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RESEARCH ARTICLE

Preformulation, Characterization, Estimation and Method Validation Studies of Esomeprazole Magnesium Trihydrate by UV –Visible Spectrophotometry

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ABSTRACT

The present paper comprises Preformulation characterization and Method Validation of UV-visible Spectrophotometric method for the estimation of Esomeprazole Magnesium trihydrate. It is a very simple, accurate, sensitive, economical and reproducible UV-spectrophotometric method. Esomeprazole Magnesium trihydrate (EMT) is a proton pump inhibitors which is used against peptic ulcer disease to prevent excess amount of acid secretion in the stomach .The standard and sample solutions of Esomeprazole Magnesium trihydrate were prepared in simulated salivary fluid pH 6.8 and in 0.1N NaOH solution. Physico- chemical characterization studies showed that EMT has showed a melting point of 174-176°C. The maximum solubility of Esomeprazole Mg trihydrate was found in Dichloromethane. The Partition Coefficient of drug was found to be 613.75 .The analytical method developed for the estimation of Esomeprazole Magnesium trihydrate shows maximum absorbance λmax of 301nm in simulated salivary fluid pH 6.8 and 305 nm in 0.1N NaOH. The linear regression analysis data for the calibration plots showed a good linear relationship over the concentration range of 2-20µg/ml for Esomeprazole Magnesium trihydrate. The developed method was validated according to ICH guidelines. The interday and intraday precision of EMT was found to be within limits (less than 2). The developed method has precise sensitivity and specificity for the determination of Esomeprazole Magnesium trihydrate and is found to be cost effective. Accuracy (80%, 100%, 120%) and reproducibility were found to be satisfactory. Statistical analysis data showed that the method is repeatable, sensitive and selective for the estimation of Esomeprazole Magnesium trihydrate. The above analytical parameters indicated that the developed UV Spectrophotometric method was simple, accurate and reproducible.

KEYWORDS

Esomeprazole, UV-Visible spectrophotometer, Estimation**,** Simulated salivary fluid (6.8 pH), Validation

INTRODUCTION

Esomeprazole is s-isomer of omeprazole and it is a mixture of the S- and R- isomers. It is benzimidazole derivative of H2 receptor blocker. Its molecular formula is $(C_{17}H_{18}N_3O_3S)_2$ Mg x 3 H_2O and having molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis. Generally proton pump inhibitors are administered as an inactive prodrug form because these are acid suppressive drugs. These drugs will be degraded when present in the gastric fluids, so enteric-coating is done to avoid the acid degradation. When the enteric coating formulations are passing through the stomach into the proximal intestine the drug will release immediately in duodenum part of intestine by

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this formulation. For the treatment of NSAIDs-associated peptic ulcer disease, its site of targeting is intestine. It is also cost effective in the treatment of gastric esophageal reflux diseases. Its half-life is 1.25 ± 0.25 hours and has a bioavailability of 50%-90%.So, it will be degraded by the gastric enzymes when conventional dosage form reaches to the gastric fluids. This problem is avoiding by the enteric coated formulation. Esomeprazole is official in The Merck Index, Martindale.[1] It provides better acid control than current racemic proton pump inhibitors and has a favorable pharmacokinetic profile relative to Omeprazole. It is a drug that blocks excessive amount of acid secretion in the stomach. Esomeprazole magnesium is being studied in the prevention of esophageal cancer and in the treatment of other conditions, including side effects of chemotherapy. It is a type of anti-ulcer agent. It is also called Esomeprazole and Nexium.[2,3]

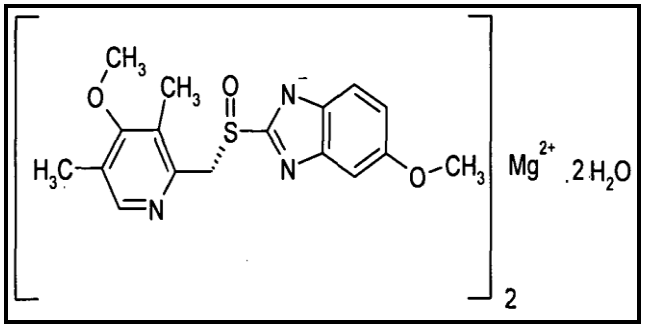

Figure 1: Structure of Esomeprazole Magnesium Trihydrate[4]

MATERIAL AND METHODS

Instruments and Apparatus

A double beam UV-Visible spectrophotometer, spectral band width of 1nm, wavelength accuracy ± 0.5nm and a pair of 1cm matched quartz cells was used to measure absorbance of the resulting solution and connected with computer loaded UV Probe software. Calibrated electronic single pan balance Shimadzu AY 220, Sonicator, pH Meter, Heating Mantle, Filter Paper 0.45 microns. All the glassware are calibrated before use.

Chemicals and Reagents

Esomeprazole Magnesium Trihydrate was received as a gift samples from Suven Pharmaceuticals (Vadodara, Gujrat, India).All the solvents and chemicals like Methanol, Dichloromethane, PEG 400, PEG 200, Propylene Glycol, Ethanol, Tween 80, Acetone, Iso-Propyl Alcohol, Cremophor RH 40, n-octanol, Sodium dihydrogen phosphate, Di-sodium hydrogen phosphate etc were gifted by Merck, Mumbai, India. All other ingredients used were of analytical grade.

Compatibility Studies

Powder X-ray diffraction patterns were recorded using x-ray diffractometer to check the compatibility between Drug and excipients. under the following conditions : target C4 , Filter Ni , voltage 45kv, current 40 mA receiving slit 91mm.

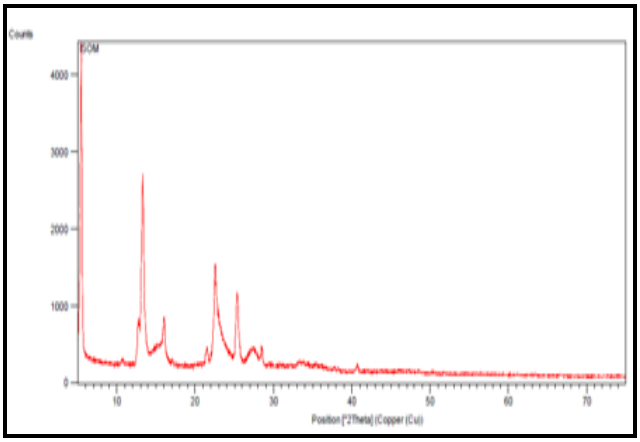

Figure 2: XRD Pattern of Pure Drug Esomeprazole Magnesium Trihydrate

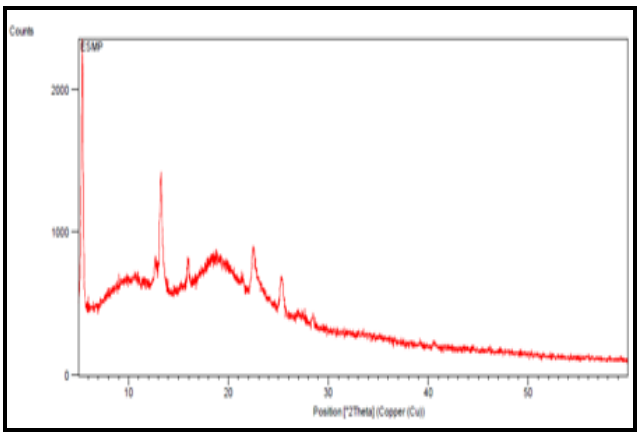

Figure 3: XRD Pattern of Physical Mixture of drug and Excipients

The powder XRD pattern of pure drug (Fig-2) and with the excipients (Fig-3) showed that drug

was highly crystalline in nature as indicated by the distinctive peaks. The degree of crystallinity of pure drug does not change in its mixture form.

Preformulation Characterization of the Drug

Melting Point

Small amount of drug was placed into a sealed capillary tube. Then this tube was placed in the melting point apparatus.[4]The temperature in the apparatus was gradually increased. Note down the observation of temperature at which drug started to melt and the temperature when the entire drug gets melted. Average of triplicate readings was noted.[5]

Table 1: Melting point of drug

Drug	Observation
Esomeprazole Mg Trihydrate	174-176°C

Solubility Study of Esomeprazole Mg Trihydrate in Different Organic Solvents[3]

Took 2ml of each solvent in culture tube. Then Add excess amount of drug in 2ml solvent by using vortex shaker. Put down the solution into water bath shaker for 48 hr at 37°C.[3] Centrifuged the sample at 10000 rpm after 48 hrs. Now take the supernatant liquid and diluted with methanol.[4] Scan the sample in UV spectroscopy between 200-400nm. From the tale it was found that maximum solubility of Esomeprazole Mg trihydrate was found in Dichloromethane.

Table 2: Solubility of Esomeprazole Magnesium Trihydrate

S.No.	Name of Solvent	Solubility in Mg*
1	Dichloromethane	4.958333333±0.0043
2	PEG 200	1.441666667±0.0076
3	PEG 400	0.329166667±0.0023
4	Propylene Glycol	0.53125±0.0008
5	Simulated saliva	0.477083333±0.0028
6	Ethanol	3.291666667±0.0098
7	Tween 80	0.995833333±0.012
8	Acetone	0.391666667±0.0065
9	Iso Propyl Alcohol	0.808333333±00.71
10	Acetonitrile	0.179166667±0.0083
11	Cremophor RH 40	0.9375±0.0090

*Milligrams

Appearance of Esomeprazole Mg Trihydrate using Optical Microscope

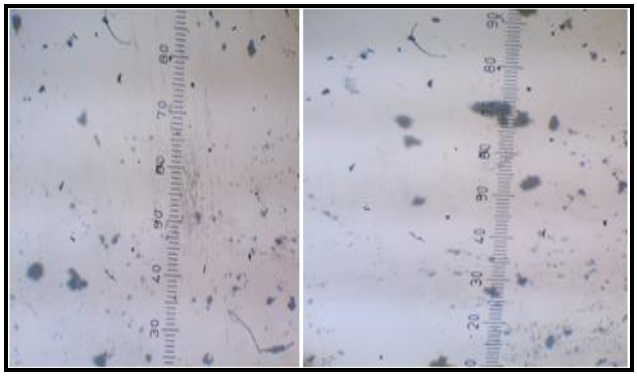

Figure 4: Different Images of Esomeprazole Magnesium Trihydrate[1]

Particle Size of Drug[8, 9]

System Calibration

A microscope slide micrometer is placed on the microscope stage. Then the microscope is adjusted to adjusted Koehler illumination at desired magnification. The drawing tube is focused to the surface of brightly illuminated graphic tablet, providing a clear, superimposed image of the micrometer slide and graphic of the microscope ocular. After that least count was calculated.

Particle Size Measurement

A sample of drug is sprinkled on a microscope slide. Then allowing the particle to position themselves, yielding the maximum projected area. The slide is placed under the microscope and a particle to be measured is chosen

randomly. The slide is rotate to measure the 10 articles.

Average Particle size of the Esomeprazole Mg Trihydrate was found to be 5.475±0.35.

Partition Coefficient of Esomeprazole Mg Trihydrate[10,12]

Partition coefficient (oil/water) is a measure of a drug's lipophilicity and an indication of distributed between the organic and aqueous phases at equilibrium. Partition coefficient provides a means of characterizing the lipophilic/hydrophilic nature of the drug. Partition coefficient of Esomeprazole Mg trihydrate was determined at 37 ± 0.5 °C by taking 5 ml of n-octanol and 5ml of water. After shaking, the system remained undisturbed for half an hour. Then prepared a saturated solution of Esomeprazole Mg trihydrate. And the above formed mixture was left undisturbed for about 24hrs. Two layers were separated by means of separating funnel and the amount of Esomeprazole Mg trihydrate solubilized, was determined by measuring the absorbance at 245 nm against simulated salivary fluid as a blank through double beam UV/Vis spectrophotometer (Shimadzu) in both the solution. Partition coefficient was determined as ratio of concentration of drug in n-octanol to the concentration of drug in water. Partition Coefficient value was reported as log P. It was found that Esomeprazole Mg trihydrate was Lipophilic in nature.

Solubility of Esomeprazole Mg Trihydrate in Octanol and Water

$$\text{Log P} = \frac{\text{Concentration in octanol}}{\text{Concentration in water}}$$

Log P= 0.491/0.0008 = 613.75

Partition Coefficient of Esomeprazole Mg Trihydrate was found to be 613.75

Calibration Curve of Esomeprazole Mg Trihydrate in Simulated Salivary Fluid pH 6.8 Buffer Solution Using UV Spectroscopy

Selection of Wavelength Range

10mg of Esomeprazole Mg trihydrate was weighed accurately and transferred into a 100 ml volumetric flask. Then the solution was sonicated for 5 min. and then the volume was made up with further quantity of simulated salivary fluid to get 100µg/ml stock solution of Esomeprazole Mg trihydrate. This solution was further diluted with simulated salivary fluid 6.8pH to get 2-20µg/ml of Esomeprazole Mg trihydrate by serial dilution method and samples are analyzed by UV spectrophotometer using simulated salivary fluid pH 6.8 as blank.

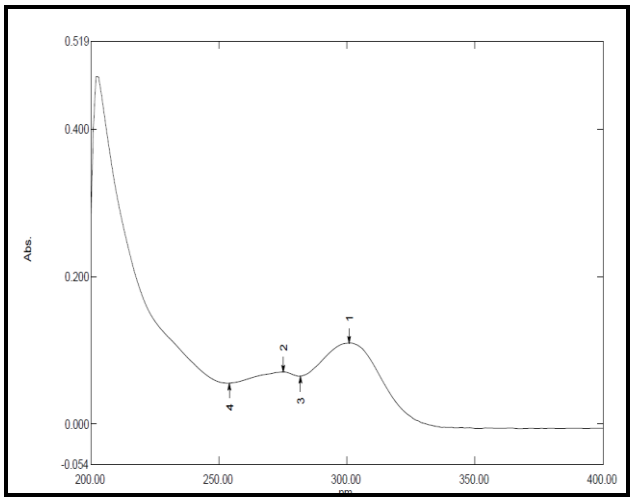

Figure 5: Absorption Maxima was found to be 301nm in simulated salivary fluid pH 6.8

Development and Validation of UV Spectrophtometric Method of Esomeprazole Mg Trihydrate in Simulated Salivary Fluid pH 6.8 (Absorption Maxima 301nm)

Validation

Validation can be defined as (ICH) Establishing documented evidence, which can provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics.[15] The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection (LOD), Limit of quantification (LOQ) according to ICH guidelines.[13]

Linearity

The linearity of the analytical method was its ability to elicit test results which are directly

proportional to analyte concentration in samples within a given range.[11] To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analyzed. The drug showed linearity in the range of 2-20µg/ml with correlation coefficient 0.999. Linearity data are shown in Table 3(n=3).

Table 3: Linearity table of Esomeprazole Mg Trihydrate in Simulated Salivary Fluid pH 6.8

S.No.	Con.*(µg/ml)	Absorbance
1	0	0±0
2	2	0.06±0.001
3	4	0.138±0.001
4	6	0.216±0.004
5	8	0.2995±0.004
6	10	0.3925±0.009
7	12	0.464±0.001
8	14	0.5455±0.003
9	16	0.6225±0.003
10	18	0.705±0.001
11	20	0.785±0.002

Intercept = 0.014

Slope = 0.039

Straight line equation: Y=0.039x-0.014

Regression coefficient: R^2 = 0.999

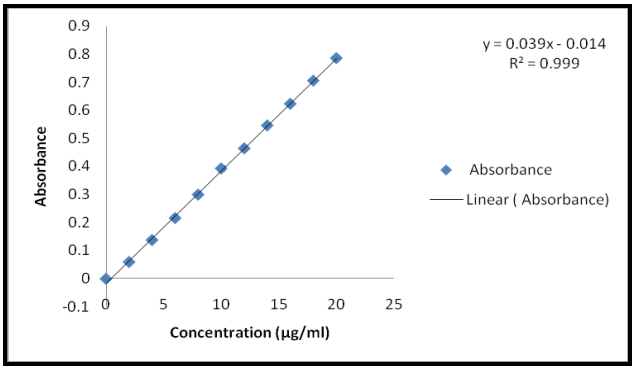

Figure 6: Standard plot of Esomeprazole Mg Trihydrate in Simulated Salivary Fluid pH 6.8 at Absorption Maxima at 301nm

Precision

Precision studies were carried out to ascertain the reproducibility of the proposed method.[16]Repeatability was determined by preparing six replicates of same concentration of the sample and then the absorbance was measured. Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision. The results were reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2.

Table 4: Intraday Precision in Simulated Salivary Fluid pH 6.8

Intraday precision	
Concentration(µg/ml)	**Absorbance**
10	0.386
10	0.390
10	0.392
10	0.399
10	0.386
Mean	0.3906
STD	0.005366563
% RSD	1.373928097

Accuracy

The accuracy of a method is the degree to which the observed results correspond to the true value of the analyte in the sample.

Robustness

Analysis was carried out at two different temperatures, room temperature and at 4°C to determine the robustness of the method and then the absorbance was measured at two different temperatures.[6]

Limit of Quantification and Limit of Detection Studies

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected.[7,11] Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy.

Table 5: Interday Precision in Simulated Salivary Fluid pH 6.8

Interday Precision						
Conc (µg/ml)	**Abs.1**	**Abs.2**	**Abs.3**	**Mean**	**Std***	**RSD**
10 (Day 1)	0.386	0.39	0.392	0.389333333	0.003055	0.78468
10 (Day 2)	0.392	0.386	0.386	0.388	0.003464	0.89281
10 (Day 3)	0.39	0.392	0.386	0.389333333	0.003055	0.78468
Average % RSD						0.82078

Table 6: Accuracy readings of Esomeprazole Mg trihydrate in simulated salivary fluid pH 6.8

Accuracy				
Level of Addition	**Absorbance**	**Mean**	**STD**	**% RSD**
80%	0.303	0.298666667	0.0038	1.26761347
80%	0.297			
80%	0.296			
100%	0.392	0.389333333	0.0031	0.784687619
100%	0.386			
100%	0.39			
120%	0.465	0.463333333	0.0015	0.329681705
120%	0.463			
120%	0.462			

Table 7: Change in Temperature in Simulated Salivary Fluid pH 6.8

Room Temperature				
Concentration(µg/ml)	**Absorbance**	**Mean**	**STD**	**% RSD**
10	0.49	0.490666667	0.0020817	0.42425251
10	0.489			
10	0.493			
Temperature 4 °C				
Concentration(µg/ml)	**Absorbance**	**Mean**	**STD**	**%RSD**
10	0.493	0.494666667	0.0015273	0.30879891
10	0.496			
10	0.495			

LOQ and LOD was determined using the following equation LOQ-10s/m, LOD-3.3s/m where(s) is the standard deviation of the response and (m) is the slope of the related calibration curve. The values of LOQ and LOD were found to be 0.1772 µg/ml and 0.05848µg/ml respectively.

Table 8: Summary of the Method Developed of Esomeprazole Mg Trihydrate in Simulated Salivary fluid pH 6.8 at Absorption Maxima at 301 nm

Parameter	Result
Absorption Maxima	301
Conc. Range	2-20 µg/ml
Correlation Coefficient	0.999
Regression Equation	Y=0.039x-0.014
Slope	0.039
Intercept	0.014
Accuracy (%RSD)	80% (1.26), 100% (0.78), 120% (0.32)
Precision (%RSD)	Repeatability (0.69), Intraday (1.37), Interday (0.82)
LOD (µg /ml)	0.058
LOQ (µg /ml)	0.1772

Calibration Curve of Esomeprazole Mg Trihydrate in 0.1N NaOH Using UV Spectroscopy

Selection of Wavelength Range

10mg of Esomeprazole Mg trihydrate was weighed accurately and transferred into a 100 ml volumetric flask. Then the solution was sonicated for 5 min. and then the volume was made up with further quantity of 0.1N NaOH to get 100µg/ml stock solution of Esomeprazole Mg trihydrate. This solution was further diluted with 0.1N NaOH to get 2-20µg/ml of Esomeprazole Mg trihydrate by serial dilution method and samples are analyzed by UV spectrophotometer using 0.1N NaOH as blank.

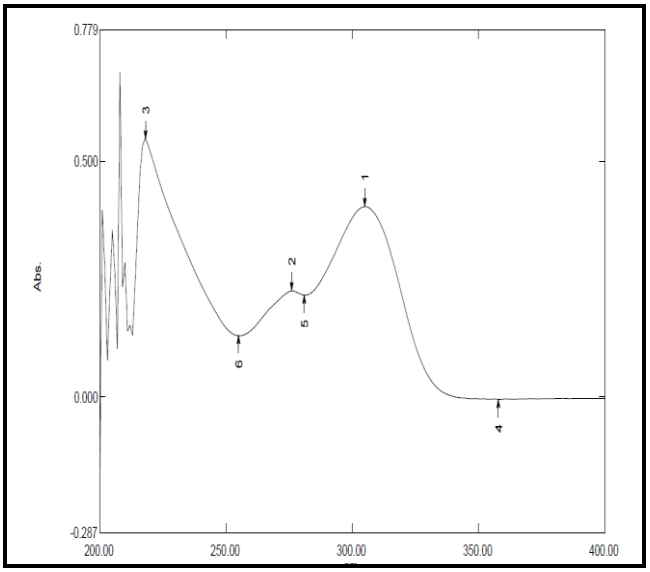

Figure 7: Absorption maxima was found to be 305 nm in 0.1N NaOH

Development and Validation of UV Spectrophtometric Method of Esomeprazole Mg Trihydrate in 0.1N NaOH at Absorption Maxima at 305nm

Validation

Validation can be defined as (ICH) Establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics. The method was validated for several parameters like linearity, accuracy, precision, Ruggedness, Robustness, Limit of detection (LOD), Limit of quantification (LOQ) according to ICH guidelines.[10]

Linearity

The linearity of the analytical method was its ability to elicit test results which are directly proportional to analyte concentration in samples within a given range. To establish the linearity of the proposed method, various aliquots of the standard solution of the drug were prepared from stock solution and analyzed. The drug showed linearity in the range of 2-10µg/ml with correlation coefficient 0.999.

Table 9: Linearity table of Esomeprazole Mg Trihydrate in 0.1N NaOH at Absorption Maxima at 305 nm

S.No.	Con.(µg/ml)	Absorbance
0	0	0±0
1	2	0.0885±0.004
2	4	0.164±0.008
3	6	0.2425±0.007
4	8	0.325±0.001
5	10	0.409±0.007
6	12	0.4995±0.003
7	14	0.585±0.005
8	16	0.6815±0.004
9	18	0.7465±0.004
10	20	0.8285±0.003

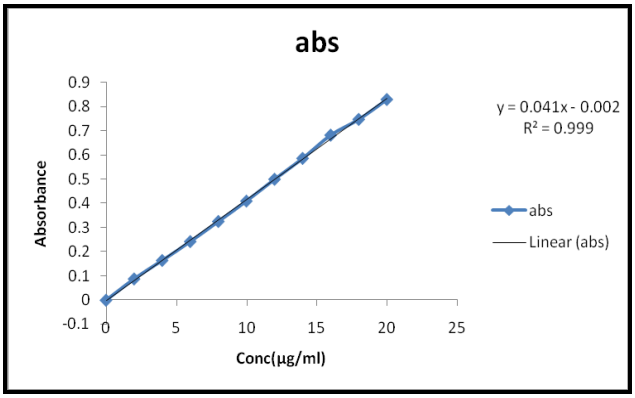

Figure 8: Standard plot of Esomeprazole Mg trihydrate in 0.1N NaOH at absorption maxima at 305 nm

Precision

Intraday precision study was carried out by preparing drug solution of same concentration and analyzing it at three different times in a day. The same procedure was followed for three different days to determine interday precision. The results were reported as %RSD. The precision result showed a good reproducibility with percent relative standard deviation less than 2.

Table 10: Intraday Precision in 0.1N NaOH at Absorption Maxima at 305 nm

S.No.	Con.(µg/ml)	Absorbance
1	10	0.489
2	10	0.49
3	10	0.494
4	10	0.491
5	10	0.493
6	10	0.495
Mean		0.492
STD		0.002366432
% RSD		0.480982096

Accuracy

The accuracy of a method is the degree to which the observed results corresponds to the true value of the analyte in the sample.[15]

Robustness

Analysis was carried out at two different temperatures, room temperature and at 4°C to determine the robustness of the method and the respective absorbance was measured.

Limit of Quantification and Limit of Detection Studies

Limit of detection (LOD) is the lowest amount of analyte in the sample that can be detected. Limit of quantification (LOQ) is the lowest amount of analyte in the sample that can be quantitatively determined by suitable precision and accuracy.[14] LOQ and LOD was determined using the following equation LOQ-10s/m, LOD-3.3s/m where (s) is the standard deviation of the response and (m) is the slope of the related calibration curve. The values of LOQ and LOD were found to be 0.29166 µg /ml and 0.09625µg/ml respectively.

Table 11: Interday Precision in 0.1N NaOH at Absorption Maxima at 305 nm

Conc (µg/ml)	Abs.1	Abs.2	Abs.3	Mean	Std	RSD
10 (Day 1)	0.489	0.49	0.494	0.491	0.002646	0.53885
10 (Day 2)	0.49	0.493	0.496	0.493	0.003	0.60859
10 (Day 3)	0.49	0.489	0.491	0.49	0.001	0.20402
Average of Percentage RSD						**0.4504**

Table 12: Accuracy Readings of Esomeprazole Mg Trihydrate in 0.1N NaOH at Absorption Maxima at 305 nm

Level of addition	Absorbance	Mean	STD	% RSD
80%	0.391	0.3923	0.001	0.29
80%	0.393			
80%	0.393			
100%	0.494	0.4933	0.003	0.61
100%	0.496			
100%	0.49			
120%	0.595	0.593333333	0.00152753	0.25
120%	0.592			
120%	0.593			

Table 13: Change in Temperature in 0.1N NaOH at Absorption Maxima at 305 nm

Room Temperature				
Concentration(µg/ml)	**Absorbance**	**Mean**	**STD**	**% RSD**
10	0.49	0.490666667	0.0020817	0.42425251
10	0.489			
10	0.493			
Temperature 4 °C				
Concentration(µg/ml)	**Absorbance**	**Mean**	**STD**	**%RSD**
10	0.493	0.494666667	0.0015273	0.30879891
10	0.496			
10	0.495			

Table 14: Summary of the method developed of Esomeprazole Mg trihydrate in 0.1N NaOH using UV Spectroscopy

Parameter	Result
Absorption Maxima	305 nm
Beers law Range	2-20 µg/ml
Correlation Coefficient	0.999
Regression Equation	y = 0.0417x - 0.002
Slope	0.0417
Intercept	0.002
Accuracy (%RSD)	80% (0.29), 100% (0.61), 120% (0.25)
Precision (%RSD)	Repeatability (0.72), Intraday (0.48), Interday (0.45)
LOD (µg /ml)	0.09625
LOQ (µg /ml)	0.29166
Robustness (%RSD)	Room Temperature (0.42), Freeze Temperature (0.309

RESULTS AND DISCUSSION

The wavelength corresponding to maximum absorbance in simulated salivary fluid pH 6.8 buffer solution was found at 301nm and 305 nm in 0.1N NaOH. Beers law was obeyed in the concentration range of 2-20µg/ml with correlation coefficient 0.999 both in simulated salivary fluid pH 6.8 buffer solution and in 0.1N NaOH. Accuracy (%RSD) and Precision (%RSD) in simulated salivary fluid was found to be 80% (1.26), 100% (0.78), 120% (0.32) and Repeatability (0.69), Intraday (1.37), Interday (0.82) and in 0.1N NaOH was found to be 80% (0.29), 100% (0.61), 120% (0.25) and Repeatability (0.72), Intraday (0.48), Interday (0.45). The limit of detection and limit of quantification of the proposed method was found to be 0.058 µg/ml and 1.772 µg/ml in simulated salivary fluid pH 6.8 buffer solution and 0.09625 µg/ml and 0.29166 µg/ml in 0.1N NaOH indicating that the method developed is sensitive.

CONCLUSION

The proposed methods provides a simple, accurate, economical and convenient methods for the analysis of Esomeprazole Mg trihydrate in simulated salivary fluid pH 6.8 buffer solution and in 0.1N NaOH using UV spectrophotometer. The both methods were found to be precise as %RSD values for interday and intraday was found to be less than 2. These methods were also found to be rugged and robust as the % RSD values were found to be less than 2. Accuracy of the proposed methods was determined by the recovery studies, and good % recovery of the drug obtained indicates that the methods are accurate and sensitive.

ACKNOWLEDGEMENT

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REFERENCES

1. Michael, E. A., & Gregory E. S. (1992). Microscopic Characterization of Particle Size and Shape: An Inexpensive and Versatile Method. *International Journal of Biological and Pharmaceutical Research*, 9(7), 856-859.
2. Saeed, A. S., Najma, S. R., & Saima, S. B. (2007). Multivariate calibration in UV Spectrophotometric analysis. *Journal of Ethnopharmacology*, 20(2), 163-74.
3. Kapil, K., Naik, S., &Mishra, J. N. (2009). Spectrophotometric method for Simultaneous Estimation of Paracetamol and Domperidone in Tablet formulation. *Asian Research Chem*, 2(2), 45-47.
4. Nayak, S. F., Ranjan, R. S., Panda, S. T., Dash, A., & Rath, S. (2011). UV Spectrophotometric Method for Simultaneous estimation of Paracetamol and

Domperidone in bulk and their formulations. *International Journal of Biological and Pharmaceutical Research*, 2(7), 49-51.

5. Pawar, P. Y., Hapse, S. B., Salve, D. J., & Agarkar, S. H. (2013). Spectrophotometric Estimation and Validation of Paracetamol and Domperidone by Different Method from Pure and Tablet Dosage Form. *International Journal of Drug Research and Technology*, 3(2), 37-40.
6. Harshini, B. F., Alekhya, S. V., Manasa, G. T., & Vanitha P. K. (2011). Extractive Spectrophotometric Estimation of Prasugrel in Pharmaceutical Formulation. *International Journal of Biological & Pharmaceutical Research*, 2 (3), 426-430.
7. Bambhrolia, S. F., & Rajput, S. J. (2013). Simultaneous estimation of Paracetamol and Pamabrom in bulk drugs and in pharmaceutical formulation by Spectrophotometry. *International Journal of Chem Tech Research*, 5(4), 1802-1807.
8. Padaliya, H. J., Bhati, S. U., Padhiyar, P. &, Patel, H. T. (2012). Development and Validation of Second order derivative spectrophotometry method for simultaneous estimation of Paracetamol and Pamabrom in pharmaceutical dosage form. *American Journal of Pharmatech Research*, 3(2), 4-6.
9. Dogrukol, A. K., Tunalier, Z. J., & Tuncel, M. G. (1998). TLC densitometric determination of omeprazole in pharmaceutical preparations. *Indian journal of pharmaceutical sciences*, 74(2), 163-167.
10. Srividya, A. R., Shalom, A. G., Chandrasekhar, R., Vijayan, P., & Vishnuvarthtan, V. J. (2012). Cytotoxic, Antioxidant and Antimicrobial Activity of Polygonum Chinensis Linn. *International Journal of Pharmaceutical Sciences and Nanotechnology*, 4(4), 1569-74.
11. Rewar, S., Bansal, B. K., Singh, C. J., & Sharma A. K. (2014). Approach for quantitative estimation of Eprosartan mesylate by U.V. spectrophotometer. *International Journal of Research and Development in Pharmacy & Life Sciences*, 3(6), 1300-1303.
12. Kumara, S. D., Gupta, M., & Punna, R. R. (2010). New validated Spectrophotometric method for the estimation of Fenofibrate in bulk and dosage forms. *International Journal of Biological & Pharmaceutical Research*, 1(2), 131-136.
13. Prathyusha, P. H., Anupama, B., Jagathi, V. J, & Sai, P. P. (2010). Spectrophotometric method for the determination of Fenofibrate. *International Journal of Pharmacy & Technology*, 2(3), 661-664.
14. Gaikwad, P. V., Sawant, S. D., Ghante, M. R. &, Munot, N. M. (2011). Three simple spectrometric methods for Fenofibrate in tablet form. *Journal of Pharmaceutical Research*, 4(6), 1741-1742.
15. Arpan, P. K., Jawed, A. H., & Chirag, S. K. (2012). Spectrophotometric estimation of Dronedarone in pure drug and pharmaceutical formulation. *Asian Journal of Biomedical Pharma Research*, 2(1), 266-71.
16. Dinesh, S. N., & Ghazal, K. H. (2011). RP-HPLC method development and validation of Dronedarone HCl in its pure form and tablet dosage form. *Asian Journal of Biomedical Pharma Research*, 1(2), 352-354.

