

Zero order and area under curve spectrophotometric methods for determination of Omeprazole capsules in pharmaceutical formulation

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Abstract

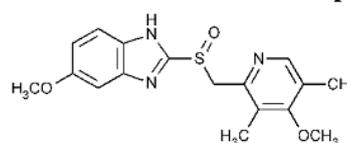
Simple, fast and reliable spectrophotometric methods were developed for determination of Omeprazole in bulk and pharmaceutical dosage forms. The solutions of standard and the sample were prepared in Methanol. The quantitative determination of the drug was carried out using the zero order derivative values measured at 303 nm and the area under the curve method values measured at 300-305 nm (n=2). Calibration graphs constructed at their wavelengths of determination were linear in the concentration range of Omeprazole using 2-10µg/.ml ($r^2=0.9985$ and $r^2=0.9959$) for zero order and area under the curve spectrophotometric method. All the proposed methods have been extensively validated as per ICH guidelines. There was no significant difference between the performance of the proposed methods regarding the mean values and standard deviations. Developed spectrophotometric methods in this study are simple, accurate, precise and sensitive to assay of Omeprazole in tablets.

Keywords: Omeprazole, UV visible spectrophotometry, AUC, Method Validation.

1. Introduction

Omeprazole is chemically known as 6-methoxy-2-[(4-methoxy-3, 5- dimethylpyridin-2-yl) methylsulfinyl]-1Hbenzimidazole. Omeprazole is a used as an antiulcer drug and against other acid-related diseases.[1] This blocks the final and common step in gastric acid secretion. Literature survey reveals that USP 2007 and IP 2007[2][3] report HPLC method for assay of omeprazole. Analytical methods reported for the estimation of omeprazole is HPLC[4-9], LC-MS[10-11]. To our notice, noUV-spectrophotometric method using Zero Order and Area under Curve (AUC) has been reported for the determination of Omeprazole in bulk and tablets.Hence an attempt has been made to develop new Zero Order and Area under Curve Spectrophotometric method for estimation of Omeprazolein bulk and pharmaceutical formulations with good accuracy simplicity, precision and economy.

Fig. 1: Chemical structure of Omeprazole



2. Materials and Methods

2.1 Apparatus and instrumentation: A shimadzu 1800 UV/VIS double beam spectrophotometer with 1cm matched quartz cells was used for all spectral measurements. Single Pan Electronic balance (CONTECH, CA 223, India) was used for weighing purpose. Sonication of the solutions was carried out using an Ultrasonic Cleaning Bath (Spectra lab UCB 40, India).Calibrated volumetric glassware (Borosil®) was used for the validation study.

2.2 Materials: Reference standard of Omeprazole API was supplied as gift sample by Lupin Laboratory Park Aurangabad. Tablet sample with label claim 20mg per tablet were purchased from local market Pune.

2.3 Method development

2.3.1 Preparation of Standard and Sample Solutions

Stock solution of 10µg/ml of Omeprazole was prepared in Methanol, for zero order and area under the curve spectrophotometric analysis. The

standard solutions were prepared by dilution of the stock solution with Methanol in a concentration range of 02, 04, 06, 08, and 10µg/ml with Methanol for zero order and area under the curve spectrophotometric methods. Methanol was used as a blank solution.

Figure 2: Zero order derivative spectrum of Omeprazole in Methanol (10µg/ml).

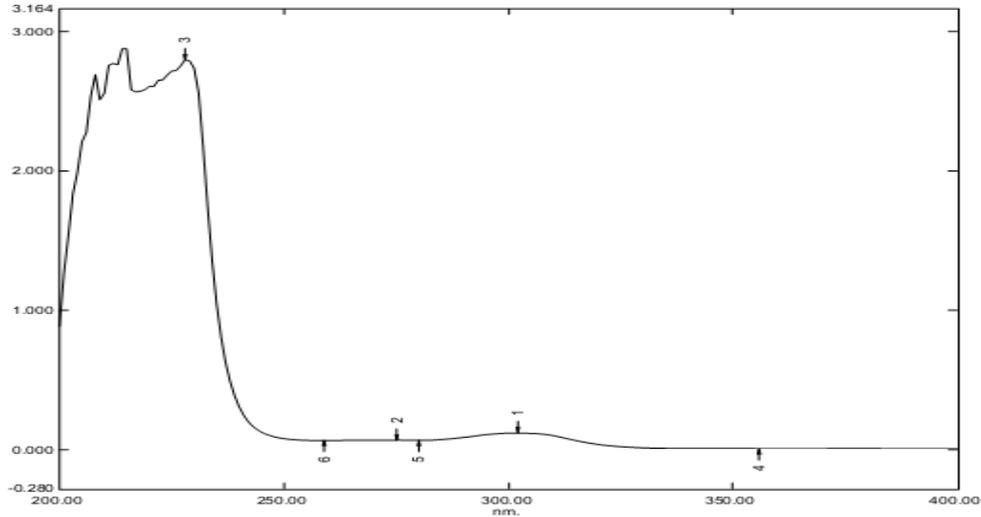
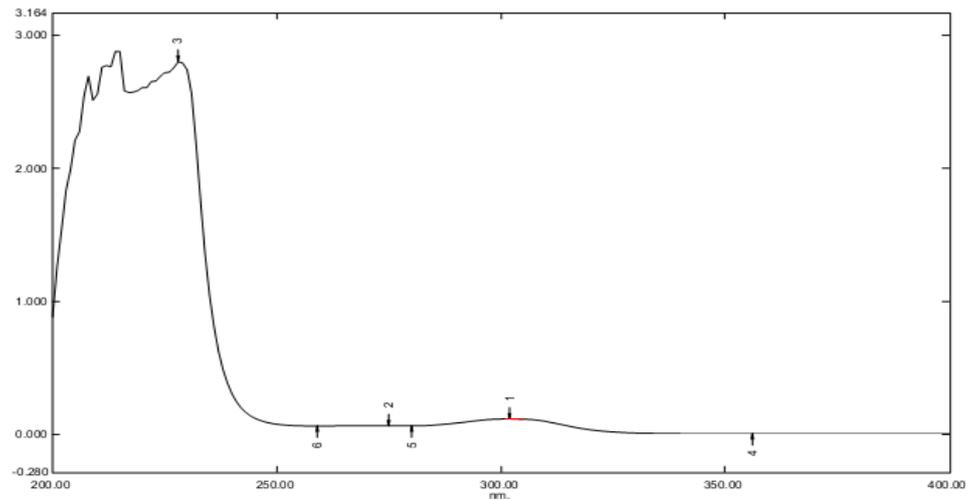


Figure 3: UV AUC spectrum of Omeprazole in Methanol (10µg/ml).



2.3.2 Area under curve (Area calculation)

Area under curve method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths such as λ1 and λ2 representing start and end point of curve region. The area under curve between λ1 and λ2 was calculated using UV probe software. In this study area was integrated between wavelength ranges from 300 to 305 nm.

$$\text{Area calculation: } (\alpha+\beta) = \int_{\lambda_2}^{\lambda_1} A d\lambda$$

Where, α is area of portion bounded by curve data and a straight line connecting the start and end point, β is the area of portion bounded by a straight line connecting the start and end point on curve data and horizontal axis, λ1 and λ2 are

wavelength range start and end point of curve region[12].

2.3.3 Assay Procedure

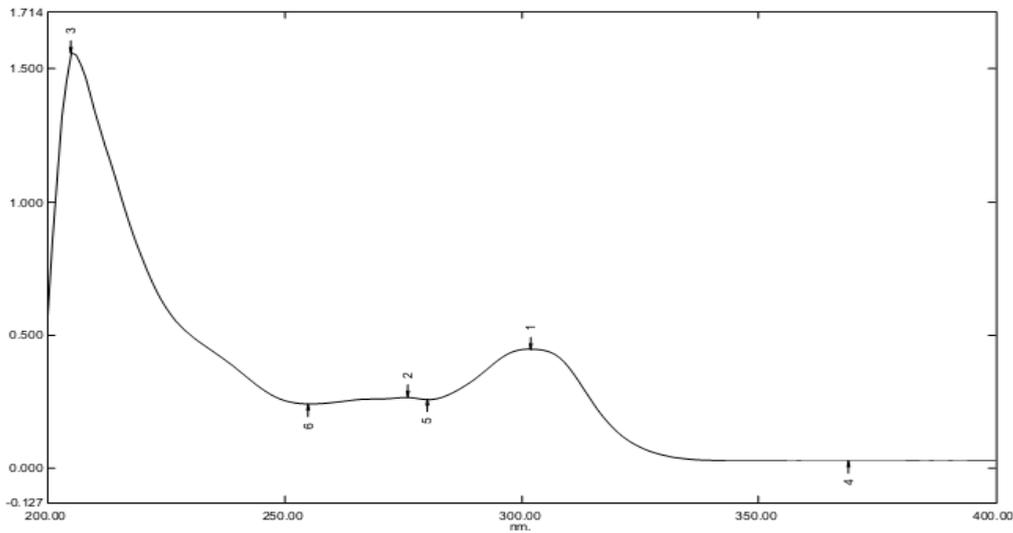
Twenty tablets each containing 20mg of Omeprazole were weighed crushed to powder and average weight was calculated. Powder equivalent to 10mg of Omeprazole was transferred in 100 ml of volumetric flask. A 50 ml of Methanol was added and sonicated for 15minutes. Then solution was further diluted up to the mark with Methanol. The solution was filtered using Whatmann filter paper no. 41; first 5 ml of filtrate was discarded. This solution was further diluted to obtain 15µg/mL solution with water subjected for UV analysis using Methanol as blank. Appropriate dilutions were made with methanol from stock solution for both zero order and area under the curve spectrophotometric methods.

Table 1: Assay of tablet dosage form

Sr.No.	Sample Solution Concentration (µg/ml)	Amount found (%)* Zero derivative	Amount found (%)* Auc	Mean % Found zero derivative	Mean % Found Auc	%RSD zero derivative	%RSD Auc
1	15	98.91	99.21				
2	15	98.89	98.13	98.84	98.54	0.1114	0.5914
3	15	98.71	98.29				

*n=3, % RSD = % Relative Standard Deviation.

Fig. 4 Zero order derivative spectrum of Omeprazole in Methanol dosage form (15µg/ml).



3. Results and Discussion

The zero order and area under the curve spectra for Omeprazole were recorded at the wavelength of 303nm and 300-305nm respectively [Fig. 2 and 3].

3.1 Linearity and Range

Under the experimental conditions described, the graph obtained for zero order and area under the curve spectra showed linear relationship.

Regression analysis was made for the slope, intercept and correlation coefficient values. The regression equations of calibration curves were $y=0.0056x+0.0097$ ($r^2=0.9985$) at 303nm for zero order derivative spectrophotometry and $y=0.0005x-0.0009$ ($r^2=0.9959$) at 300-305nm for area under the curve spectrophotometry. The range was found to be 2-10µg/ml for both zero order and area under the curve spectrophotometric methods.

Fig.5 Linearity of Omeprazole by Absorbance

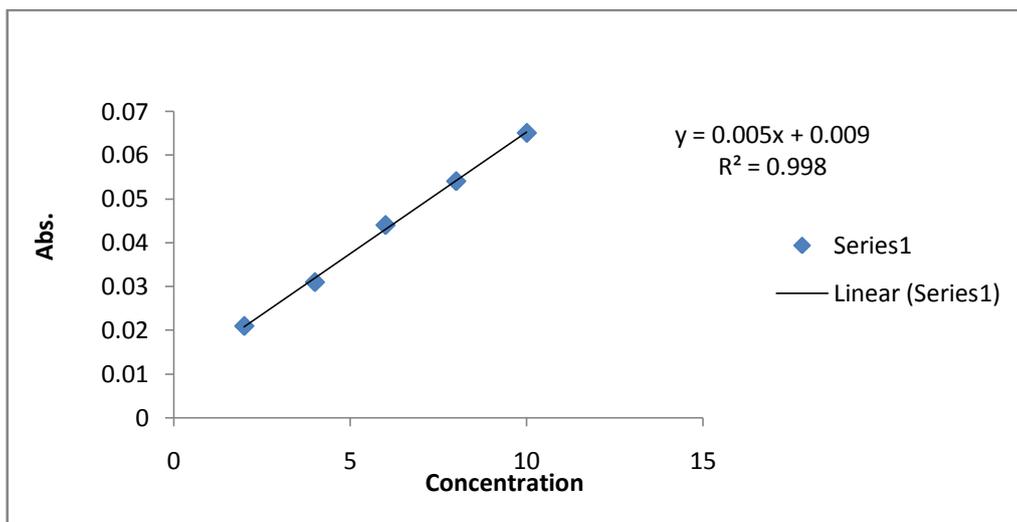


Fig.6 Linearity of Omeprazole by AUC

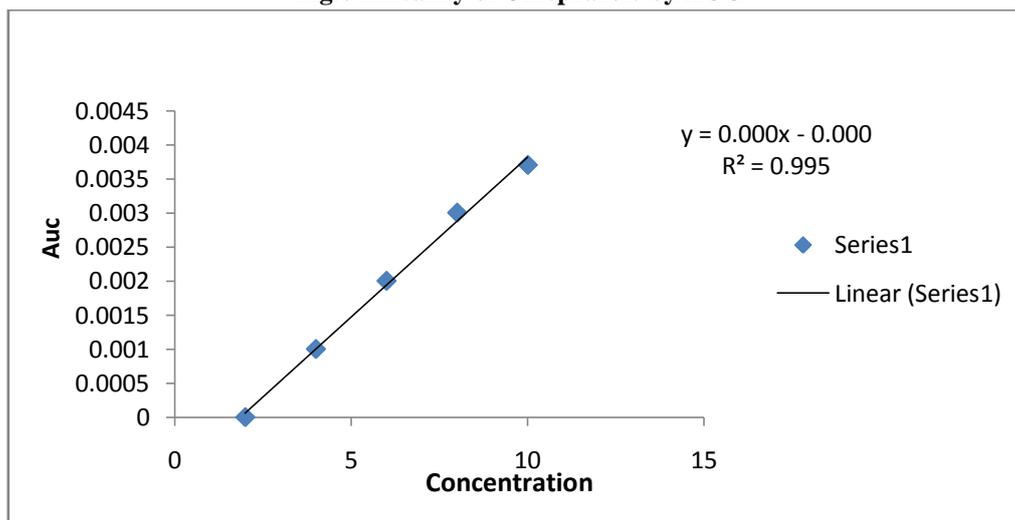


Fig. 7 Zero order derivative overlay of Omeprazole at diff. Concentration

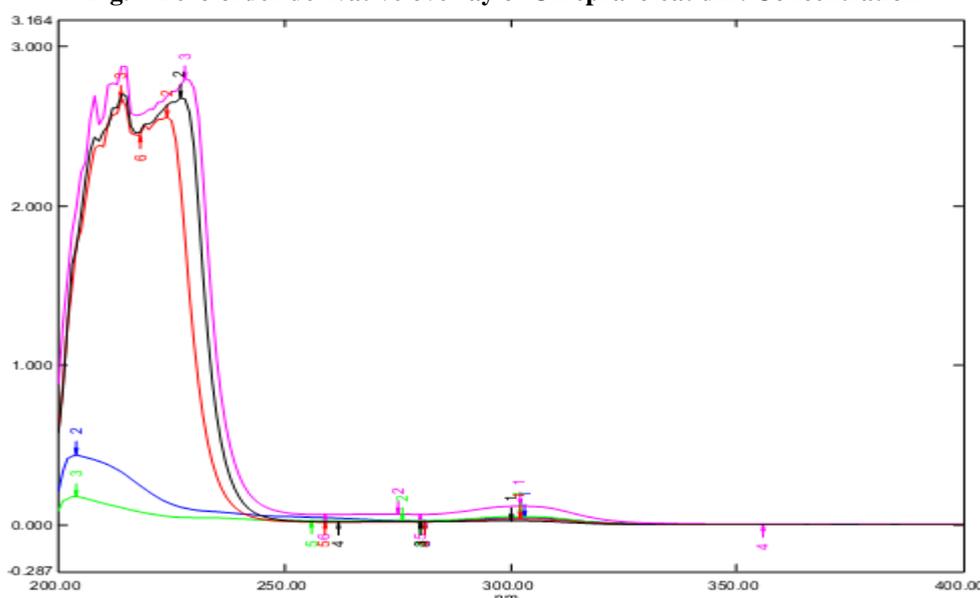


Table 2: Stastical data for the calibration graphs for determination of Omeprazole by Proposed methods

Parameters	Zero order derivative	Area Under the Curve
Linearity range (µg/ml)*	2-10	2-10
r ² ± S.D*	0.9957	0.9983

3.2 Accuracy

To study the accuracy of the proposed methods, and to check the interference from excipients used in the dosage forms, recovery experiments were carried out by the standard addition method. The accuracy for the analytical method was evaluated at 80%, 100% and 120% levels of 15µg/ml

standard solution. For Area under curve (AUC) was measured in wavelength range 300-305 nm and For Zero order derivative at 303nm and results were obtained in terms of percent recovery. Three determinations at each level were performed and % RSD was calculated for each level.

Table 3: Accuracy results for Omeprazole

Accuracy level	Sample conc (µg/)	Std. conc	Total amnt. Added (µg/m)	%Recovery zero derivatie	% Recovery Auc*	Mean of Zero derivative*	Mean of Auc*	% RSD Zero derivative	% RSD Auc
80	15	12	27	99.18	98.11				
100	15	15	30	98.14	98.86	99.01	98.40	0.806	0.407
120	15	18	33	99.71	98.24				

*n=3, % RSD = % Relative Standard Deviation.

3.3 Precision

To determine the precision of the method, Omeprazole solutions at a concentration of 10µg/ml were analysed each three times for both zero order

and area under the curve spectrophotometric methods. Solutions for the standard curves were prepared fresh everyday (Table 4).

Table 4: Results of Intra and Inter Day Precision

Parameters	Intra Day Precision		Inter Day Precision	
	S.D*	% RSD*	S.D*	% RSD*
Zero derivative	0.0005	0.8093	0.0011	1.1863
Area under the curve	0.000	0	1.0623	1.7704

3.4 Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations $LOD = 3x\sigma/S$ and $LOQ = 10x\sigma/S$, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 0.4731µg/ml and 1.4339µg/ml respectively for zero order derivative and The LOD and LOQ were found to be 0.7691µg/ml & 2.3307µg/ml for area under the curve methods respectively.

3.5 Analysis of the Marketed Formulation

There was no interference from the excipients commonly present in the tablets. The drug content was found to be 99.50% and 97.94% zero order and area under the curve spectrophotometric methods respectively. It may therefore be inferred that degradation of Omeprazole had not occurred in the marketed formulations that were analysed by this method. The low % R.S.D. value indicated the suitability of this method for routine analysis of Omeprazole in pharmaceutical dosage form (Table 4).

Table 5: Summary of validation parameters

Parameter	Zero derivative	AUC
λ range	200-400 nm	300-305nm
Regression Equation (y=mx+c)	Y=0.0056x+0.0097	Y=0.0005x-0.0009
Measured wavelength	303 nm	234nm
Linearity range	2-10µg/ml	2-10µg/ml
Slope	0.0056	0.0005
Intercept	0.0097	0.0009
Correlation coefficient (R ²)	0.9985	0.9959
Limit of Detection (LOD) µg/ml	0.4731	0.7691
Limit of Quantitation (LOQ) µg/ml	1.4339	2.3307
Accuracy (Mean % Recovery)	99.01	98.40
Precision (%RSD)	0.8093	0

4. Conclusion

No UV or Area under Curve spectrophotometric methods have been described for the determination of Omeprazole. Therefore simple, fast and reliable derivative spectrophotometric methods were developed for the routine determination of Omeprazole. The developed methods can be concluded as accurate, sensitive and precise and can be easily applied to the pharmaceutical formulation.

Acknowledgement

The authors are highly thankful to the Sahyadri College of Pharmacy, Methwade, Sangola, Solapur, Maharashtra, India for providing all the facilities to carry out the research work.

References

- [1] Stenhoff H, Blomqvist A, Lagerstrom PO. Determination of the enantiomers of omeprazole in blood plasma by normal-phase liquid chromatography and detection by atmospheric pressure ionization tandem mass spectrometry. *J Chromatography B* 1999; 734: 191–201.
- [2] The United States Pharmacopoeia, Asian edition, Rockville, MD: USP Convention Rockville, MD, USP Convention Inc, 2007, 2796-2798.
- [3] Indian Pharmacopoeia, Volume II, Published by the controller of Publication, Delhi, 2007; 1473-1475.
- [4] Dubuc MC, Hamel C, Caubet MS, Brazier JL. Method for separation and determination of Omeprazole extracted from human plasma. *J Liq Chromatogr & Related Tech* 2001; 24: 1161-1169.

- [5] El-Sherif ZA, Mohamed AO, El-Bardicy MG, El-Tarras MF. Reversed phase high performance liquid chromatographic method for the determination of lansoprazole, Omeprazole and pantoprazole Sodium sesquihydrate in presence of their Acid Induced degradation products. *Chem & Pharm Bull* 2006; 54: 6814.
- [6] Murakami FS, Cruz AP, Pereira RN, Valente BR, Silva MAS. Statistical assurance of process validation by analytical method development and validation for Omeprazole capsule. *J Liq Chromatogr & Related Tech* 2007; 30: 113-121.
- [7] Sivasubramanian L, Anilkumar V. Simultaneous HPLC estimation of Omeprazole and Domperidone from tablets. *Indian J Pharm Sci* 2007; 69: 674-676.
- [8] Sluggett GW, Stong JD, Adams JH, Zhao Z. Optimization and validation of Optimisation and validation of HPLC-UV method for simultaneous determination of Omeprazole and its Metabolites In human Plasma : effects of various experimental conditions and parameters. *J Pharm Biomed Anal* 2001; 25: 357-61.
- [9] Junior IJSde, Sartor JP, Rosa PCP, Veredas Vde, Júnior AGB, Santana CC. A validated normal phase HPLC method for simultaneous determination of drotaverine HCl and omeprazole in pharmaceutical formulation. *J Chromatogr A* 2007; 1162: 97-102.
- [10] Ribani M, Collins CH, and Bottoli CBG. Validation of chromatographic methods: Evaluation of detection and quantitation limit in the determination of Impurities in Omeprazole. *J Chromatogr A* 2007; 1156: 201-205.
- [11] Raval PB, Puranik M, Wadher SJ, Yeole PG. A validated HPLC method for determination of ondansetron with omeprazole or rabeprazole in solid dosage form. *Indian J of Pharm Sci* 2008; 70: 386-390.
- [12] International Conference on Harmonization (ICH) of Technical Requirements for the registration of Pharmaceuticals for Human use, Validation of Analytical Procedures Methodology; ICH-Q2(R1), Geneva, 1-8, 1996.