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DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND PIPERINE IN BULK FORM

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ABSTRACT

A new simple, accurate, rapid and precise isocratic High performance liquid chromatographic (HPLC) method was developed and validated for the determination of Omeprazole and Piperine in bulk form.The Method employs Waters HPLC system on XTerra RP8Column (4.6 x 150 mm and 3.5µm) and flow rate of 1ml/min with a load of 20µl. Acetonitrile and Phosphate buffer was used as mobile phase in the composition of 55:45. The Detection was carried out at 240nm. Linearity ranges for Omeprazole and Piperine were 10-50µg/mlrespectively. Retention Time of Omeprazole and Piperine were found to 2.767 min and Piperine 4.029 min respectively. Percent recovery study values of Omeprazole and Piperine were found to be within 98-102 %.This newly developed method was successfully utilized for the Quantitativeestimation of Omeprazole and Piperine in bulk form. This method was validated for accuracy, precision, linearity andRobustness as per ICH guidelines.

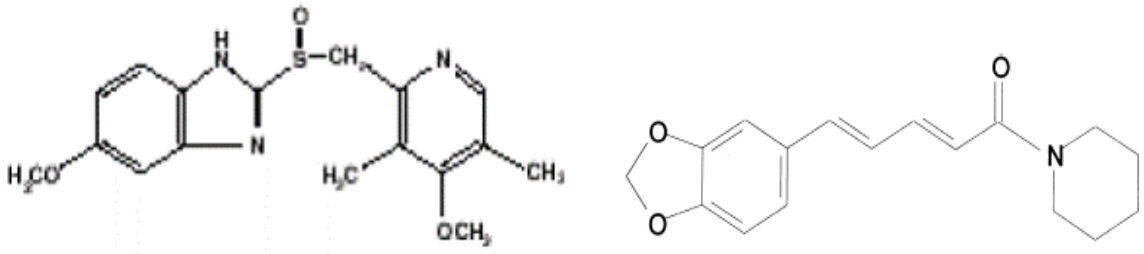
KEYWORDS: Omeprazole, Piperine, RP-HPLC, validation, simultaneous estimation.

INTRODUCTION

Omeprazole is a substituted benzimidazole and acts as proton pump inhibitor, which control gastric acid secretionby inhibition of gastric H +, K + -ATPase, the enzyme responsible for the final step in thesecretion of hydrochloric acid by the gastric parietalcell.[1] Omeprazole is an acid-labile lipophilicweak base (pKa, =4.2; pKa=9). Unprotectedexposure to acidic gastric contents results in inactivation of >50% of an oral dose (20-40mg daily) leading to poor

bioavailability.[3] Followingadsorption, omeprazole is eliminatedrapidly and almost completely metabolized in theliver. Eighty percent of the metabolites (omeprazolesulphoxide and hydroxy omeprazole) is excreted in the urine while the other 20% is excretedin the feces after biliary secretion.[2,3]

Piperine, a major alkaloid of black and long Peppers, possesses several pharmacological actions. In addition, it has been reported to enhance the bioavailability of several drugs.Recently, it has been patented as bioavailability enhancer, non-nicotine smoking cessation aid and as an important ingredient of incapacitating composition.[4,5]



Omeprazole **Piperine**

Fig 1: Chemical structures of Omeprazole and Piperine

The extensive literature survey carried out and revealed that there are no methods reported for the simultaneous estimation of these drugs. Hence an attempt was made to develop a specific, precise, accurate, linear, simple, rapid, validated and cost effective RP-HPLC method for the simultaneous estimation of Omeprazole & Piperine in bulk form.

MATERIAL AND METHODS

Instrumentation

A High performance liquid chromatography (WATERS) equipped with AutoSampler and UV detector with the software of EMPOWER Version 2. Allweighing's are done on single pan balance (Shimadzu).

Reagents and Standards

Omeprazole and Piperine standards were obtained from Piramal, Mumbai, India. Analytical grade methanol and Acetonitrilewere purchased from Merck Specialties Private Ltd., Mumbai. Double distilledwater was used throughout the experiment.

Optimized chromatographic conditions

Chromatographic conditions were optimized after using mobile phase, acetonitrile:buffer (Phosphate buffer: pH 6.5 ± 0.1) (55:45). The flow rate was 1ml/min and the detector was set at 240nm. The separation was achieved onXTerra RP8 Column (4.6 x 150 mm and 3.5µm).

Preparation of standard stock solution

Accurately weighed about 10 mg of Omeprazoleand 10 mg of Piperine working standards and transferred into 10ml cleandry volumetric flask, added about 7ml of Diluent and sonicated to dissolve itcompletely and make the volume up to the mark with Mobile phase (Stocksolution). Further pipette out 0.3 ml of the above stock solution (Omeprazole& Piperine) into a 10ml volumetric flask anddiluted up to the mark with same mobile phase to get the concentrations of 30µg/ml respectively. These stock solutions were filteredthrough 0.45µm membrane filter paper by using vacuum filter.

METHOD DEVELOPMENT

Inject 20 mL of the standard solution into the chromatographicsystem and measure the areas and system suitability parameters for the Omeprazole &Piperinepeaks (Fig.2).

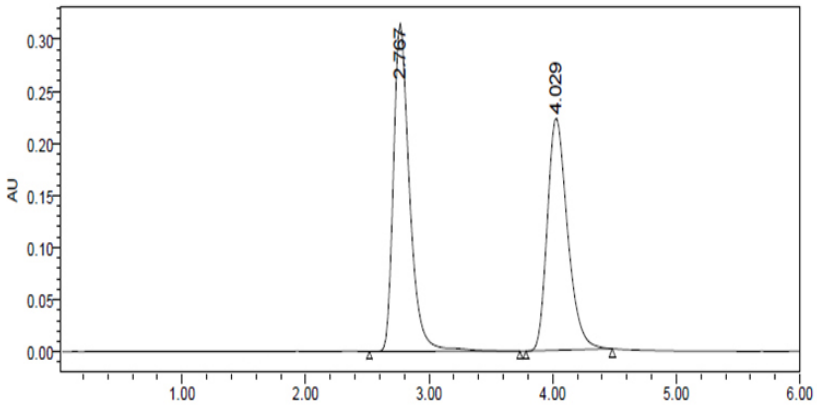


Fig 2: Chromatogram of Standard solution (Omeprazole 30µg/ml&Piperine30µg/ml)

VALIDATION OF ANALYTICAL METHOD

Precision

The precision of the method was performed by intra-day variation studies. In the intra-day precision, five repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated and present in **Table. 1.** In the same manner for the Inter –day precision, five repeated injections of standard solution were made and the response factor of drug peak and % RSD were calculated and present in **Table. 2.**

Table 1: Precision results for Omeprazole& Piperine

Injection	Area of Omeprazole	Area of Piperine
Injection-1	2849314	2534539
Injection-2	2860134	2539247
Injection-3	2861298	2544661
Injection-4	2863959	2548839
Injection-5	2874416	2558822
Average	2861824	2545221
Standard Deviation	8983.0	9330.0
%RSD	0.31	0.37

Table 2: ID Precision results for Omeprazole& Piperine

Injection	Area of Omeprazole	Area of Piperine
Injection-1	2837703	2540424
Injection-2	2837396	2545953
Injection-3	2800105	2552894
Injection-4	2864566	2514155
Injection-5	2859837	2558072
Average	2839922	2542300
Standard Deviation	25498.3	17102.9
%RSD	0.90	0.67

Linearity

The linearity study was made from a series of standard solutions of Omeprazole& Piperine. For Omeprazole & Piperine suitable volumes of stock solution of 1000µg/ml wasdiluted to obtain a series of solutions having concentrations of 10-50µg/ml of Omeprazole & Piperine.Each solution wasinjected and chromatograms were recorded. The peak areas were plotted againstconcentration to obtain calibration curves forOmeprazole& Piperine. Thecalibration curves were linear in the range 10-50µg/ml of Omeprazole & Piperine.

Table 3: Linearity results for Omeprazole& Piperine

S.No	Linearity Level	Omeprazole		Piperine	
		Conc. (µg/mL)	Area	Conc. (µg/mL)	Area
1	I	10	894043	10	920032
2	II	20	1913389	20	1752782
3	III	30	2906620	30	2521426
4	IV	40	3800672	40	3326009
5	V	50	4738193	50	4217393
Correlation coefficient		0.999		0.999	

ACCURACY

To check the accuracy of the method, recovery studies were carried out at three different levels of 80%, 100% and 120% solutions made from standard solutions of Omeprazole & Piperine and calculate the individual recovery and mean recovery values. The% mean recoveries were in between 99.7-99.9and were given in Table 4&5.

Table 4: Accuracy data for Omeprazole (analyte recovery)

% Concentration at specification level	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
80%	4306922	7.58	7.43	98.0%	99.7%
100%	5784168	10.0	9.98	99.8%	
120%	7162858	12.2	12.3	101.3%	

Table 5: Accuracy data for Piperine (analyte recovery)

% Concentration at specification level	Area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
80%	3769304	7.63	7.48	98.1%	99.9%
100%	5023657	10.0	9.98	99.8%	
120%	6311333	12.3	12.5	101.9%	

Detection limit and quantification limit

Limit of detection (LOD) and Limit of quantification (LOQ) were estimated from the signal-to-noise ratio. The detection limit was defined as the lowest concentration level resulting in a peak height of three times the baseline noise. The quantification limit was defined as the lowest concentration level that provided a peak height with a signal-to-noise ratio higher than 10.

LODand LOQ values of Omeprazole& Piperine were reported in Table.6&7.

Table 6: LOD results for Omeprazole&Piperine

Parameters	Omeprazole	Piperine
Concentration (µg/mL)	0.012	0.016
Retention time(R_t)	2.772	4.043
Height (µv)	128	132
Area	1147	1512

Table 7: LOQ results for Omeprazole & Piperine

Parameters	Omeprazole	Piperine
Concentration (µg/mL)	0.042	0.053
Retention time(R_t)	2.771	4.044
Height (µv)	429	427
Area	3844	4892

Robustness

The robustness of a method is its ability to remain unaffected by small deliberate changes in optimized method parameters like variation of flow rate (+1ml/min), mobile phase composition and temperature. There is no significant impact on retention time and peak area was found.

System suitability parameters

These are the tests to ensure that the method can generate results of acceptable accuracy and precision. System suitability was assessed by injecting mixedstandard preparation in replicate. Parameters such as Tailing factor, Theoreticalplates and resolution etc. were determined. The system suitabilityparameters for the method are listed below in the Table.8.

Table 8: LOD results for Omeprazole&Piperine

S.No	Parameters	Omeprazole	Piperine
1	Area	2849708	2534375
2	Retention time (R_t)	2.776	4.042
3	Resolution (R_s)	-	4.7
4	Tailing factor (T)	1.4	1.3
5	No. of theoretical plates (N)	2313	2979

RESULTS & DISCUSSION

The present study was carried out to develop a sensitive, precise and accurateRP-HPLC method for the analysis of Omeprazole and Piperine in bulk form. The retention times for Omeprazole & Piperine were found to be 2.767&4.029min respectively.Each standard was injected five times and the peak area for drugsolution was reproducible as indicated by low coefficient of variation. A good linear relationship (r = 0.999) was observed between the concentrations andrespective peak areas. Precision was determined and the results are presentedin the form of %RSD which is below 1.00 and shows that the proposed HPLC method was highly precise. The amount of drug recovered was shown inTable 4&5.The method was robust as observed from insignificant variation in theresults of analysis by changes in flow rate, Mobile phase composition andtemperature. The proposed reversed phase HPLCmethod was found to be simple, precise, highly accurate, specific and less timeconsuming.

CONCLUSION

Thus the proposed RP-HPLC method for the simultaneous estimation of Omeprazole & Piperine in bulk form was accurate,precise, linear, robust, simple & economic.

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