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Simultaneous Estimation of Rabeprazole Sodium and Diclofenac Sodium by Rp-Hplc Method in Combined Tablet Dosage Form

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Abstract: The simple, sensitive, accurate, precise, rapid and economical reverse phase high pressure liquid chromatographic method was developed for the simultaneous estimation of Rabeprazole Sodium and Diclofenac Sodium in combined tablet dosage form. The method was developed using a HiQ SiL C18 (250 mm · 4.6 mm i.d.) column with a mobile phase consisting of water: methanol: acetonitrile (20:40:40 v/v), at a flow rate of 1.2 mL/ min and detection was carried out at 284 nm. Retention times were found to be 2.823 min and 5.083 min for Rabeprazole Sodium and Diclofenac Sodium respectively. The linearity were found to be in the concentration ranges of 2-20 µg/ml & 10-60 µg/ml for Rabeprazole Sodium and Diclofenac Sodium respectively. The proposed method can be used for the estimation of these drugs in combined tablet dosage form.The results of analysis have been validated statistically and by recovery studies.

Keywords: Rabeprazole Sodium, Diclofenac Sodium.

INTRODUCTION

Rabeprazole Sodium (RAB) is chemically, 2-[[[4-(3-methoxypropoxy) -3-methyl-2-pyridinyl]-methyl] sulfinyl] -1H–benzimidazole sodium[1]. Rabeprazole, a substituted benzimidazole, inhibits gastric acid secretion, used as an antiulcerative in treatment of duodenal ulcers, gastroesophageal reflux disease (GERD), Zollinger-Ellison syndrome etc. Diclofenac Sodium (DCL) is chemically, Sodium 2-[2-(2, 6-dichloroanilino) phenyl] acetate1. Diclofenac Sodium, derived from benzeneacetic acid, is a NSAID (nonsteroidal anti inflammatory drug), used in the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis and also for a variety of nonrheumatic inflammatory conditions. Diclofenac Sodium is official in IP, BP and USP. The IP[2] describes titrimetry method, BP[3] and USP[4] describes potentiometric method for estimation of DCL. Literature survey reveals HPLC[5-6] methods for determination of DCL in pharmaceutical dosage forms. Rabeprazole Sodium is not official in IP, BP or USP. Literature survey reveals HPLC[7-8] methods for determination of RAB in pharmaceutical dosage forms and in human plasma. The combination of these two drugs is not official in any pharmacopeia; hence no official method available for estimation of RAB and DCL in their combined dosage forms.

MATERIALS AND METHOD

Instruments and Reagents

The chromatographic separation was performed on a Jasco chromatographic system equipped with a Jasco PU–1580 plus HPLC pump, Jasco UV-1575 plus UV–Vis detector and Rheodyne injector with 20 lL loop volume. HiQ SiL C18 (250 mm · 4.6 mm i.d.) (Merck, Mumbai, India) was used for the separation. HPLC grade methanol, Acetonitrile- procured from Merck, India. High pure water was prepared by using Millipore Milli Q plus purification system. Rabeprazole Sodium was obtained as a gift sample from Zydus Cadila, ahmedabad and Diclofenac Sodium was obtained as a gift sample from Ipca Lab.

Marketed formulation available is R Clonac tab. (Lupin.) having content Rabeprazole-20 mg and Diclofenac-100 mg.

Optimised Chromatographic conditions

HiQ SiL C18 (250 mm · 4.6 mm i.d.) column with a mobile phase consisting of water: methanol: acetonitrile (20:40:40 v/v), at a flow rate of 1.2 mL/ min and detection was carried out at 284 nm The mobile phase was filtered through a 0.45 µm membrane filter and degassed under ultrasonic bath prior to use. The elution was monitored at 284nm and the injection volume was 20µL.

Preparation of Standard Drug Solutions

Standard stock solutions of a concentration of 100 µg/ml of RAB and DCL were prepared separately using methanol. The mixed standard solution was prepared in the ratio of 1:5 using same solvent to contain 20 µg/ml of RAB and 100 µg/ml of DCL.

Preparation of mobile phase

Prepare a filtered and degassed mixture of water: methanol: acetonitrile in the ratio of 20:40:40.

Preparation of Calibration Curves

In a series of 10 ml volumetric flask several dilutions of RAB (2-20µg/ml) and DCL (10-60µg/ml) were prepared in methanol. Each solution was injected into HPLC system and the chromatograms were recorded. The peak areas of both drugs were calculated and the respective calibration curves were plotted against ratio of area under curve and concentration of drug.

The equations of the regression lines obtained are

For RAB:

$Y=27576x+33904$ ($R^2=0.9995$)

For DCL:

$Y=34688x+10660$ ($R^2=0.9990$)

Estimation of Rabeprazole Sodium and Diclofenac Sodium in tablet

Twenty tablets (trade name: R Clonac), each containing 20 mg RAB and 100 mg DCL were weighed and finely powdered. A quantity of powder equivalent to 120 mg of drugs were transferred to 100 ml volumetric flask, and 70 ml of HPLC grade methanol was added and solution was sonicated for 15 minutes, there after volume was made up to 100 ml with same solvent. Then 10 ml of the above solution was diluted to 100 ml with HPLC grade methanol. The solution was filtered through a membrane filter (0.45 µm) and sonicated to degas. From this stock solution (3.5 ml) was transferred to five different 10 ml volumetric flasks and volume was made up to 10 ml with same solvent system. The resultant solution was 35µg/ml

The solution prepared was injected in five replicates into the HPLC system and the observations were recorded. The peak area of each of the drugs was calculated and the amount of each drug present per tablet was estimated from the respective calibration curve.

The results of analysis of tablet formulation are shown in Table 1.

Method Validation

As per the ICH guidelines[9], the method validation parameters checked were linearity, accuracy, precision, limit of detection, limit of quantitation.

Linearity and Range

The linearity of the method was determined at five concentration levels ranging from 2-20 µg/ml for Rabeprazole Sodium and 10-60 µg/ml for Diclofenac Sodium.

Accuracy and Precision

The accuracy of the method was determined by recovery experiments. The recovery study was carried out by the standard addition method at three levels of 80, 100 and 120%. Each solution was injected in triplicate and the percentage recovery was calculated. Recovery was within the range of 100 ± 2% which indicates accuracy of the method. The data for recovery study is shown in table 2.

The precision of the method was demonstrated by intra-day and inter-day variation studies. For intra-day studies the drug having concentration value 80%, 100 % & 120% of the target concentration (n = 3), were injected in triplicate into the HPLC system and for inter-day studies the drug at above three concentrations were injected in triplicate into the HPLC system for three days. Data were subjected to statistical treatment for the calculation of SD and %RSD. The value of %RSD for RAB and DCL were found to be 0.505 and 0.201 respectively for intra-day studies. The values for inter-day studies were 0.373 and 0.211 respectively. This shows that values are not more than 2%, indicates that the developed method is precise.

Limit of Detection and Limit of Quantification

The LOD was found to be 0.377 µg/ml and 2.081 µg/ml and LOQ was found to be 1.143 µg/ml and 6.307 µg/ml for Rabeprazole Sodium and Diclofenac Sodium respectively which represents that sensitivity of the method is high.

RESULTS AND DISCUSSION

For the RP-HPLC method, chromatographic conditions were optimized to achieve the best resolution and peak shape for RAB and DCL.

Different mobile phases containing water methanol and acetonitrile were examined and the mobile phase containing water: methanol: acetonitrile in the ratio of 20:40:40 was selected as optimal for obtaining well-resolved peaks with acceptable system suitability parameters (theoretical plates, resolution factor and asymmetry).The optimum wavelength for detection and quantitation was 284 nm, at which the best detector response was obtained for both the substances.

The method was found to be linear in the concentration range of 2-20 µg/ml for Rabeprazole Sodium and 10-60 µg/ml for Diclofenac Sodium. It was also found to be accurate, precise with acceptable values of LOD and LOQ. Table 3 shows the validation parameters for the method.

CONCLUSION

The method described for simultaneous estimation of Rabeprazole Sodium and Diclofenac Sodium are found to be simple, sensitive, accurate, precise, rapid, economical and rapid. Hence method could be successfully employed for routine analysis of RAB & DCL in their combined tablet dosage form.

Table 1: Estimation of Rabeprazole Sodium and Diclofenac Sodium in R Clonac tablet

S. No.	Peak Area (µV*sec)		Conc. Found in µg/ml		Amount found in (mg/tablet)	
	RAB	DCL	RAB	DCL	RAB	DCL
1	227080	1221893	7.01	35.01	20.02	99.77
2	226107	1223026	6.97	34.95	19.91	99.85
3	227398	1224429	7.02	34.99	20.05	99.97
4	225862	1223971	6.96	34.97	19.89	99.91
5	226316	1224165	6.98	34.98	19.94	99.94
Mean			6.97	35.01	19.92	99.89

Table 2: Data of recovery studies

S.No.	Conc. before spiking C_1 (µg/ml)	Reference Std. added C_2 (µg/ml)*	Conc. after spiking C_3 (µg/ml)*	Percent recovery $(C_3-C_1)*100/C_2$
1	2.079	2	4.016	100.55
	9.996	10	19.854	99.25
2	2.079	4	6.008	100.60
	9.996	20	29.692	98.48
3	2.079	6	8.117	99.03
	9.996	30	40.035	99.47
Mean ± SD	RAB	100.060 ± 0.892		
	DCL	99.067 ± 0.519		

* Mean of three triplicate determinations

Table 3:- Summary of validation parameters by RP-HPLC method

Validation parameters		RAB	DCL
Specificity		% interference <0.5 %	
Range (µg/ml)	Linear range	2-20	10-60
	Working range	0.377-20	2.081-60
	Target range		28,35,42
	Target concentration	7	35
Accuracy (% Recovery)		100.060	99.067
Precision (% RSD)	Repeatability	0.721	0.974
	Intra day	0.545	0.201
	Inter day	0.373	0.211
LOD (µg/ml)		0.377	2.081
LOQ (µg/ml)		1.143	6.307

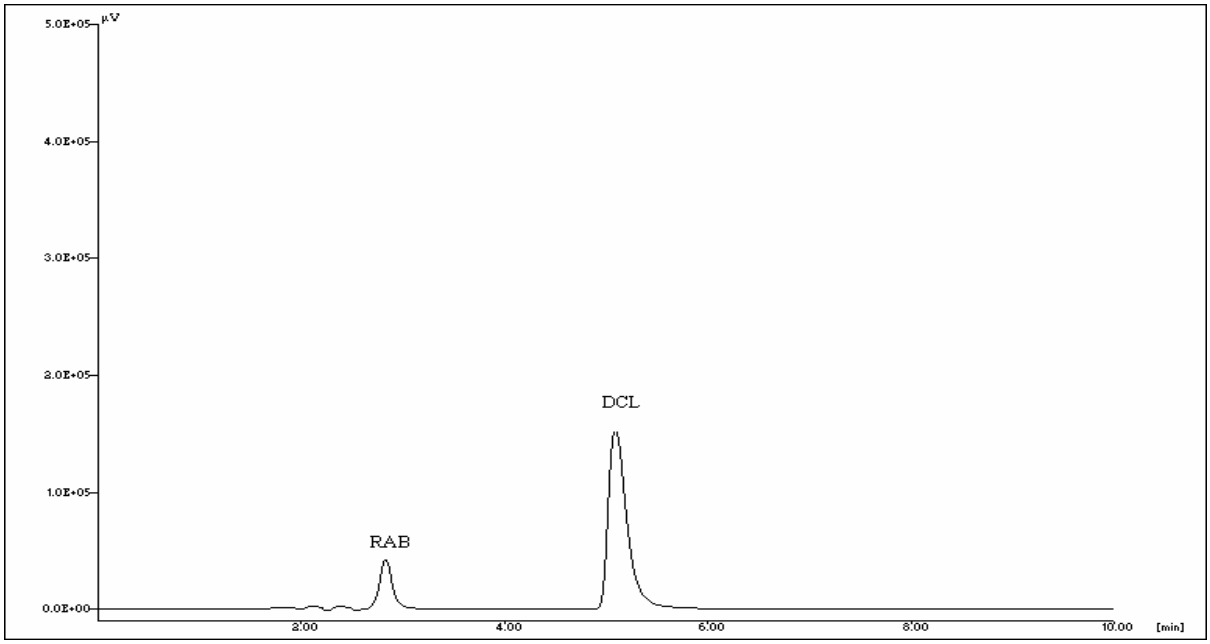

Fig. 1. Chromatogram of Rabeprazole Sodium (2.823 min) and Diclofenac Sodium (5.083 min)

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