

ANALYTICAL METHODS DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF ESOMEPRAZOLE MAGNESIUM TRIHYDRATE AND METFORMIN HYDROCHLORIDE IN SYNTHETIC MIXTURE

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ABSTRACT

The objective of this study was to develop and validate accurate, precise, and reliable RP-HPLC methods for the simultaneous estimation of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in a synthetic mixture as per ICH guidelines. Identification tests such as melting point, solubility, FT-IR were performed. In the RP-HPLC method, a mobile phase of phosphate buffer and acetonitrile (60:40 v/v, pH 3) was used. Retention times were found to be 2.3 min for Esomeprazole and 5 min for Metformin. The method showed excellent linearity, precision, and accuracy with correlation coefficients close to 1. Recovery studies were within acceptable limits. Low LOD and LOQ values indicated high sensitivity. Percentage assay values were near 100%, confirming the reliability of the developed method. No interference from excipients was observed. The developed RP-HPLC method was found to be accurate, precise, economical,

and reproducible. This method is suitable for routine quality control analysis of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in synthetic mixtures.

KEYWORDS: Esomeprazole Magnesium Trihydrate, Metformin Hydrochloride, Synthetic Mixture, RP-HPLC Method Development and Validation.

INTRODUCTION^[1,8]

Esomeprazole Magnesium Trihydrate is a proton pump inhibitor used for the treatment of acid-related disorders such as gastroesophageal reflux disease (GERD), heartburn, and gastric ulcers by reducing gastric acid secretion. Metformin Hydrochloride is a biguanide antidiabetic drug widely used for the management of type 2 diabetes mellitus by decreasing hepatic glucose production and improving insulin sensitivity.

The combination of these drugs has shown potential therapeutic benefits, as both drugs may exhibit synergistic effects in certain pathological conditions such as pre-eclampsia by reducing endothelial dysfunction and specific protein secretion.

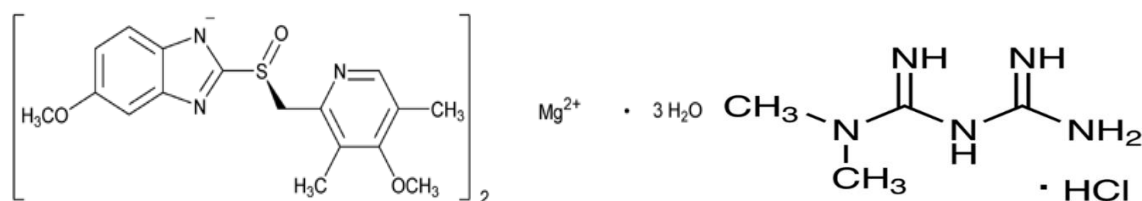


Fig. 1: structure of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride.^[7,8]

Literature review reveals that various analytical methods such as RP-HPLC^[9-13] have been reported for individual drugs. However, no validated method has been reported for the simultaneous estimation of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in synthetic mixture.

Therefore, the present study aims to develop and validate simple, precise, and accurate RP-HPLC methods for simultaneous estimation of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in synthetic mixture, suitable for routine quality control analysis.

MATERIALS AND METHODS

Instruments and apparatus

- A Systronic RP-HPLC (LC-20-AD) (SPD-20 A) Instrument [Clarify]
- Column: Kromstar C₁₈ (250 × 4.6 mm, 5 μm)
- Digital Analytical Balance: Scale tec (India)

- pH meter (Systronic, Naroda, Ahmedabad)
- Sonicator (Equitron, India)
- Volumetric flask: 10, 50 and 100 ml (Borosil)
- Pipettes: 1, 2, 5 and 10 ml (Borosil)
- Beaker: 50, 100 and 150 ml (Borosil)
- Hamilton syringe

CHEMICALS AND MATERIALS

- Acetonitrile, Methanol and Water (HPLC grade) (Finar Chemicals Pvt. Ltd., India)
- Ortho Phosphoric Acid (AR Grade) (Astron Chemical India)
- Esomeprazole Magnesium Trihydrate (BLD Pharmatech Pvt. Ltd., Hyderabad)
- Metformin Hydrochloride (Stallion Pharmaceuticals Pvt. Ltd., Ahmedabad)

Selection of Detection Wavelength

The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 252 nm both drugs gave remarkable absorbance, good peak height and shape. So, 252 nm was selected for simultaneous estimation of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in synthetic mixture.

Mobile phase selection

The composition and flow rate of mobile phase were changed to optimize the separation condition using combined solution. After number of trial experiments, it was established that the mobile phase Phosphate buffer (pH 3 adjusted with Ortho phosphoric acid): Acetonitrile (60:40 %v/v) shows good peak shape and resolution.

Chromatographic condition

Column: Kromstar C₁₈ (250 mm × 4.6 mm, 5 μm)

Mobile phase: Phosphate buffer (pH 3 adjusted with Ortho phosphoric acid): Acetonitrile (60:40 %v/v)

Flow rate: 1 ml/min

Run time: 10 min

Detection wavelength: 252 nm

Detector: U.V Detector

Injection volume: 20 μL

Syringe: Hamilton

Preparation of Mobile phase

- **Preparation of 10% Orthophosphoric acid** 10% ortho phosphoric acid was prepared by diluting 1.0 ml of concentrated ortho phosphoric acid in 10 ml HPLC grade water.
- **Preparation of buffer (10 mM phosphate buffer)** Accurately weighed 0.272 gm potassium dihydrogen phosphate (KH_2PO_4) was transferred it in 200 ml HPLC grade water and allowed it to dissolve. It was filtered through 0.45 μm membrane filter and sonicated for about 10 min. Buffer pH was adjusted to 4.0 with 10% ortho phosphoric acid.
- **Preparation of Mobile phase:** Phosphate buffer (pH 3 adjusted with Ortho phosphoric acid): Acetonitrile (60:40 %v/v). Mobile phase was used after filtered it through 0.45 μm membrane filter and sonication.

Preparation of standard stock solution

a. Esomeprazole Magnesium Trihydrate (100 $\mu\text{g/ml}$)

Accurately weighed Esomeprazole Magnesium Trihydrate (10 mg) was transferred to a 100 ml volumetric flask, and diluted up to the mark with mobile phase to obtain a standard stock solution (100 $\mu\text{g/ml}$).

b. Metformin Hydrochloride (1000 $\mu\text{g/ml}$)

Accurately weighed Metformin Hydrochloride (100 mg) was transferred to a 100 ml volumetric flask, and diluted up to the mark with mobile phase to obtain a standard stock solution (1000 $\mu\text{g/ml}$).

Preparation and analysis of synthetic mixture

- The synthetic mixture of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride was prepared in the ratio of 40:1000.
- Common excipients such as MCC (Microcrystalline Cellulose) (10 mg), Lactose (24 mg), Talc (14 mg) and Magnesium Stearate (15 mg), Croscarmellose Sodium (18 mg), Starch (12 mg), Glyceryl Monostearate (26 mg), Hydroxypropyl Methyl Cellulose (20 mg), Triethyl Citrate (21 mg) were added in the motor pestle along with the drug Esomeprazole Magnesium Trihydrate (40 mg) and Metformin Hydrochloride (1000 mg).
- This solution was filtered through Whatmann filter paper. The filtrate was diluted to the mark with Methanol. The mixture contains 400 $\mu\text{g/ml}$ of Esomeprazole Magnesium

Trihydrate and 10000 µg/ml of Metformin Hydrochloride.

- From above synthetic mixture solutions Accurately 0.1 ml of the above mixture solution of (Esomeprazole Magnesium Trihydrate 400 µg/ml and Metformin Hydrochloride 10000 µg/ml) was pipetted out into 10 ml volumetric flask and the volume was adjusted up to the mark with Methanol.
- Final concentration of Esomeprazole Magnesium Trihydrate was 4 µg/ml and Metformin Hydrochloride 100 µg/ml.

METHOD VALIDATION

The developed method was validated with respect to specificity, linearity, range, accuracy, precision, limit of detection and limit of quantification in accordance with the ICH guideline.

a. Specificity

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

b. Linearity & Range

The linearity of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride was found to be in the range of 2-10 µg/ml and 50-250 µg/ml respectively. Plot the calibration curve of Peak area Vs Concentration (µg/ml). Linearity of both the drugs was checked in term of slope, intercept and correlation coefficient.

Preparation of calibration curve

Aliquots of stock solution of Esomeprazole Magnesium Trihydrate (100 µg/ml) 0.2, 0.4, 0.6, 0.8 and 1.0 ml and Metformin Hydrochloride (1000 µg/ml) 0.5, 1.0, 1.5, 2.0 and 2.5 ml were pipetted out in five different 10 ml volumetric flasks and further diluted with mobile phase to obtain the concentration of about 2, 4, 6, 8 and 10 µg/ml for Esomeprazole Magnesium Trihydrate and 50, 100, 150, 200 and 250 µg/ml for Metformin Hydrochloride. 20 µL of each solution were injected into RP-HPLC system by Hamilton syringe and analyzed. Calibration curve was obtained by plotting respective Peak area Vs Concentration in µg/ml and Regression equation was obtained.

c. Precision

Precision may be considered at three levels: Intermediate (Intraday) precision,

Reproducibility (Interday precision), Repeatability.

1) Intraday Precision: (n=3)

Solutions containing 2, 4, 6 µg/ml of Esomeprazole Magnesium Trihydrate and 50, 100, 150 µg/ml of Metformin Hydrochloride were analysed three times on the same day and %RSD was calculated.

2) Interday Precision: (n=3)

Solutions containing 2, 4, 6 µg/ml of Esomeprazole Magnesium Trihydrate and 50, 100, 150 µg/ml of Metformin Hydrochloride were analysed on three different successive days and %RSD was calculated.

3) Repeatability: (n=6)

Solutions containing 4 µg/ml of Esomeprazole Magnesium Trihydrate and 100 µg/ml of Metformin Hydrochloride were analysed for six times and %RSD was calculated. RSD was not more than 2%.

d. Limit of Detection (LOD)

Limit of detection can be calculated using following equation as per ICH guidelines.

$$\text{LOD} = 3.3 \times (\sigma / S)$$

where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

e. Limit of Quantification (LOQ)

Limit of quantification can be calculated using following equation as per ICH guidelines.

$$\text{LOQ} = 10 \times (\sigma / S)$$

where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

f. Accuracy

Accuracy of the developed method was confirmed by doing recovery study as per ICH guideline at three different concentration levels 50%, 100%, 150% and the values were measured for Esomeprazole Magnesium Trihydrate (**4 µg/ml**) and Metformin Hydrochloride (**100 µg/ml**). This performance was done in triplicate.

g. Robustness

➤ In case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate

h. System suitability tests

- A system suitability test is an integral part of liquid chromatography.
- They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done.
- The test includes the Resolution, Column efficiency, Tailing factor and Theoretical plates.

RESULTS AND DISCUSSION

Selection detection wavelength

- The sensitivity of RP-HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 252 nm both drugs give good peak height and shape. So, 252 nm was selected for simultaneous estimation of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in synthetic mixture.
- Overlay UV spectra of Esomeprazole Magnesium Trihydrate (4 µg/ml) and Metformin Hydrochloride (100 µg/ml) in Methanol has been shown in Figure 1.1.

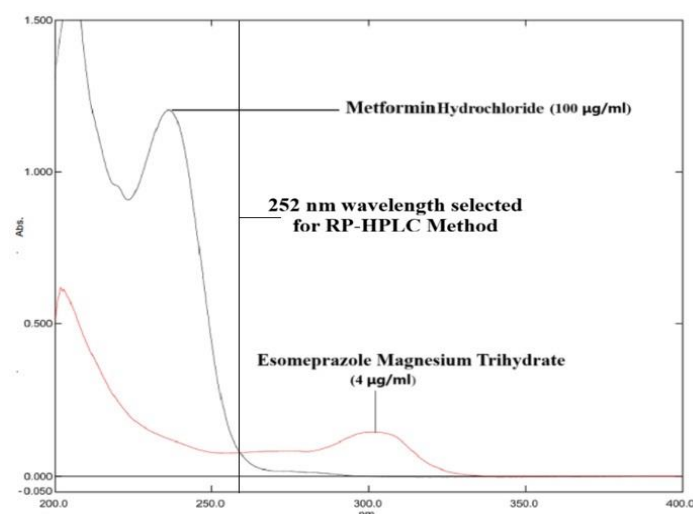


Figure 2: Overlay UV spectra of Esomeprazole Magnesium Trihydrate (4 µg/ml) and Metformin Hydrochloride (100 µg/ml) in Methanol at 252 nm.

Optimization of Chromatographic conditions

The mobile phase Phosphate buffer (pH 3 adjusted with Ortho phosphoric acid): Acetonitrile

(60:40 %v/v) was selected because it was found to ideally resolve the peaks with retention time 2.3 min and 5 min for Metformin Hydrochloride and Esomeprazole Magnesium Trihydrate, respectively. Kromstar C₁₈ (250×4.6 mm, 5 μm) column was used for separation of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride with flow rate of 1.0 ml/min.

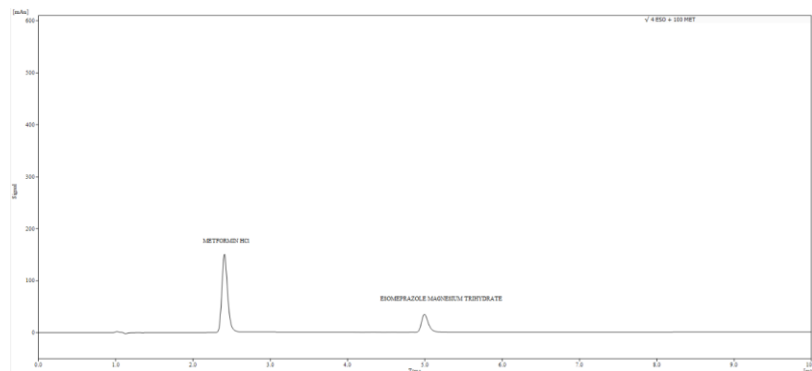


Figure 3: RP-HPLC Chromatogram of Esomeprazole Magnesium Trihydrate (4 μg/ml) and Metformin Hydrochloride (100 μg/ml) in in Phosphate buffer (pH 3 adjusted with OPA): Acetonitrile (60:40 %v/v) at 252 nm {Run time: 10 min, Flow rate: 1 ml/min}.

Table 1: System suitability parameter.

Parameters	Retention Time	Tailing Factor	Number of Theoretical plate	Resolution
Metformin Hydrochloride	2.3 min	0.8	8437	3.0
Esomeprazole Magnesium Trihydrate	5 min	1.1	6926	

Method Validation of RP-HPLC method

a. Specificity

Typically, these might include impurities, degradants, matrix, etc. It was proved by comparing the chromatogram of mobile phase, test preparation solution to show that there was no interference of mobile phase and excipients peaks with peak of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride shown in figure 1.8, 1.9 and 1.10.

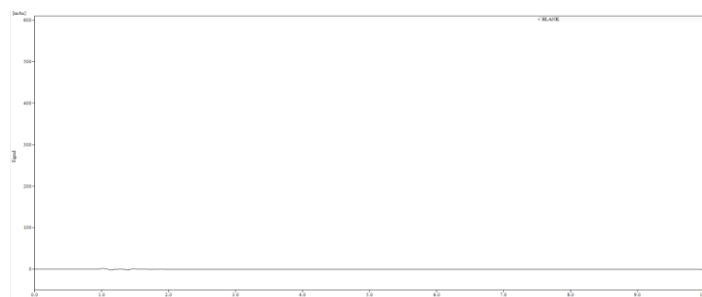
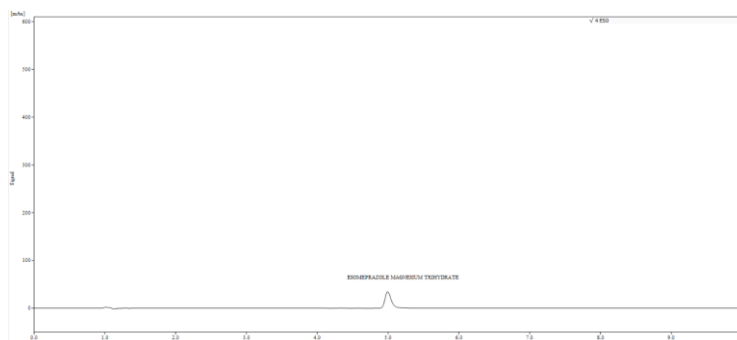
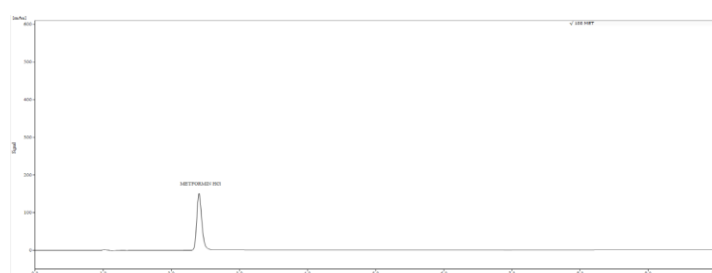
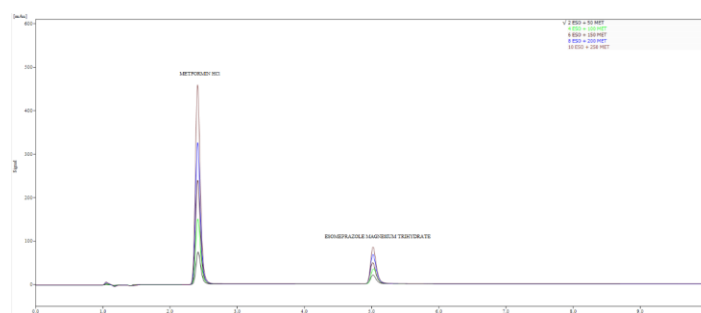


Figure 4: RP-HPLC Chromatogram of Blank.**Figure 5 RP-HPLC Chromatogram of Esomeprazole Magnesium Trihydrate (4 µg/ml).****Figure 6: RP-HPLC Chromatogram of Metformin Hydrochloride (100 µg/ml).****b. Linearity**

The linearity of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride was found to be 2-10 µg/ml and 50-250 µg/ml, respectively.

**Figure 7: Overlain RP-HPLC chromatogram of Esomeprazole Magnesium Trihydrate (2-10 µg/ml) and Metformin Hydrochloride (50-250 µg/ml).****Table 2: Calibration data for Esomeprazole Magnesium Trihydrate (2-10 µg/ml) and Metformin Hydrochloride (50-250 µg/ml).**

Sr. No	Concentration (µg/ml)		Mean Peak area (mAu*sec) ± S. D. (n=6)		% RSD	
	ESO	MET	ESO	MET	ESO	MET
1	2	50	125.266±1.3233	677.996±9.3336	1.06	1.38
2	4	100	214.208±1.6197	1349.933±15.9036	0.76	1.18
3	6	150	307.267±1.9857	2025.813±21.6323	0.65	1.07

4	8	200	411.044±2.1137	2660.233±22.5733	0.51	0.85
5	10	250	505.039±1.8013	3267.433±18.4541	0.36	0.56

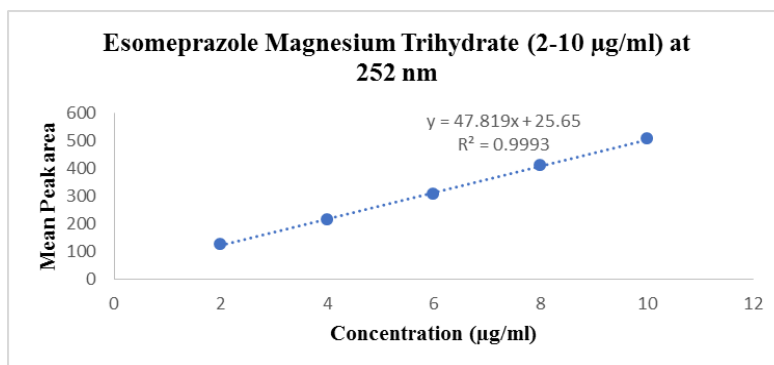


Figure 8: Calibration curve of Esomeprazole Magnesium Trihydrate (2-10 µg/ml).

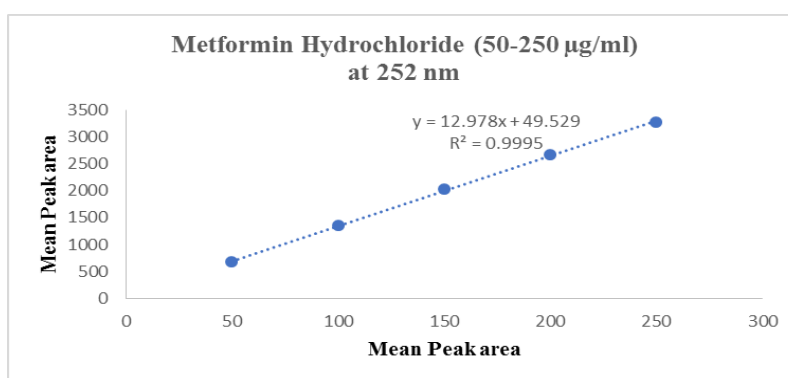


Figure 9: Calibration curve of Metformin Hydrochloride (50-250 µg/ml).

c. Precision

Table 3: Precision study for Esomeprazole Magnesium Trihydrate.

Esomeprazole Magnesium Trihydrate (252 nm)		
Intraday precision of Esomeprazole Magnesium Trihydrate		
Conc. (µg/ml)	Mean peak area (mAu*sec) ± S.D (n=3)	% RSD
2	122.297±1.3257	1.08
4	213.331±1.8088	0.85
6	305.571±1.9411	0.64
Interday precision of Esomeprazole Magnesium Trihydrate		
Conc. (µg/ml)	Mean peak area (mAu *sec) ± S.D (n=3)	% RSD
2	121.033±1.3589	1.12
4	216.725±1.8877	0.87
6	308.296±2.0446	0.66
Repeatability of Esomeprazole Magnesium Trihydrate		
Conc. (µg/ml)	Mean peak area (mAu *sec) ±SD (n=6)	% RSD
4	213.541±1.8776	0.88

Table 4: Precision study for Metformin Hydrochloride.

Metformin Hydrochloride (252 nm)

Intraday precision of Metformin Hydrochloride		
Conc. ($\mu\text{g/ml}$)	Mean peak area (mAu*sec) \pm SD (n=3)	% RSD
50	683.735 \pm 8.9938	1.32
100	1360.120 \pm 15.4132	1.13
150	2032.693 \pm 19.8176	0.97
Interday precision of Metformin Hydrochloride		
Conc. ($\mu\text{g/ml}$)	Mean peak area (mAu *sec) \pm SD (n=3)	% RSD
50	684.035 \pm 9.3738	1.37
100	1360.053 \pm 15.4961	1.14
150	2032.727 \pm 19.8625	0.98
Repeatability of Metformin Hydrochloride		
Conc. ($\mu\text{g/ml}$)	Mean peak area (mAu *sec) \pm SD (n=6)	% RSD
100	1356.600 \pm 15.8966	1.17

d. Accuracy (Recovery study)

Table 5: Recovery study data.

Name of Drug	%Level Of Recovery	Test Amount ($\mu\text{g/ml}$)	Amount of drug Spiked ($\mu\text{g/ml}$)	Total Std Amount ($\mu\text{g/ml}$)	Total amount Recovered ($\mu\text{g/ml}$)	% Recovery \pm S. D (n=3)
Esomeprazole Magnesium Trihydrate	50	4	2	6	5.985	99.75 \pm 0.1417
	100	4	4	8	7.984	99.80 \pm 0.1609
	150	4	6	10	9.993	99.93 \pm 0.1823
Metformin Hydrochloride	50	100	50	150	149.83	99.88 \pm 0.0400
	100	100	100	200	199.82	99.91 \pm 0.0642
	150	100	150	250	249.89	99.95 \pm 0.0832

e. LOD and LOQ

Table 6: LOD and LOQ data.

Drug Name	Esomeprazole Magnesium Trihydrate	Metformin Hydrochloride
LOD ($\mu\text{g/ml}$)	0.09	2.37
LOQ ($\mu\text{g/ml}$)	0.28	7.19

f. Analysis of synthetic mixture

Table 7: Analysis of synthetic mixture.

Drug Name	Amount in synthetic mixture ($\mu\text{g/ml}$)	Amount found ($\mu\text{g/ml}$)	% Assay \pm S.D. (n=3)
Esomeprazole Magnesium Trihydrate	4	3.98	99.50 \pm 0.105
Metformin HCl	100	99.94	99.94 \pm 0.053

g. Robustness

Table 8: Robustness data.

Condition	Variation	Esomeprazole Magnesium Trihydrate	Metformin Hydrochloride
		% Assay \pm SD (n=3)	% Assay \pm SD (n=3)
Flow rate (1 ml \pm 0.2 ml/ min)	0.8 ml/min	99.42 \pm 3.5166	98.45 \pm 1.3730
	1.0 ml/min	99.65 \pm 5.2691	99.75 \pm 2.5545
	1.2 ml/min	99.76 \pm 7.4770	99.95 \pm 4.0286
Detection wavelength (252 nm \pm 2 nm)	250	98.58 \pm 4.1268	99.65 \pm 2.9454
	252	99.92 \pm 4.4267	99.85 \pm 2.5055
	254	100.02 \pm 6.0256	99.99 \pm 5.3762
Mobile Phase (PO ₄ Buffer: Acetonitrile (60:40 \pm 2 % v/v)	58:42	99.34 \pm 3.0784	99.47 \pm 4.1116
	60: 40	99.83 \pm 4.9421	99.65 \pm 1.0552
	62: 38	99.64 \pm 5.0143	100.05 \pm 4.1845

h. Summary of Validation Parameters

Table 9: Summary of Validation Parameters.

Sr. No.	Parameters	Esomeprazole Magnesium Trihydrate	Metformin Hydrochloride
1	Detection wavelength (nm)	252 nm	
2	Linearity Range (μ g/ml)	2-10	50-250
3	Regression equation (y = mx + c)	y = = 47.819x + 25.65	y = 12.978x + 49.529
4	Correlation Coefficient (r ²)	0.9993	0.9995
5	Intraday Precision (%RSD, n=3)	0.64-1.08	0.97-1.32
6	Interday Precision (% RSD, n=3)	0.66-1.12	0.98-1.37
7	Repeatability (% RSD, n=6)	0.88	1.17
8	Accuracy (% Recovery, n=3)	99.75%-99.93%	99.88%-99.95%
9	LOD (μ g/ml)	0.09	2.37
10	LOQ (μ g/ml)	0.28	7.19
11	% Assay	99.50%	99.94%

CONCLUSION

- A rapid, sensitive, accurate and precise RP-HPLC method has been developed and validated for routine analysis of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in Synthetic mixture.

- The RP-HPLC method is suitable for simultaneous estimation of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in Synthetic mixture in without interference of each other. The developed method was successfully applied in Synthetic mixture. The proposed method can be utilized for the routine analysis of Esomeprazole Magnesium Trihydrate and Metformin Hydrochloride in Synthetic mixture.

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