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DEVELOPMENT AND VALIDATION OF ULTRA PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR ASSAY OF OMEPRAZOLE BLEND

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ABSTRACT

Aim of the present work was to develop simple, shorter and effective UPLC method with UV detection (305nm) and subsequent validation for the determination of omeprazole in omeprazole magnesium blend. The method uses isocratic the mobile phase mixture of buffer and methanol in the ratio 40:60(v/v) on Hypersil GOLD, 50 x 4.6 mm, 3µ column. The buffer was prepared by dissolving 10 ml of triethylamine in 1000ml of water and adjusts the pH to 7.4 with dilute othophosphoric acid. The RSD for five injections was observed to 0.1% .The linearity of test method was performed with 25 – 150 percentages of label claims and correlation coefficient was found to be 0.9999correlation. The observed result shows that the method was rapid, precise, accurate and simple. The method was validated as per ICH guidelines.

KEY WORDS

Omeprazole, Method development, UPLC, Validation.

INTRODUCTION

Omeprazole is a proton pump inhibitor used in the treatment of dyspepsia, peptic ulcer disease (PUD), gastro esophageal reflux disease (GORD/GERD), laryngo pharyngeal reflux (LPR) and Zollinger–Ellison syndrome and is one of the most widely prescribed drugs internationally. Omeprazole suppresses gastric acid secretion. By acting specifically on the proton pump, omeprazole blocks the final step in acid production, thus reducing gastric acidit [1]. Omeprazole inhibits CYPs 2C9 and 2C19 and in those with the extensive metaboliser phenotype there is evidence that omeprazoleadministration results in significant decreases in the clearance of diazepam, phenytoin, and possibly carbamazepine and S-warfarin [2, 3] . Omeprazole is chemically designated as "1H-Benzimidazole,5- methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulfinyl]-5-Methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl] sulfinyl]benzimidazole" with the empirical formula C 17 H19 N3O3S and its molecular weight was 345.42 and it was freely soluble in 0.1N sodium hydroxide solution. Omeprazole was a race mate. It contains a tricoordinated sulfinyl sulfur in a pyramidal structure and therefore can exist in equal amounts of both (S) - and (R)- enantiomers. Omeprazole chemical structure is as shown in **Fig.1**. Literature search revealed that, papers on degradation of omeprazole [4], determination by UV spectrophotometer method [5], omeprazole in human plasma & urine by LC-MS-MS [6], determination of S-omeprazole, R-omeprazole and racemic omeprazole [7], are available. But as such there is no validated method available, which is having short run time of 2 minutes to estimate the Assay of Omeprazole. A key benefit of the method is that the less run time which will also save the solvents consumption. The present work describes a simple, isocratic method for the determination of Omeprazole in Omeprazole Magnesium blend as for ICH guidelines [8-11].

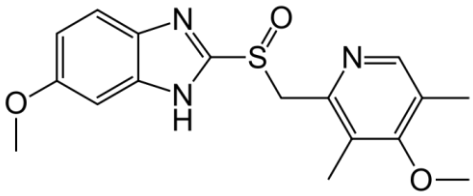

Figure: 1 Structure of Omeprazole

MATERIALS AND METHODS

Chemicals

Qualified standards and samples of Omeprazole were obtained from local laboratories and were used without any further purification. The chemicals like triethylamine, othophosphoric acid and methanol were purchased from Merck, Mumbai. Millipore water generated from TK water system. The analytical column used was Hypersil GOLD, 50 x 4.6 mm, 3µ.

Instruments

An Acquity UPLC system manufactured by Waters which consist of Photo Diode Array (PDA) detector, Quaternary solvent manager, sample manager, column heating compartment was used for determination of Omeprazole in Omeprazole Magnesium blend. UPLC instrument was controlled by Waters Empower chromatographic software. Hypersil GOLD, 50 x 4.6 mm, 3µm column was used for chromatographic separation and determination of Omeprazole.

Standard preparation

Weighed accurately 90.0 mg of Omeprazole magnesium working standard and transfer into a 200 ml volumetric flask. Add about 100 ml of diluent-1, sonicate to dissolve and dilute to volume with diluent-1. Pipette out 10 ml of this solution into a 25 ml volumetric flask, dilute to volume with diluents-2 and mix.

Sample preparation

Weighed and transferred an amount of enteric coated pellets equivalent to 200 mg of Omeprazole into a 500 ml volumetric flask, add 250 ml of diluents-1. Sonicate for 30 minutes with intermediate shaking and dilute to volume with diluent-1. Centrifuge a portion of this solution in a test tube with cap at 4000 RPM for about 10 minutes. Pipette out 10 ml of the clear centrifuged solution into a 25 ml volumetric flask, dilute to volume with diluents-2 and mix. Filter though 0.45 µm filter.

Chromatographic conditions

The chromatographic column used Hypersil GOLD with dimensions of 50 x 4.6 mm with 3µm particle size. The isocratic method was employed with the mobile phase mixture of mixture of buffer and methanol in the ratio 40:60(v/v). The column temperature was maintained at 25.0^{o}C and detection was monitored at a wavelength of 305nm. Injection volume was 5µl and the mobile phase flow was set at 1.0mL/min.

METHOD VALIDATION

The developed method for determination of Omeprazole in Omeprazole Magnesium blend was validated for system suitability along with method selectivity, specificity, linearity, precision, accuracy, robustness according to the ICH guidelines.

Method validation parameters

The system suitability was conducted using standard preparation and evaluated by injecting five replicate injections. Specificity is the ability of analytical method to assess un equivocally the analyte in the presence of component that may be expected to be present. Performed the specificity parameter of the method by injecting Diluent, placebo into the chromatographic system and evaluated by show any peak at the retention time of analyte. Performed the linearity with Omeprazole in the range of 25 to 150% of specification limit. Recorded the area response for each level and calculated slope, intercept & correlation coefficient. Also performed precision at higher level by injecting six times into the chromatographic system

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple sampling of homogeneous sample. The precision of analytical method is usually expressed as the standard deviation or relative standard deviation of series of measurements. The system precision was conducted

using Omeprazole and evaluated by making six replicate injections. The Accuracy of the method by recoveries of Omeprazole sample solutions at different concentration levels ranging from 25 to 150%.The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions:

Method development includes selection of appropriate chromatographic conditions/factors like detection wave length, selection and optimization of stationary and mobile phases. The wavelength of 305nm was selected due to it produces less noise, which minimizes problems that may exhibit around the active ingredient when attempting to quantify Omeprazole. Preliminary development trials were performed with various columns of different types and dimensions from different manufacturers were tested for the peak shape and the number of theoretical plates for specification concentrations. Finally by switching to Hypersil GOLD, 50 x 4.6 mm and 3µ column there a significant improvement in the peak shapes with 1.1 tailing factor.

System suitability:

The RSD from five replicate injections of standard preparation was 0.1 %. Tailing factor for Omeprazole peak was 1.1.

Selectivity:

Performed the specificity parameter of the method by injecting diluent, standard preparation sample preparation and placebo preparation into the chromatographic system and recorded the retention times. Specificity study of the method proved no peak observed at retention time of Omeprazole. Specificity results of Omeprazole given in the below **Table-2**.The selectivity chromatograms shown in the **Figures 2-5.**

Table 2: Selectivity results of Omeprazole

S.No.	Sample	Retention time
1	Blank	-
2	Placebo	-
3	Standard	1.246
4	Sample	1.243

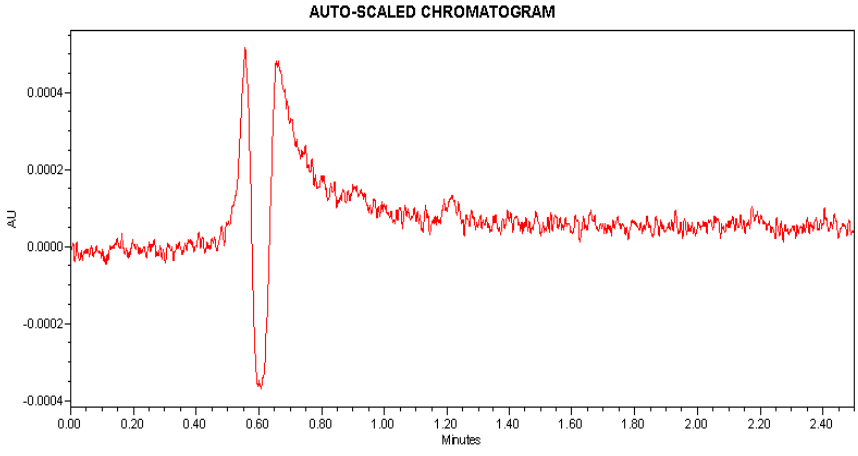

Fig: 2 Chromatogram of blank

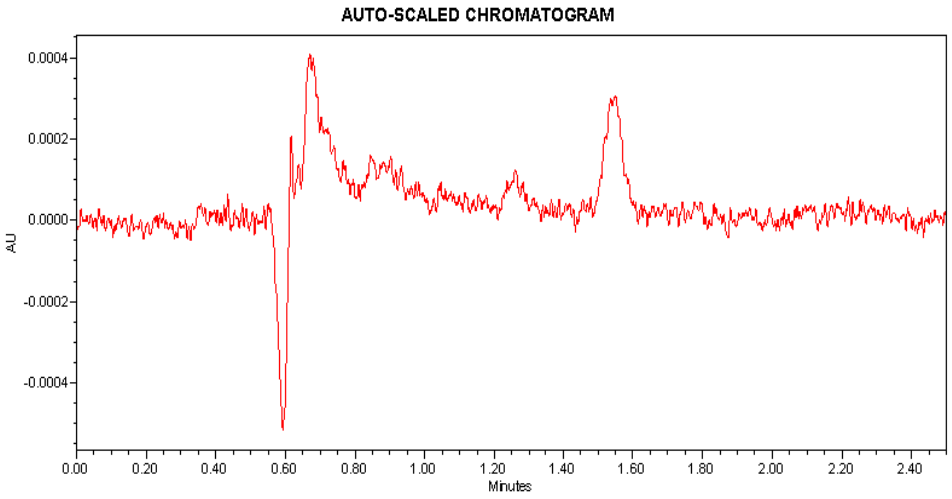

Fig: 3 Chromatogram of placebo

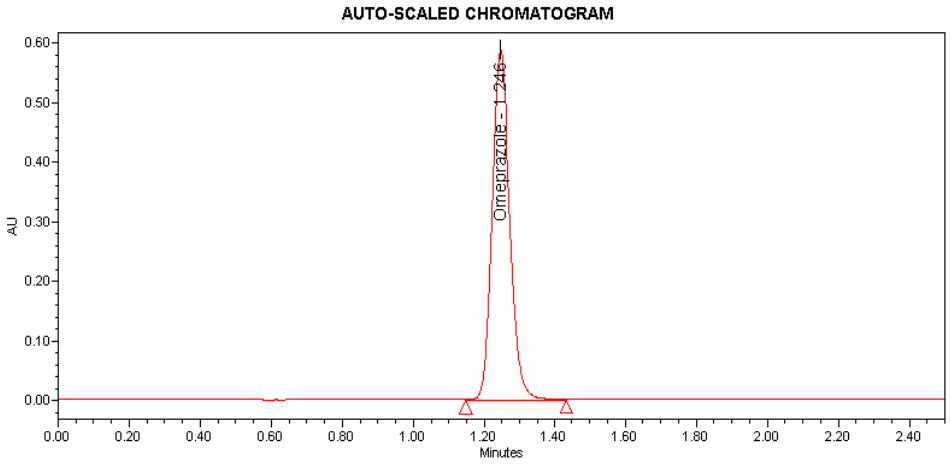

Fig: 4 Chromatogram of Omeprazole

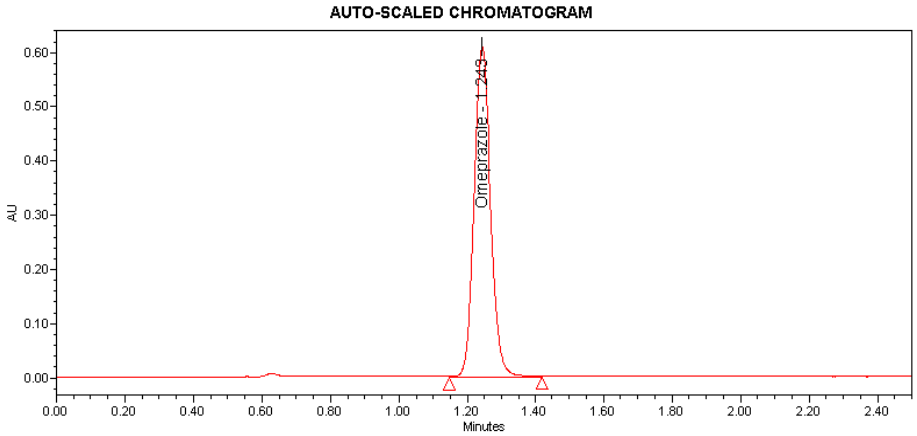

Fig: 5 Chromatogram of Omeprazole sample

Linearity:

To demonstrate the linearity with Omeprazole standard in the range of 25 to 150% of specification limit**.** Correlation coefficient of Omeprazole was 0.9999.The linearity results shown in the below Table -3.The linearity plot of omeprazole shown in the **Figure-6.**

Table 3: Linearity results of Omeprazole

S.No.:	Spike level	'mg/ml' of Omeprazole added	'mg/ml' of Omeprazole found
1	25%	0.04011	0.04007
2	50%	0.07922	0.07868
3	75%	0.11969	0.11973
4	100%	0.15877	0.15882
5	125%	0.19869	0.19902
6	150%	0.23797	0.23672
Coefficient of correlation		0.99997	

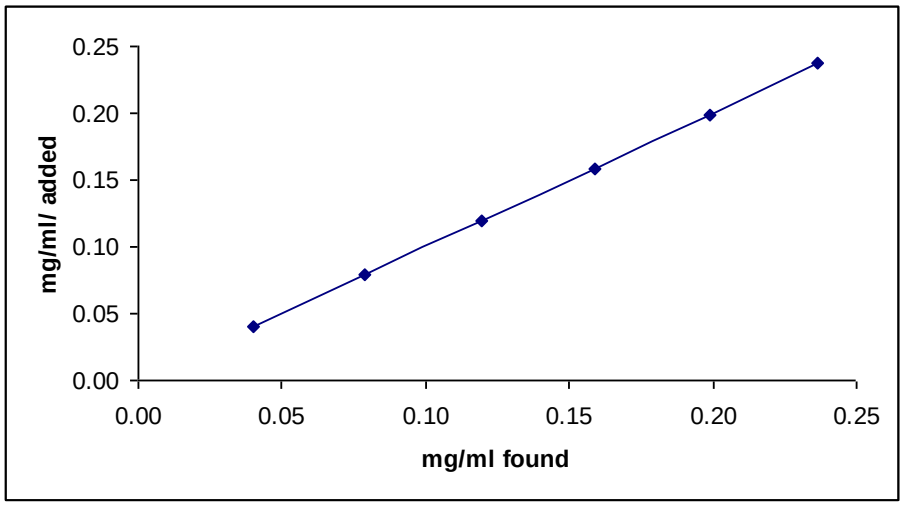

Figure-6 Linearity plot of omeprazole

Accuracy:

Accuracy study found that the mean % of recovery was more than 97.0% and less than 103.0% at each level 25 to 150% of concentration levels, hence method is accurate. The accuracy results are given **Table-4**.

Table4: Accuracy results

S.No	Level in %	% Mean Recovery
1.	25	99.89
2.	50	99.33
3.	75	100.04
4.	100	100.03
5.	125	100.17
6.	150	99.47

Precision:

The precision of test method was validated by assaying six samples prepared on Omeprazole and calculate relative standard deviation of Assay results. The precision results are given **Table-5**.

Table: 5 Precision results

Sample No	% Assay of Omeprazole
1	99.23
2	99.43
3	98.85
4	99.95
5	98.99
6	98.69
Average	99.19
% RSD	0.46

Robustness

The method robustness was studied by injecting the system suitability solution at change in the percentage of organic modifier (methanol), flow rate, and column temperature. The results were obtained as shown in the below **Table-6**.

Table: 6 Robustness results

Condition	Tailing factor	% RSD
Limits	NLT 2.0	NMT 2.0
Normal Condition	1.1	0.1
Flow rate 0.8mL/min	1.2	0.4
Flow rate 1.2mL/min	1.1	0.2
Column Temperature 20°C	1.2	0.7
Column Temperature 30°C	1.2	0.5
Organic phase +10.0%	1.2	0.1
Organic phase -10.0%	1.3	0.1

CONCLUSIONS

A simple isocratic UPLC method has been developed and validated for the determination of assay of Omeprazole in Omeprazole Magnesium blend tablets. The developed method has been found to selective, sensitive, precise, robust and stability indicating. The method can be directly adopted in quality control laboratories for routine analysis with respect to determination and quantification of Omeprazole in Omeprazole Magnesium blend and also for the analysis of stability samples.

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