAN (IS) standard stock solution:

An accurately weighed quantity of LAN [= 20 mg] was dissolved in methanol and volume was made up to 25 ml with methanol [0.8 µg/µl].

Aliquot portions of standard stock solutions of ESO and LAN were further diluted with methanol to get 0.08µg/µl of ESO and 0.08µg/µl for LAN (IS), respectively. Solution of ESO was applied as bands ranging from 2-10 µl on TLC plate with Linomat V. The solution of LAN (IS) was spiked 4 µl on each band of ESO on TLC plate with Linomat V. The plates were developed in Twin trough chamber, already saturated with mobile phase for 30min. After drying it was evaluated densitometrically. The observations are shown in Table No.3 and graph was plotted as concentration of drug Vs ratio of peak area of ESO/LAN as shown in Fig. No.3

Table No.3: Observations for standard calibration curve.

Sr. No.	Volume applied [µl]	Conc./ spot ESO [µg]	Peak area ESO	Peak area LAN [4µl]	Ratio ESO/LAN
1	2	0.16	2513.1	4578.7	0.545887
2	4	0.32	5117.7	4775.2	1.024763
3	6	0.48	6740.6	4726.8	1.46417
4	8	0.64	8505.6	4609.3	1.847557

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5	10	0.8	9923.1	4328.5	2.155462

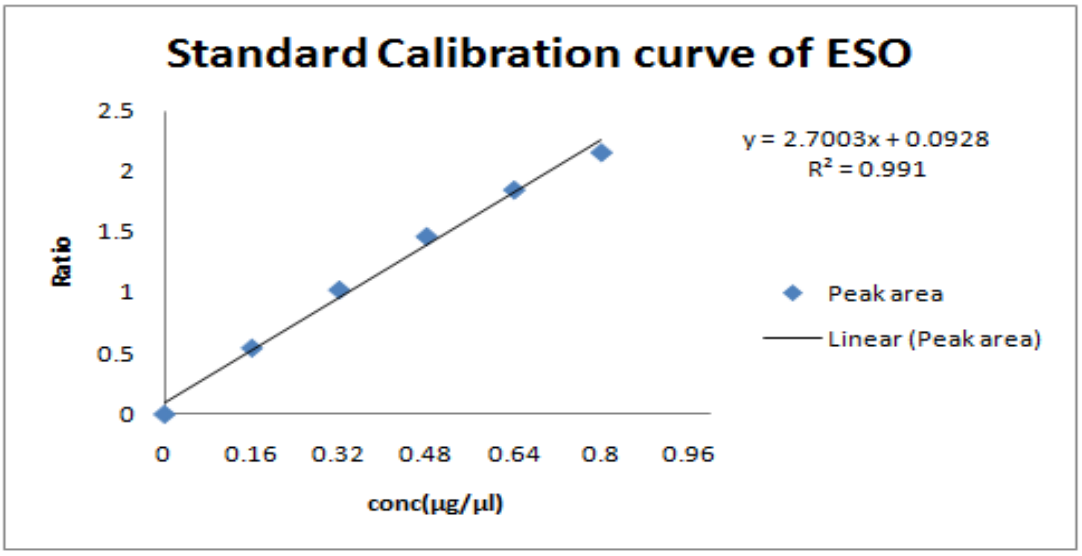

Fig.No.3: Standard calibration curve of Esomeprazole Magnesium

5. System Suitability Test

System suitability test is a pharmacopoeial requirement and is used to verify, whether the proposed chromatographic system is suitable for analysis of drugs or not. It is used to check whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The tests were performed by collecting data from five replicate injections of standard drug solution.

a] Preparation of standard drug solution: Aliquot portions of standard stock solution of ESO and LAN(IS) were prepared and diluted appropriately with methanol to get final concentration of 0.08 µg/µl for ESO and 0.08µg/µl for LAN(IS), respectively.

b] Procedure: A 3µl of standard stock solution of ESO was applied as a bands of 6mm five times separately on TLC plate and 4µl of standard stock solution of LAN(IS) was spiked on each band of ESO and their system suitability parameters were calculated. The results are shown in Table No.4.

Table No.4: System Suitability Parameters

Sr. No.	Asymmetry		Selectivity	Retention Factor		Resolution	Capacity Factor	
	ESO	LAN		ESO	LAN		ESO	LAN
1.	0.9262	1.0961	1.83	0.44	0.56	2.739	3.406	4.608
2.	1.0	0.9314	1.85	0.43	0.55	2.863	3.314	4.513

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3.	1.0	1.0	1.83	0.44	0.56	2.628	3.406	4.608
4.	0.9262	1.0961	1.83	0.44	0.55	3.148	3.406	4.513
5.	0.9262	1.0	1.85	0.43	0.56	2.948	3.314	4.608
Mean	0.95572	1.02472	1.838	0.436	0.556	2.8252	3.3692	4.57
± S.D.*	0.0456	0.0709	0.010954	0.005477	0.005481	0.219036	0.05039	0.052034
R.S.D.*	0.0480	0.06343	0.00596	0.012562	0.009851	0.077529	0.014956	0.011386
C.V.	4.806	6.343	0.596	1.2562	0.9851	7.7729	1.45956	1.1386

Where, S.D.*=Standard Deviation, R.S.D.*= Relative Standard Deviation, C.V. = Coefficient Variance

6. **Analysis of laboratory mixture by proposed method**

Preparation of laboratory mixture [Standard]:

Accurately weighed quantity of 20 mg ESO [99.5 %, Themis Laboratories, Mumbai, India] was dissolved in methanol in 25 ml volumetric flasks. Volume was made up to the mark with methanol. The aliquot portion of the solution was further diluted to get final concentration of 0.08µg/µl for ESO.

Preparation of laboratory mixture [Sample]

Five different laboratory mixtures of ESO [Zim Laboratories Ltd, Kalmeshwar, India] were prepared by same procedure as for laboratory mixture [standard] so as to get the final concentration of 0.08µg/µl for ESO.

Preparation of Internal standard

Accurately weighed quantity of 20 mg LAN [99.43 %, Blue Cross Laboratories, Nasik, India] was dissolved in methanol in 25 ml volumetric flasks. Volume was made up to the mark with methanol. The aliquot portion of the solution was further diluted to get final concentration of 0.08µg/µl for LAN.

Procedure

3 µl of standard and sample laboratory mixtures of ESO were applied as 6 mm bands on the TLC plate. 4 µl solution of LAN (IS) was spiked on each bands of ESO on the TLC plate. The plate was developed in Twin trough chamber, already saturated with mobile phase for 30 min. After development the plate was dried with the help of hot air drier and evaluated densitometrically at wavelength of 294 nm.

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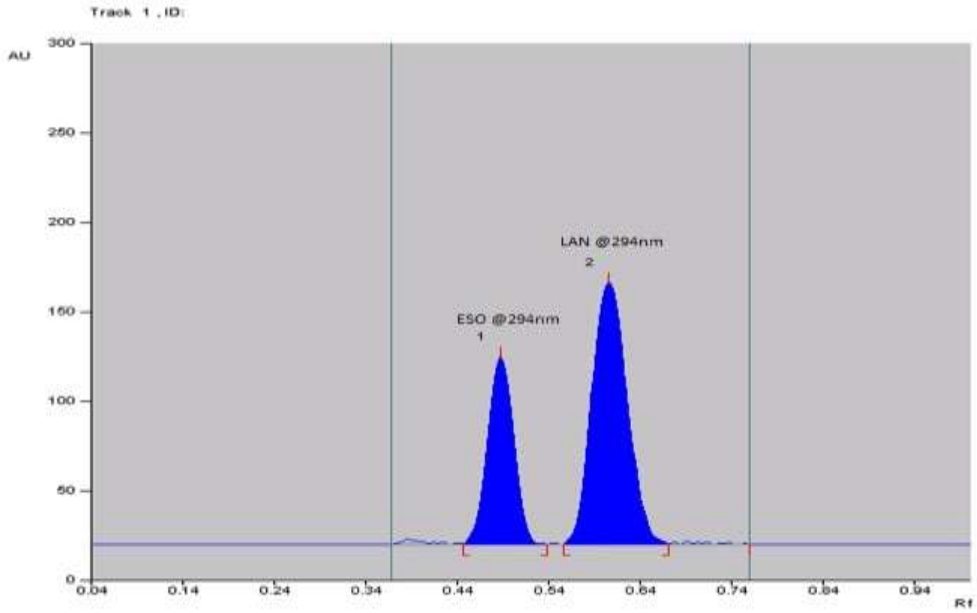

Fig No.4: Chromatogram of Esomeprazole Magnesium [Rf 0.44] and Lansoprazole (IS) [Rf 0.56] in laboratory mixture.

Table No. 5: Results of % estimation of drugs in laboratory mixture.

Sr. No	Laboratory mixture	Wt. taken [g]		Peak area		% estimated
		ESO	LAN	ESO	LAN	ESO
1	Standard	0.0201	0.02	3380.2	4625.9	-
2	Sample	0.020		3336.3	4717.3	99.189
		0.0202		3354.9	4654.1	98.77
		0.0201		3327.7	4691.4	98.48
		0.0200		3342.3	4673.9	99.37
		0.0200		3367.1	4598.1	100.10
					Mean	98.18

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± S. D	0.6206
R. S. D	0.000973
C. V	0.0973

7. Application of proposed method for estimation of ESO in Tablet formulation:

Standard Solution:

Accurately weighed quantity of 20 mg ESO [99.5 %, Themis Laboratories, Mumbai, India] was dissolved in methanol in 25 ml volumetric flask. Volume was made up to the mark with methanol. The aliquot portion of the solutions was further diluted to get final concentration of 0.08µg/µl for ESO.

Sample Preparation:

Twenty tablets (ESOZ-20) were weighed and powdered finely. An accurately weighed quantity of powder equivalent to 20 mg of ESO was taken in 25 ml volumetric flask. Then about 15 ml of methanol was added to the flask and sonicated for 30 min, finally volume was made up to the mark with methanol. The extracts were filtered through Whatmann filter paper no 41 and required dilutions were made to get the final concentration of 0.08 µg/µl for ESO.

Preparation of Internal standard :

Accurately weighed quantity of 20 mg LAN [99.43 %, Blue Cross Laboratories, Nasik, India] was dissolved in methanol in 25 ml volumetric flasks. Volume was made up to the mark with methanol. The aliquot portion of the solution was further diluted to get final concentration of 0.08µg/µl for LAN.

Procedure:

3 µl of standard and Sample of ESO were applied as 6mm bands on the TLC plate. 4 µl of LAN (IS) was spiked on each bands of ESO on the TLC plate. The plate was developed in Twin trough chamber, already saturated with mobile phase for 30 min. After development the plate was dried with the help of hot air drier and evaluated densitometrically at wavelength of 294 nm.

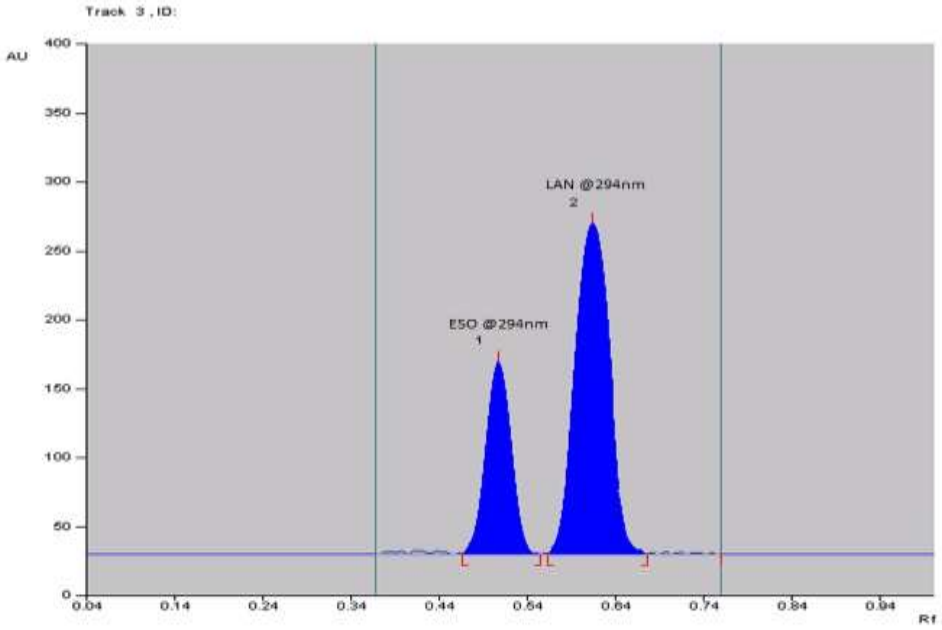

Fig No.5: Chromatogram of Esomeprazole Magnesium [Rf 0.45] and Lansoprazole (IS) [Rf 0.57] in marketed formulation.

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Table No. 6: Results of % estimation of drugs in tablets.

Brand Name: ESOZ-20						**Average Wt.:0. 14083 g**
Sr. No	**Laboratory mixture**	**Wt. taken [g]**		**Peak area**		**% estimated**
		ESO	**LAN**	**ESO**	**LAN**	**ESO**
1	**Standard**	0.0200	0.0201	3315.6	4618.9	-
2	**Sample**	0.1408		3243.7	4697.1	99.42
		0.1403		3267.6	4564.3	98.64
		0.1404		3291.3	4593.7	100.36
		0.1406		3309.2	4659.5	99.01
		0.1405		3293.3	4713.3	99.76
					Mean	99.438
					± S. D	0.66597
					R. S. D	0.00669
					C. V	0. 6697

8. **Validation parameters:**

8.1. Accuracy

Accuracy of proposed method was ascertained on the basis of recovery study performed by standard addition method.

On plate recovery study

An accurately weighed quantity of pre-analysed tablet powder equivalent to 20 mg ESO (n=3) was taken in 25 ml volumetric flask and to it 80%, 100% and 120% of labelled claim of ESO was added. Then to each flask about 15 ml of methanol was added and sonicated for 45 min, finally volume was made up to the mark with the same. The extracts were filtered through Whatman filter paper no. 41 and required dilutions were made.

Procedure:

3 µl of sample preparation of ESO was applied [three times] as band of 6 mm. These bands of ESO was spiked with 4 µl of LAN (IS) solution and the plate was developed in Twin trough glass chamber, already saturated with mobile phase for 30 min. After development the plate was removed and dried under hot air and then evaluated densitometrically at 294 nm. The % recovery was then calculated by using formula.

Table No.7: Results of recovery study

Sr. No	**Wt. of tablet powder taken [g]**	**Amount of pure drug added each [g]**	**Wt. taken (g)**	**% of drug found on preanalysed basis**	**Sample Peak area**	**% Recovery**

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		ESO	LAN(IS)	ESO	ESO	LAN(IS)	ESO
1	0.1407	0.0163	0.0201	99.58	6329.2	4627.6	99.22
2	0.1409	0.0201	0.020	99.85	6990.3	4745.7	99.35
3	0.1408	0.0241	0.0202	100.006	7587.7	4586.4	99.70
						Mean	99.42
						± S.D.	0.248261
						C.V	0.2579
						R.S.D	0.002497

8.2. Precision:
Precision of an analytical method is the degree of agreement among individual test results. It was ascertained by replicate estimation of marketed formulation [five times] and expressed as the S.D. and R.S.D. of the series of measurements. The results are shown in Table No.8.

Table No.8: Precision Study.

Brand Name: ESOZ-20		**Average Wt.:0. 14083 g**				
Sr. No	**Laboratory mixture**	**Wt. taken [g]**		**Peak area**		**% estimated**
		ESO	**LAN**	**ESO**	**LAN**	**ESO**
1	**Standard**	0.0200	0.0201	3315.6	4618.9	-

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2	**Sample**	0.1408	3243.7	4697.1	99.42
		0.1403	3267.6	4564.3	98.64
		0.1404	3291.3	4593.7	100.36
		0.1406	3309.2	4659.5	99.01
		0.1405	3293.3	4713.3	99.76
				Mean	99.438
				± S. D	0.66597
				R. S. D	0.00669
				C. V	0. 6697

8.3. Linearity and range:
According to USP 80 % to 120 % of test concentration was taken and dilution was done appropriately. The observations are shown in Table No.9 and graphs are depicted in Fig. No.6

Fig.No.6: Plot of linearity and range of Esomeprazole

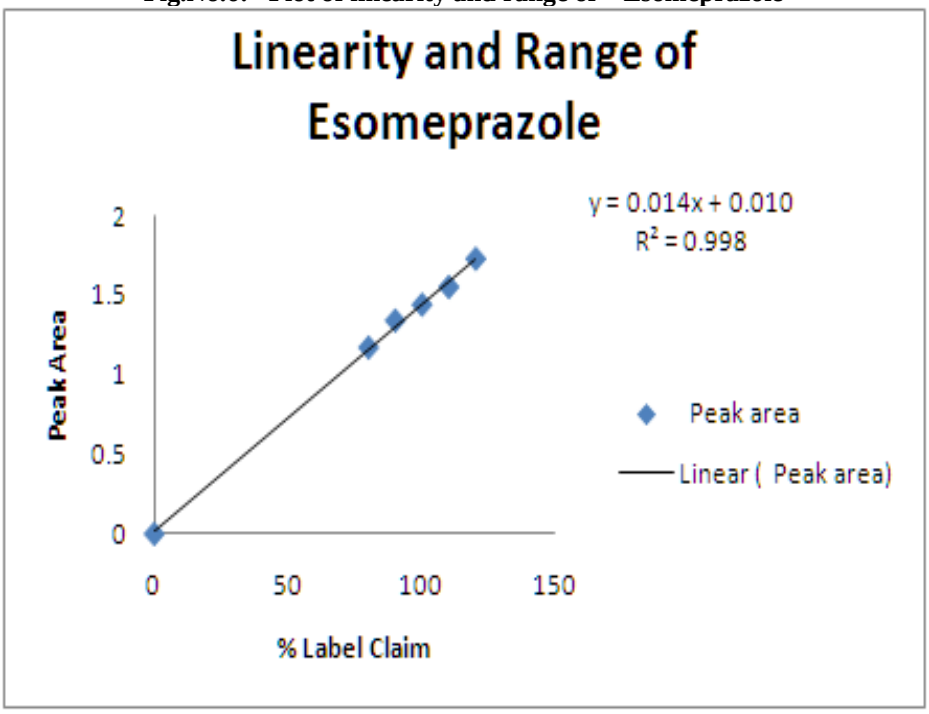

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Table No.9: Observations of linearity and range study

Sr. No.	% test concentration	Peak area		Ratio
		ESO	LAN(IS)	
1	80	5380.2	4678.2	1.1686
2	90	6158.9	4723.6	1.3378
3	100	6617.7	4875.1	1.4374
4	110	7129.1	4563.8	1.5485
5	120	7945.4	4634.3	1.7258

The graph was plotted as % test concentration Vs Peak area and correlation coefficients were calculated

8.4. Ruggedness:

The study of ruggedness was carried out under two different conditions.

8.4.1. Different days:

a) **Interday study:**

The interday study was performed by applying the proposed method on same sample of tablet on different days. The percent label claim was calculated using same formula as in analysis of tablet. The results are shown in Table No 10.

Table No. 10: Results of Interday study

Day	Wt. of tablet powder taken (g)	Wt. of std taken [g]		Standard peak area		Sample peak area		% Label claim
		ESO	LAN(IS)	ESO	LAN(IS)	ESO	LAN(IS)	ESO

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1	0.1407	0.0200	0.0202	3313.4	4631.2	3286.9	4543.4	99.21
2	0.1408	0.0201	0.0200	3289.6	4783.7	3247.5	4716.3	99.26
3	0.1406	0.0202	0.0201	3327.5	4574.4	3293.7	4662.5	99.73
							Mean	99.40
							± S.D.	0.28688
							C.V.	0.288
							R.S.D.	0.002886

- **Intraday study:**

The intraday study was performed by applying the proposed method on same sample of tablet on same day at two hours interval. The percent label claim was calculated using same formula as in analysis of tablet. The results are shown in Table No. 11.

Time [hour]	**Wt. of tablet powder taken (g)**	**Wt. of std taken [g]**		**Standard peak area**		**Sample peak area**		**% Label claim**
		ESO	**LAN(IS)**	**ESO**	**LAN(IS)**	**ESO**	**LAN(IS)**	**ESO**
0	0.1407	0.0202	0.0201	3418.5	4587.9	3346.2	4672.9	98.11

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2	0.1408	0.0201	0.0200	3391.8	4652.6	3324.7	4738.5	98.52
4	0.1406	0.020	0.0202	3386.6	4724.4	3359.5	4565.6	99.80
							Mean	98.81
							± S.D.	0.881533
							C.V.	0.892
							R.S.D.	0.008921

8.4.2. Different analysts:
The sample and standard solutions were prepared by three different analysts and analysis was done by proposed method. The percent label claim was calculated using same formula as in analysis of tablet. The results are shown in Table No.12.

Table No.12: Results of different analysts

Analyst	**Wt. of tablet powder taken (g)**	**Wt. of std taken [g]**		**Standard peak area**		**Sample peak area**		**% Label claim**
		ESO	**LAN(IS)**	**ESO**	**LAN(IS)**	**ESO**	**LAN(IS)**	**ESO**
Analyst 1	0.1406	0.0200	0.0201	3317.8	4751.1	3273.2	4761.2	99.11

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Analyst 2	0.1408	0.0201	0.0201	3312.2	4757.7	3258.9	4716.8	99.14
Analyst 3	0.1407	0.0200	0.0202	3337.6	4634.5	3289.4	4682.7	98.94
							Mean	99.06
							± S.D.	0.107858
							C.V.	0.1088
							R.S.D.	0.001089

III. DISCUSSION AND CONCLUSION

Market survey revealed that tablet formulation containing Esomeprazole Magnesium Trihydrate was available especially for the treatment of peptic ulcer disease , Gastric Esophageal Reflux Disease [GERD][14].

Literature survey revealed that very few analytical methods are so far been indicated for the Esomeprazole Magnesium Trihydrate, so an attempt has been made to develop precise, accurate, simple, reliable, economic and validated HPTLC method for these formulation using internal standard.

Esomeprazole Magnesium Trihydrate and Lansoprazole (IS)

Both the drugs show solubility in methanol, hence methanol was selected for HPTLC study. UV overlain spectra of these drugs was taken and detection was carried out at 294 nm. some chromatographic conditions of these drugs are depicted in Table No. 13

Initially, various mobile phase compositions were tried, but only Ethyl Acetate: n-Hexane : methanol [8:1:1v/v] was found to be quite robust. The average Rf values for Esomeprazole Magnesium Trihydrate and Lansoprazole (IS) were found to be 0.44 ± 0.0054 and 0.56 ± 0.00548. The calibration curve was found to be linear in concentration range of 0.016-0.8 µg/spot for Esomeprazole Magnesium Trihydrate.

Table No.13: chromatographic conditions.

Sr. No.	Parameters	HPTLC
1	**Stationary phase**	Silica Gel 60 F_{254} HPTLC Aluminium sheets

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2	**Mobile phase**	Ethyl Acetate: n-Hexane : methanol [8:1:1v/v]
3	**Detection wavelength**	294 nm
4	**Sample size**	3 µl

The limit of detection for Esomeprazole Magnesium Trihydrate was found to be 0.8151 ng/spot . While the limit of quantitation for Esomeprazole Magnesium Trihydrate was found to be 2.4453 ng/spot .

System suitability tests are used to verify the reproducibility of the chromatographic system. The tests were carried out on freshly prepared standard stock solutions. The parameters obtained are shown in Table No 14.

Table No.14: System Suitability Parameters[29,30]

Parameters*	**ESO**	**LAN**
Asymmetry	0.95572	1.02472
Rf values	0.436	0.556
Resolution	2.8252	
Selectivity	1.838	
Capacity factor	3.3692	4.57

***** Mean of five determinations

In the next step the proposed method was applied on the standard laboratory mixture and then to the marketed formulation.

Recovery studies were carried out to study accuracy of the methods. These studies were carried out at three levels i.e. **Multiple level recovery studies**. The results of recovery studies for Esomeprazole Magnesium Trihydrate was found to be within acceptable limit.[29,30]

Ruggedness of the proposed method was determined by analysis of three samples of three different concentrations, which were prepared and analyzed by intraday, by interday and by different analysts. The results show the accuracy and reproducibility of the method. The % RSD was calculated, that was found to be within range.[9,10,11]

Table No. 15: Results for laboratory mixture, marketed formulation and validation

Sr. No.	**Parameters**	**Statistical data**	**HPTLC**
			ESO
1	**Standard Laboratory mixture**	**Mean [%]**	98.18

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		S.D.	0.6206
		%R.S.D.	0.000973
2	**Marketed formulation**	**Mean [%]**	99.438
		S.D.	0.66597
		%R.S.D.	0.00669
3	**Recovery study**	**Mean [%]**	99.42
		S.D.	0.248261
		%R.S.D.	0.002497
4	**Interday study**	**Mean [%]**	99.40
		S.D.	0.28688
		%R.S.D.	0.002886
5	**Intraday study**	**Mean [%]**	98.81
		S.D.	0.881533
		%R.S.D.	0.008921
6	**Different analysts**	**Mean [%]**	99.06
		S.D.	0.107858
		%R.S.D.	0.001089

CONCLUSION

- The results of analysis by this method are computed to know the accuracy, precision and ruggedness of method. The small values of standard deviation (S.D.) in HPTLC method suggest that the method has high precision.
- The results of recovery study show that the method is accurate. Method was found to be rugged under two different conditions i.e. days and analysts.
- On the basis of above study and results, it can be concluded that the proposed HPTLC method is specific, accurate and precise for the determination of Esomeprazole Magnesium Trihydrate using internal standard from tablet dosage form.
- The results obtained by use of the method, with an internal standard, were comparable with those from reported methods for analysis of Esomeprazole Magnesium Trihydrate in pharmaceutical formulations.

CONCLUSION

On the basis of above study and results, it can be concluded that the proposed HPTLC method is simple, accurate, economic, reliable, and precise and so it can be used for routine estimation of Esomeprazole Magnesium Trihydrate in

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pharmaceutical dosage form and can be used for routine analysis in Quality Control Laboratory.

Probable Outcome:

The validated HPTLC method can be used for the determination of Esomeprazole Magnesium Trihydrate in different brands using an internal standard and can be conveniently used for quality control of drugs in pharmaceutical dosage form in quality control laboratories.

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