

A VALIDATED ULTRA FAST LIQUID CHROMATOGRAPHY METHOD FOR ASSAY DETERMINATION OF OMEPRAZOLE

SUBHAKAR NANDIPATI^{1*}, V KRISHNA REDDY¹, SREENIVAS UBA²

¹Department of Chemistry, Sri Krishnadevaraya University, Ananthapur, A.P, India, ²Department of Chemistry, Ideal College, Kakinada, A.P, India. Email: nsubhakar@yahoo.com

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ABSTRACT

A Rapid, selective and specific 'Ultra Performance Liquid Chromatography' (UPLC) method was developed for Assay determination of Omeprazole. The chromatographic separation was performed on waters UPLC PDA system and Zorbax SB C18, Agilent column using isocratic elution of mobile phase with the composition of 0.02M Potassium dihydrogen phosphate (KH₂PO₄), 0.003M Dipotassium hydrogen phosphate (K₂HPO₄) buffer and acetonitrile in 60:40 v/v ratio, adjusted pH 7.4 with potassium hydroxide solution. The flow rate of isocratic program was set as 1.0ml min⁻¹. Ultraviolet detection was performed at 302 nm. Omeprazole was well separated with a total run time of 2 minutes. Calibration shows that the response of omeprazole was a linear function of concentration over the range 10–60 µg mL⁻¹ ($r^2 \geq 0.999$) and the method was validated over this range for precision, intermediate precision, accuracy, linearity and specificity. For precision study, percentage relative standard deviation of Omeprazole was <2.0% ($n = 6$). The method was found to be precise, accurate, linear and specific. The proposed method was successfully employed for estimation of Omeprazole Assay in pharmaceutical applications.

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 acidit¹. Omeprazole inhibits CYPs 2C9 and 2C19 and in those with the extensive metaboliser phenotype there is evidence that omeprazole

administration results in significant decreases in the clearance of diazepam, phenytoin, and possibly carbamazepine and S-warfarin^{2,3}. Omeprazole is chemically designated as "1*H*-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 C₁₇H₁₉N₃O₃S 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

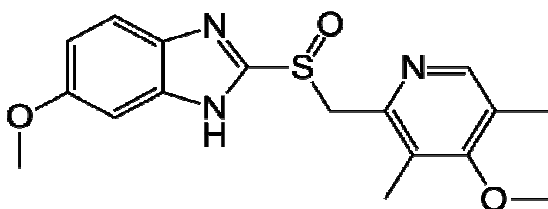
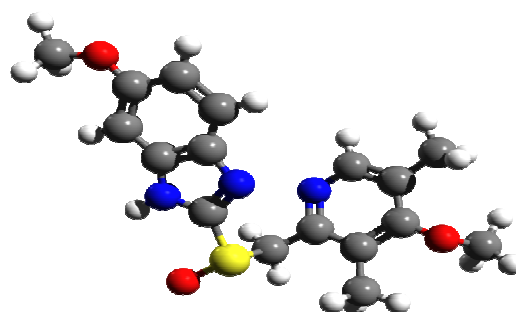


Fig. 1:



Literature search revealed that, papers on degradation of omeprazole⁴, determination by UV spectrophotometer method⁵, omeprazole in human plasma & urine by LC-MS-MS⁶, determination of S-omeprazole, R-omeprazole and racemic omeprazole⁷, 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.

MATERIALS AND METHODS

All experiment was performed using "class A" volumetric glassware, pharmaceutical grade omeprazole. Analytical grade potassium dihydrogen phosphate, dipotassium hydrogen phosphate was used for mobile phase preparation and potassium hydroxide was used for pH adjustment to 7.4 for the buffer. Using HPLC grade Acetonitrile, and highly pure HPLC grade Milli Q water (Millipore, Bedford, MA, USA), mobile phase was prepared and filtered through 0.22µm membrane filter (millipore, Barcelona) and degassed under vacuum by filtering assembly, prior to use. Standard and samples were

dissolved in 0.1N Sodium hydroxide and further diluted with mobile phase.

Equipment

Omeprazole assay analysis was performed by using Waters UPLC(Milford, MA, USA) PDA system consists of a Quaternary solvent manager, a sample manager, column-heating compartment, and Photodiode array detector. This system was controlled by Waters Empower software. Zorbax SB C18, Agilent, 4.6 x 50mm 1.8µm employed as stationary phase for chromatographic separation. Sartorius semi micro balance with model ME235S was used for all weighing and Thermo Orion pH meter was used for buffer pH adjustment. Sonication carried out with Bandelin sonicator and Rotary shaker used for shaking of samples during preparation. All samples were centrifuged by Hermle centrifuge machine.

Standard and sample preparation

The standard stock solution of Omeprazole was prepared by dissolving an accurately weighed amount of Omeprazole working

standard in 0.1N sodium hydroxide solution, resulting in a concentration of 0.4 mg mL⁻¹. Then above solution was further diluted in diluent (mobile phase) to get a final solution of 0.04 mg mL⁻¹.

The test solution was prepared by dissolving an accurately weighed portion of the Omeprazole enteric coated pellets, equivalent to 100 mg of Omeprazole in 150mL of 0.1N sodium hydroxide. After sonicating for around 15 minutes, shake the solution for 20 minutes and the volume was made up to 250 ml with 0.1N sodium hydroxide solution. A portion of above solution was centrifuged at 3000 rpm for 15 minutes in order to eliminate insoluble excipients. 2mL of the above supernatant solution was further diluted up to 20mL volume with the diluent (mobile phase) and the same was used for chromatographic analysis.

Chromatographic Conditions

The analysis carried out by using advanced chromatographic techniques of Ultra Performance Liquid Chromatography (UPLC). The omeprazole was separated on an Extend C18, Agilent, 4.6 x 50mm 1.8µm column at ambient column oven temperature with an isocratic run program at a flow-rate of 1.0mL min⁻¹. The separation was achieved by isocratic elution with run time of 2 minutes. The mobile phase was filtered through a 0.45µm Millipore filter, before use. UV detection was performed at 302 nm. The sample injection volume was 5µL.

Method Validation

The method was validated for specificity, linearity, precision, accuracy, range, ruggedness and robustness as per the International Conference on Harmonization (ICH) guidelines^{8,9}.

Specificity

A study was conducted to demonstrate the effective separation of Omeprazole and its degradation products, impurities. Also study was intended to ensure the effective separation of degradation peaks of formulation ingredients at the retention time of omeprazole and its impurities. Separate portions of drug product and ingredients were exposed to following stress conditions to induce degradation. The drug product was subjected to acid hydrolysis using 0.1 N Hydrochloric acid, base hydrolysis with 0.1 N Sodium hydroxide, Oxidation study was performed with 1 % Peroxide solution for 25 minutes at 25°C temperature and neutral hydrolysis with water at 60°C temperature for duration of 1 hour. On photo stability study, drug product was sufficiently spread on Petri plates (1mm thick layer), exposed to sunlight and UV light (1.2 million lux hours) at ambient conditions for 7 days. Humidity study was performed separately by exposing the drug product to humidity at 25°C, 90% RH for 7 days. Thermal degradation study was performed by heating drug product at 105°C for 6 hours. Similarly placebo samples were prepared as like as drug product by exposing formulation matrices without drug substance. Stressed samples were injected into the UPLC system with PDA detector by following test method conditions.

Precision

The precision of test method was evaluated by using six sample preparations of omeprazole enteric coated pellets and analyzed as per test method. Intermediate precision was also studied using different Instrument, column and with different analysts.

Accuracy

To confirm the accuracy of the proposed method, recovery studies were carried out by standard addition technique. Samples were prepared six times at lower (50%) and higher (150%) level concentrations and in triplicate at 75%, 100%, 125% (A nominal concentration of about 0.02mg mL⁻¹ to 0.06mg mL⁻¹) of the test concentration.

Linearity of Detector Response

To demonstrate the linearity of detector response of assay method, prepared not less than five standard solutions with concentration ranging from 25% to 150% of Omeprazole to cover Omeprazole concentration 10ppm to 60ppm.

Range

Range of the method was established with respect to lower concentration level (50%) and higher concentration level (150%) of the test concentration. Injected the known concentration of drug solution at 50%, 100%, 150% levels and verified the method precision at above concentration ranges.

Ruggedness

As a part of ruggedness study, established the bench top stability, Refrigerator stability for omeprazole standard and test preparations and bench top stability of mobile phase preparation.

Robustness

To demonstrate the robustness, checked the system suitability parameters by injecting standard preparation; with various mobile phase compositions (one containing 110% of the method organic phase Acetonitrile composition and other contains 90% of the method organic phase Acetonitrile composition), with various flow rates (0.8mL min⁻¹ and 1.2 mL min⁻¹), with various mobile phase pH (7.2 and 7.5) and with various column temperature at 30°C. To check the filter interference, carried out filter validation using three different filters namely, 0.22µm Durapore hydrophilic membrane filter, 0.22µm Nylon 66 membrane filter and 0.22µm nylon 66 syringe SY25NH. Centrifuge and filter different portions of the test preparation and inject in to the system along with unfiltered standard.

Application of Developed Method

The method suitability was verified for omeprazole assay determination by using advanced chromatographic technique with short run time which supports to the process, cost reduction by reduced solvent consumption and less analysis time. The method is more precise and accurate through out its range with good separation between degradation products and omeprazole.

RESULT AND DISCUSSION

A reversed-phase chromatographic technique was developed to quantify Omeprazole at 302nm. The presence of non-aqueous solvents in the mobile phase, such as Acetonitrile, was studied. Acetonitrile is used as organic modifier. Satisfactory separation between process impurities, degrade impurities from Omeprazole was achieved when the acetonitrile concentration was 40% in mobile phase with 60% of phosphate buffer. Buffer strength selected to cope up for the injection load on the column to reduce peak tailing due to changes in ionic form. pH (7.4) of the buffer solution described based on the pKa value of Omeprazole and which decided the peak symmetry and separations.

Experiments conducted by using C18 stationary phase as Omeprazole is having polar nature. By selecting Zorbax SB C18 column achieved more selectivity, sensitivity and separations from all the process impurities, degrade impurities from Omeprazole peak within short period of run time and which is rugged for mobile phase variation.

The test concentration established based on the detector response at 302nm which is the λ_{max}¹⁰ of Omeprazole and also supports the recovery study for selected test concentration. Use of UPLC was given a good result in reduced run time of 2 minutes without any compromising of separation, symmetry of omeprazole peak. No interference of placebo, degrade impurities and impurities with the omeprazole peak were observed with the short run time. The developed method was used for further validation of method as per the ICH guide lines. The method was validated to ensure the suitability of the method for its intended purpose. The results of system suitability parameters of tailing factor, theoretical plates and relative standard deviation for omeprazole peak were found satisfactory as shown in Table 1.

Specificity study of the method proved the separation of impurity peaks from omeprazole peak and the degradation study of all exposed samples were found spectrally pure as evidenced that the purity angle was less than the Purity threshold of principle peak and also there was no Flag in purity results observed in degradation studies as shown in Table 2.

Table 1: System Suitability results and criteria for omeprazole peak

Parameter	Observed Results	Acceptance criteria
Tailing Factor	1.1	Not more than 2.0
Theoretical Plates	7206	Not less than 2500
% RSD of five replicates	0.3%	Not more than 2.0%

Table 2: Specificity study with degradation impurities

Stress condition	Purity angle	Purity Threshold	Purity Flag
Acid hydrolysis	0.736	1.119	No
Base hydrolysis	0.664	1.100	No
Peroxide oxidation	0.122	0.318	No
Water stress	0.376	0.669	No
Heat stress	0.277	0.636	No
Humidity stress	0.049	0.245	No
Sunlight stress	0.050	0.244	No
UV light stress	0.049	0.245	No

The above values described by using the Empower 2 software with waters PDA detector.

The method is more precise as evidenced that the relative standard deviation of omeprazole peak area was found less than 2.0% in both precision and intermediate precision study. The % recovery from the

accuracy study found not less than 97.0% and not more than 103.0% at each level from 50% to 150% of concentration level and hence the method is accurate at each concentration level as shown in Table 3.

Table 3: Results of Precision and Accuracy

S. No.	Level in %	Mean % Recovery	% of RSD
1	50%	102.51%	0.41%
2	75%	100.81%	0.48%
3	100%	101.65%	0.48%
4	125%	101.63%	1.19%
5	150%	101.02%	0.52%

% RSD and Mean Recovery calculated with six sample preparations at 50%, 100%, 150% and with three sample preparations at 75% and 125% levels.

The calibration curve obtained by plotting the omeprazole peak area versus concentration over the range of about 10- 60µg/mL and were

found to be linear ($r^2 = 0.999$). The data of regression analysis of the calibration curves are shown in Table 4 and Fig. 2.

Table 4: Linearity results of concentration and peak area

S. No.	Linearity Level	Concentration (µg/ml)	Peak Area
1	25%	10.0075	84616
2	50%	20.0150	179364
3	75%	30.0225	251867
4	100%	40.0300	344565
5	125%	50.0375	429747
6	150%	60.0450	516881

The resulted correlation coefficient from above data is 0.9993

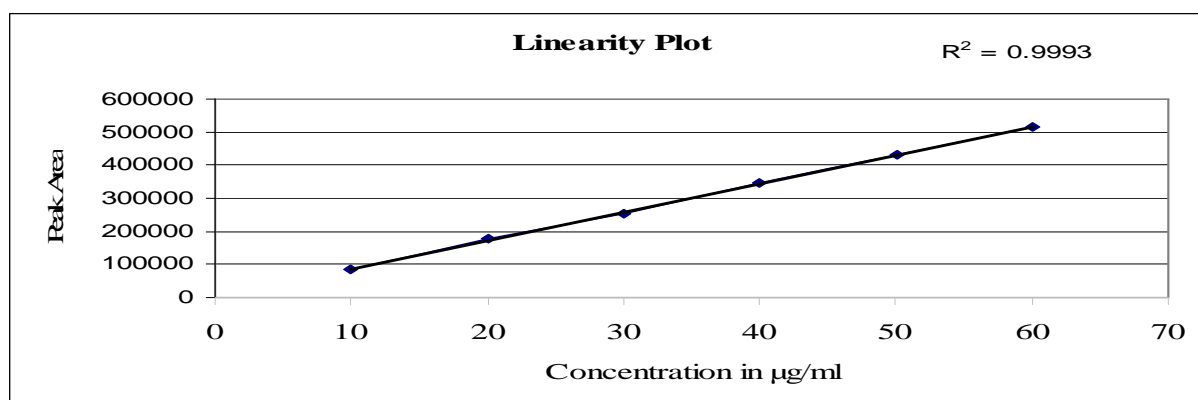


Fig. 2: Linearity plot between concentration and peak area

The chromatograms obtained for Blank, Standard and Sample injections from the validated method were shown in Fig. 3, Fig. 4 and Fig. 5 respectively. Based on the data, the method is more

precise and accurate through out its range and commercially more useful with respect to lesser run time and reduced solvent consumption.

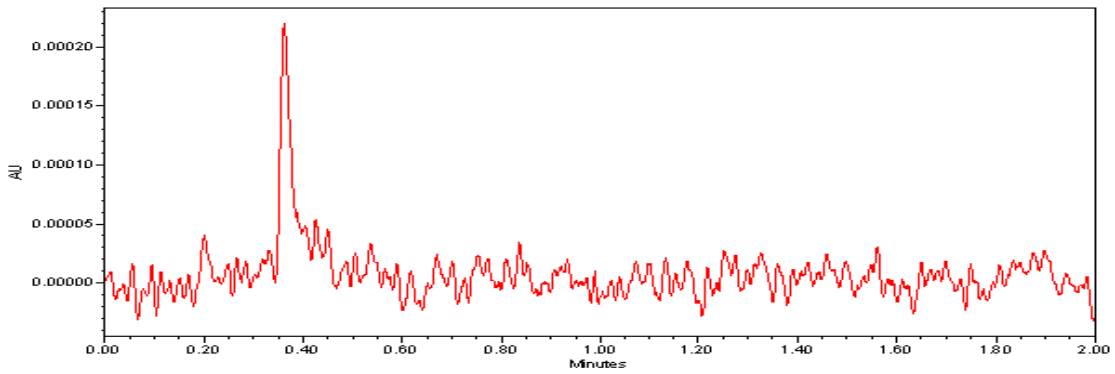


Fig. 3: Specimen Chromatogram of Blank Injection

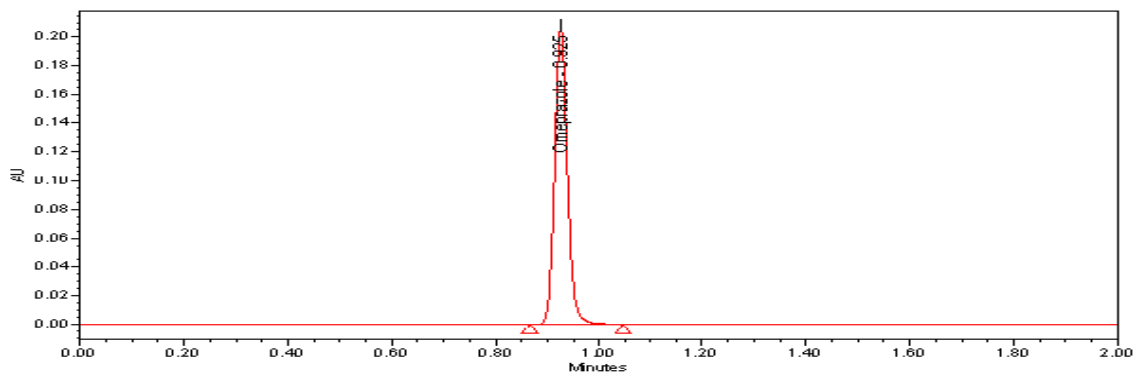


Fig. 4: Specimen Chromatogram of Standard Injection

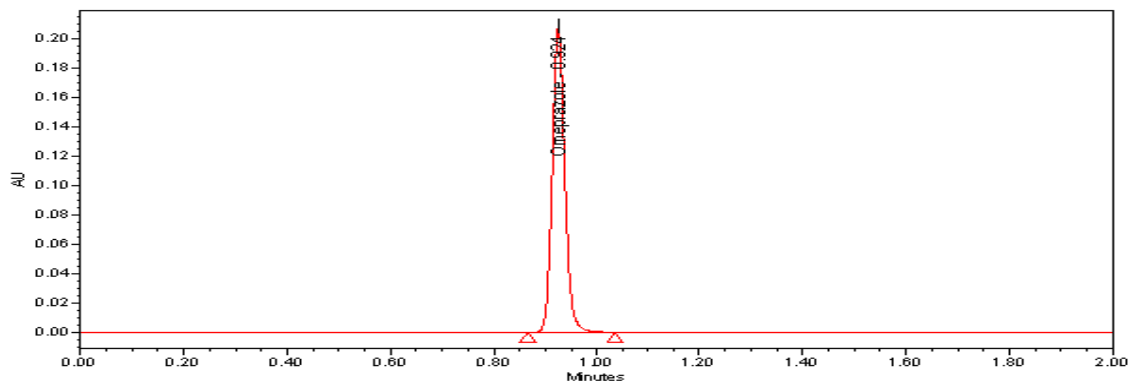


Fig. 5: Specimen Chromatogram of Sample Injection

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