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Artículos originales

Development and validation of a high-performance liquid chromatography method for the estimation of esomeprazole in bulk and tablet dosage form

Desarrollo y validación de un método por cromatografía líquida de alta resolución para la estimación de esomeprazol en forma de dosificación a granel y en tableta

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Conflict of interest

The authors have no conflicts of interest regarding this investigation.

Resumen

Introducción: Se desarrolló y validó un método de cromatografía líquida de alta resolución de fase reversa exacto, simple, preciso, rápido, económico y reproducible para la estimación de esomeprazol (ESO) en forma de dosificación a granel y en tabletas.

Método: La separación se llevó a cabo en columna Finepak SIL C18T-5 (250 × 4,6 mm; 5,0 µm i.d.) utilizando tampón fosfato dihidrógeno de potasio (0,025 M): ACN (20:80 v/v) y a un caudal de 1,0 ml/min. utilizando un detector UV a 302 nm con un tiempo de ejecución de 10 min. El método fue validado para exactitud de linealidad, exactitud, precisión, límite de detección (LOD), límite de cuantificación (LOQ) y robustez.

Resultados: La curva de calibración estándar fue lineal con R2 = 0,995. El LOD y el LOQ obtenidos para esomeprazol fueron 0,0001 y 0,0004 µg/mL respectivamente. El método se encontró robusto para posibles cambios. Los resultados del análisis de otros parámetros también se probaron y validaron según las pautas de ICH y los estudios de recuperación confirmaron la precisión del método propuesto. Los estudios de validación mostraron que el método HPLC desarrollado es simple, reproducible, rápido, preciso y confiable. La alta recuperación y la baja desviación estándar relativa confirman la idoneidad del método desarrollado para la determinación de esomeprazol en forma de dosificación en tabletas.

Conclusión: Este método puede ser utilizado como una opción más conveniente y eficiente para el análisis de esomeprazol para establecer la calidad de la sustancia durante el análisis de rutina con resultados consistentes y reproducibles.

Palabras clave: Esomeprazol; Desarrollo de métodos; Parámetros de idoneidad del sistema; Validación

Abstract

Introduction: An accurate, simple, precise, rapid, economic and reproducible reverse-phase high-performance liquid chromatography method was developed and validated for the estimation of Esomeprazole (ESO) in bulk and tablet dosage form.

Method: The separation was carried out on Finepak SIL C18T-5 column (250 × 4.6 mm, 5.0 µm i. d.) using potassium dihydrogen phosphate buffer (0.025M): ACN (20:80 v/v) and at a flow rate of 1.0 mL/min. using UV detector at 302 nm with a run time of 10 min. The method was validated for accuracy for linearity, accuracy, precision, limit of detection (LOD), limit of quantification (LOQ) and robustness.

Results: The standard calibration curve was linear with $R^2 = 0.995$. LOD and LOQ obtained for esomeprazole were 0.0001 and 0.0004 µg/mL respectively. The method was found robust for possible changes. Results of analysis of other parameters were also tested and validated as per ICH guidelines and recovery studies confirmed the accuracy of the proposed method. validation studies showed that the developed HPLC method is simple, reproducible, rapid, precise and reliable. The high recovery and low relative standard deviation confirm the suitability of the developed method for the determination of esomeprazole in the tablet dosage form.

Conclusion: This method may be used as a more convenient and efficient option for the analysis of esomeprazole to establish the quality of the substance during routine analysis with consistent and reproducible results.

Keywords: Esomeprazole; Method development; System suitability parameters; Validation

Highlight

Esmoprazole is most widely used drug for the treatment of gastric acidity and it is necessary to quantify it in pure as well as tablet dosage form.

The current article focused on development of robust method of analysis of the pure esmoprazole and its dosage form by HPLC method.

Introduction

Esomeprazole (ESO) is a potent gastric proton-pump inhibitor used in the treatment of gastric-acid related disorders, it acts by inhibition of gastric acid secretion. Esomeprazole shows its pharmacological action by reducing the concentration of gastric acid by hindering enzyme action in gastric parietal cells, thus reducing the movement of hydrogen ions into the gastric lumen. It is well-tolerated, hence avail-

able in the market widely[1,2]. Chemically Esomeprazole is 5-Methoxy-2- (S) [(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl]-1H-benzimidazole magnesium salt trihydrate with molecular formula $C_{34}H_{36}MgN_6O_6S_2 \bullet 3H_2O$ $C_{17}H_{18}N_3O_3S \bullet Na$[3-4]. Esomeprazole is an optical S-isomer of omeprazole which provides better acid control than existing forms of proton pump inhibitors and has a superior pharmacokinetic profile in comparison to omeprazole[5]. Esomeprazole is having greater, reliable and more stable bioavailability than omeprazole. The drug is having a better pharmacokinetic profile, confirming an improved systemic exposure and fewer inter-individual variability as compared with omeprazole, and more effective destruction of gastric acid production compared with other proton pump inhibitors[6].

Literature survey reveals that so many analytical methods were reported for estimation of Esomeprazole individually or in combination with other drugs from the bulk and pharmaceutical dosage form by using different mobile phase ratios, column temperature and pH[7-11]. Previously reported methods of analytical estimation of ESO by HPLC technique showed lesser sensitivity and high noise in the baseline indicating a need to develop a more sensitive, accurate, simple and rapid method. Many HPLC methods were developed by using methanol along with water as a mobile phase whereas acetonitrile was also used in specific cases. As we have selected mobile phase acetonitrile over methanol due to its advantages in several features viz. its lower absorbance than methanol leading to lower noise in the HPLC chromatogram, the lower pressure experienced by the column than that of methanol and the elution strength is also superior in case of acetonitrile. Present research work represents a convenient, accurate, simple, precise, rapid, economic and reproducible RP-HPLC method for estimation of ESO in bulk and tablet dosage form.

Material and Methods

Reagents and Materials

Chemicals and reagents: The working standard gift sample of Esomeprazole was received from Cipla Ltd. Kurkumbh, Pune, Maharashtra. Acetonitrile, Methanol and Water (HPLC grade) were procured from Merck Laboratories Pvt. Ltd., Mumbai. Potassium dihydrogen phosphate was of high purity analytical grade. The Nexium Tablets (Glenmark Pharmaceutical Ltd.) equivalents to 40 mg were purchased from the local pharmaceutical market.

Instrumentation

The instrument used for analysis was the HPLC system (Agilent technology) accompanied by Borvin software. Finepak SIL C18T-5 column (250 × 4.6 mm dimensions) with 5 µm as an internal diameter was used as the stationary phase. A precision water bath equipped with MV controller (Biomedica, India) was used to carry out selected reactions in solution during the stress degradation study.

Chromatographic conditions

The HPLC system was controlled at ambient temperature and a flow rate of 1 mL/min. The measurements were done with a UV detector at 302 nm. The mobile phase was composed of potassium dihydrogen phosphate buffer (0.025M): Acetonitrile (ACN) (20:80 v/v). This mobile phase was ultrasonicated for 10 min and then it was filtered through a 0.22 µ membrane filter. The run time was set at 10 min for this research work at a flow rate of 1.00 mL/min.

Selection of Analytical Wavelength

The prepared standard stock solution of concentration (100 µg/mL) was scanned using a double beam UV-Visible Spectrophotometer (Shimadzu-1700) in the spectrum mode between the wavelength range 400 nm to 200 nm against the mobile phase as blank, and their spectra was overlaid. The wavelength selected for analysis was 302 nm, as the drug showed significant absorbance at this wavelength[12].

Preparation of standard stock solution

Accurately weighed quantity (10 mg) of Esomeprazole was transferred to a 10.0 mL volumetric flask, dissolved and diluted up to the mark with the mobile phase. The 0.22 µ membrane filter was used to filter the solution. (Concentration: 1000 µg/mL). From standard stock solution, 0.2, 0.3, 0.4, 0.5 and 0.6 mL were transferred individually to 10.0 mL volumetric flask and diluted to the mark with mobile phase (Concentration 20, 30, 40, 50 and 60 µg/mL respectively). The diluted solutions were filtered through a 0.22 µ membrane filter.

Calibration curve of Esomeprazole:

Then each solution (20 µl) was injected into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and the area of each peak for ESO was measured at 302 nm. Each sample solution was chromatographed in triplicate and the mean peak area for ESO was calculated[13].

System Suitability Parameters

To ascertain the resolution and reproducibility of a proposed chromatographic system for estimation of ESO in tablets, system suitability parameters like tailing factor (T), resolution (R), column efficiency (number of theoretical plates, N) and system precision were studied[14].

Linearity and Range

Linearity and range were determined by 5 times repeating the procedure for the calibration curve[15].

Accuracy

To ascertain the accuracy of the proposed method, recovery studies were carried out at three different levels viz. 80 % 100 % and 120 % by standard addition method as per ICH guidelines. An accurately weighed quantity of pre-analyzed tablet powder equivalent to about 10 mg ESO was transferred in nine different 10.0 mL volumetric flasks separately. To each of the flask containing ESO, the following quantities of pure ESO were added: Flask No. 1: 8.0 mg ESO, Flask No. 2: 8.1 mg ESO, Flask No. 3: 8.1 mg ESO, Flask No. 4: 10.1 mg ESO, Flask No. 5: 10.0 mg ESO, Flask No. 6: 10.1mg ESO, Flask No. 7: 12.3 mg ESO, Flask No. 8: 12.2 mg ESO, Flask No. 9: 12.25 mg ESO. Then, 8 mL mobile phase was added to each flask, and the content of the flask was ultrasonicated for 10 min, volume was then made up to the mark with the mobile phase. The solution was mixed thoroughly and filtered by using no. 42 Whatman filter paper. From the filtrate 0.4 mL solution was diluted to 10 mL with the mobile phase. The diluted solution was filtered through a 0.22 µ membrane filter. Then solution (20 µL) was injected into the column and chromatographed using optimized chromatographic conditions. The individual solution was injected and in triplicate chromatographed. The corresponding chromatograms were recorded and the area of each peak was measured at 302 nm. The total amount of ESO in the sample was calculated, by comparing the mean peak area for standard and sample solutions.

Precision

Intra-day and inter-day precision were determined by analyzing tablet sample solutions containing Esomeprazole (10 mg) at three different time intervals on the same day and three different days respectively. Tablet sample solutions were prepared and analyzed similarly as described under the analysis of tablet formulations[16].

Limit of detection (LOD) and Limit of quantitation (LOQ)

The LOD and LOQ were separately determined based on the standard deviation of the response of the calibration curve. The slope of the calibration curves and standard deviation of the y-intercept were used to calculate the LOD and LOQ.

The ruggedness of method

The ruggedness of the method was checked by analyzing tablet formulation by different analysts under the same experimental conditions.

Robustness of method

To evaluate the robustness of the planned method, minor but intentional deviations in the method parameters were done. The effect of change in flow rate, mobile phase ratio, wavelength, etc., on retention time and tailing factor were studied. The tablet sample solution containing 100 µg/mL of ESO was injected (in triplicate) into the HPLC system under varied conditions.

Forced degradation studies

Tablet powder equivalent to about 10 mg ESO were separately transferred to five different 10 mL volumetric flasks (Flask No. 1, 2, 3, 4 and 5) added 3.0 mL of 0.1 N HCl, 0.01 N NaOH and 1 % H_2O_2 to Flask No. 1, 2 and 3 respectively. Contents of Flask No. 1 and 2 were heated in a water bath for 3 h at 80° C. Flask No. 3 was heated in a water bath for 1 h. Flask No. 4 containing tablet powder was kept at 50° for 24 h to study the effect of heat on tablet sample (heat / thermal degradation). Flask No. 5 containing tablet powder sample was exposed to UV radiations at 254 nm for 24 h. Samples were withdrawn at appropriate times, allowed to cool and then a selected mobile phase was added to each flask. The samples were then analyzed similarly as described under the analysis of tablet formulation[17].

Analysis of tablet formulation

Twenty tablets were weighed and the average weight was calculated. Tablets were crushed to convert into fine powder. Accurately weighed quantity of tablet powder equivalent to about 10 mg ESO was transferred to 10.0 mL volumetric flask, followed by mobile phase and ultrasonicated for 20 min, the mobile phase was then used to make to make the final volume. The resulting solution was shaken and filtered through No. 42 Whatman filter paper. From the filtrate, 0.2, 0.3, 0.4, 0.5, 0.6 mL solution was diluted to 10 mL with mobile phase and filtered using 0.22 µ membrane filter.

An equal volume of standard stock solution and sample solution (20 µL) was injected into the column and chromatographed using optimized chromatographic conditions. The corresponding chromatograms were recorded and the area of each peak for sample and ESO was measured at 302 nm. The individual solution was injected and in triplicate chromatographed. The amount of ESO in the sample (mg) was calculated by comparing the mean peak area of the sample with that of the standard.

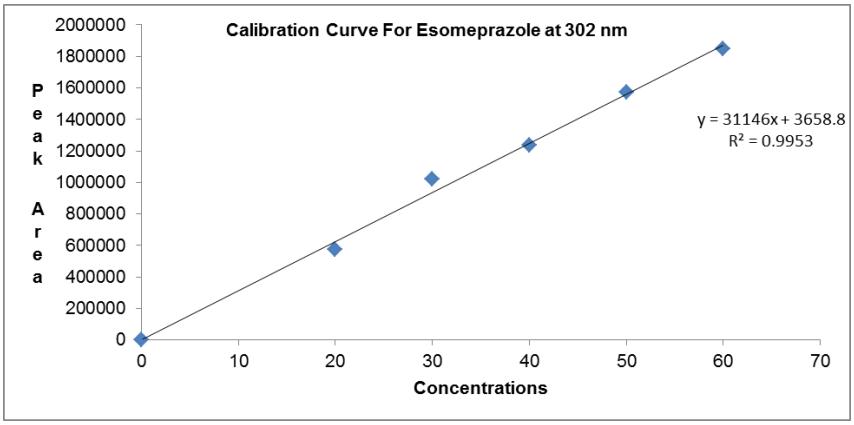
Results and Discussion

Esomeprazole is the most widely used drug to counteract hyperacidity. In the present study, efforts have been made to develop a stability indicating HPLC method to estimate Esomeprazole in bulk drug and in tablet formulation. Various parameters according to the ICH guideline Q2 (R1) were followed during the study. During development the method type and concentration of the mobile phase very important role. Various composition of mobile phases was tried to develop a chromatogram out of which potassium dihydrogen phosphate buffer (0.025M): ACN (20:80 v/v) was found most optimum. Linearity is the ability to detect the test results that are proportional to concentration. Linearity of detector response was studied by plotting a graph of concentration v/s peak area. Linearity was observed in the concentration range of 20 – 60 µg/mL for Esomeprazole, the results were found near about in agreement with the study carried out by Sojitra and Rajput (2012)[18]. The coefficient of correlation was found to be 0.995 for Esomeprazole. The calibration curve and chromatogram for esomeprazole was shown in Table 1 and Figure 1.

Table 1. Linearity Concentration Range and Peak Area Data for esomeprazole

Concentration (µg/mL)	Peak Area (mm^2)*
20	553263
30	1032340
40	1235925
50	1570302
60	1849245

* Denotes average of three determinations.



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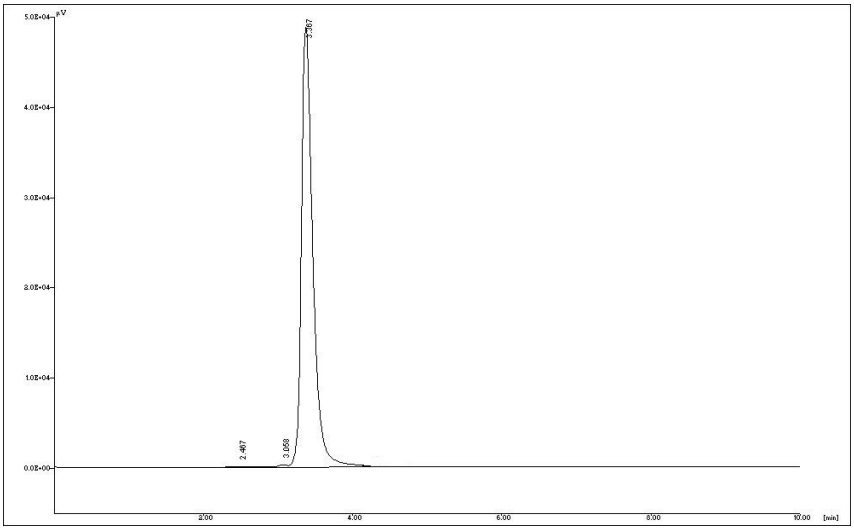


(B)

Figure 1. (A) Standard calibration curve for esomeprazole, (B) Chromatogram of esomeprazole

To ascertain the resolution and reproducibility of the chromatographic system suitability tests and system precision were carried out. Results of system suitability parameters and system precision were shown in Table 2.

Table 2. System Suitability Parameters

Parameter	Results
Resolution (R)	3
Tailing Factor (T)	1.10
No. of Theoretical Plates (N)	3177
Precision % RSD (n = 6)	0.0149

From the above results, it was evident that the peaks were almost symmetrical having satisfactory resolution and precision. The efficiency of the column was also found satisfactory. To ascertain the accuracy of the proposed method, recovery studies were carried out by the standard addition method at three different levels (80 %, 100 % and 120 %). Results of recovery studies were summarized in Tables 3.

Table 3. Recovery Studies

Level of recovery	Weight of tablet powder taken (mg)	Amount of drug added (mg)	Amount of drug recovered (mg)	% Recovery
80 %	122.5	8.1	07.91	98.87
	121.0	8.0	08.05	100.62
	122.25	8.1	07.86	98.25
100 %	121.25	10.1	10.06	99.60
	122.0	10.0	09.83	98.30
	121.25	10.1	09.94	98.41
120 %	121.0	12.3	12.34	100.32
	122.25	12.2	12.37	101.39
	122.25	12.25	12.22	99.75
Statistical Validation for Recovery Study				
Level of recovery	**% Recovery ***		**C. V.**	
80 %	99.24 ± 1.22		1.22	
100 %	98.77 ± 0.72		0.72	
120 %	100.48 ± 0.83		0.83	

*Denotes the average of three determinations.

The percent recovery was observed at around 100 % representing the accuracy of the proposed method and besides, excipients did not show any interference during the estimation of drug results of intra-day and inter-day precision were summarized in Tables 5. The results of the percent label claim and the standard deviation indicated the repeatability and reproducibility of the method under different conditions. LOD and LOQ are referred to as the ability of the analytical method to detect and quantify the least amount of drug respectively. For the estimation of ESO LOD and LOQ were performed and the results of LOD and LOQ studies were summarized in Table 4. The ruggedness of the method was performed by three different analysts under the same experimental and environmental conditions. The results of ruggedness studies were summarized in Table 4. The robustness of the planned method was studied by small but intentional alterations in the method parameters. The effect of change in flow rate, mobile phase ratio, wavelength etc. on retention time and tailing factor were shown in Table 4.

Table 4. Data of Intra-day Precision, Inter-day Precision, LOD and LOQ

Intra-day Precision Data		**Inter-day Precision Data**		**LOD (µg/mL)**	**LOQ (µg/mL)**
% Label Claim*	C. V.	% Label Claim*	C. V.	0.000141	0.000429
99.17 ± 0.320	0.3226	99.28 ± 0.1819	0.1832		
Results of Ruggedness Study					
Amount of drug estimated (mg/tablet)*		% Label Claim		C. V.	
39.64		99.12 ± 0.8158		0.8230	
Result of Robustness Studies					
Factor	mL/min	Level	Retention Time	Tailing Factor	
Flow Rate			ESO	ESO	
	0.9	- 0.1	3.63	1.05	
	1.0	0.0	3.44	1.10	
	1.1	+ 0.1	3.07	1.16	
Mean			3.38± 0.284	1.10± 0.05	
Wavelength	nm	Level	ESO	ESO	
	300	-2	3.57	1.23	
	302	0	3.41	1.09	
	304	+2	3.36	1.0	
Mean			3.44± 0.109	1.10± 0.11	
Mobile Phase Composition Change (Potassium dihydrogen phosphate: ACN) (v/v)			ESO	ESO	
78: 22			3.83	0.9	
80: 20			3.48	1.16	
82: 18			3.36	1.13	
Mean			3.556 ± 0.244	1.06 ± 0.14	
ESO: esomeprazole					

The method was found to withstand the deliberate deviations in method parameters. The standard deviation of a set of results indicated the robustness of the method. The assay of tablet was performed and the results of the amount of drug estimated in mg/tablet and percent label claim were mentioned in Table 5.

Table 5. Statistical validation of analysis of tablet formulation

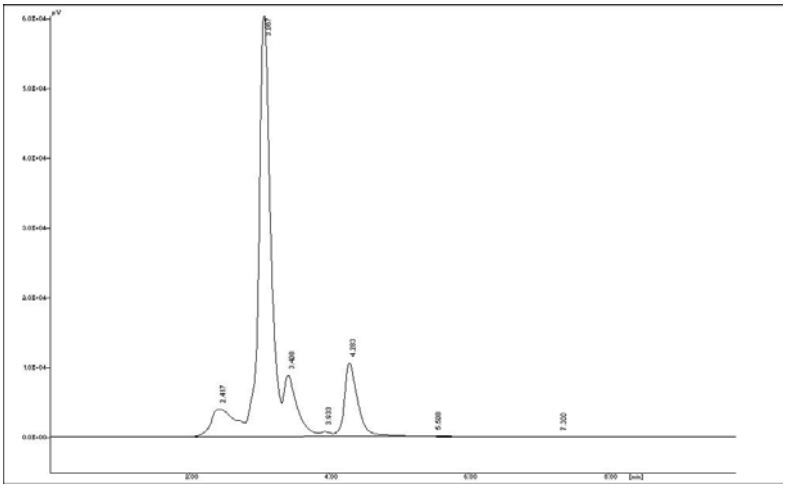
Amount of Drug Estimated (mg/tablet)*	**% Label Claim**	**C. V.**
39.61	98.90 ± 0.8869	0.8967

* Denotes average of three determinations

The percentage label claim was found 98.90 %, and the results were found in agreement with the study carried out by Jain et al. (2019)[7]. Forced degradation studies were carried out to detect the degradation of ESO in the presence of various stress conditions such as acidic, alkaline, oxidative, thermal, and photolytic stress. Esomeprazole was found to be susceptible to acid and oxidative stress conditions whereas it was found to be stable under alkaline, heat (thermal) and photodegradation stress conditions[19]. The method was able to resolve the peaks of degraded products from the drug peaks indicating the selectivity and specificity of the proposed RP-HPLC method. Results of forced (stress) degradation studies were shown in Table 6 and Figure 2.

Table 6. Result of Forced Degradation Studies

Stress Condition	Temperature and Time	Assay of the active substance (%)
Acid (0.1N HCl)	80° for 3 h	54.53
Alkali (0.01 N NaOH)	80° for 3 h	63.20
Oxide (1 % H_2O_2)	80° for 1 h	78.35
Heat (Thermal)	50° for 24 h	99.60
UV exposure	254 nm for 24 h	98.30



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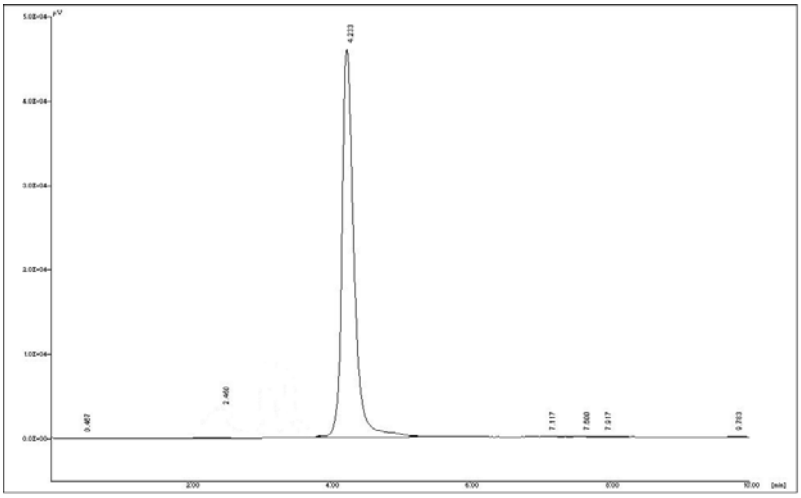

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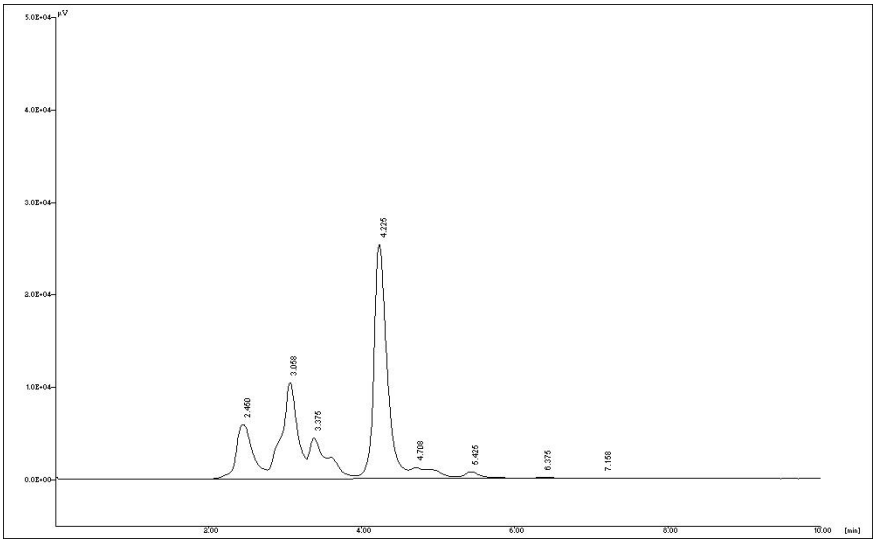
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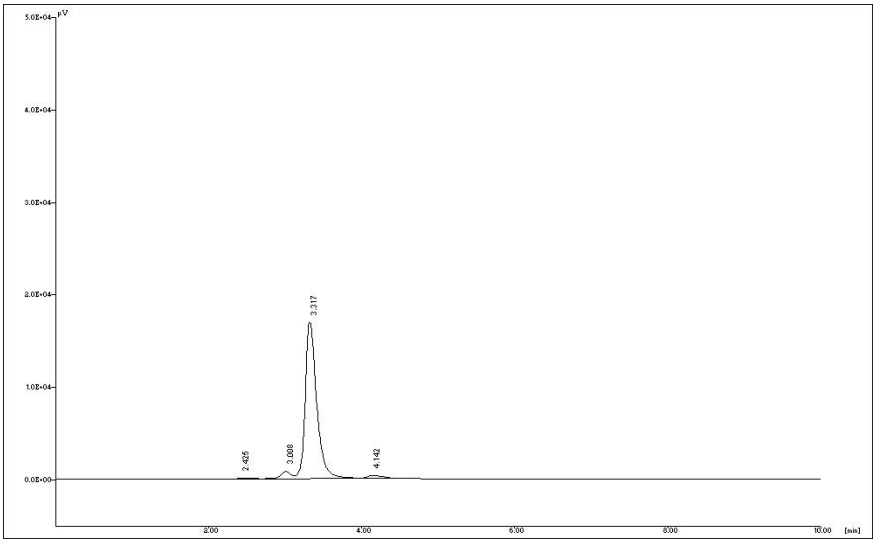
(C)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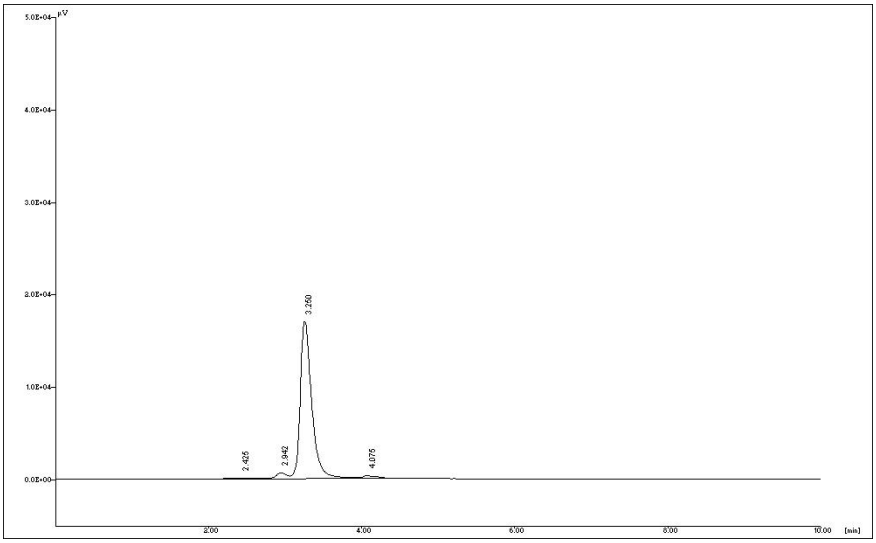

(E)

Figure 2. (A) Chromatogram of Acid (0.1 N HCl) Treated Sample, (B) Chromatogram of Alkali (0.1 N NaOH) Treated Sample, (C) Chromatogram of Oxide (1 % H_2O_2) Treated Sample, (D) Chromatogram of Sample Exposed to Thermal Process, (E) Chromatogram of Sample Exposed to UV – radiations

Conclusion

A simple, linear, robust, rugged, accurate, precise, convenient and reproducible RP-HPLC method has been developed for estimation of esomeprazole in bulk and tablet dosage form using a UV detector. Esomeprazole was found to be unstable at acidic, alkaline, oxidative stress conditions whereas it was found to be stable under heat and photodegradation stress conditions. The method was able to resolve the peaks of degraded products from the drug peaks indicating the selectivity and specificity of the proposed RP-HPLC method. Therefore, this method might have the potential to be used as a more convenient and robust alternative for the analysis of esomeprazole to establish the quality of the substance during routine analysis with consistent and reproducible results.

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