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Analytical Method Development and Validation for Dissolution Method of Esomeprazole Magnessium Gastro Resistant Tablets by Using HPLC

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ABSTRACT:

Dissolution testing includes biopharmaceutical characterization of the drug product, as a tool to make sure consistent product quality and to predict in vivo drug bioavailability. Dissolution is precise test method used for evaluating drug release of solid and semisolid dosage forms. It is the process by which a solid solute enters a solution. A reversed-phase high performance liquid chromatography (RP-HPLC) method was developed and validated for the estimation of esomeprazole in bulk and tablet dosage forms. The separation was achieved on Waters XterraRP18 (150X4.6) mm, 5µm analytical column using Mix 350 mL of Acetonitrile and 500 mL of the Buffer(pH 7.1 phosphate buffer). Dilute with water to 1000 mL as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a UV detector at 302nm. Injection volume of sample was 20ul.The total chromatographic analysis time per sample was about 10 min with esomeprazole eluting at retention time of about 4.8min. The method was validated for accuracy, precision, specificity, linearity and Filteration. Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curve was linear over the concentration range of 10%-150% corresponded with 4µg/mL to 60 µg/mL with R2 close to one (0.999). The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of esomeprazole in tablets dosage form.

1. Introduction:

Substituted benzimidazoles, later known as proton pump inhibitors, PPIs have widely been used as anti-ulcer drugs with high and long lasting antisecretory activity. It suppresses gastric acid secretion by specific inhibition of the Hþ/Kþ ATPase enzyme system at the secretory surface of the gastric parietal cell. Esomeprazole magnesium (ES) [Fig.1] is a proton pump inhibitor. Chemically it is bis(5-methoxy-2-[(S)-[(4-methoxy-3,5-dimethyl-2- pyridinyl) methyl]sulfinyl]-lH-benzimidazole-1-yl) magnesium trihydrate. Esomeprazole is the S-isomer of omeprazole. The empirical formula is $(C_{17}H_{18}N_3O_3S)_2$ Mg $3H_2O$, representing a molecular weight of 767.2 as a trihydrate and 713.1 on an anhydrous basis. The stability of esomeprazole magnesium trihydrate decreases with a corresponding decrease in the pH of the media. Hence, the exposure of the drug to the acidic contents of the stomach would lead to significant degradation of the drug and would result in reduced bioavailability. Few attempts have been made to deliver this drug by per oral route in the form of enteric coated granules, solid dispersion, and suspension and matrix tablets. A number of enteric coating polymers are available and capable of protecting the drug core from the aggressive environments of the stomach. Being soluble at higher pH values, these polymers dissolve in the intestine and release the core for ready action. The polymers which said above include all the several synthetic polymers like Polymethacrylates (Eudragits), Cellulose Acetate Phthalate (CAP), Hydroxy Propyl Methyl Cellulose Phthalate (HPMCP).

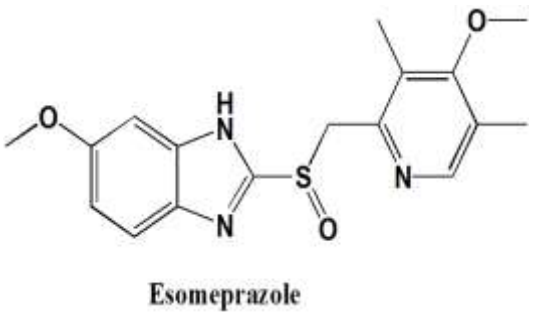


Figure.1 Structure of Esomeprazole

Literature survey reveals that Esomeprazole is estimate in dosage forms individually or in combination with naproxen, diclofinac sodium, levosulpiride, aspirin etc. It is also estimated in human plasma, in pellet form, in micro pellet formulation, in tablets dosage form and in bulk drugs and it is estimated and validated in tablets dosage form by HPLC, UV-Spectrophotometry, RP-HPLC by using different mobile phase ratio, column temperature etc. So the

present work is aimed to develop a simple feasible and sensitive RP-HPLC method for the qualitative Esomeprazole magnesium trihydrate in tablets dosage form. This proposed method was validated in accordance with International Conference on Harmonization (ICH) guidelines.

2. Materials and Methodology:

Reference standard material: Drug Substance of Esomeprazole from hetero labs

Chemicals: Methanol(HPLC grade), Acetonitrile(HPLC grade), Hydrochloric acid(AR), Sodium hydroxide(AR) were purchased from Rankem and disodium hydrogen phosphate dihydrate, Sodium di hydrogen phosphate and tri sodium phosphate dodecanhydrate all are Emplura grade purchased from Merck

Instruments: Lab India Dissolution Test Apparatus(Auto DS 8000),HPLC(Waters Alliance Separation Module 2695 Detector, PDA 2996 / UV 2489), UV-Visible Spectrophotometer (Perkin Elmer, Lambda 25), pH Meter(Lab India, PICO +), Milli-Q-Water system(Millipore, Elix – Gradient), Semi Micro Balance(Radwag, XA 82/220/2X), Pan Balance(Radwag, PS600.R2), Ultrasonic Bath(PCI analytics), Centrifuge(Eltek, TC8100F), Water bath(VLS)

Preparation of reagents and solutions

A. Preparation of mobile phase:

1. Preparation of 1.0 M Sodium dihydrogen phosphate dihydrate buffer: Dissolve about 15.6 g of Sodium dihydrogen phosphate dihydrate in 100 mL of ultrapure water and mix well.

2. Preparation of 0.5 M Disodium hydrogen phosphate anhydrous buffer: Dissolve about 14.2 g of Disodium Hydrogen phosphate anhydrous in 200 mL ultrapure water and mix well.

3. Preparation of 10% (v/v) Orthophosphoric acid solution: Dilute 10 mL of Orthophosphoric acid to 100 mL with ultrapure water and mix well.

4. Preparation of pH 7.1 phosphate buffer: Mix 10.5 mL of 1.0 M Sodium dihydrogen phosphate dihydrate buffer and 60 mL of 0.5 M Disodium hydrogen phosphate anhydrous buffer. Make up to 1000 mL with ultrapure water, if necessary adjust the pH of solution to 7.1 ± 0.05 with 10 % Orthophosphoric acid solution. Filter through 0.45 µ membrane filter.

5. Preparation of mobile phase: Mix pH 7.1 phosphate buffer, Acetonitrile and ultrapure water in the ratio of 500:350:150(v/v/v) respectively and sonicate.

B. Dissolution medium preparation:

1. Preparation of 0.1N HCl: Dilute 86 mL of Hydrochloric acid to 10,000 mL of ultrapure water.

2. Preparation of 0.086M Disodium hydrogen phosphate anhydrous buffer: Dissolve about 122 g of Disodium hydrogen phosphate anhydrous in 10,000mL of ultrapure water and mix well.

3. Preparation of 2N Sodium Hydroxide solution: Dissolve about 16 g of NaOH in 200 mL of ultrapure water and mix well.

4. Preparation of 0.25N Sodium Hydroxide solution: Dilute 25 mL of 2N NaOH solution to 200 mL with ultrapure water and mix well.

5. Preparation of 0.1N Sodium Hydroxide solution: Dilute 50 mL of 2N NaOH solution to 1000 mL with ultrapure water and mix well.

6. **Preparation of blank solution:** Transfer 5 mL of Dissolution medium into 2 mL of 0.25 N NaOH solution mix well.Filter through 0.45µ nylon syringe filter, discard about first 2 mL of filtrate.

C. Preparation of standard solutions:

1. Preparation of standard stock solution: Weigh and transfer about 50 mg of Esomeprazole working standard into 100 mL volumetric flask. Add 30 mL of Methanol, sonicate for about 10 minutes to dissolve the material completely. Dilute to volume up to the mark with Methanol and mix well.

2. Preparation of standard solution for 40 mg strength (about 33.3 ppm): Pipette out 4 mL of the standard stock solution into a 50 mL volumetric flask. Dilute to volume with dissolution medium. Immediately mix 5 mL of above solution with 1 mL of 0.25N sodium hydroxide solution. Filter through 0.45 µm nylon filter and collect the filtrate after discarding initial 2 -3 mL of filtrate.

D. Optimization of Chromatographic Conditions:

After several trails with the method was optimized with chromatographic parameters Waters XterraRP18 (150X4.6) mm, 5µm analytical column using Mix 350 mL of Acetonitrile and 500 mL of the Buffer(pH 7.1 phosphate buffer). Dilute with water to 1000 mL as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a UV detector at 302nm. Injection volume of sample was 20ul.The total chromatographic analysis time per sample was about 10 min with esomeprazole eluting at retention time of about 4.8min.

E. Optimization of Dissolution Conditions

After trying many buffers, finally pH 6.8 phosphate buffers is optimized for dissolution test of esomeprazole tablets with paddle (USP apparatus II) at 100rpm and volume of buffer taken is 1000 mL (300mL acid medium + 700mL buffer medium),temperature maintained was 37±ºC and the test time 30mins.

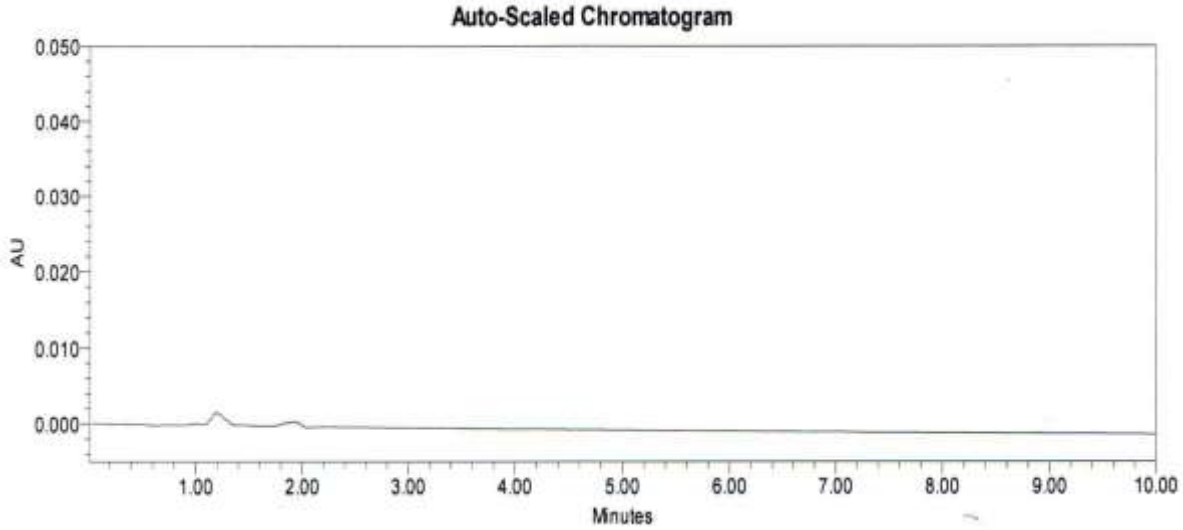
Results and Discussion:

Statistical evaluation of analysis and recovery study was carried out. The data obtained from the proposed method showed accuracy of method. The values of standard deviation and coefficient of variation were satisfactorily low. The percentage recovery of 95 % to 105% was indicative of accuracy of method

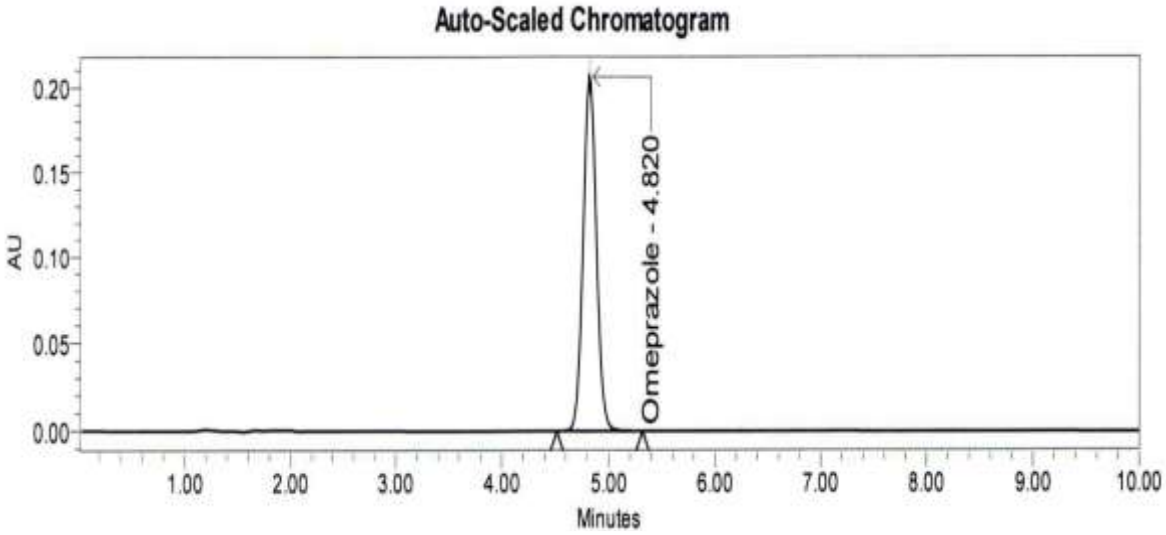
A. Specificity / Placebo Interference:

It is necessary to demonstrate that dissolution results are not affected by placebo constituents, other active ingredients in the drug product. Placebo interference was evaluated by weighing samples of placebo blend and dissolving or dispersing it into the dissolution medium at concentrations that would normally be encountered during testing. The chromatograms of blank, placebo, test sample and standard are used to justify the specificity of target analyte.

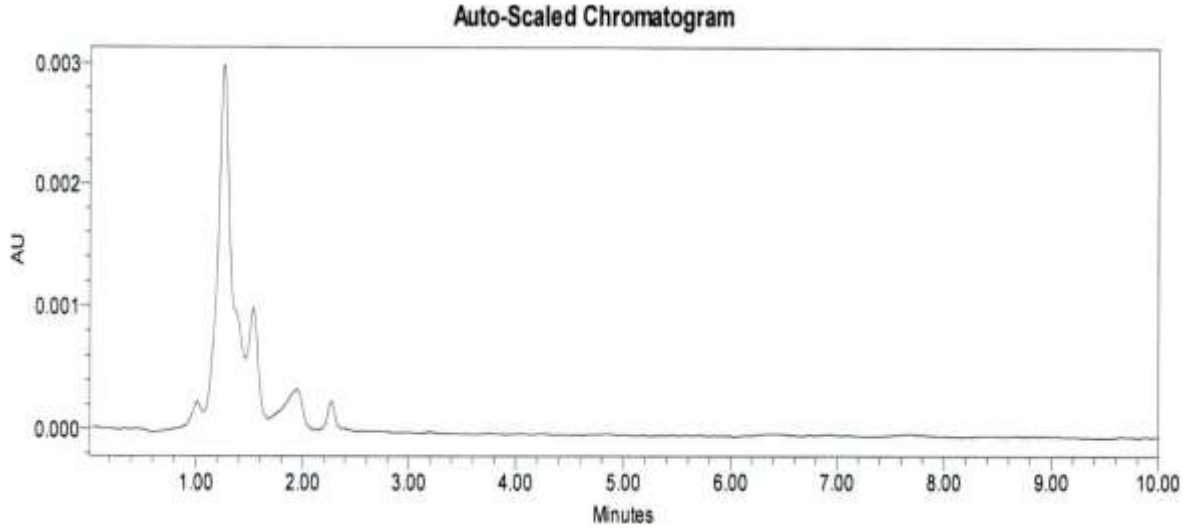
Chromatogram of blank



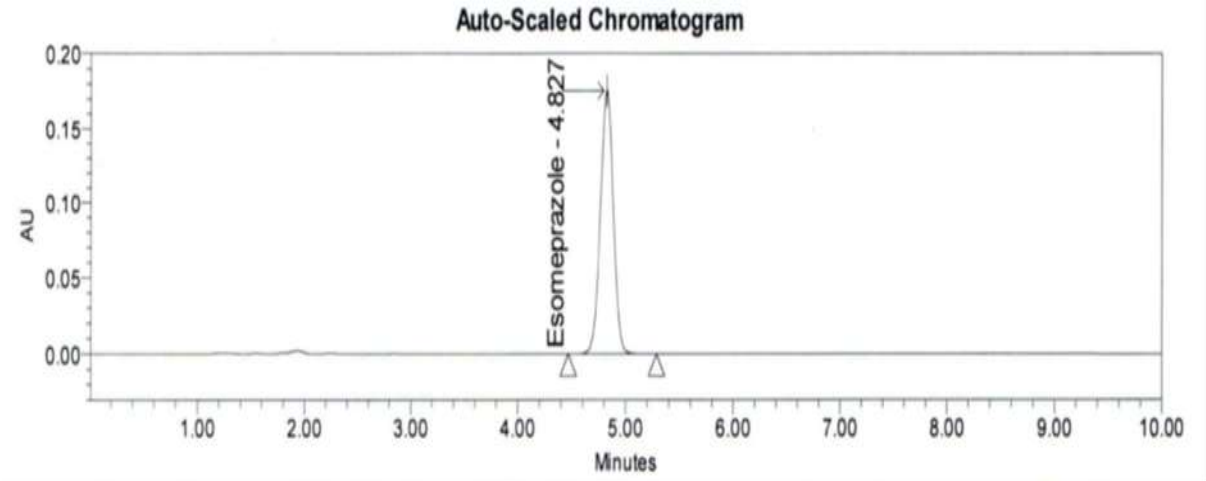
Chromatogram of standard



Chromatogram of placebo



Chromatogram of sample



Conclusion: No interference was found in the blank at the retention time of Omeprazole No interference was found in the Placebo at the retention time of Omeprazole

B. Linearity:

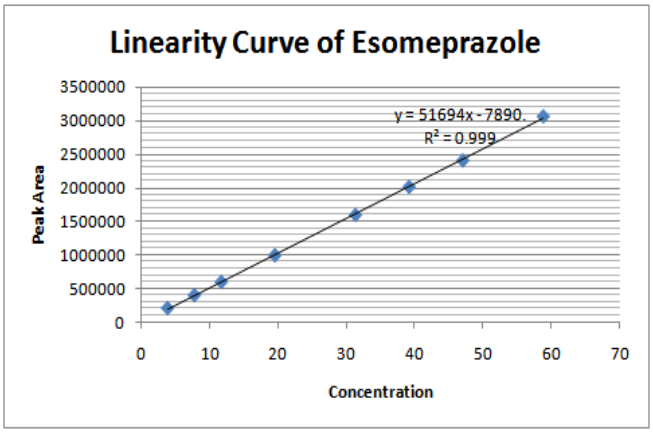
Measurements using clean standard preparations were performed to demonstrate method linearity. The solutions for different levels of drug (10% to 150% of target concentration of Esomeprazole standard) comprising of 8 levels were prepared and analyzed and the average peak areas were plotted against the concentration to determine the concentration range with linearity. The data is given below:

S.No	Level (%)	Weight of API (mg)	Weight as Esomeprazole (mg)	Dilution (mL)	Volume taken (mL)	Dilution (mL)	Volume taken (ml)	Dilution (ml)
1	10	61.21	54.874	100	1.0	100	5	7
2	20				1.0	50	5	7
3	30				1.5	50	5	7
4	50				5.0	100	5	7
5	80				4.0	50	5	7
6	100				5.0	50	5	7
7	120				6.0	50	5	7
8	150				3.0	20	5	7

Note: Convert the API weight taken to Esomeprazole by applying 89.65% potency (Weight taken X 89.65 / 100).

Acceptance criteria: Correlation coefficient should be not less than 0.999

S. No	% Level	Concentration (ppm)	Area
01	10	3.92	210265
02	20	7.84	403429
03	30	11.76	600142
04	50	19.60	996218
05	80	31.36	1595146
06	100	39.20	2013561
07	120	47.04	2405704
08	150	58.79	3059869
Slope			51497
Correlation coefficient (r)			1.000
Regression coefficient (R^2)			0.999



Conclusion: Correlation coefficient between Esomeprazole concentration and peak area was calculated by linear regression and was found to be within the acceptance criteria.

C. Accuracy:

Accuracy parameter was determined through recovery test by adding known amounts of Esomeprazole Mg Trihydrateto the placebo samples at four different levels, 50%, 100%, 150% and 200% of the target concentration.

Dissolution parameters

S. No	Parameter	
01	Medium	pH 6.8 Phosphate Buffer
02	Apparatus	Paddle (USP Apparatus-II)
03	RPM	100
04	Volume	900 mL
05	Temperature	37°C±0.5°C
06	Spectrometric Mode	HPLC by PDA/UV
07	Analytical Wavelength	302 nm
08	Time	30 minutes

Accuracy results of Esomeprazole Mg Trihydrate :

S.No	Strength	% level	Response	% recovery
01	40 mg	50%	778943	103.8
02	40 mg	100%	1446187	98.9
03	40 mg	150%	2272065	102.9
04	40 mg	200%	3083048	104.3

Conclusion: Recovery for analyte was within acceptance criteria of NLT 95% and NMT 105 %.

D. Precision:

Precision of the method refers to the reproducibility of value on repeated measurements. Six samples were injected and RSD of the values is estimated to understand the precision of the method.

Procedure for Sample preparation:

Transferred 1000 mL of pH 6.8 phosphate buffer (300 mL of 0.1 N HCl +700 mL of 0.086M Disodium hydrogen phosphate anhydrous buffer) into each dissolution vessel, which was preheated to 41°C. Transferred one capsule into each dissolution vessel. After 120 minutes, added 700 mL of 0.086 M Sodium dihydrogen phosphate solution to each dissolution vessel which was preheated to 41°C and adjustwith2 Hydrochloric acid or 2 N sodium hydroxide solutions, if necessary, to pH 6.80 ±0.05. After specified time intervals withdrawn 10 mL of sample by auto sampler with 10 mm polypropylene filters from each dissolution vessel. Replaced aliquots withdrawn for analysis with equal volumes of dissolution medium and maintained at 37±0.5°C. Immediately, Pipetted 5 mL of above sample into a test tube containing 1 mL of 0.25NNaOH and mixed well. Filtered the samples through 0.45 µm nylon filter, discarded about first 2 mL of filtrate.

For 40 mg Tablets: Pipetted 2 mL of the above solution to 20 mL volumetric flask and made up the volume with diluent. Filtered the samples through 0.45µm nylon filter (Make: Chromsource), discarded about first 2 mL of filtrate.

Precision of the Dissolution method:

S. No	Parameter	
01	Medium	pH 6.8 Phosphate Buffer
02	Apparatus	Paddle (USP Apparatus-II)
03	RPM	100
04	Volume	1000 mL
05	Temperature	37°C±0.5°C
06	Spectrometric Mode	HPLC by PDA/UV
07	Analytical Wavelength	302 nm
08	Q point time	30 minutes

S. No	% of drug release	% RSD
01	95.5	**7.4**
02	93.6	
03	89.2	
04	79.7	
05	97.2	
06	85.5	
Average	90.1	

Conclusion:

Precision data at Q point time was within acceptance criteria of NLT 80% and RSD below 10%.

E. Filter Compatibility:

Filter compatibility was performed by preparing standard and sample solutions and comparing the results for unfiltered and filtered solutions.

Compatibility of 0.45µm nylon filter and PVDF filter was studied. Standard and dissolution samples were filtered and analyzed along with unfiltered solutions. The % assay and difference were calculated.

Make: Chrome source

Diameter: 25 mm

Results for sample:

Time point	Centrifuge	0.45µ nylon	Difference	0.45µ PVDF	Difference
15 mins	87.3	86.9	0.4	83.9	3.4
30 mins	94.5	93.9	0.6	93.8	0.7
45 mins	91.7	91.5	0.2	91.3	0.4

Conclusion:

After the analysis it was found that nylon filter was suitable for filtration as the filter interference observed is below ± 2%. Precision data at Q point time was within acceptance criteria of NLT 80% and RSD below 5%

F. Solution Stability:

Solution stability is important for the given conditions and length of time of dissolution test. The standard and sample solution was stored at Bench top and Refrigeration temperature conditions up to 48 hours, and later analyzed using freshly prepared solutions for comparison

Parameters	Initial	24 h RT	24 h Refrigerator	48 h RT	48 h Refrigerator
Weight (mg)	56.64	56.64	56.64	56.64	56.64
Area	883011	884519.500	885871.500	997560.500	939856.500
% RF Ratio	101	100	100	104	98
Diff w.r.t initial	NA	1	1	-3	3

Dissolution Sample Solution Stability

24Hrs Samples Solution stability by Area:

	Initial area	24 hr RT	24 hr Freezer	Diff w.r.t initial B.T	Diff w.r.t initial Fridge
Sample-1	818379.000	819922.000	824711.000	0.2	-0.8
Sample-2	794519.000	800483.000	798549.000	0.7	-0.5
Sample-3	809837.000	812209.000	811042.000	0.3	-0.1
Sample-4	825201.000	829798.000	826741.000	0.6	-0.2
Sample-5	802678.000	810240.000	809095.000	0.9	-0.8
Sample-6	802549.000	803144.000	810197.000	0.1	-1.0

5. Conclusion:

An isocratic and fast HPLC method was developed and validated to determine Esomeprazole in tablet containing gastro-resistant pellets. The separation was achieved on Waters XterraRP18 (150X4.6) mm, 5µm analytical column using Mix 350 mL of Acetonitrile and 500 mL of the Buffer(pH 6.8 phosphate buffer). Dilute with water to 1000 mL as mobile phase and at a flow rate of 1.0 mL/min. Detection was carried out using a UV detector at 302nm. Injection volume of sample was 20ul.The total chromatographic analysis time per sample was about 10 min with esomeprazole eluting at retention time of about 4.8min. The method was validated for accuracy, precision, specificity, linearity and Filter compartability. Validation studies demonstrated that this HPLC method is simple, specific, rapid, reliable and reproducible. The standard curve was linear over the concentration range of 10%-150% corresponded with 4µg/mL to 60 µg/mL with R2 close to one (0.999). The high recovery and low relative standard deviation confirm the suitability of the proposed method for the determination of esomeprazole in tablets dosage form. This rapid method is of great feasibility since it can be used to determine esomeprazole in the quality control routine for assay, content uniformity, as well as for dissolution test of esomeprazole tablets.

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