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METHOD DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF OMEPRAZOLE AND DICYCLOMINE HYDROCHLORIDE IN COMBINED TABLET DOSAGE FORM

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ABSTRACT

The main objective of the work is to develop a new, simple and economical method for the simultaneous estimation of omeprazole and dicyclomine hcl by using RP-HPLC technique In this method mobile phase of composition Phosphate buffer 0.02M,p^H6.5 and acetonitrile in the ratio of 65:35 was pumped with a flow rated of 1ml/min through C18 250mm column which was maintained at 25°C temperature. The wavelength 223nm was optimized as the both drugs have optimum absorbance. Volume of injection was 10µl. The retention time of the omeprazole and dicyclomine hcl was 2.02min and 9.93min respectively with appreciable resolution of 9.15. Using the above method the run was performed and the system suitability parameters were calculated and were within the limits. So the method was set for validation, the method was found to be specific in the determination of drug without any interference. The method was observed to be precisied as the %RSD was 0.59 and 0.63 for omeprazole and dicyclomine hcl respectively. Recovery studies were performed and found to be 99.45% for omeprazole and 100.35% for dicyclomine hcl. Calibration curve was plote and correlation coefficient was 0.999. The method was also found to be robust. The method was valid as all the parameters were passed. This method was precise, accurate and accurate, this method can be used in the regular assay of the formulations.

Key words: Omeprazole, Dicyclomine hcl, ICH guidelines, RP-HPLC.

INTRODUCTION

Omeprazole is used in the treatment of peptic ulcer, dyspepsia and gastro esophageal reflux. The IUPAC name is 6-methoxy-2-[(4-methoxy-3,5-dimethylpyridin-2-yl)methanesulfinyl]-1*H*-1,3-benzodiazole. Its molecular formula is $C_{17}H_{19}N_3O_3S$. omeprazole is soluble in water, methanol, and DMSO and its pKa(strongest acidic) value is Strongest basic 6.86. Dicyclomine hcl also known as dicycloverine is used in the treatment of irritable bowel syndrome to decrease the hypermotility of the intestine. IUPAC name of dicyclomine hcl was 2-(diethylamino) ethyl 1-cyclohexylcyclohexane-1-carboxylate. Its molecular formula is $C_{19}H_{35}NO_2$. dicyclomine hcl was soluble in water, ethanol, chloroform and ether and its pKa(strongest basic) value is 8.96. According to literature survey there was one method for the simultaneous estimation of omeprazole and dicyclomine hcl by Chaitany A. Dave et al., (2013). Many other methods were available for estimation of dicyclomine hcl and omeprazole individually and with other drug combinations.

EXPERIMENTAL WORK

Materials and reagents: Renol the formulation of omeprazole and dicyclomine hcl bought from the local retail shop. Bulk drugs omeprazole and dicyclomine hcl are gift samples from spectrum research solutions, all the solvents used in this method were HPLC grade and chemicals like orthophosphoric acid was of analytical grade.

Instruments: HPLC used in this method was of Hitachi lachrome model integrated with quaternary pumps delivery system. Sampling was done by

automatic sampler, detector was a photo diode array detector 2990, column maintained at required temperature by oven. Digisun ph meter7007, Labmann ultrasonic cleaner, shimadzu weighing balance.

Preparation of Buffer: Potassium dihydrogen phosphate buffer was prepared by dissolving 2.72grams of KH_2PO_4 in 700ml HPLC grade water, sonicated for 15min and made up to 1000ml with same water. pH was adjusted to 6.5 with dilute sodium hydroxide.

Preparation of standard working solution: About 25 mg of pure samples of Omeprazole and Dicyclomine hcl hydrochloride were accurately weighed and transferred into a 25ml clean dry volumetric flask containing mobile phase .The solution was sonicated to dissolve and the volume was made up to mark with mobile phase. 5 ml of above solution was diluted to 50 ml with mobile phase. From this 3ml of solution was taken into separate 10ml volumetric flask and made up the volume to the mark with mobile phase.

Preparation of sample working solution: 20 tablets were taken and average weight of tablet was found. Tablet powder weight equivalent to 25 mg of Omeprazole and Dicyclomine hcl hydrochloride were accurately weighed and transferred into a 25ml clean dry volumetric flask containing mobile phase .The solution was sonicated to dissolve and the volume was made up to mark with mobile phase. 5 ml of above solution was diluted to 50 ml with mobile phase . From this 3ml of solution was taken into separate 10ml volumetric flask and made up the volume to the mark with mobile phase which is 30ppm.

Chromatographic conditions: Twenty micro liters of the sample was injected and the chromatogram was developed using buffer: acetonitrile (65:35) as a mobile phase. The flow rate was maintained at 1ml/min and column temperature was maintained at 25°C. Total run time was fixed as 15 minutes. Detector wavelength was fixed at 223 nm.

Method Validation: Validation was performed for the developed method to confirm the standard of the method and whether performed method suites to estimate the omeprazole and dicyclomine hcl combination tablets in the regular assay.

Specificity: This study was to check whether the drug was estimated by the method without any interference. Blank, placebo and standard solutions were injected such that the retention time in the blank and placebo were interfering the retention time of the drugs.

Linearity: Six different concentrations were taken as beers range 10µg/ml, 20µg/ml, 30µg/ml, 40µg/ml, 50µg/ml, of omeprazole and dicyclomine hcl. calibration curve was obtained from the concentration versus response by the injection of particular concentrations.

Precision:

Repeatability: It was also called as intraday precision multiple injection of multiple sampling solutions with in the same day by the same person on the same instrument should give the agreements closer to each other. Working sample solution was prepared and 10µl of the sample was injected in the replicate of six. %RSD should be within the range.

Intermediate: It was also called as inter day precision multiple injection of multiple sampling solutions were injected in the replicate of six. The results obtained between the instruments, between the analyst or days, should have their results close to each other. %RSD should be within the range.

Accuracy: Accuracy represents the closeness of the agreement to the conventional true value. The pure drug was spiked to the placebo in three levels 80%, 100% and 120%. The resulted solutions were injected to the HPLC in a triplicate, manner. The % recovery should be calculated.

LOD: LOD was calculated by standard deviation method. The concentration at which the ratio of signal to the noise was 3:1 is called Limit of detection otherwise the minimum concentration that can be detected by the detector in this particular method.

LOQ: LOD was calculated by standard deviation method. The concentration at which the ratio of signal to the noise was 10:1 is called Limit of detection otherwise the minimum concentration that can be quantified by the detector with and accuracy and precision in this particular method.

Robustness: Small changes in the optimized method was done such as change in flow rate of 10% (±),change in temperature 5°C (±). Maintaining these conditions samples were injected and %RSD was reported.

System suitability: System suitability for that method was tested by six replicate injections of

standard preparation. Plate count, tailing factor, resolution and %RSD were calculated and reported.

Assay: Percentage labeled amount was found by performing assay for Renol tablet formulation. Sample and standard solutions of same concentrations were prepared and injected to HPLC the obtained sample peak area was compared to the standard peak area.

RESULTS AND DISCUSSION

Method was developed by changing column, mobile phase ratio, flow rate, different buffers and their pH. But the results were optimum when 10µl of standard working solution was injected in to the mobile phase flow of composition buffer and acetonitrile in the ratio of 65:35 was pumped with a flow rated of 1ml/min through C18 250mm column which was maintained at 25°C temperature. The wavelength 223nm was optimized as the both drugs have optimum absorbance. Volume of injection was 10µl. The retention time of the omeprazole and dicyclomine hcl was 2.02min and 9.93min respectively with appreciable resolution of 9.15. Using the above method the run was performed and the system suitability parameters were calculated and were within the limits. So the method was set for validation as per ICH guidelines. The method was found to be very specific without any placebo interference. The method was observed to be precisied as the %RSD was 0.59 and 0.63 for omeprazole and dicyclomine hcl respectively. Recovery studies were performed and found to be 99.45% for omeprazole and 100.35% for dicyclomine hcl. Calibration curve was plote and correlation coefficient was 0.999 and 0.999 for omeprazole and dicyclomine hcl respectively. Linearity equation obtained was y = 409.0x + 621.1 for omeprazole and y = 1526.x + 2028 for dicyclomine hcl. The method was robust and %RSD was found to be 1.49 and 1.13 for omeprazole and dicyclomine hcl respectively.

CONCLUSION

This method was precise, accurate and accurate, this method can be used in the regular assay of the formulations.

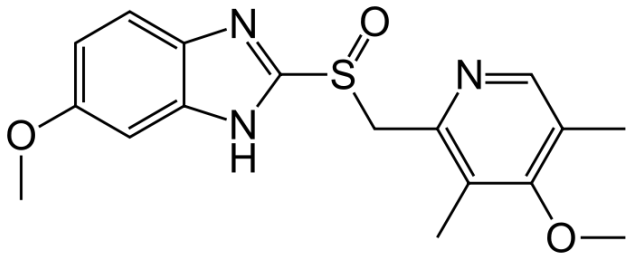

Fig.1: Omeprazole

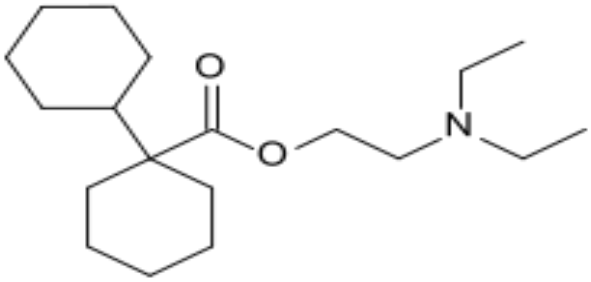

Fig.2: Dicyclomine hcl

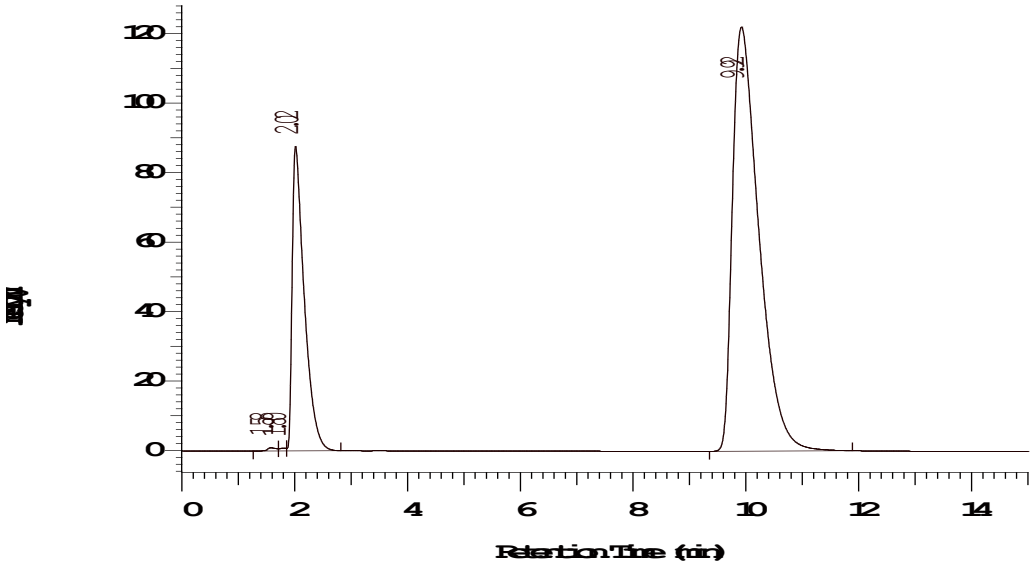

Fig.3: Chromatogram of Standard working solution

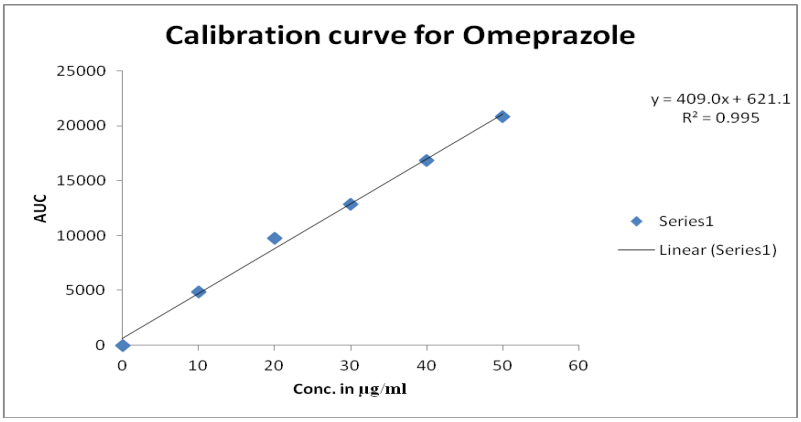

Fig.5: Calibration curve of Omeprazole

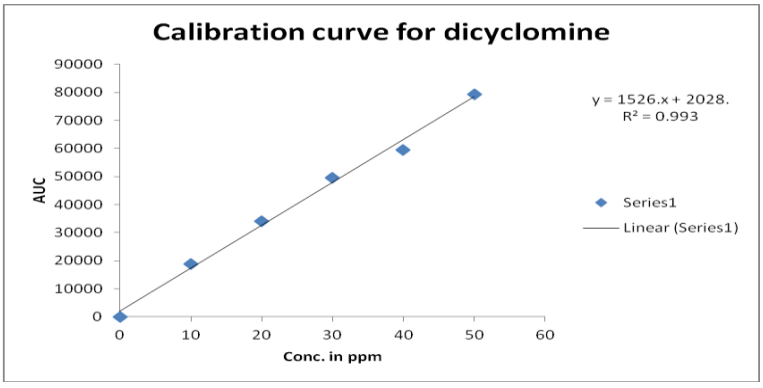

Fig.6: Calibration curve of Dicyclomine hcl

Table.1: Validation parameters

Parameters	Omeprazole	Dicyclomine hcl
Recovery	99.45%	100.35%
Intraday precision	0.59	0.63
Inter day precision	0.85	0.65
LOD	0.02µg/ml	0.06µg/ml
LOQ	0.04µg/ml	0.18µg/ml
Specificity	Specific	Specific
Robustness	1.32	1.16

Table.2: Calibration Data

Parameters	Omeprazole	Dicyclomine hcl
Optimized Wavelength	223nm	223nm
Linearity range	10ppm-50ppm	10ppm-50ppm
Intercept	621.1	2028
Slope	409.0	1526
Correlation Coefficient	0.999	0.999
Linearity Equation	y = 409.0x + 621.1	y = 1526.x + 2028

Table.3: Robustness Data

Parameters	Omeprazole	Dicyclomine hcl
Flow minus	1.43	0.46
Flow Plus	0.75	1.67
Temperature minus	1.74	0.46
Temperature Plus	0.36	0.92

Table.4: Recovery Data

Parameters	Omeprazole			Dicyclomine hcl			
Level of Recovery	80%	100%	120%	80%	100%	180%	
%Recovery	99.68	99.19	99.49	99.92		100.72	100.40
STDEV	0.223	0.060	0.219	0.469	1.570	1.398	
%RSD	0.22	0.06	0.22	0.46	1.55	1.39	

Table.5: Assay table

	Lable claim		Amount recovered		% Assay	
Formulation	AZIL	CHLO	AZIL	CHLO	AZIL	CHLO
Edarbyclor	10mg	10mg	10.09mg	9.94mg	100.9	99.4

Table.6: System suitability table

Parameters	Omeprazole	Dicyclomine hcl
Retention time	2.02±0.3min	9.93±0.3min
Plate count	3658	4258
Tailing Factor	0.12	0.5
Resolution		9.15
%RSD	0.59	0.63

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