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Research Article

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Characterization and quantification for sulfide impurity of Esomeprazole by RP-HPLC

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ABSTRACT

A novel gradient reversed-phase High performance liquid chromatographic method has been developed for quantitative determination sulfide impurity of esomeprazole. The method display quantification potential related substances Sulfide (Impurity C) of Esomeprazole. Mobile phase A, B consists of 0.01M Disodium hydrogen phosphate: acetonitrile (80:20, v/v) and (30:70, v/v) respectively, pH-7.6 with orthophosporic acid, which were run as per the optimized gradient program. Zorbax SB C-8, (150cm×4.6 mm, 5µm) was used as the stationary phase. 1.0 mL per minute was employed as the flow rate and injection volume was kept as 40 µL. Detection wavelength was kept as 280 nm. The method was validated as per ICH Q2 (R1) guidelines &confirmed a good performance with respect to specificity, linearity, sensitivity, accuracy, precision, selectivity. So the proposed method can be used in routine quality control laboratories.

Keywords: Development, Validation, RP-HPLC, Esomeprazole, Impurities

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INTRODUCTION

Esomeprazole is a proton pump inhibitor, belonging to the benzimidazole group of drugs [1]. Esomeprazole is the prototype member of substituted benzimidazoles which reduce the final common step in gastric acid secretion and have overtaken H_2 blockers for acid-peptic disorders [2]. It is a potent inhibitor of gastric acid: can totally eliminate HCl secretion, both resting as well as that stimulated by any of the secretal glands. Esomeprazole is the prototype member of substituted benzimidazoles which reduce the final common step in gastric acid secretion and have overtaken H_2 blockers for acid-peptic disorders. It is a potent inhibitor of gastric acid: can totally eliminate HCl secretion, both resting as well as that stimulated by any of the secretal glands [3]. Esomeprazole inhibits oxidation of certain drugs: diazepam, phenytoin and warferin levels may be increased. In the present study, an HPLC method for the estimation of related substances of Esomeprazole has been developed and validated according to ICH guidelines [4].

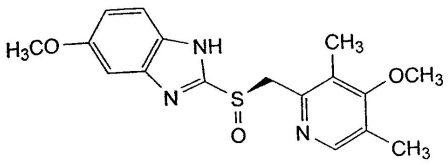

Fig. 1 Structure of Esomeprazole

EXPERIMENTAL SECTION

Chemicals and Reagents

Esomeprazole provided as a gift Sample by Switzer Life sciences Pvt. Ltd. Ahmedabad, HPLC grade acetonitrile was purchased from Rankem, India, disodium hydrogen phosphate anhydrous, orthophosphoric acid from Qualigens and HPLC grade water from central drug house New Delhi.

HPLC instrument and chromatographic conditions

A chromatographic system used was Waters 2695 separations module with 2487 dual wavelength absorbance detector and 2996 Photodiode Array Detector equipped with Empower chromatographic software. Coloumn Zorbax SB C-8, (150 x 4.6) mm, 5µm at temperature 25° C, with mobile phase at the flow rate of 1.0 ml/min.

Preparation of buffer solution for mobile phase: About 1.46gm of disodium hydrogen phosphate anhydrous was weighed accurately and transferred the material into a 1 L bottle added 1 L of HPLC grade water. Mixed well & pH was checked and adjusted to 7.6 ±0.05 with orthophosphoric acid. The solution was filtered through a 0.45 µm nylon membrane filter.

Preparation of mobile phase A: Mobile phase-A was prepared by mixing 800 ml of buffer solution and 200 ml of acetonitrile in a 1 L bottle and sonicated for 5 minutes.

Preparation of mobile phase B: Mobile phase-B was prepared by mixing 300 ml of buffer and 700 ml of acetonitrile solution in a 1L bottle and sonicated for 5 minutes.

Preparation of diluent: mobile phase-A was used as diluent for sample preparation.

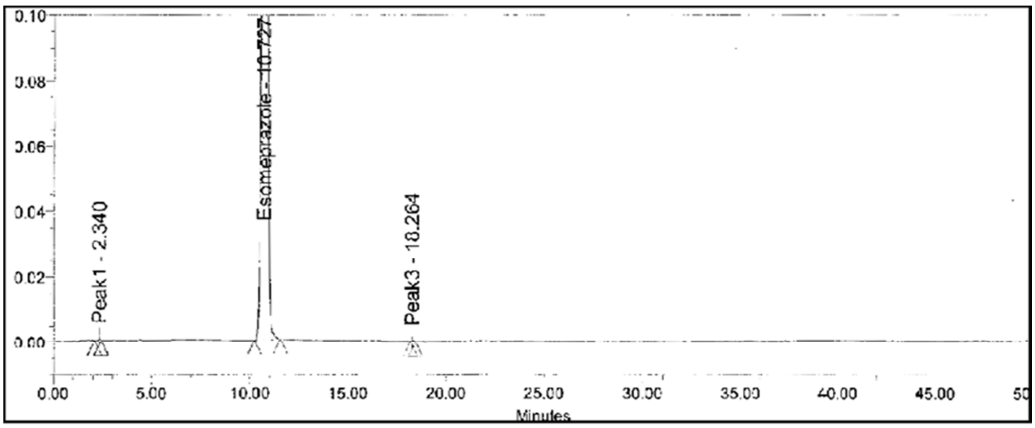

Fig. 2. Chromatogram for standard Esomeprazole

RESULTS AND DISCUSSION

Method Development and Optimization

Simple, sensitive, accurate, characterization, quantification for sulfide impurity of esomeprazole was developed by RP-HPLC method. Challenges were observed in the selection of the stationary phase and mobile phase. Mobile phase A, B consists of 0.01M Disodium hydrogen phosphate: acetonitrile (80:20, v/v) and (30:70, v/v) respectively,

pH-7.6 with orthophosporic acid, which were run as per the optimized gradient program. Zorbax SB C-8, (150cm×4.6 mm, 5µm) was used as the stationary phase. 1.0 mL per minute was employed as the flow rate and injection volume was kept as 40 µL. Detection wavelength was kept as 280 nm.

Accuracy

Accuracy (recovery) was carried out at QL level, 100% and 120% of target limit. Accuracy solutions were prepared in triplicate. % Recovery of results found to be in the range of 80-120 and individual and cumulative (overall) % RSD of % Recovery was well within the limit i.e. Not more than 10.0.

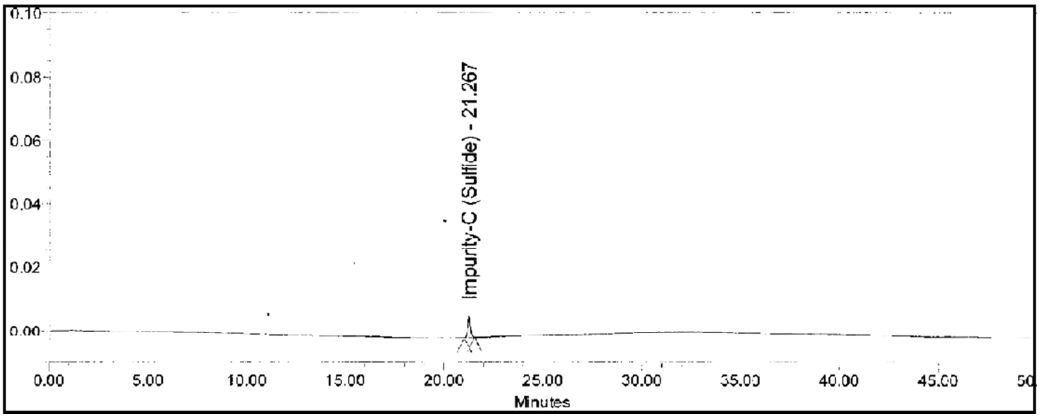

Fig.3. Typical Chromatogram Representing Retention Time of Sulfide Impurity-C

Table1. Recovery studies data for sulfide Impurity C

Recovery Level	Conc. (µg/mL)	Amount Added (%w/w)	Amount Found (%) w.r.t RF	% Recovery	Mean	SD	% RSD
LOQ	0.043	0.031	0.033	106.45	**106.45**	**0.000**	**0.00**
		0.031	0.033	106.45			
		0.031	0.033	106.45			
100%	0.144	0.103	0.102	99.03	**99.68**	**0.560**	**0.56**
		0.102	0.102	100.00			
		0.102	0.102	100.00			
120%	0.173	0.123	0.118	95.93	**98.09**	**2.047**	**2.09**
		0.121	0.119	98.35			
		0.119	0.119	100.00			
Overall					**101.41**	**3.988**	**3.93**

Table 2. System Precision

Injection No.	**1**	**2**	**3**	**4**	**5**	**6**
Resolution	7.72	7.71	7.74	7.72	7.74	7.73
Mean	7.73					
S.D	0.01					
%RSD	0.16					

Table 3. Intermediate Precision

Impurity-Sulfide		
Sample No.	**Day 1**	**Day 2**
1	0.10	0.10
2	0.09	0.10
3	0.10	0.10
4	0.10	0.10
5	0.10	0.10
6	0.10	0.10
Mean	**0.10**	**0.10**
SD	**0.004**	**0.000**
% RSD	**4.00**	**0.00**

Precision,
The study system precision (repeatability), of test results obtained by six replicate injections of reference solution and intermediate precision(ruggedness) standard solutions were analyzed 6 times on the different time interval of 24 hours. Resolution was found greater than 3.0 & % RSD of impurity Sulfide was found less than 10.0 which were found to be well within the limit.

Linearity
Linearity solutions were injected in triplicate and correlation coefficient was calculated for all known impurities and Esomeprazole. Response factor for all known impurities against Esomeprazole was calculated. The linearity of response for Esomeprazole and all related substances were determined in the range from LOQ to 120% of specification levels. Correlation coefficient found to be more than 0.990. Hence the method was found to be linear.

Table 4.Standard calibration curve data for Impurity (Sulfide)

Impurity (Sulfide)		
Conc. Level	**Conc. (µg/mL)**	**Area counts**
LOQ	0.043	2057
80%	0.115	5613
90%	0.130	6281
100%	0.144	6969
110%	0.159	7700
120%	0.173	8386
Slope		**48602**
Intercept		**-21**
Correlation coefficient		**0.9999**
Response Factor		**1.10**

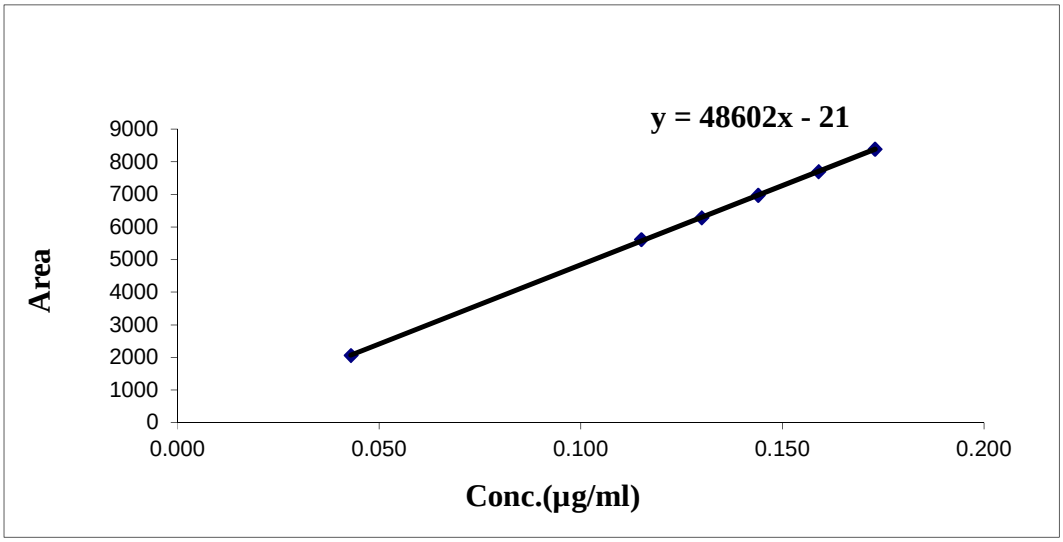

Fig. 4. Standard calibration curve for Impurity sulfide

Limit of Detection and limit of Quantitation (LOD-LOQ)
For the determination of LOD and LOQ, impurity sulfide and Esomeprazole solutions at 0.01%, 0.02%, 0.03%, 0.04% and 0.05% of sample concentration were prepared. %RSD for LOQ and LOD were within the limit as shown in Table 5.

Robustness
Sample solution spiked related substances Impurity sulfide was analyzed under different conditions and relative retention time was calculated at each variable condition. Method illustrated robust result at different deliberate changes in chromatographic conditions. Robustness data are shown in table, Table 6.

Table 5. LOD and LOQ for Impurity C (Sulfide)

Impurity C (Sulfide)	**LOD**	**LOQ**
Conc. (µg/mL)	0.015	0.044
% w/w	0.011	0.031
Injection	**Area counts**	
1	510	2088
2	833	2021
3	751	2103
4	665	2124
5	706	2092
6	702	2019
Mean	**695**	**2075**
SD	**107.2**	**44**
RSD (%)	**15.42**	**2.12**

Table 6. Results for Robustness

Robustness Parameter	**RRT Impurity C(Sulfide)**
Control (Specificity)	1.97
Flow rate (–10%)	1.85
Flow rate (+10%)	2.09
Column Oven Temp. (-5°C)	1.98
Column Oven Temp. (+5°C)	2.04
pH Change (– 0.2 pH units)	2.01
pH Change (+ 0.2 pH units)	2.11
Mobile Phase composition change(–2 %)	1.67
Mobile Phase composition change(+2 %)	2.43
Wavelength(–5 nm)	1.99
Wavelength(+5 nm)	1.99

CONCLUSION

The RP-HPLC method for the determination of related substances in Esomeprazole has been developed and was specific, sensitive, precise, accurate, rapid and robust. The mobile phase A, B consists of 0.01M Disodium hydrogen phosphate: acetonitrile (80:20, v/v) and (30:70, v/v) respectively, pH-7.6 with orthophosporic acid, which were run as per the optimized gradient program. Zorbax SB C-8, (150cm×4.6 mm, 5µm) was used as the stationary phase. 1.0 mL per minute was employed as the flow rate and injection volume was kept as 40 µL. Detection wavelength was kept as 280 nm. The method was validated as per ICH Q2 (R1). The developed method can be conveniently used by quality control department to determine the related substances in regular Esomeprazole production samples.

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