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A Validated StabilityIndicating High Performance Liquid Chromatographic Assay Method to Investigate Stability of Omeprazole Injection in Injectable Solutions (5 % Dextrose and 0.9% Sodium Chloride)

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Abstract
Intravenous Omeprazole injection is an alternative short-term treatment for patients (with reflux oesophagitis, duodenal and benign gastric ulcers) who cannot take Omeprazole orally. The stability of reconstituted solution in 5% Dextrose and 0.9% Sodium Chloride is critical before intravenous infusion. An improved isocratic, reversed-phase liquid chromatographic (HPLC) method was developed and validated for the determination of Omeprazole. Each reconstitution solution was kept under storage at room temperature (25°C ±2°C) and analysed as per method at 0,4,8,12,18 & 24 hours. Separation was achieved with ACE C8, 250 x 4.6mm, 5µ column and flow rate of 1mL min^{-1}. UV detection was performed at 302 nm. The data analysis concluded that novel stability-indicating assay method is suitable to assess stability and reconstituted solutions of Omeprazole injection are stable for up to 24 hours at 25°C.

Keywords-Omeprazole injection• 5% Dextrose injection• 0.9% Sodium Chloride injection• Chemical and Physical Stability• Stability-indicating HPLC assay method

INTRODUCTION

Omeprazole is a substituted benzimidazole, 5-methoxy2-[[(4-methoxy-3, 5-dimethyl-2-pyridinyl) methyl] sulfinyl] 1 *H*-benzimidazole (Fig. I), a compound that inhibits gastric acid secretion in the stomach by specific action on the proton pumps of the parietal cells. Its empirical formula is $C_{17}H_{19}N_3O_3S$, with a molecular weight of 345.42. . Omeprazole is white to off-white crystalline powder that melts with decomposition at about 155°C. It is a weak base, freely soluble in ethanol and methanol, and slightly soluble in acetone and isopropanol and very slightly soluble in water. The stability of Omeprazole is a function of pH it is rapidly degraded in acid media, but has acceptable stability under alkaline conditions [1].

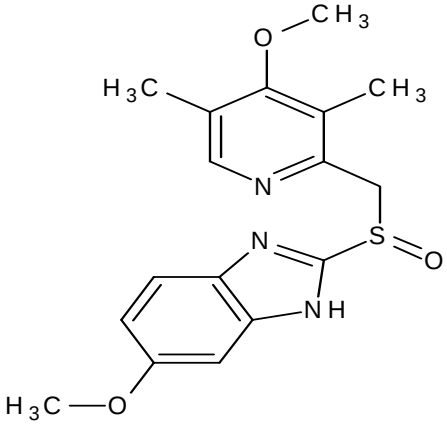

Fig I: Structure of Omeprazole

The concentration of Omeprazole injection after reconstitution to Dextrose and Sodium chloride solutions is extremely low. The literature survey resulted into absence of guidance and method for the quantitation of Omeprazole injection after dilution. Ribani [2] et al (2008) developed and validated a chromatographic method for quantification of Omeprazole in Tablets. Lobenhoffer et al (2007) [3] developed LC-MS/MS method for quantitation of Omeprazole and metabolites in human plasma, Shimizu et al (2006) [4] developed HPLC method for quantitation of Omeprazole and metabolite in human plasma. Bonato et al (2003) [5] developed chiral HPLC method for enantiomeric separation of Omeprazole. Rajic et al (2003) [6] developed UV method for quantitation of Omeprazole and impurities. Subramanian et al (2007) [7] developed method for quantitation of Omeprazole and Domperidone in Tablets. Methods with Spectroscopic techniques, Electrochemical methods, HPLC, LC-MS , Supercritical fluid chromatography & TLC are described in literature reference [8], Omeprazole determination from Plasma by LC-MS method is described in reference by Ute Hoffman et al [9] and Kanazawa et al [10]. Omeprazole determination in rat plasma by HPLC is described in reference by Huijian Jia et al [11]. The other references Rezk et al (2006) [12], Dubuc et al (2001) [13], Tata and Bramer et al (1999) [14] developed method for quantitation of Omeprazole and metabolites in human plasma. Jane et al (2006) reported method for Omeprazole sodium and Pantoprazole sodium stability in infusion [15].

Omeprazole injection is currently marketed in glass vial of strength 40mg. A series of stability studies was conducted to evaluate the stability of Omeprazole injection in 5% Dextrose injection and 0.9% Sodium chloride injection. The reversed phase High-Performance liquid chromatographic (HPLC) assay method was developed and validated as per ICH guideline Q2 (R1) [16] over the concentration range after dilution in the diluents (i.e. concentration of 0.4 mg/ml, it was further diluted for analysis purpose to 0.02mg/ml).

MATERIALS AND METHODS

Materials

Materials like Omeprazole drug substance, Omeprazole injection, The impurities 5-Methosy-1H-Benzoimidazole-2-Thiol(Impurity A), 5-Methoxy-{[(4-Methoxy-3,5-Dimethyl-Pyridine-2-yl)methyl]sulphonyl}-1H-Benzoimidazole (Impurity D) and 4-Methoxy-2{[(RS)-5-Methoxy-1H-Benzoimidazole-2-yl)sulphonyl]methyl}-3,5-Dimethylpyridine 1 oxide (Impurity E) were obtained from Cadila Healthcare ltd. All chemicals required to perform the analytical research were obtained as HPLC grade reagents from various sources. The HPLC column of brand ACE C8 was used. HPLC instrument of Agilent 1200 Series with VWD detector and Waters instrument with Photo-diode array detector was used.

Sample preparation and storage

Two vials of Omeprazole injection (strength 40 mg per vial) were taken and reconstituted with 10ml of 5% Dextrose injection and 0.9% Sodium chloride injection separately. This reconstituted solution then transferred to two separate 100 ml volumetric flasks and diluted to volume with respective diluents. The concentration of Omeprazole in each solution was around 0.4 mg/ml. This solution was subsequently diluted to obtain concentration of Omeprazole for assay method as 0.02 mg/ml. A quantification of Omeprazole was done immediately after the preparation of each solution and subsequent assays were performed after storage for 4, 8, 12, 18 & 24 hours at room temperature 25°±2°C.

Method development

A novel assay method was developed by taking into consideration the aqueous solubility and UV absorbance at 302 nm of Omeprazole. The reverse-phase was preferred as an elution mode [17]. The commonly employed Potassium dihydrogen phosphate solution (0.025M) was selected as a buffer and the pH of buffer solution was adjusted to 7.0 by using 10% Sodium hydroxide solution. Acetonitrile was used as an organic solvent to modify the polarity of mobile phase. The octysilane (C8) stationary phase column of Brand ACE C8 with 250mm length and 4.6mm diameter was used. The particle size of stationary phase was 5µ. The column temperature was kept at 30°C. The flow rate was kept as 1 ml/min. Injection volume of 20µl was used. Different compositional trials of buffer and acetonitrile were conducted to optimize the elution pattern of Omeprazole peak. The optimized mobile phase composition was Acetonitrile-Potassium phosphate (0.025 M) (32:68, v/v).

The known impurities 5-Methosy-1H-Benzoimidazole-2-Thiol(Impurity A, Fig II), 5-Methoxy-{[(4-Methoxy-3,5-Dimethyl-Pyridine-2-yl)methyl]sulphonyl}-1H-Benzoimidazole (Impurity D, Fig III) and 4-Methoxy-2{[(RS)-5-Methoxy-1H-Benzoimidazole-2-yl)sulphonyl]methyl}-3,5-Dimethylpyridine 1 oxide (Impurity E, Fig IV) of Omeprazole were checked for elution in the assay method. All impurity peaks are well separated from the Omeprazole peak.

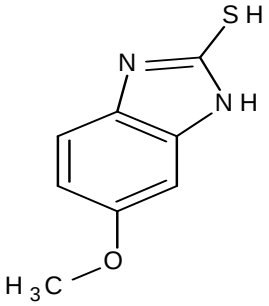

Fig II: Structure of 5-Methoxy-1H-Benzoimidazole-2-Thiol (Impurity A)

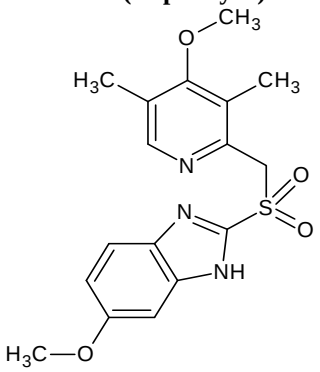

Fig III: Structure of 5-Methoxy-{[(4-Methoxy-3,5-Dimethyl-Pyridine-2-yl)methyl]sulphonyl}-1H-Benzoimidazole (Impurity D)

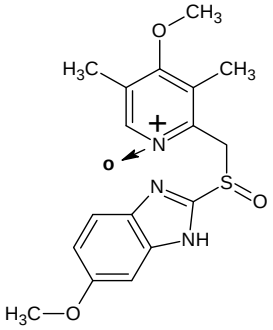

Fig IV: Structure of) 4-Methoxy-2{[(RS)-5-Methoxy-1H-Benzoimidazole-2-yl)sulphonyl]methyl}-3,5-Dimethylpyridine 1 oxide (Impurity E)

The Omeprazole working standard injection was injected five times to check the system suitability performance. The standard injections were followed by Omeprazole injections in 5% Dextrose injection and 0.9% Sodium chloride injection for the respective storage stability time points of Initial, 4, 8, 12, 18 & 24 hours.

The concentration of Omeprazole (expressed as %) in each sample was calculated by using the equation.

% of Omeprazole

$$= (A_{sample}/ A_{standard}) \times (W) \times (D_{sample})/(D_{standard}) \times (P/100) \times 100/Dose$$

Where, A_{sample} and $A_{standard}$ are the peak areas of Omeprazole in sample and standard Omeprazole preparations respectively. W is the mg of Omeprazole taken, D_{sample} and $D_{standard}$ are dilutions of test and standard preparations, P is the % potency of Omeprazole and Dose strength is 40 mg.

System Suitability

The Omeprazole working standard injection was injected five times to check the system suitability performance. In this the Omeprazole peak area response for five injections was precise (within 2 % Relative Standard Deviation).

RESULTS AND DISCUSSION

Method Validation of the assay method

The assay method to quantitate Omeprazole in 5% Dextrose injection and 0.9% Sodium chloride injection was validated as per ICH guideline Q2 (R1) [15] to demonstrate that it is suitable for its intended purpose. The method was validated for performance characteristics like accuracy, precision, specificity (stability-indicating capability), linearity, solution stability and robustness. Method was not evaluated for its limit of Detection and limit of Quantitation as it determines the major constituent (i.e. Omeprazole) in a solution.

Precision

The precision of an assay method was evaluated through analyses of six replicate preparations in diluents at 0.4mg/ml level. Samples at target concentration were independently prepared and analyzed after further dilution to 0.02mg/ml according to the method, and the results of these analyses are summarized in Table 1. The precision of the assay values (as measured by the relative standard deviation of the six independent preparations) was well within 2% for each diluent (5% Dextrose injection and 0.9% Sodium chloride injection).

Table 1, Results of Precision studies

Precision (n=6)	Mean assay (%) / % R.S.D
Set 1 in 5 % Dextrose	98.6 / 0.4
Set 2 in 0.9 % Sodium Chloride	99.0 / 0.5

Accuracy of the method

Accuracy solutions of the Omeprazole injection were prepared by spiking with known concentrations of Omeprazole at target concentrations of 50, 100 & 150% of the intended working concentration of the method. The solutions were analyzed by HPLC method and concentration of each solution was determined. The accuracy was evaluated as the percentage recovery from these solutions. The accuracy results are reported in Table 2 & 3. The average recovery observed between 98- 102 % over the range studied.

Table 2, Results of Accuracy experiment in 5% Dextrose

Accuracy level	Amount Spiked (mg)	Amount Recovered (mg)	% Recovery
Level 1(50)(n=3)	19.05	18.79	98.64
Level 2(100)(n=3)	38.69	38.07	98.40
Level 3(150)(n=3)	58.97	58.18	98.66
Mean % Recovery(n=9)± R.S.D	98.57± 0.147		

Table 3, Results of Accuracy experiment in 0.9% Sodium Chloride

Accuracy level	Amount Spiked (mg)	Amount Recovered (mg)	% Recovery
Level 1(50)(n=3)	19.08	18.81	98.58
Level 2(100)(n=3)	39.33	38.92	98.97
Level 3(150)(n=3)	59.87	59.48	99.35
Mean % Recovery(n=9)± R.S.D	98.97± 0.389		

Specificity

The specificity of an assay method was evaluated by preparing the placebo solutions of Omeprazole injection and by preparing forced degradation solutions of Omeprazole injection (Acid and Alkali Hydrolysis, Oxidation with Peroxide, Thermal degradation, Photolytic degradation and moisture hydrolysis) and analyzing by HPLC. There were no interfering peaks observed at the retention time of Omeprazole (peak purity analysis performed) proving the assay method as specific to quantitate Omeprazole in 5% Dextrose injection and 0.9% Sodium chloride solutions. The known impurities of Omeprazole were checked for elution in an assay method. All impurity peaks are well separated from the Omeprazole peak indicating Specificity of the method. The impurities 5-Methosy-1H-Benzoimidazole-2-Thiol(Impurity A), 5-Methoxy-{[(4-Methoxy-3,5-Dimethyl-Pyridine-2-yl)methyl]sulphonyl}-1H-Benzoimidazole (Impurity D) and 4-Methoxy-2{[(RS)-5-Methoxy-1H-Benzoimidazole-2-yl)sulphonyl]methyl}-3,5-Dimethyl pyridine 1 oxide (Impurity E) were evaluated for Specificity of method. The results are summarized in table 4.

Table 4, Results of forced degradation studies for assay method

Degradation condition	% Omeprazole content
Acid Hydrolysis	96.7
Alkali Hydrolysis	96.7
Oxidation	90.
Thermal Degradation	99.4 1
Photolytic Degradation	98.9
Moisture Degradation	98.7

Linearity

The linearity of method was evaluated by performing the linear regression analysis between the analytical results and known concentrations of Omeprazole in test solutions prepared for linearity studies. The results are tabulated in Table 5 & 6. The correlation coefficient of 0.999 in each of 5% Dextrose injection and 0.9% Sodium chloride injections obtained during validation indicates that the assay method has an acceptable linearity.

Table 5, Regression analysis in 5% Dextrose

Linearity (n=5)	Results
Range (mg/ml)	0.01004-0.03147
Slope	45889.21
Intercept	7.05
Correlation coefficient	0.999

Table 6, Regression analysis in 0.9% Sodium Chloride

Linearity (n=5)	Results
Range (mg/ml)	0.01004-0.03147
Slope	47475.97
Intercept	5.51
Correlation coefficient	0.999

Robustness

The robustness of the method was determined by small deliberate changes in flow rate, pH of mobile phase, temperature and mobile phase composition. The peak area of five replicate injections in all these changed conditions remained within the system precision limit of % RSD of not more than 2.0% indicating that the method is robust. The summary results is reported in Table 7.

pH measurements

The pH of the reconstituted solutions was determined using a glass combination electrode. The pH measurement was performed immediately after preparation of the solutions and of each storage sample for 24 hours. The pH measurements are tabulated in Table 8

Table 8, pH measurements

Time of Stability (in hours)	pH of Omeprazole injection in 5% Dextrose	pH of Omeprazole injection in 0.9% Sodium Chloride
Initial	9.88	9.61
4	9.82	9.59
8	9.80	9.57
12	9.77	9.54
18	9.72	9.54
24	9.66	9.52

Table 7, Summary of Robustness studies

Parameter modified	Condition setting	% RSD of peak response for Omeprazole	Retention time of Omeprazole peak (in minutes)
Temperature	30°C	0.07	10.3
	25°C	0.07	10.3
	35°C	0.05	10.1
pH	7.0	0.15	9.4
	6.8	0.17	8.8
	7.2	0.07	10.0
Flow rate	1.0 ml/min	0.07	10.3
	0.8 ml/min	0.10	12.8
	1.2 ml/min	0.06	8.1
Mobile phase composition	Buffer 68 : ACN32	0.07	10.3
	Buffer 70 : ACN30	0.05	10.8
	Buffer 66 : ACN34	0.13	10.1

Stability study results

The results of stability studies in each 5% Dextrose injection and 0.9% Sodium chloride injection solutions are summarized in Table 9 and HPLC chromatogram obtained is shown in (Fig V). The concentration of Omeprazole in both diluents was not dropped below 98% by the end of 24 hours storage period proving chemical stability of reconstituted solutions.

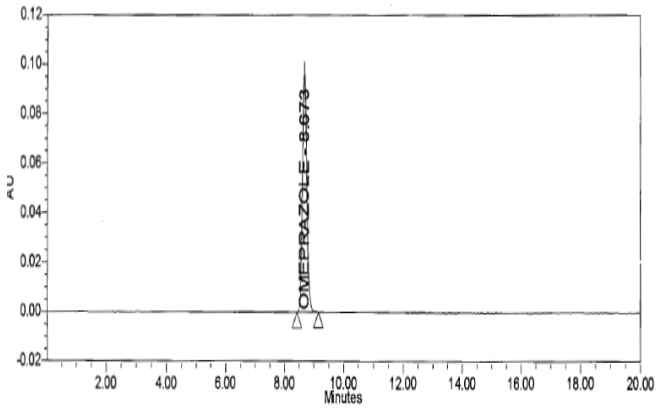

Fig V: HPLC chromatogram of Omeprazole injection

Table 9, Stability study results

Stability time (in hours)	Content of Omeprazole injection in 5% Dextrose	Content of Omeprazole injection in 0.9% Sodium Chloride
Initial	99.4	99.2
4	98.7	97.8
8	98.0	97.7
12	97.5	97.9
18	97.3	97.8
24	98.4	97.4

CONCLUSION

The novel HPLC stability-indicating assay method developed conforms to validation parameters as per ICH guideline and is suitable to quantitate the stability assay of Omeprazole in injection diluents of 5% Dextrose injection and 0.9% Sodium chloride injection.

As the Omeprazole content in each of 5% Dextrose injection and 0.9% Sodium chloride injection solution over storage period of 24 hours was within the stability acceptance criteria of NLT 97.0% Omeprazole content, it was concluded that Omeprazole is stable in each of 5% Dextrose injection and 0.9% Sodium chloride injection.

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