

A Validated Stability of Omeprazole Sodium Injection in 0.9% Sodium Chloride and 5% Dextrose Solutions by RP-HPLC

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ABSTRACT: A specific, accurate, precise and reproducible stability indicating investigation of RP-HPLC method has been developed for omeprazole sodium injection in 0.9% sodium chloride and 5% dextrose solutions. The chromatographic separation was achieved by using the Zorbax Eclipse C₈ column (250 mm X 4.6 mm, 5 μm) with a mixture of phosphate buffer (pH 7.2): acetonitrile in the ratio of 75:25 (v/v) as mobile phase at a flow rate of 1.5 ml/min. The UV detection was performed at 280 nm. It was subsequently validated according to ICH and USP guidelines. The described method was linear over the concentration range of 80-240 μg/ml. The specificity of the method shows that the analyte was not interfered by the presence of excipients in both 0.9% sodium chloride and 5% dextrose solutions and the stability of the analyte after reconstitution was found stable for 24 hours in both 0.9% sodium chloride and 5% dextrose solutions. Robustness studies were found satisfactory; hence it proves that the method was robust. The developed method was successfully employed for the determination of omeprazole sodium injection, when it reconstituted with 0.9% sodium chloride and 5% dextrose solutions.

KEY WORDS: Omeprazole, RP-HPLC, Sodium Chloride, Dextrose, and Validation

I. INTRODUCTION

Omeprazole ((RS)-5-Methoxy-2-[[[4-methoxy-3, 5-dimethyl-2-pyridinyl)-methyl]sulfinyl]-1H-benzimidazole or C₁₇H₁₉N₃O₃S) (Fig. 1) is widely used as proton pump inhibitor and it indicated for the conditions of dyspepsia, peptic ulcer disease (PUD), gastroesophageal reflux disease, laryngopharyngeal reflux (LPR) and Zollinger–Ellison syndrome^{1,2}. The mechanism involves with the suppression of gastric acid secretion by specific inhibition of the H⁺/K⁺-ATPase in the gastric parietal cell leads to reducing the gastric acidity³. The physical characteristics show it is of white and soluble in water, DMSO and ethanol⁴. The literature survey reveals various methods like Spectrophotometric⁵⁻¹⁰, TLC densitometry¹¹, HPLC¹²⁻¹⁹, LC/MS²⁰⁻²² have been preceded for omeprazole but no method have been developed related to stability of omeprazole injection in 0.9% sodium chloride and 5% dextrose solutions. Hence, it is needed to know the stability of omeprazole injection in the solutions of 0.9% sodium chloride and 5% dextrose solutions; however it is essential to investigate a suitable RP-HPLC method for the stability of omeprazole in commercial formulations. The present method was aimed to develop a simple, rapid, precise, accurate and selective reversed phase chromatographic method to verify the omeprazole sodium stability. The proposed procedure was optimized and validated according to International Conference on Harmonization (ICH) guidelines^{23,24} and USP guidelines²⁵.

II. MATERIALS AND METHODS

2.1. Chemicals

The gift sample of omeprazole sodium injection and Placebo was obtained from Caplin Point Laboratories Limited, Chennai. The HPLC grade of acetonitrile, methanol was from Qualigens Fine Chemicals, Mumbai, India. The experimental procedure was carried out completely using Millipore (Bangalore, India) Milli-Q-Water purifier. The chemicals of monobasic sodium phosphate (LR), Rankem; anhydrous dibasic sodium phosphate (LR), sodium chloride (AR), dextrose (AR) and phosphoric acid (AR), and other reagents of analytical reagent grade from Merck were used for the analysis.

2.2. Instrumentation and Analytical Conditions

The chromatographic method carried out using Agilent 1200 series HPLC equipped with DAD & VWD detector. The omeprazole separation was achieved by using the Zorbax Eclipse C₈ column (250 X 4.6 mm, 5 μ) with a mixture of phosphate buffer (pH 7.2): acetonitrile in the ratio of 75:25 (v/v) as mobile phase at a flow rate of 1.5 ml/min. The λ_{max} was monitored by UV at 280 nm. The software of open lab software was

used to monitor the data acquisitions and other proceedings. The run time was fixed as 18 min. and the room was air-conditioned at temperature of 25 ± 2 °C. The freshly prepared mobile phase was vacuum filtered through a 0.45 μ m Millipore nylon filters and it was used for the complete analysis.

2.3. Preparation of Phosphate Buffer

Dissolve 0.2 g of monobasic sodium phosphate and 1.120 g of anhydrous dibasic sodium phosphate in 300 mL of water, mix and dilute with water to 1000 mL; adjust the pH with ortho-phosphoric acid to 7.2, and filter through 0.45 μ m filter.

2.4. Preparation of Standard Solutions

Accurately weigh about 40 mg of Omeprazole standard and transfer into a 25 mL volumetric flask, dissolve in suitable volume with mobile phase and make up to the final volume using the same. Transfer 5 mL of this above solution into 50 mL volumetric flask, dilute and make up to the mark with mobile phase.

2.5. Preparation of 0.9% Sodium Chloride Solution

Transfer 0.9 g of sodium chloride into 100 mL volumetric flask and dissolve it in 50 ml of water and make up to the mark using the same.

2.6. Preparation of 5% Dextrose Solution

Transfer 5 g of dextrose into 100 mL volumetric flask and dissolve it in 50 ml of water and make up to the mark using the same.

2.7. Sample

Omeprazole sodium injection [10ml injection vial contains 40 mg equivalent of omeprazole as omeprazole sodium].

2.8. Placebo Sample

Placebo injection [10 ml injection vial contains placebo mixture without drug].

2.9. Preparation of Sample Solution (0.9% NaCl solution)

Take 10 numbers of sample vials and reconstitute each vial with 0.9% NaCl solution. Pooled all the vials and transfer 10 ml (equivalent to 40 mg of omeprazole) into a 25 ml volumetric flask and make up to mark with mobile phase. Transfer 5 ml of the above solution to a 50 ml standard flask and make up the volume with mobile phase.

2.10. Preparation of Sample Solution (5% Dextrose)

Take 10 numbers of sample vials and reconstitutes each vial with 5% Dextrose solution. Pooled all the vials and transfer 10 ml (equivalent to 40 mg of omeprazole) into a 25 ml volumetric flask and make up to mark with mobile phase. Transfer 5 ml of the above solution to a 50 ml standard flask and make up the volume with mobile phase.

2.11. Procedure for Analysis

The following procedure was recommended for the analysis of omeprazole estimation. Inject the volume of 20 μ l of blank (mobile phase) and placebo once and inject the standard preparation 6 times followed by sample preparation twice into the HPLC. After the completion of injection integrate and calculate the assay of omeprazole.

III. RESULTS AND DISCUSSION

3.1. Development of the method

A simple and rapid RP-HPLC method for the estimation of omeprazole sodium injection was developed, which can be effectively employed for quality control comparative analysis. The stationary phase of Zorbax Eclipse C₈, (250 X 4.6 mm, 5 μ) was used. The mobile phase was confirmed after conducting the different trials using phosphate buffer, methanol and acetonitrile. The proportion of phosphate buffer: methanol (50:50 v/v) showed more asymmetric factor with poor peak shape. Hence, decided to use phosphate buffer: instead of methanol and it found that peak with short retention time. The present method was aimed to conduct the comparative stability study of omeprazole sodium injection and it need somewhat high retention time for the better separation of omeprazole from degradation or matrix peaks while conducting the stability of omeprazole sodium injection in 0.9% sodium chloride and 5% dextrose injection. The various trials were conducted with

different proportions of phosphate buffer: acetonitrile like 50:50 v/v, 60:40 v/v, 70:30 v/v, 75:25 v/v, and 80:20 v/v. The proportion of 75:25 v/v yield a good peak shape but little peak fronting. The different pH conditions were used to eliminate the peak fronting by altering the pH from 5.0, 6.0, 6.5, 7.0, 7.2, and 7.4. At pH 7.2 the peak symmetry was achieved with for 0.9% sodium chloride was 11.147 ± 0.12 and 5% dextrose was to be 11.053 ± 0.18 . The chromatograms were showed in Fig. 2A. 0.9% Sodium chloride and Fig. 2B. 5% dextrose. All the above studies were monitored at 280 nm with 1.5 ml/min flow rate. The method was found to be specific, and it shows none of the excipients interfered with the analyte of interest. Hence, the method can be successfully employed for the assay of commercially available formulations.

3.2. Validation of the Method

The validation of the optimized carried out method was performed according to the ICH guidelines with respect to following parameters:

3.3. Linearity

Linearity graph was obtained from peak area response against the injected concentrations of the drug. It was performed over the concentration range of 80-240 $\mu\text{g/ml}$. Each, concentration of the injection was injected three times individually and carried out in the HPLC by keeping the constant injection volume. The regression and correlation coefficients are calculated. The results showed a good linear relationship and the peak responses of omeprazole were strictly linear ($r^2 > 0.99$) in the range of concentrations. The slope of the omeprazole in plain omeprazole standard solution, omeprazole in 0.9% NaCl and in 5% dextrose solution was found 2147967, 2158745 and 2093624. The intercept of omeprazole in plain omeprazole standard solution, omeprazole in 0.9% NaCl and in 5% dextrose was found 8560197, 9154275 and 7691475 respectively. The Linearity plots are showed in Fig. 3A, 3B and 3C.

3.4. Precision

The intra-day precision was carried out in both samples solutions with each six sample preparations as per method. After completion of the analysis the chromatograms are integrated and calculated. The intermediate precision was performed for three consecutive days for both solutions. The % RSD was found to be less than 2 and it shows the method was precised one. The results are summarized in Table 1.

3.5. Accuracy

The accuracy was performed by standard addition method. The drug recovery was determined in triplicate by spiking with equivalent amount of placebo into each 50%, 80%, 100%, 120% and 150% level of the standard concentration and reconstituted with 0.9% NaCl and 5% dextrose solution separately and further dilutions are made as per method. The average percentage recovery of omeprazole was found to be within the limits and it was highly accurate. The accurate responses of the drug are shown in Table 2.

3.6. Limit of Detection (LOD) and Limit of Quantification (LOQ)

From the linearity curve the LOD and LOQ was evaluated. The LOD and LOQ of omeprazole sodium were found to be 0.493 $\mu\text{g/ml}$ and 1.414 $\mu\text{g/ml}$ respectively. It shows that the method can be efficiently used for the estimation of omeprazole sodium injection.

3.7. Robustness

The robustness of the method was performed by deliberate changes in the mobile phase composition (77:23 & 73:27), wavelength (± 5 nm), column oven temperature (± 5 °C), pH (± 0.2) and flow rate (± 0.15). These studies were carried out as per the above changes and the results are found (Table 3) within the acceptance limits. Hence, the method was found to be robust.

3.8. Specificity

The method was found to be specific. Peak purity of the omeprazole was analyzed and found 1.0 for standard omeprazole and omeprazole in both 0.9% sodium chloride and 5% dextrose reconstituted sample solutions, it proves that there is no interference and co-elution of any other peaks found in the retention time of Omeprazole and the results are showed in Table 4. It shows that there is no interference in the presence of excipients or placebo. The methodology can be suitably employed for the commercial injectable formulation of omeprazole sodium injection to evaluate the compatibility studies.

3.9. Stability of solution

The prepared drug sample solution was found to be stable, and it was performed by injecting the sample solution up to 24 hours in interval of every 4 hours. From the study it was concluded that the results are found within the acceptance and hence the analyte was stable for 24 hours in both 0.9% sodium chloride and 5% dextrose reconstituted solutions. The results are showed in Table 5.

IV. CONCLUSION

Stability of omeprazole sodium injection in 0.9% sodium chloride and 5% dextrose solution was estimated along with method validation by Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) technique and carried out successfully. As per the validated study it has been concluded that the present study will be effectively employed to determine the omeprazole in omeprazole sodium injection while reconstituted with 0.9% sodium chloride and 5% dextrose solutions. From the validated results it was found that the analyte omeprazole was stable in both solutions.

V. ACKNOWLEDGEMENTS

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Table 1: Precision data for the analysis

0.9% Sodium Chloride				
Concentration	Intra-mediate Precision	Intermediate Precision		
		Day-1	Day-2	Day-3
40 µg/ml	343748847	333261693	343494986	343488334
40 µg/ml	342947053	340142352	342483475	342518135
40 µg/ml	343130789	340357288	342562442	332446036
40 µg/ml	343729793	340686732	338150805	338216816
40 µg/ml	348573950	340125624	342885997	342986003
40 µg/ml	343447877	340664884	340972192	340957101
Mean	344263052	339206928	341758316	340102070
Std Dev	2135931	2922751	1954087	4207644
% RSD	0.62	0.86	0.57	1.23
5% Dextrose				
40 µg/ml	315384991	321193443	320266147	320155139
40 µg/ml	315219348	322267800	320817790	321876648
40 µg/ml	315871848	321240300	319906614	320665489
40 µg/ml	315622423	320990875	320209663	321568452
40 µg/ml	316102768	321471220	320926240	321658423
40 µg/ml	314100427	319468879	321314019	325489761
Mean	315383634	321105419	320573412	321902319
Std Dev	705143	916762	529915	1877431
% RSD	0.22	0.29	0.16	0.58

Table 2: Summarized Analysis of Accuracy study

Accuracy Level (%)	Amount Added (mg)		Amount Recovered (mg)		*Mean Recovery (%)	
	0.9% NaCl	5% Dextrose	0.9% NaCl	5% Dextrose	0.9% NaCl	5% Dextrose
50	20.17	19.42	20.12	19.56	99.85	100.85
	20.07	19.61	20.15	19.83		
	20.22	19.46	20.10	19.60		
80	32.71	32.24	32.24	32.24	101.39	100.41
	32.68	31.17	32.17	31.30		
	32.58	31.22	32.22	31.49		
100	39.68	39.55	39.75	39.55	99.45	99.74
	39.58	39.05	40.00	39.05		
	39.67	39.04	39.84	39.04		
120	48.42	47.93	48.23	47.76	100.77	99.67
	48.56	47.84	48.14	47.60		
	48.60	47.09	48.09	47.03		
150	60.02	59.02	59.16	58.82	98.62	99.51
	59.84	59.14	59.13	58.67		
	60.03	59.27	59.11	59.05		

*Average of three observations

Table 3: Summarized data of Robustness

Parameter Name	Condition	Tailing factor		%RSD	
		%0.9 NaCl	5% Dextrose	%0.9 NaCl	5% Dextrose
Wave Length (nm)	282	1.12	1.10	0.84	0.21
	278	1.15	1.13	0.83	0.29
Mobile Phase variation (v/v)	77:23	1.19	1.17	0.06	0.22
	73:27	1.07	1.06	0.06	0.32
pH	7.0	1.06	1.08	0.11	0.16
	7.4	1.14	1.017	0.10	0.28
Flow Rate (ml)	1.35	1.21	1.24	0.01	0.25
	1.65	1.10	1.09	0.10	0.22
Column Temperature (°C)	25	1.21	1.23	0.02	0.10
	35	1.09	1.11	0.03	0.27

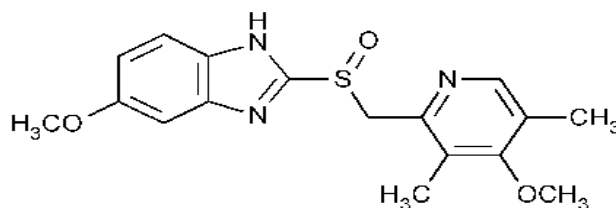
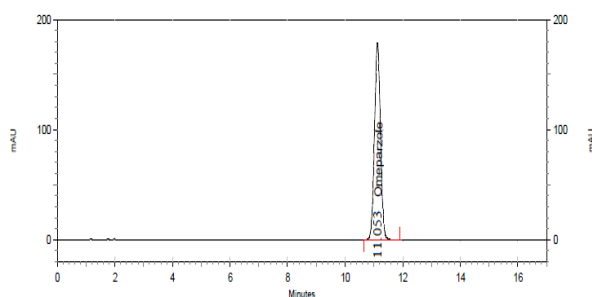
*Average of three determinations

Table 4: Specificity study of Omeprazole

S. No.	Name	RT	Peak purity
1	Standard Omeprazole	11.053	1
2	Omeprazole sodium in 0.9% NaCl	11.147	1
3	Omeprazole sodium in 5.0% Dextrose	11.174	1

Table 5: Summarized study of Stability investigation

S. No.	Time point	Peak Area		Difference in Area%	
		%0.9 NaCl	5% Dextrose	%0.9 NaCl	5% Dextrose
1	Zero hour	374217950	315050108	0.000	0.000
2	4 th hour	377688378	317367956	0.927	0.735
3	8 th hour	378607640	318170768	1.173	0.990
4	12 th hour	379479037	318729180	1.406	1.167
5	16 th hour	378267415	318877459	1.082	1.214
6	20 th hour	379852454	318138456	1.506	0.980
7	24 th hour	380222381	318271366	1.605	1.022

**Fig. 1: Structure of Omeprazole****(2A)**

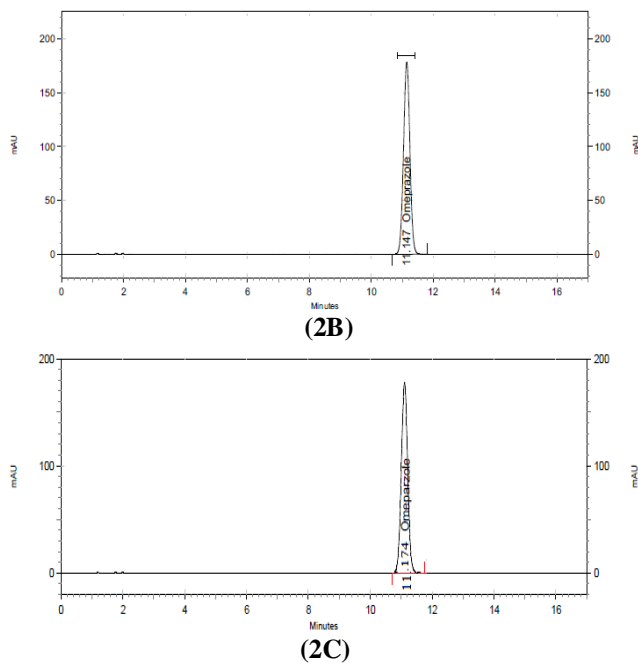


Fig. 2: Chromatogram of Omeprazole with (2A) Standard Chromatogram (2B) 0.9% Sodium Chloride, and (2C) 5% Dextrose

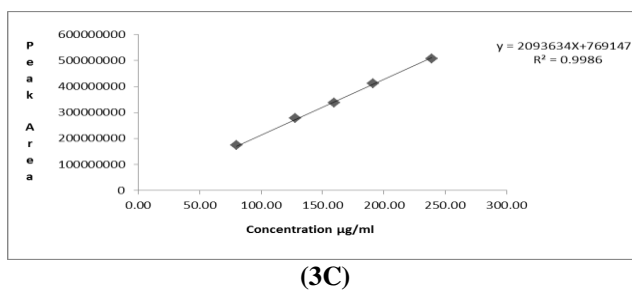
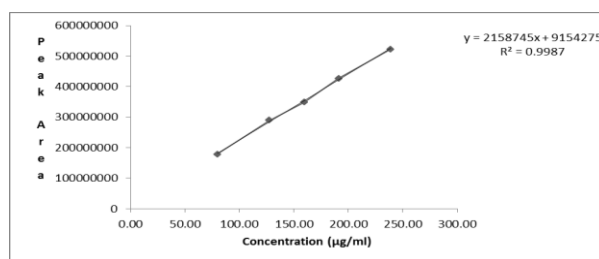
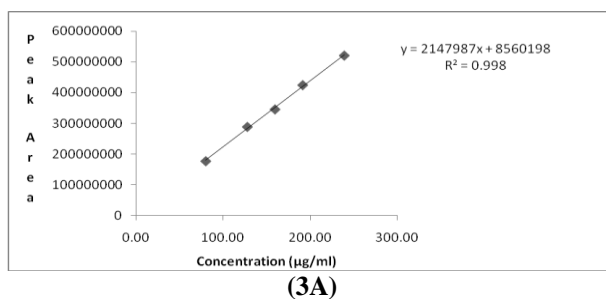


Fig. 3: Linearity of Omeprazole in (3A) Standard Solution (3B) 0.9% Sodium Chloride solution, and (3C) 5% Dextrose solution