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Development and validation of RP-HPLC method for the estimation of omeprazole in bulk and capsule dosage forms

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ABSTRACT

A method for the determination of omeprazole in bulk and capsule dosage form by reverse phase high performance liquid chromatography has been developed. This is a simple, rapid, precise and an accurate method. The method was developed on a Novapak C_{18}, (250 x 4.6 mm, 5µ) column using phosphate buffer (pH 7.4) and acetonitrile in the ratio of 60:40, v/v as a mobile phase which was pumped at a flow rate of 1.0 ml/min and detection was done at 302 nm. The retention time of the drug was found to be 7.71 min. The method was validated for accuracy, precision, linearity, specificity, robustness. The linearity was observed in the range of 20-60 ppm. The results of recovery studies indicated that the method was accurate. Hence the developed method was found to be suitable for the estimation of omeprazole in bulk and capsule dosage forms.

***Key Words*:** Omeprazole, RP-HPLC, determination, capsule dosage form, C_{18}, linearity.

INTRODUCTION

Omeprazole (Namrata singh *et al.*, 2012; Indian Pharmacopoeia., 2010; United States of Pharmacopoeia 2009) (RS)-6-methoxy-2-((4-methoxy-3, 5-dimethylpyridin-2-yl) methyl sulfinyl)-1H-benzo (d) imidazole is a proton pump inhibitor (PPI) and an anti-secretory compound. It suppresses gastric acid secretion by inhibiting the gastric H^+/K^+ATPase (hydrogen-potassium adenosine triphosphatase) at the secretory surface of the gastric parietal cell. Omeprazole has been characterized as a gastric acid-pump inhibitor, in that it blocks the final step of acid production. After rapid disappearance from plasma, Omeprazole can be found within the gastric mucosa for a day or more. It is freely soluble in ethanol and methanol, slightly soluble in acetone and isopropanol and very slightly soluble in water.

Omeprazole is useful in combination with the antibiotics clarithromycin and amoxicillin (or metronidazole in penicillin-hypersensitive patients) in the 7-14 day eradication triple therapy for Helicobacter pylori. Infection by H. pylori is the causative factor in the majority of peptic and duodenal ulcers. As it is a proton pump inhibitor it is used to treat ulcers, heartburn, gastroesophageal reflux, or Zollinger-Ellison syndrome. It works by blocking acid production in the stomach (Martindale: The Extra Pharmacopoeia, 2011).

Literature survey revealed that there are methods based on UV (Stephen *et al.*, 2006) HPLC (Harshal and Mukesh, 1997; Motevalian *et al.*, 2008; Dong-Seok Yim *et al.*, 2001; Cristina *et al.*, 2008; Inger *et al.*, 1986; Naser *et al.*, 2006; Kirti *et al.*, 2010) for the determination of omeprazole individually and in combination with other drugs in dosage forms and biological fluids. Here a new reverse phase high performance liquid chromatographic method has been proposed for the estimation of omeprazole in bulk and capsule dosage form.

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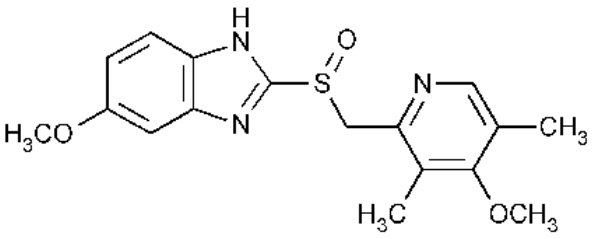

Figure 1: Structure of omeprazole.

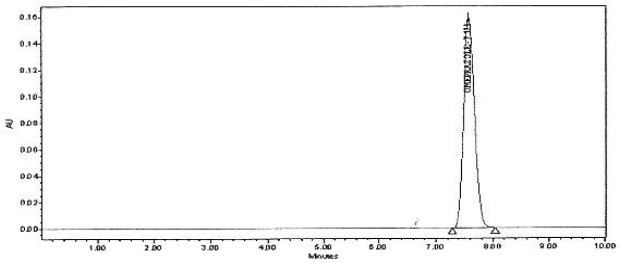

Figure 2: A typical chromatogram of omeprazole standard.

EXPERIMENTAL

Instruments and reagents

A prominence isocratic HPLC system (youngling HPLC YL 9000 series) with YL 9110 pump with autochro 3000 software and UV-VIS detector YL 9120 was used. Potassium dihydrogen orthophosphate and dipotassium hydrogen phosphate were of AR grade and acetonitrile and water of HPLC grade were used. A 20 µl hamitton injection syringe was used for sample injection.

Chromatographic conditions

A Novapak C_{18} (250 x 4.6mm, 5µ) column and a mobile phase comprising of phosphate buffer (pH 7.4) and acetonitrile in the ratio of 60:40, v/v were used. The flow rate was maintained at 1.0 ml/min.

Selection of analytical wavelength

From standard stock solution further dilutions were prepared using mobile phase and scanned over the range of 200-400 nm and spectrum was overlain. It was observed that 302 nm was λ_{max} of omeprazole and it was preferred as suitable wavelength for detection.

Selection of mobile phase and column

During the development the method was tried with various columns such as Kromasil C_{18} (150mm x 4.6 mm i.d, 5µm), Novapak C_{18}, (250 x 4.6 mm, 5µ) and various mobile phase compositions with methanol, water, phosphate buffer (pH 7.4) and acetonitrile and finally Novapak C_{18}, (250 x 4.6 mm, 5µ) was selected and phosphate buffer (pH 7.4) and acetonitrile in the ratio of 60:40, v/v was selected as mobile phase.

Preparation of mobile phase

Accurately weighed and transferred 2.72g of potassium dihydrogen orthophosphate and 0.525g of dipotassium hydrogen phosphate to a 1000 ml volumetric flask, 300 ml water was added and the volume was made up to 1000 ml with water. The pH was adjusted to 7.4. Buffer and acetonitrile were mixed in the ratio of 60:40, v/v. The solution was filtered through a 0.45 µm membrane filter and degassed.

Preparation of stock and standard solutions

40 mg of omeprazole was weighed and transferred to a 100 ml volumetric flask containing 30 ml of 0.1N NaOH. The drug was allowed to dissolve and the Volume was made up to 100 ml with 0.1 N NaOH. The above solution was diluted with mobile phase to obtain a working standard solution of 40µg/ml. The Blank solution was prepared and injected. The prepared standard solution was injected and the chromatogram was recorded. A typical chromatogram was shown in Figure 2.

Construction of calibration curve

Solutions containing 20-60 ppm of omeprazole were prepared. 20µl of each concentration were injected for 5 times and the mean area was calculated. A calibration plot was constructed between peak area and concentration. The regression equation obtained was y = 50417.3x + 2579.4 and the regression coefficient was found to be 1.0000. This equation was used later to estimate the amount of omeprazole in capsule dosage form.

Assay of omeprazole in capsule dosage form

Quantity of pellets (from a blend of 20 capsules) equivalent to 100 mg of omeprazole were weighed and transferred to a 250 ml Volumetric flask containing 150 ml of 0.1 N NaOH. The solution was sonicated for 15 min under controlled temperature not exceeding 30°C. The solution was made up to the Volume with 0.1 N NaOH. Centrifuge a portion of the sample at 3000 RPM for 15 minutes. The solution was diluted with the mobile phase to obtain a sample of 40µg/ml.

Table 1: Results of precision studies.

Amount of omeprazole (ppm)	Intra-day precision		Inter-day precision	
	Mean (n=5)	%RSD	Mean (n=5)	%RSD
30	29.90	0.501	29.80	0.268
40	39.78	0.274	39.76	0.150
50	49.77	0.301	49.81	0.160

Table 2: Results of accuracy studies.

Amount taken (ppm)	Mean % recovery	% RSD
20+10	99.10	0.35
20+15	99.48	0.45
20+20	99.47	0.22

Table 3: Linearity values of omeprazole.

Concentration in ppm	Omeprazole peak area
20.0040	1010996
30.0060	1517841
40.0080	2022465
50.0100	2511583
60.0120	3035496

Table 4: Results of robustness.

Result	Flow rate (ml/min)			pH of the buffer			Organic phase proportion of the mobile phase		
	0.9	1.0	1.1	7.2	7.4	7.6	55	60	65
Tailing factor	1.22	1.19	1.26	1.17	1.19	1.21	1.30	1.19	1.25
Retention time	8.12	7.71	7.5	7.69	7.71	7.7	7.7	7.71	8.1

Validation of the proposed method

The precision, accuracy, linearity specificity, robustness and system suitability parameters were studied systematically to validate the proposed RP-HPLC method for the estimation of omeprazole. Solutions containing 30, 40, 50 ppm of omeprazole were subjected to intra-day and interday precision. The results were furnished in Table 1. The accuracy was of the method was determined by performing accuracy studies. The results of accuracy studies were furnished in Table 2. Solutions ranging from 20-60 ppm were prepared and chromatograms were recorded by injecting the solutions. The mean area was calculated and a graph was constructed between concentration and peak area. The calibration plot was shown in figure 3. The results of linearity studies were shown in Table 3.

Specificity of the method was validated by forced degradation studies. The drug was subjected to acid degradation, base degradation, UV light stress, humidity stress. The method was found to be specific for the determination of omeprazole as no interference was observed from excipients and degradation products. The robustness of the method was studied by changing the organic phase proportion of mobile phase, flow rate, pH of the mobile phase. The results were furnished in Table 4.

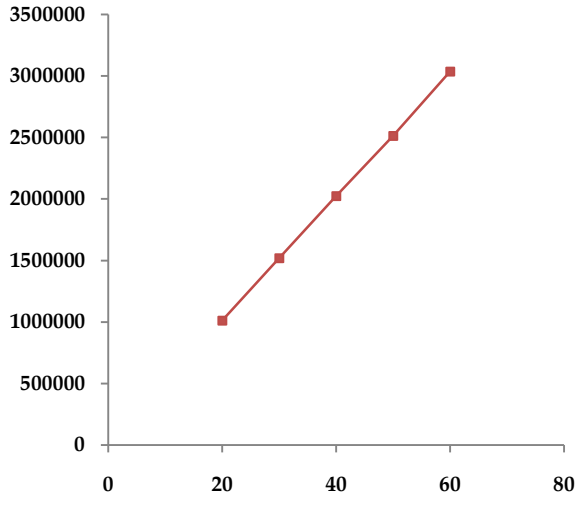

Figure 3: Calibration plot of omeprazole.

RESULTS AND DISCUSSION

In the present study a simple, reproducible and stability indicating RP-HPLC method has been developed for the determination of omeprazole by using a Novapak C_{18}, (250 x 4.6 mm, 5µ) and phosphate buffer of pH 7.4 and acetonitrile in the ratio of 60:40 v/v as mobile phase. The flow rate was maintained at 1.0 ml/min and the eluents were monitored at 302 nm. With the above parameters peaks were obtained with good resolution.

In the proposed method the retention time was found to be 7.71 min. The method was linear in the range of 20-60 ppm. The number of theoretical plates calculated was 7911, which is indicating efficient performance of the column. The high

percentage of recovery indicates that the method is highly accurate. No interference was observed from excipients and degradation products.

Thus the proposed HPLC method is rapid, precise and accurate for the estimation of omeprazole and can be reliably adopted for routine analysis of omeprazole in capsule dosage forms.

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