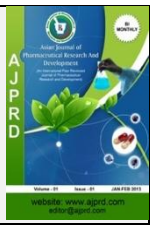
Available online on 15.04.2021 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

Open Access to Pharmaceutical and Medical Research



Open Access

Research Article

Stability Indicating Method Development and Validation for Assay of Omeprazole Sodium for Injection by a Rapid RP-HPLC

Aishwarya Dande, Gummadi Rishitha Reddy, Gaddam Saithya

Department of Pharmaceutical Analysis, G. Pulla Reddy College of Pharmacy (Ou), Mehidipatnam, Hyderabad, Telangana.

A B S T R A C T

Omeprazole,[13] a member of proton pump inhibitor, used for the treatment of peptic ulcers, it supresses gastric acid secretion by inhibiting H^+/K^+ ATPase. A new fundamental, rapid and sensitive HPLC method was developed for the assay of Omeprazole sodium for injection. It was validated according to ICH and FDA guidelines [2]. The HPLC analysis was performed on Dionex ultimate 3000 system equipped with C-18 (150cm*4.6mm) 5µ column, with a mixture of ammonium acetate and acetonitrile buffer in the ratio 65:35 v/v as the mobile phase at the wavelength (λ) 305nm. The total run time was 8 minutes. The calibration curve was linear for 50-150% range of the analytical concentration of 40mg/ml, r^2 was found to be 0.9998. The precision was calculated and % RSD was 0.1 % and the recovery of omeprazole was within the range of 98-102%. Validation parameters like robustness, solution stability studies, specificity and forced degradation studies were performed and found to be within acceptance limits. As compared to other methods, this method was found to be more accurate, linear, precise and specific, with short run time. All the results were acceptable and confirmed that the method is suitable for its intended use in routine quality control analysis.

Key Words: Omeprazole, Omeprazole sodium for injection, HPLC.

A R T I C L E I N F O: Received 19 Jan. 2021; Review Complete 15 March 2021 Accepted 08 April 2021; Available online 15 April. 2021

Cite this article as:

Aishwarya Dande A, Reddy GR, Saithya G, Stability Indicating Method Development and Validation For Assay of Omeprazole Sodium For Injection By A Rapid RP-HPLC. Asian Journal of Pharmaceutical Research and Development. 2021; 9(2):18-32.
DOI: http://dx.doi.org/10.22270/ajprd.v9i2.943

***Address for Correspondence:**

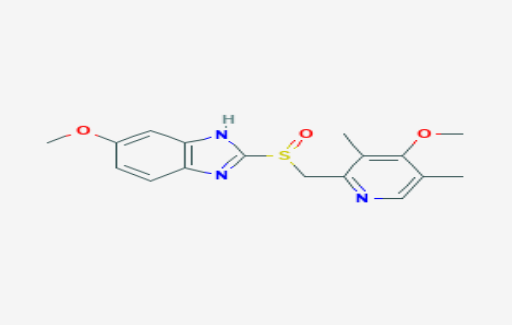
Aishwarya Dande, Department of Pharmaceutical Analysis, G. Pulla Reddy College of Pharmacy (Ou), Mehidipatnam, Hyderabad, Telangana, India

INTRODUCTION

Omeprazole, a white crystalline powder, which is slightly soluble in acetone and isopropanol, freely soluble in methanol and ethanol, very slightly soluble in water, is a member of proton pump inhibitors, which is used for treatment and management of medical conditions like- Peptic ulcers, Gastro Oesophageal Reflux Disease (GERD), Zollinger- Ellison syndrome and forms of Esophagitis (erosive and eosinophilic)[1-6-7]

It is also utilized as prophylactic to prevent upper gastro intestinal bleeding. As selective and irreversible proton pump inhibitors (PPI), within gastric mucosa at secretory surface of gastric parietal cells.

Omeprazole functions by inhibiting H^+/K^+ ATPase to supress production of stomach acid or gastric acid secretion. There are various analytical techniques, which are been used for quantification of omeprazole. High Pressure Liquid Chromatography (HPLC) is the most predominant method for the determination of Omeprazole. Analytical method validation was the crucial step in developing Omeprazole in Omeprazole sodium for injection, as it provided information about accuracy, linearity, precision, specificity, range, robustness, solution stability and forced degradation studies. All the above validation parameters were successfully reported. [7-8]



Molecular formula $C_{17}H_{19}N_3O_3S$

Molecular weight - 345.4 g/mol

Physical properties:

Colour White to off-white crystalline powder

Boiling point 599.991 °C at 760 mmHg

Melting point 155 °C-156 °C

Solubility Freely soluble in ethanol and methanol, and slightly soluble in acetoneand isopropanol and very slightly soluble in water.

MATERIALS AND METHODS

INSTRUMENTATION

Dionex Ultimate 3000 Thermoscientific HPLC system was used for analytical method development and validation for assay of Omeprazole in Omeprazole sodium for injection, and autosampler, a Gemini NX C-18 (150*4.6mm) 5 micron column(Phenomenex), and the detector containing UV Visible which was operated at 305nm. Chromeleon software version 7.2 was used for data processing and evaluation. [9-10]

CHEMICALS AND REAGENTS-

Omeprazole sodium for injection (purity), was obtained from Dr. Reddys Laboratories. Omeprazole sodium for injection 40mg/ml containing Omeprazole was purchased from local market (RDL)

Acetonitrile HPLC grade (Merck), methanol HPLC grade (Merck), Ammonium acetate Lichropurgrade (Merck), sodium phosphate tribasic Dodecahydrate AR grade(SRL**).**[7]

Water was deionised and purified using a Milli Q reagent grade water system (Millipore cor).

CHROMATOGRAPHCIC CONDITIONS

The mobile phase was prepared by dissolving 1.54gm of Ammonium acetate in 1000 ml of Milli Q water then filter through 0.45 microns membrane filter. From the prepared solution measure 650ml ammonium acetate buffer and add 350ml of HPLC grade ACN, transfer into mobile phase bottle, mixed well and sonicated for 10 minutes.

The analysis was carried out on Dionex Ultimate 3000 (Thermoscientific) HPLC system. Gradient flow was maintained through C-18 column with Ammonium acetate buffer:HPLC grade ACN (65:35) as mobile phase at a flow rate of 1ml/min and run time was 20 microliter and detection was at 305nm. The HPLC system was operated at 25 degree centigrade. Data was collected by Chromeleon software.

PREPARATIONS

Preparation of Mobile phase:

Accurately weighed 1.54 gm of Ammonium acetate in to a beaker and dissolved in 1000 ml of MilliQ water and then filtered through 0.45µ membrane filter.Mixed 650 ml of Ammonium acetate buffer and 350 ml of HPLC grade Acetonitrile, transferred into a mobile phase bottle, mixed well and sonicated for 10 minutes.

Preparation of Diluent:

Accurately weighed 3.8 gm of Sodium Phosphate Tribasic Dodecahydrate in to a beaker and dissolved in 1000 ml of MilliQ water then filtered through 0.45µ membrane filter. Mixed 600 ml of Sodium Phosphate Tribasic Dodecahydrate buffer and 400 ml of HPLC grade Methanol, transferred into a mobile phase bottle, mixed well and sonicated for 10 minutes.

Preparation of Blank preparation:

Use Diluent as Blank.

Preparation of Standard solutions

Preparation of Standard Solution-01:

Omeprazole stock solution:

Weigh accurately about 20 mg of Omeprazole and transfer into a 50 mL of volumetric flask, add about 30 mL of Diluent. Sonicated to dissolve completely, then made up to the mark with diluent and mixed well.

Standard solution preparation: Pipetted and transferred 5.0 mL of above stock solution into a 50 mL volumetric flask. Made up to the mark with diluent and mixed well.

Preparation of Standard Solution-02:

Omeprazole stock solution:

Weigh accurately about 20 mg of Omeprazole and transfer into a 50 mL of volumetric flask, add about 30 mL of Diluent. Sonicated to dissolve completely, then made up to the mark with diluent and mixed well.

Standard solution preparation:

Pipetted and transferred 5.0 mL of above stock solution into a 50 mL volumetric flask. Made up to the mark with diluent and mixed well.

Preparation of Sample

Reconstitute 3 vial of Omeprazole Sodium for Injection each with 10 mL of sterile water for injection and pooled the reconstituted solutions.Transferred 10 mL of the reconstituted solution into a 50 mL volumetric flask, added about 30 mL of diluent, and swirl to mix. Dilute to volume with diluent and mix well. Further dilute 5 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent and mix well.

Preparation of Placebo

Reconstitute 3 vial of Placebo for Omeprazole Sodium for Injection each with about 10 mL of sterile water for injection and pool the reconstituted solutions.Transfer 10 mL of the reconstituted solution into a 50 mL volumetric flask, add about 30 mL of diluent, and swirl to mix. Dilute to volume with diluent and mix well.Further dilute 5 mL of the above solution into a 100 mL volumetric flask and dilute to volume with diluent and mix well.

Procedure

Equilibrate the column Inject 1 Blank, Standard (5 replicate injections) and Check Standard (2 replicate Injections) into the Chromatographic system and check the system suitability criteria, if system suitability meets inject samples into HPLC.

System suitability criteria

The USP Asymmetry for Omeprazole peak from first injection of standard should be NMT 2.0. The Theoretical plates of Omeprazole peak from first injection of standard should be NLT 2000. The % Relative Standard Deviation for the peak area for Omeprazole peak from standard should be NMT 2.0 %. Recovery of check standard against standard preparation should be in between 98.0 to 102.0 %.

Recovery of check standard (Similarity factor) calculation:

$$\frac{\text{Average area of standard}-1}{\text{Average area of standard}-2}\times\frac{\text{weight of standard}-2}{\text{weight of standard}-1}\times 100$$

Calculations:

$$\text{Assay (\% Label claim)} = \frac{A}{B}\times\frac{W_S}{50}\times\frac{5}{50}\times\frac{50}{V}\times\frac{100}{5}\times\frac{P}{100}\times\frac{100}{L}$$

Where,

A= Peak area of Omeprazole from test preparation

B= Average peak area of Omeprazole from Standard Solution

Ws = Weight of Omeprazole working standard taken in mg

V= Volume of Sample solution taken in mL

L= Label claim of Omeprazole for Injection in mg/mL

P= Potency of Omeprazole working Standard ($C_{17}H_{19}N_3O_3S$) on as is basis

METHOD VALIDATION

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from **method validation** can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of good analytical practice.The method was validated as per ICH and FDA guidelines, and the validation parameters included specificity, linearity, range, accuracy, precision and robustness. [2-3]

1. System suitability:

It is performed to ensure system performance before or during the analysis.System suitability parameters (Asymmetry, plate count, %RSD, %r recovery) were performed. Acceptance criteria for asymmetry-NMT 2, plate count-NLT 2000, %RSD for five replicate injections-NMT 2, % Recovery within 98%-102%.

2. Specificity:

Specificity of the analytical method is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. It includes impurities, degradants, matrixes etc.Interference for blank, standard and forced degradation products were performed and method was found to be specific. Peak purity data for all the degradation conditions shows homogenous peaks and no co-eluting peaks were observed, which indicates the method was stable and specific.

3. Linearity:

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample.It was determined using seven different concentrations (20, 32, 36, 40, 44, 48, 60 µg/ml) and average peak areas were plotted against concentrations. Linearity was determined using calibration curve to calculate correlation coefficient, % Y-intercept, slope and Y-intercept. Graph was plotted by taking concentrations (µg/ml) on X axis and response on Y axis. Acceptable criteria for regression line r2 should be greater than 0.9998.

4. Precision:

Precision of analytical method is the degree of agreement among individual tests, when the technique is applied respectively to analyse multiple replicates.System precision was performed using ten replicate injections of standard solutions, the %RSD and recovery were found to be within limits (NMT 2%). Method precision (Repeatability) was performed using six sample solutions, the %RSD and recovery were found to be within limits (NMT 2%).Intermediate precision was performed by using six sample solutions and when these samples were analysed by different analysts on different days by different instruments and columns, the results should be comparable and meet the acceptance criteria. Hence the method was precise and rugged.

5. Accuracy:

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. It was determined by preparing samples by spiking drug at different concentrations (50-150%) with respect to test concentration. Placebo concentration was kept constant. Both % Recovery (98-102%) and %RSD (NMT 2%) were found to be within the limits and the method was found to be accurate.

6. Range:

The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.The range of the analytical method for Omeprazole is 50-150% of test concentration.

7. Solution stability:

Bench top stability and refrigerator stability of standard and sample solutions, bench top stability of mobile phase, was performed for two days and the solutions were stable for 2 days.

8. Robustness:

The robustness of an analytical procedure 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.The test was performed for column temperature (plyus or mi 5 degree), flow rate (pom 0.2 ml per min), wavelength (pom 3 nm), mobile phase organic composition (pom 5%). All the above parameters were evaluated by calculating %RSD, % Recovery, % Asymmetry, plate count and were within the limits. Thus, the method was robust.

RESULTS AND DISCUSSION

Table: 1 System Suitability Parameters

System Suitability Parameters	Result
The USP Asymmetry for Omeprazolepeak from standard solution	1.06
The USP plate count for Omeprazolepeak from standard solution	9860
The % RSD of peak areas from five replicate injections of standard solution.	0.1%
Recovery of check standard preparation against standard preparation should be in between 98.0% and 102.0% for Omeprazole peak	99.80%

Acceptance Criteria:

- The USP Asymmetry for Omeprazole peaks from standard solution should be NMT 2.0.
- The USP plate count for Omeprazole peak from standard solution should be NLT 2000.
- The % RSD of Omeprazole peak areas from five replicate injections of standard solution should be NMT 2.0.
- Recovery of check standard against standard preparation should be in between 98.0% and 102.0%.

CONCLUSION:

From the above data, it can be concluded that the system is found to be suitable

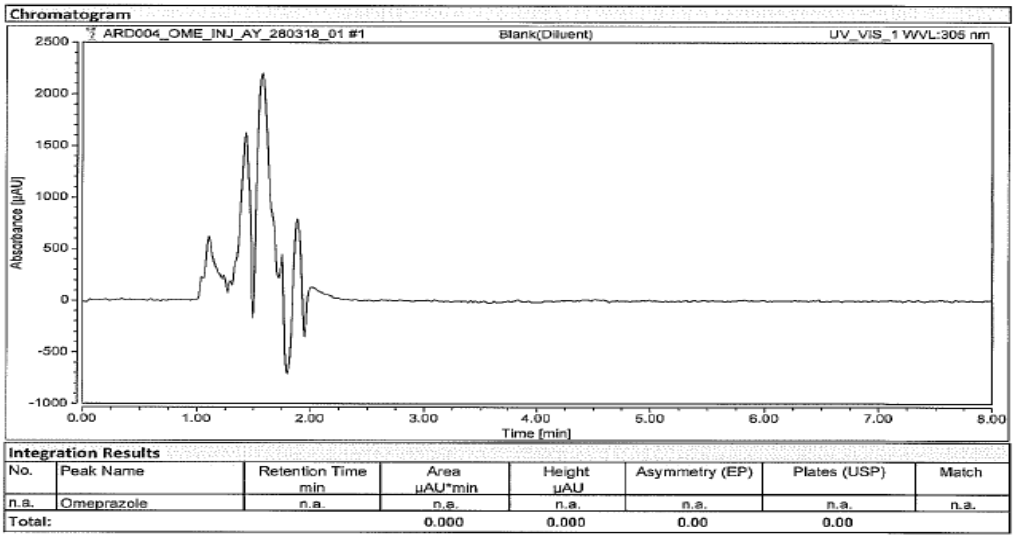


Figure: 1 Chromatogram of Blank

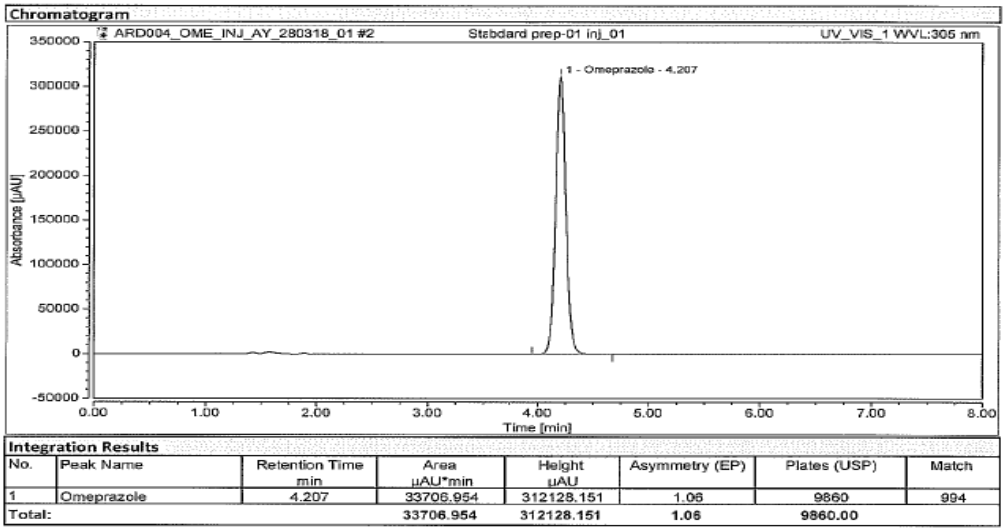


Figure: 2 Chromatogram of Standard

Specificity

Blank and Placebo Interference

A study was conducted to demonstrate the non-interference of placebo, prepared placebo for Omeprazole solution in triplicate at concentration equal to the concentration in the sample preparation as per test procedure and injected blank and placebo solutions in to the HPLC system as per methodology. Evaluated the interference of blank, placebo at the retention time of Omeprazole and found no peaks at the retention time of Omeprazole. The results are summarized in Table-1 and Figure-3&4.

Acceptance Criteria:

Blank and placebo solutions should not show any peak at the retention time (RT) of Omeprazole.

Table: 1 Results of Placebo Interference

Sample No.	Interference (Yes/No)
Interference due to the Blank	NO
Interference due to the Placebo of Omeprazole at the RT of Omeprazole.	NO

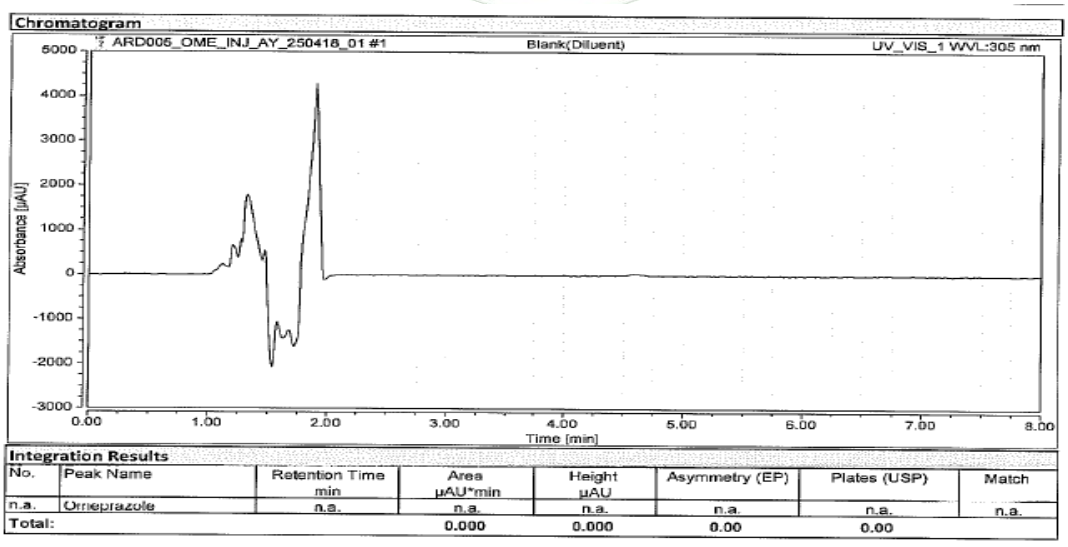


Figure: 3 Chromatogram of Blank for Omeprazole

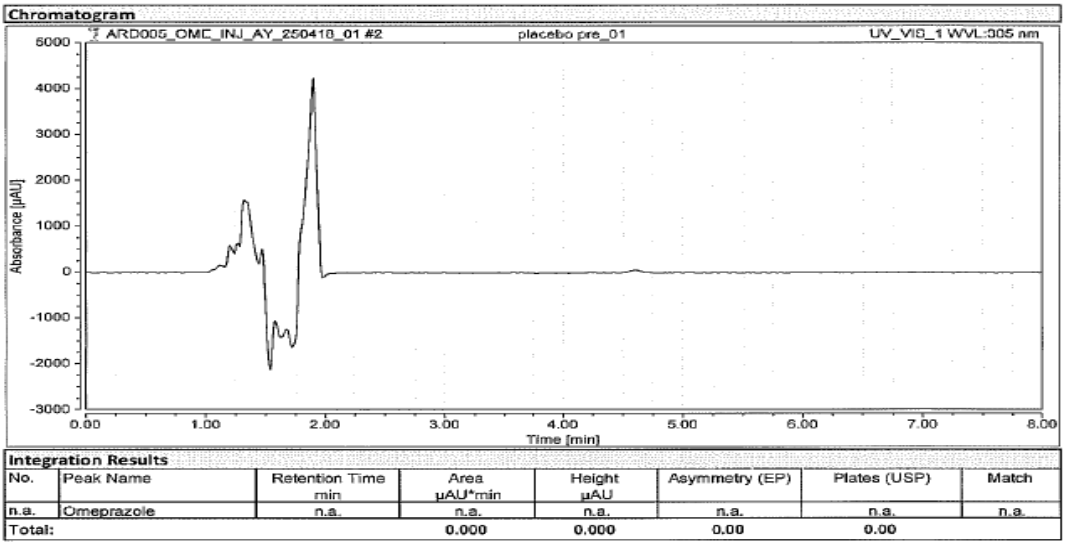


Figure: 4 Chromatogram of Placebo sample for Omeprazole

Conclusion: Method was found specific to Blank and placebo interference.

Interference from forced degradation Products

Omeprazole sodium for Injection 40 mg/vial samples and placebo were stressed under following conditions and solutions prepared as per test method and injected into HPLC system as per methodology.

Table: 2 Conditions for Forced Degradation

S. No.	Degradation / Stress Mechanism	Sample Details	Condition
1	Acid Hydrolysis	Drug Product / Placebo	0.2N HCl (10 mL at RT for 15 minutes)
2	Base Hydrolysis	Drug Product / Placebo	2N NaOH (10mL at 80°C for 45 minutes)
3	Peroxide Hydrolysis	Drug Product / Placebo	3 % H_2O_2 (10 mL at RT for 15 minutes)
4	Water Hydrolysis	Drug Product / Placebo	At 80°C for 3 hours
5	Thermal exposure	Drug Product / Placebo	In hot air oven at 60°C for 6 Days
6	Humidity exposure	Drug Product / Placebo	At 25°C and 90 % RH for 3 Days.
7	Photolytic exposure	Drug Product / Placebo	Visible light (1.2 million lux hours UV light 200 watts/m^2)

Unstressed and stressed samples (placebo and sample solutions) were injected into the HPLC system equipped with Photo diode array detector as per test method conditions. All degradants peaks were resolved from Omeprazole peak in the chromatogram of all stressed samples. The chromatogram of the stressed samples was evaluated for peak purity. The results are summarized in Table-2 & Table-3 and Figure-5 to 12.

Forced degradation for placebo:

Acceptance Criteria: Blank and Placebo solutions should not show any interfering peak at retention time (RT) of Omeprazole peak.

Table: 3 Results of Forced Degradation placebo/Diluent

Observation / Results		
Degradation mechanism	**Observation**	**Results**
Unstressed sample	No interference at RT of Omeprazole peak	Passed
Acid sample	No interference at RT of Omeprazole peak	Passed
Base sample	No interference at RT of Omeprazole peak	Passed
Peroxide sample	No interference at RT of Omeprazole peak	Passed
Water sample	No interference at RT of Omeprazole peak	Passed
Thermal sample	No interference at RT of Omeprazole peak	Passed
Humidity sample	No interference at RT of Omeprazole peak	Passed
Photolytic sample	No interference at RT of Omeprazole peak	Passed

Forced degradation for samples:

Acceptance criteria:

Peak should be homogeneous and there should be no co-eluting peaks. Peak purity of Omeprazole should pass (Match should be not less than 990 for peak purity as per Chromeleon 7.2 software).

Table: 4 Results of Forced Degradation Sample

Stressed Condition	Observation/Result		
	% degradation	Peak Purity	Mass balance
Unstressed	NA	992	NA
Acid	0.08	993	99.7
Base	4.41	993	96.4
Peroxide	1.52	992	101.9
Water	0.95	993	98.7
Thermal	0.33	992	101.3
Humidity	0.09	995	100.0
Photolytic	0.11	993	97.8

Conclusion: The peak purity data of Omeprazole peak of all degradation conditions shows that the peak was homogeneous and there were no co-eluting peaks indicating that the method was stability indicating and specific.

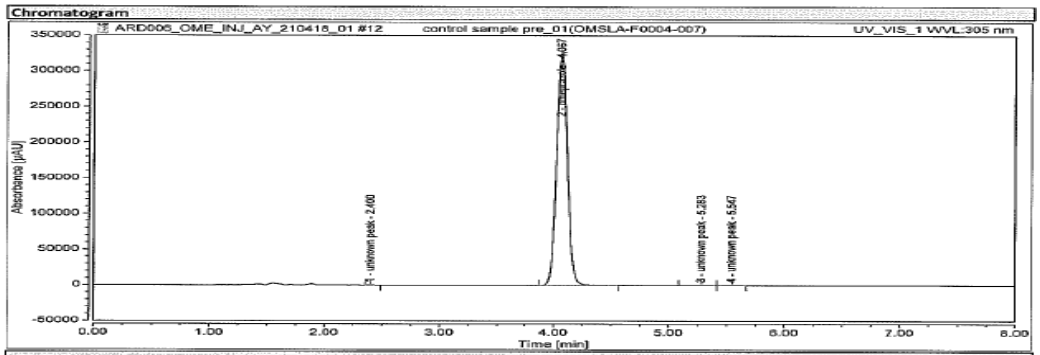


Figure: 5 Chromatogram of Unstressed sample

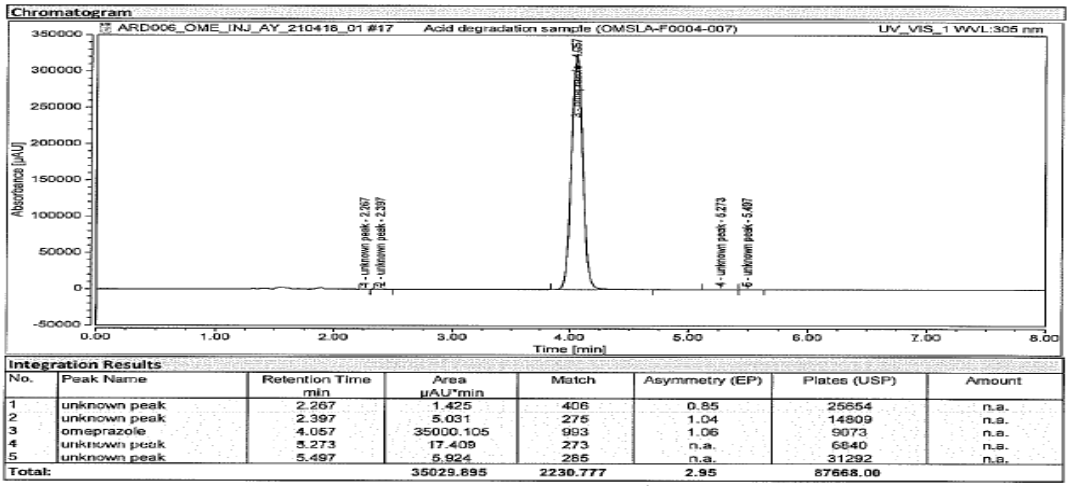
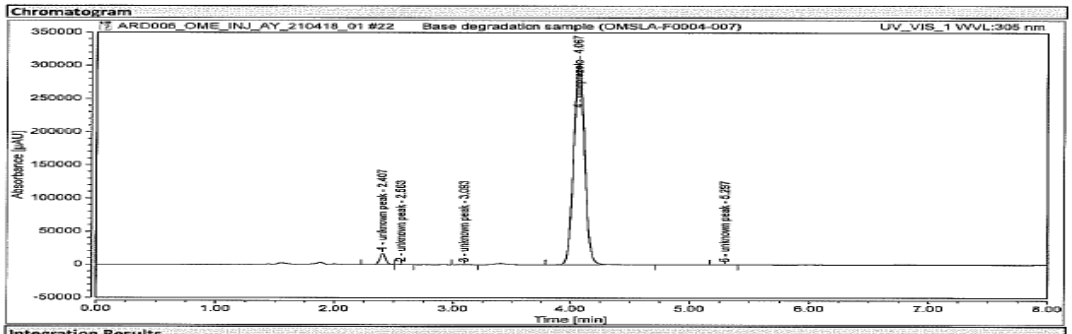


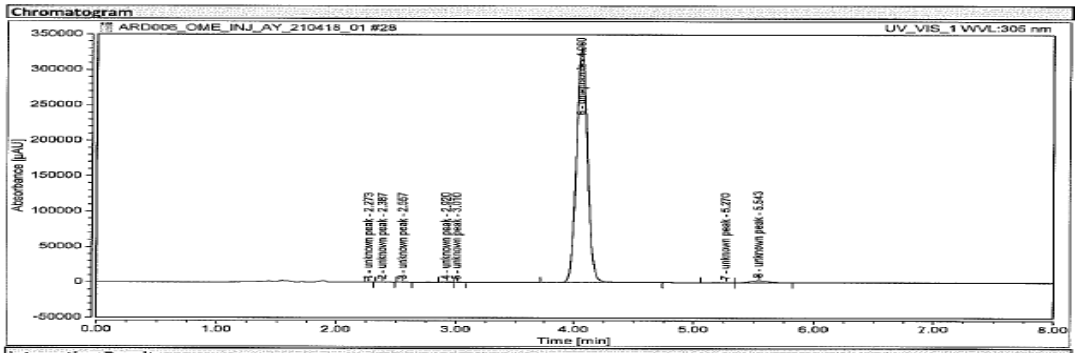
Figure: 6 Chromatogram of Acid Stress



Integration Results

No.	Peak Name	Retention Time min	Area µAU*min	Match	Asymmetry (EP)	Plates (USP)	Amount
1	unknown peak	2.407	982.440	982	1.08	11158	n.a.
2	unknown peak	2.563	35.693	372	n.a.	8837	n.a.
3	unknown peak	3.093	17.996	204	1.02	8721	n.a.
4	omeprazole	4.067	32320.284	993	1.07	9423	n.a.
5	unknown peak	5.297	20.164	528	0.89	11849	n.a.
Total:			33386.558	3078.918	4.06	49988.00	

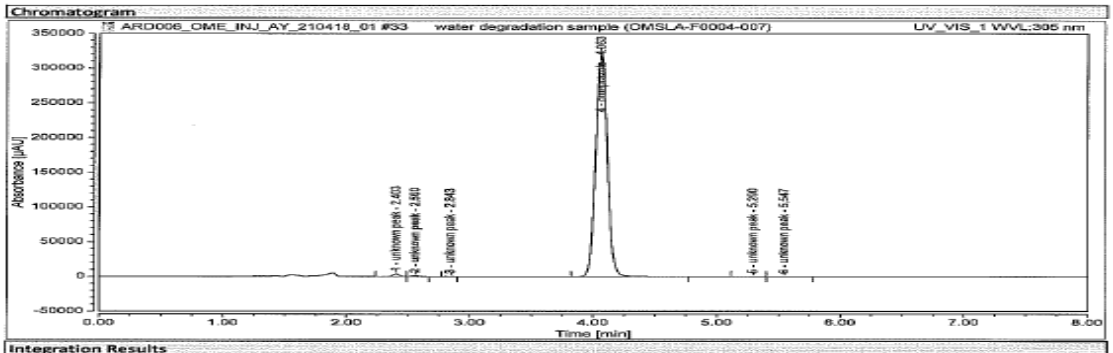
Figure-7: Chromatogram of Base Stress



Integration Results

No.	Peak Name	Retention Time min	Area µAU*min	Match	Asymmetry (EP)	Plates (USP)	Amount
1	unknown peak	2.273	1.543	322	1.12	26065	n.a.
2	unknown peak	2.387	3.487	381	1.22	n.a.	n.a.
3	unknown peak	2.557	9.125	244	1.14	10991	n.a.
4	unknown peak	2.929	8.346	489	n.a.	8591	n.a.
5	unknown peak	3.010	3.957	470	n.a.	n.a.	n.a.
6	omeprazole	4.060	35282.299	992	1.08	9365	n.a.
7	unknown peak	5.270	14.982	325	n.a.	2981	n.a.
8	unknown peak	5.543	492.250	806	1.08	7461	n.a.
Total:			35815.999	4029.668	5.64	65454.00	

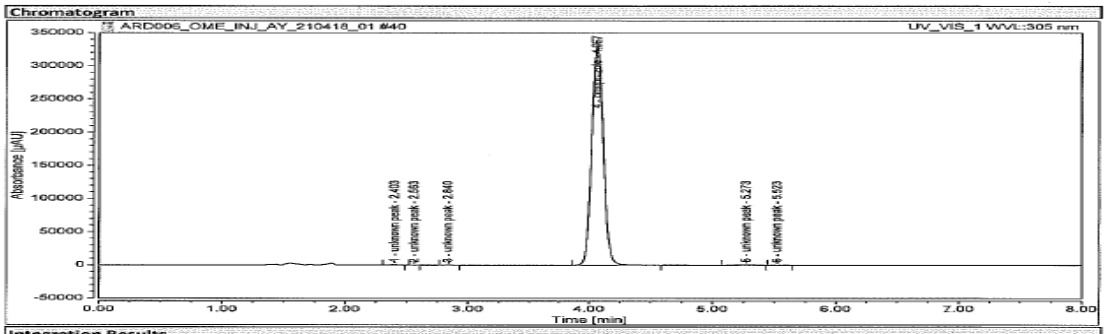
Figure-8: Chromatogram of Peroxide Stress



Integration Results

No.	Peak Name	Retention Time min	Area µAU*min	Match	Asymmetry (EP)	Plates (USP)	Amount
1	unknown peak	2.403	212.322	658	1.08	11434	n.a.
2	unknown peak	2.560	79.679	565	1.06	9561	n.a.
3	unknown peak	2.843	4.292	260	0.86	7714	n.a.
4	omeprazole	4.063	34346.994	993	1.08	9371	n.a.
5	unknown peak	5.290	21.313	350	n.a.	10656	n.a.
6	unknown peak	5.547	14.787	261	n.a.	9812	n.a.
Total:			34679.387	3086.386	4.09	58548.00	

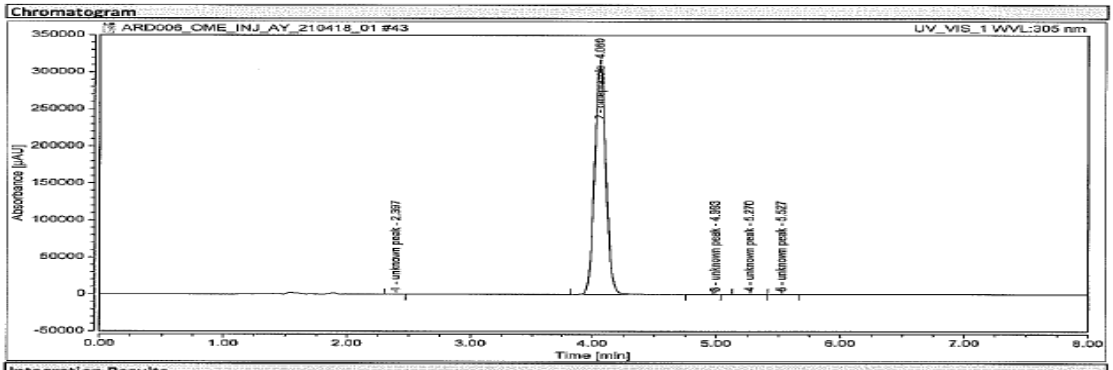
Figure-9: Chromatogram of Water Stress



Integration Results

No.	Peak Name	Retention Time min	Area µAU*min	Match	Asymmetry (EP)	Plates (USP)	Amount
1	unknown peak	2.403	36.377	371	0.90	10464	n.a.
2	unknown peak	2.563	1.030	321	1.02	n.a.	n.a.
3	unknown peak	2.840	5.098	240	1.14	10478	n.a.
4	omeprazole	4.067	35488.854	992	1.05	9356	n.a.
5	unknown peak	5.273	64.813	184	0.90	6143	n.a.
6	unknown peak	5.523	8.479	386	1.28	15387	n.a.
Total:			35604.651	2494.000	6.27	51828.00	

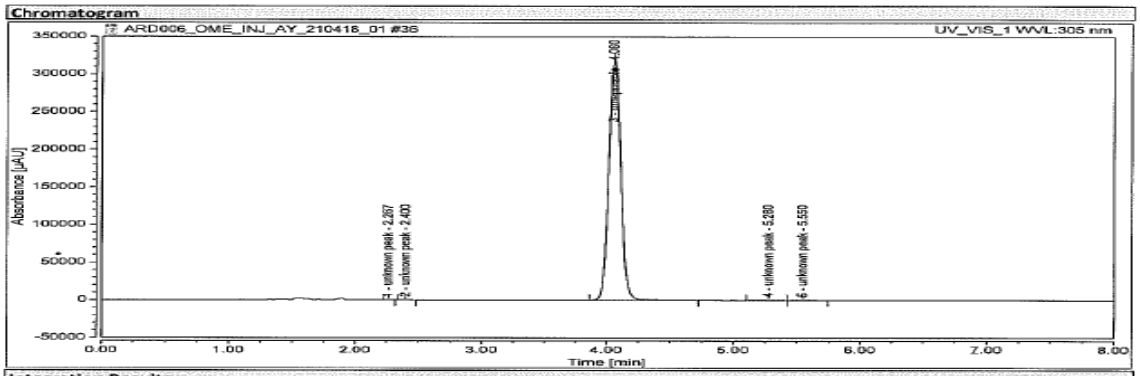
Figure-10: Chromatogram of Thermal Stress



Integration Results

No.	Peak Name	Retention Time min	Area µAU*min	Match	Asymmetry (EP)	Plates (USP)	Amount
1	unknown peak	2.397	13.497	393	0.85	10888	n.a.
2	omeprazole	4.060	32941.939	993	1.05	9351	n.a.
3	unknown peak	4.993	0.632	400	1.39	n.a.	n.a.
4	unknown peak	5.270	17.446	206	0.93	7127	n.a.
5	unknown peak	5.527	6.374	428	1.16	18703	n.a.
Total:			32979.888	2418.642	5.39	46069.00	

Figure-11: Chromatogram of Humidity Stress



Integration Results

No.	Peak Name	Retention Time min	Area µAU*min	Match	Asymmetry (EP)	Plates (USP)	Amount
1	unknown peak	2.267	2.091	172	1.09	n.a.	n.a.
2	unknown peak	2.400	5.533	421	1.05	12024	n.a.
3	omeprazole	4.060	34327.853	993	1.08	9294	n.a.
4	unknown peak	5.280	19.111	189	n.a.	9917	n.a.
5	unknown peak	5.550	10.994	250	n.a.	14141	n.a.
Total:			34365.582	2024.965	3.22	45376.00	

Figure-12: Chromatogram of Photolytic Stress

Linearity

Linearity of detector response was established by plotting a graph of concentration versus average peak area and by determining the correlation coefficient, % Y-Intercept, slope and Y-intercept. A series of solutions of Omeprazole was prepared in the concentration range of 50 % to 150 % of the target sample concentration and analyzed as per the

test method. A graph was plotted to concentration in µg/mL on X- axis versus response on Y-axis. The results are summarized in Table-4&5, and the linearity graph is presented in Figure-13.

Table: 5 Linearity of Omeprazole

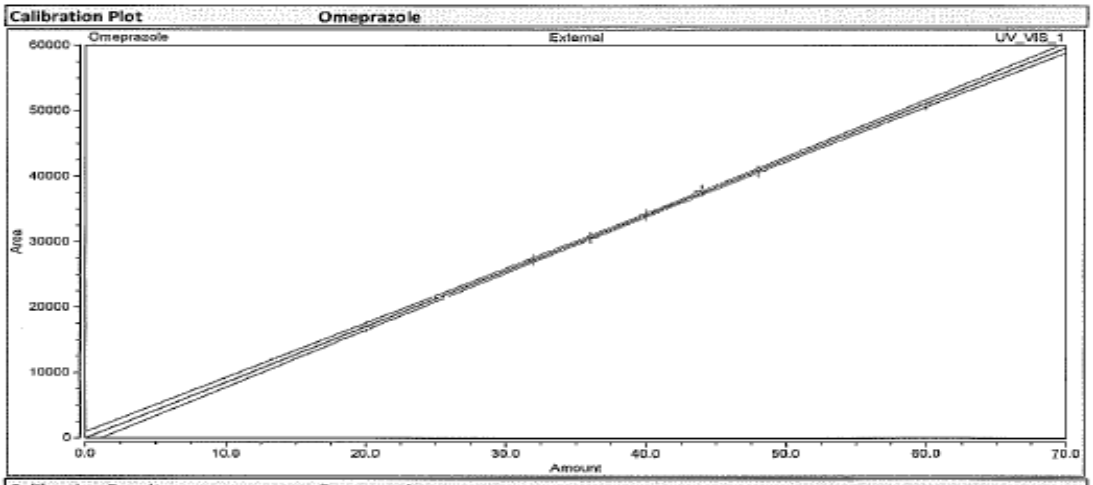
Solution No.	% level of target concentration	Concentration (µg/mL)	Average Peak Area
1	50%	20	16985.1050
2	80%	32	27230.6760
3	90%	36	30679.1157
4	100%	40	34165.0392
5	110%	44	37877.5808
6	120%	48	40842.8600
7	150%	60	51053.7030
Parameter		**Acceptance Criteria**	**Result**
Correlation coefficient (r)		NLT 0.999	0.9998
% Y-intercept		NMT 2.0	-8.483
Y-Intercept		Report the value	-0.02
Slope		Report the value	853.19

virchow

Calibration

Calibration Details	Omeprazole		
Calibration Type	Lin, WithOffset	*Offset (C0)*	-8.4833
Evaluation Type	Area	*Slope (C1)*	853.1909
Number of Calibration Points	7	*Curve (C2)*	0.0000
Number of disabled Calibration Points	0	*R-Square*	0.9998

Calibration Plot Omeprazole



Calibration Results Omeprazole

No.	Injection Name	Calibration Level	X Value UV_VIS_1 Omeprazole	Y Value UV_VIS_1 Omeprazole	Y Value UV_VIS_1 Omeprazole	Area µAU*min UV_VIS_1 Omeprazole	Height µAU UV_VIS_1 Omeprazole
2	Standard Prep 01 Inj_01		n.a.	n.a.	n.a.	33897.118	304867.367
3	Standard Prep 01 Inj_02		n.a.	n.a.	n.a.	33899.023	304984.342
4	Standard Prep 01 Inj_03		n.a.	n.a.	n.a.	33890.087	305427.868
5	Standard Prep 01 Inj_04		n.a.	n.a.	n.a.	33917.633	304898.477
6	Standard Prep 01 Inj_05		n.a.	n.a.	n.a.	33839.492	304649.170
9	Linearity_50%(20 PPM)	1	20.0000	16985.1047	16985.1047	16985.105	153859.104
10	Linearity 80%(32 PPM)	2	32.0000	27230.6757	27230.6757	27230.676	244962.347
11	Linearity 90%(36 PPM)	3	36.0000	30679.1157	30679.1157	30679.116	276376.244
12	Linearity 100%(40 PPM)	4	40.0000	34165.0392	34165.0392	34165.039	307828.733
13	Linearity 110%(44 PPM)		n.a.	n.a.	n.a.	51123.197	461811.926
16	Linearity 120%(48 PPM)	6	48.0000	40842.8602	40842.8602	40842.860	368538.980
17	Linearity 150%(60 PPM)	7	60.0000	51053.7029	51053.7029	51053.703	459543.631
19	Linearity 110%(44 PPM) pre_2	5	44.0000	37877.5808	37877.5808	37877.581	343044.973

CONCLUSION:

From the above results, it can be conclude that the method is found to be linear within the predefined limits.

PRECISION

System precision

To demonstrate system Precision the standard solution prepared as per test procedure and injected into the HPLC system as per methodology. The system Precision parameter was evaluated and found to be within the acceptance criteria. The % RSD for Omeprazole peak areas from ten replicate injections of standard solution was found to be within the limits. The results are summarized in Table-6.

Table 6: System Precision

System Precision Parameter	Observed value
The % Relative Standard Deviation (RSD) of Omeprazole peak areas from ten replicate injections.	0.1%
Recovery of check standard preparation against standard preparation.	99.80%

Method Precision (Repeatability)

Prepared six sample solutions of Omeprazole as per test procedure and injected each solution in to HPLC system as per methodology. The results are summarized in Table-7 and the test sample chromatogram is presented in Figure-14.**Acceptance Criteria:**

% RSD for assay results of six replicate preparations should be NMT 2.0.

Table 7: Method Precision (Repeatability)

Sample No.	% Assay
1	104.5
2	105.0
3	103.8
4	104.8
5	103.7
6	104.2
Average	**104.3**
% RSD (NMT 2.0)	**0.5**

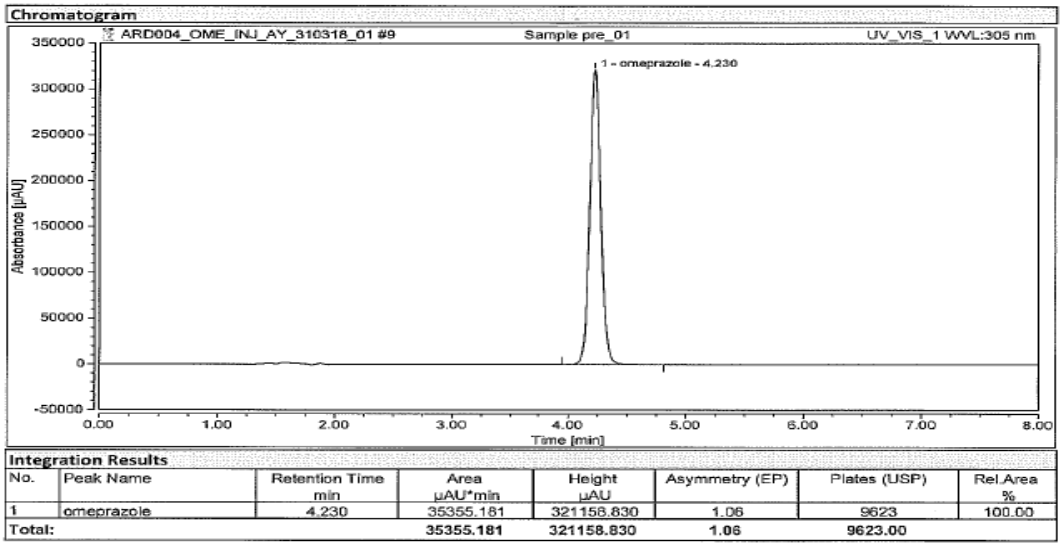


Figure: 13: Chromatogram of Sample – Repeatability

CONCLUSION: From the above results, it can be conclude that the method is found to be precise within the predefined limits.

Intermediate Precision

Perform the intermediate precision of same batch of samples analyzed by method precision by different analyst on different day, different column and on different instrument.

Prepared six sample solutions of Omeprazole Sodium for Injection 40mg/vial per test procedure and injected each solution in to HPLC system as per methodology.

The % assay and % RSD and overall % RSD were calculated and the results were found to be within the acceptance criteria. The results are summarized in Table-8 to 10 and the test sample chromatogram is presented in Figure-15.

Table 8: Intermediate Precision

HPLC System I.D No.	HPLC System – 1	HPLC System - 2
	VBPL-ARD004	VBPL-ARD002
Column I.D No.	Column – 1	Column - 2
	CLC003	CLC080

Table: 9 System suitability details

System Suitability Parameters	**Observed value**		**Acceptance Criteria**
	Method precision	**Intermediate Precision**	
The USP Asymmetry for Omeprazole peak from standard solution.	1.07	1.01	NMT 2.0
The USP Theoretical plates for Omeprazole peak from standard solution.	9773	12005	NLT 2000
The % RSD of peak areas of Omeprazole from five replicate injections of Standard solution.	0.1	1.2	NMT 2.0

Table 10: Intermediate Precision-for Omeprazole Assay

Sample No.	**% Assay**	
	Method Precision	**Intermediate Precision**
1	104.5	101.0
2	105.0	101.3
3	103.8	100.8
4	104.8	100.4
5	103.7	101.4
6	104.2	101.5
Average	**104.3**	**101.1**
Standard deviation	**0.5279**	**0.4179**
% RSD (NMT 2.0)	**0.5**	**0.4**
Overall % RSD (NMT 2.0)	**1.7**	

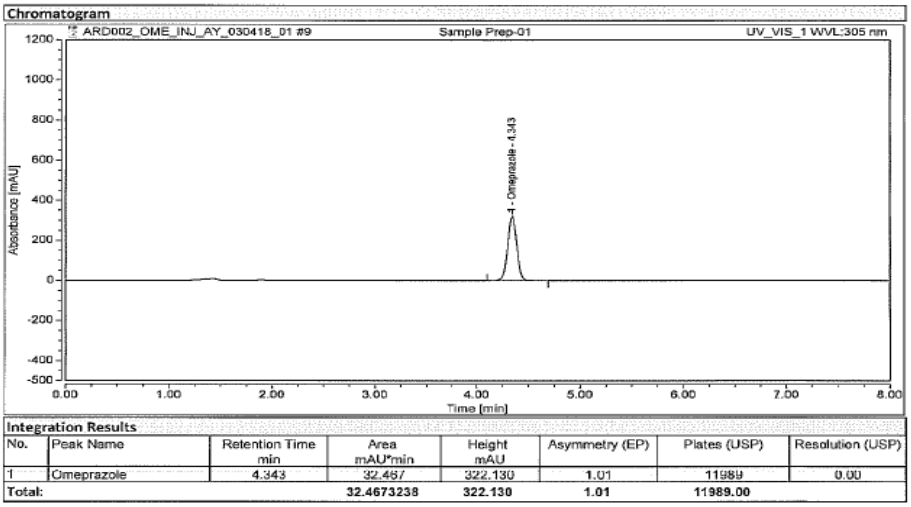


Figure-13: Chromatogram of Sample – Intermediate Precision

Note: For Analyst-1 results from Repeatability.

Conclusion: Based on the results, it can be conclude that when the samples are analyzed by different analyst on different days by using different instruments and columns, the obtained results are comparable and meeting the acceptance criteria for both individual as well as cumulative. Hence it can be concluded that the method is found to be precise and rugged.

Accuracy

Accuracy study was conducted by preparing samples by spiking Omeprazole at different concentrations (50 % to 150 %) with respect to test concentrations and kept placebo concentration constant as in target of sample in test method. Prepared 50%, 75%, 100%, 125% and 150% levels for Omeprazole. Prepared the recovery samples in triplicate for 75%, 100% and 125% levels. Prepared six recovery sample preparations at 50 % and 150 % levels. The prepared samples were injected in to HPLC system as per methodology.

Acceptance criteria is "The method is considered to be "ACCURATE" if the average recovery is between 98.0 to 102.0 %".

Calculated the mg added, mg found, % recovery and also calculated the % RSD of lower level (50 %) and higher levels (150 %) to prove the precision. The results are summarized in Table-11 and 12.

Table 11: Accuracy for Omeprazole

Recovery level	Sample No.	µg added	µg found	% Recovery	Average % Recovery	% RSD
50 %	1	20.0131	20.2807	101.3	**101.5**	**0.4**
	2	20.0131	20.4594	102.2		
	3	20.0131	20.3759	101.8		
	4	20.0131	20.2174	101.0		
	5	20.0131	20.2692	101.3		
	6	20.0131	20.2992	101.4		
150 %	1	60.0393	60.1818	100.2	**99.9**	**0.3**
	2	60.0393	59.8090	99.6		
	3	60.0393	59.8606	99.7		
	4	60.0393	60.2486	100.3		
	5	60.0393	59.7830	99.6		
	6	60.0393	59.9887	99.9		

Table 12: Accuracy for Omeprazole

Recovery level	Sample No.	µg added	µg found	% Recovery	Average % Recovery (Limit: 98.0 to 102.0)
75 %	1	30.0264	30.4394	101.4	**101.2**
	2	30.0264	30.2786	100.8	
	3	30.0264	30.4631	101.5	
100%	1	40.0351	40.4997	101.2	**101.1**
	2	40.0351	40.4572	101.2	
	3	40.0351	40.0351	100.9	
125%	1	50.0328	50.6010	100.8	**100.8**
		50.0328	50.2560	100.1	
		50.0328	50.4107	100.4	

CONCLUSION:

From the above results, the test method was found to be accurate from 50 % to 150 % of target concentration.

Range

Range of analytical method can be obtained from the linearity, precision and accuracy data.

Acceptance criteria:

Report the range in % with respect to test concentration.

Conclusion:

It can be concluded from the linearity, precision and accuracy experiments, that the range of analytical method for the assay of Omeprazole in Omeprazole Sodium for Injection 40mg/vial is 50% to 150% of test concentration.

Solution Stability:

Preparation of standard solution: Prepared standard solution as per test method.

Preparation of sample solution: Prepared sample solution as per test method.

Bench Top Stability of Standard and Sample Solution

A study to establish bench top stability of Omeprazole in sample solution and standard solution was conducted at Initial, Day 1 and Day 2. The assay of Omeprazole in sample solution and standard solution was estimated against freshly prepared standard at each time. The difference in % Assay of standard and sample solutions from initial to Day 1 and Day 2 was calculated against freshly prepared standard at each time and results were summarized in Table-13.

Table 13: Bench Top Stability of Standard and Sample Solution-for Omeprazole

Time in days	% Assay of Standard preparation	Difference from initial (NMT 2.0)	% Assay of sample preparation	Difference from initial (NMT 2.0)
Initial	99.2	NA	104.0	NA
1	99.3	0.1	103.1	-0.9
2	98.5	-0.7	102.3	-1.6

Refrigerator Stability of Standard and Sample Solution

A study to establish refrigerator stability of Omeprazole in sample solution and standard solution was conducted at Initial, Day 1 and Day 2. The assay of Omeprazole sample solution was estimated against freshly prepared standard at each time. The % difference of standard and sample solutions from initial to Day 1 and Day 2 was calculated against freshly prepared standard at each time and results are summarized in Table-14.

Table 14: Refrigerator Stability of Standard and Sample Solution-for Omeprazole

Time in days	% Assay of Standard preparation	Difference from initial (NMT 2.0)	% Assay of sample preparation	Difference from initial (NMT 2.0)
			Sample	**Sample**
Initial	99.2	NA	104.0	NA
1	99.6	0.4	103.4	-0.6
2	98.5	-0.7	103.2	-0.8

Conclusion: From the above data it was concluded that the standard solution and sample solutions were stable up to 2 days on bench top condition and in refrigerator condition.

Bench Top Stability of Mobile Phase

A study to establish the bench top stability of mobile phase was conducted at initial, Day 1, and Day 2. The mobile phase was prepared as per the test method and kept on bench top in well closed condition. Standard solution was prepared as per test method and injected into HPLC system with the same mobile phase at initial, Day 1, and Day 2. The system suitability parameters from initial were evaluated and found to be within the limits. The results are summarized in Table-15.

Table 15: Bench top stability of mobile phase

System Suitability Parameters	Initial	Day 1	Day 2	Acceptance criteria
The USP Asymmetry for Omeprazole peak from standard solution	1.06	1.05	1.05	NMT 2.0
The USP plate count for Omeprazole peak from standard solution	9860	9817	9912	NLT 2000
The % RSD of peak areas of Omeprazole from five replicate injections of Standard solution	0.1	0.2	0.1	NMT 2.0

Conclusion: From the above data it was concluded that the mobile phase was stable up to 2 days on bench top.

Robustness

A study was conducted to determine the effect of variation in chromatographic conditions of the test method. Sample solution (in duplicate) were prepared as per test method. All the solutions were injected in to the HPLC system at one variable condition at a time. The results are summarized in Table 16.

Table 16: System suitability at robustness variation conditions-for Omeprazole

Robustness/Parameters	Asymmetry		Plate count	% RSD	Recovery of check Standard	%Assay	Diff.
	Flow variation (mL/minute)						
Test method flow	1.0	1.10	9517	0.1	100.4	104.7	NA
Low flow	0.9	1.09	9842	0.1	100.1	104.6	0.1
High flow	1.1	1.10	8881	0.1	100.0	104.7	0.0
	Column oven temperature variation (°C)						
Test method temperature	30	1.10	9517	0.1	100.4	104.7	NA
Low temperature	25	1.14	9036	0.1	99.9	104.9	0.2
High temperature	35	1.07	9333	0.1	100.0	104.9	0.2
	Organic composition (Acetonitrile in Mobile phase)						
Test method organic Acetonitrile (MP)	35	1.10	9517	0.1	100.4	104.7	NA
Low organic Acetonitrile (MP)	31.5	1.08	9533	0.1	100.0	104.8	0.1
High organic Acetonitrile (MP)	38.5	1.11	9008	0.1	99.9	105.0	0.3

Acceptance criteria

- The USP Asymmetry for Omeprazole peak from standard solution should be NMT 2.0
- The USP plate count for Omeprazole peak from standard solution should be NLT 2000
- The % RSD of Omeprazole peak areas from five replicate injections of standard solution should be NMT 2.0.
- Recovery of check standard against standard preparation should be in between 98.0 to 102.%
- Difference between % assay of test method and variation condition should be NMT 2.0.

Conclusion:

From the above results it can be concluded that the method is robust with respect to flow rate, column oven temperature and mobile phase organic composition.

CONCLUSION

The test procedure was validated for specificity, linearity, precision, accuracy, solution stability and robustness were found to meet the predefined acceptance criteria.

The validated method was specific, linear, precise, stable and robust for the Assay of Omeprazole in Omeprazole sodium for Injection 40 mg/vial.

Hence the test procedure can be used for its intended purpose.

ACKNOWLEDGMENTS

This research was supported by Virchow Biotech Private Limited.

Virchow Biotech Private Limited

Survey No. 172, Gagillapur (V),Dundigal (Mndl), Medchal Dist., Telangana-500043.

CONFLICT OF INTEREST

There was no conflict of interest.

REFERENCES

1. Roberto Cirilli[1], Rosella Ferretti, Bruno Gallinella, Emiliana De Santis, Leo Zanitti, Francesco La Torre. High-performance liquid chromatography enantioseparation of proton pump inhibitors using the immobilized amylose-based Chiral peak IA chiral stationary phase in normal-phase, polar organic and reversed-phase conditions. J Chromatogr A. 2008; 1177(1):105–13.
2. ICH Harmonised Tripartite Guideline.Validation of analytical procedures:text and methodology, Q2(R1),USA,2005:1-13.
3. ICH Guideline:Validation of analytical procedures: text and methodology, in Proceedings of International Conference on Harmonization,Q2(R1), Geneva, Switzerland, 2005.
4. Harshal, K.T. and Mukesh, C.P. Development and Validation of a precise single HPLC Method for Determination of Omeprazole and its related compound in pharmaceutical formulation. Indian journal of pharmaceutical sciences. 1997; 59(3):124-127.
5. Martindale.The Complete Drug Reference, Pharmaceutical Press, London, UK, 2005; 34:1536-5050.
6. Neil MJO, Ed., The Merk Index- An Encyclopedia of Chemicals, Drugs and Biological, Merck Research Laboratories.2006; 14.
7. L.L. Brunton, J.S. Lazo, and K. L. Parker. Goodman & Gilman's the pharmacological basis of therapeutics. McGraw-Hill Professional. 2005;11: 969.
8. P. S. Bonato and F. O. Paias, J. Braz. Chem. Soc.2004; 15(2):318.
9. S. Vyas, A. Patel, K. D. Ladva, H. S. Joshi, and A. H. Bapodra, J. Pharm. Bioallied. Sci.2011; 3(2):310.
10. Kalakonda Sri Nataraj1, Mohammad Badrud Duza1, Kalyani Pragallapati1, Dussa Kiran Kumar. Development and validation of RP-HPLC method for the estimation of omeprazole in bulk and capsule dosage forms. International current pharmaceutical journal.2012; 1(11):366-369
11. Fabio S. Murakami, Ariane P. Cruz, Rafael N. pereira. Development and Validation of a RP-HPLC Method to Quantify Omeprazole in Delayed Release Tablets. Journal of liquid chromatography and related technologies. 2007; 30(1):113-12.