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IJPBS |Volume 2| Issue 3 |JULY-SEPT |2012|160-169

Research Article
Pharmaceutical Sciences

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF ACECLOFENAC AND RABEPRAZOLE SODIUM IN BULK AND CAPSULES

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ABSTRACT

This paper describes a novel, simple, precise, accurate, sensitive, rapid reversed-phase liquid chromatographic method for simultaneous estimation of Aceclofenac and Rabeprazole sodium in bulk and capsules. The chromatographic separation was achieved on WATERS E 2695 HPLC separation module equipped with SUNFIRE C_{18} column(250×4.6mm×5µ) and UV-Detector (Waters) using Ammonium acetate buffer and Acetonitrile in the ratio of (50:50v/v) as mobile phase at a flow rate of 1ml/min. The detection was carried out at 300nm.The retention time of Aceclofenac and Rabeprazole sodium was found to be 2.72 and 3.95min respectively. Linearity was observed in the concentration range of 10-60µg/ml for Aceclofenac and 1-6µg/ml for Rabeprazole sodium.%Recoveries obtained for Aceclofenac and Rabeprazole sodium were 99.62% & 99.2% respectively.The %RSD below 2.0 shows the high precision of proposed method. The method was validated for precision, Recovery, Specify and Detection and Quantification limits in accordance with ICH guidelines.

KEYWORDS

Aceclofenac, Rabeprazole, RP-HPLC, Simultaneous estimation, Validation.

INTRODUCTION

Aceclofenac (ACE) chemically,2-[(2,6-Dichloro-Phenyl)amino]Phenyl acetoxy acetic acid (**Figure 1**) is a Phenyl acetic acid derivative with potent analgesic and anti-inflammatory properties.It is largely used in the Symptomatic treatment of pain and of inflammatory or Degenerative Arthropathies like Osteoarthritis,Rheumatoid arthritis and Ankylosing Spodylities.It is official in Indian Pharmacopoeia.

Rabeprazole sodium is chemically known as 2-[[4-(3-methroxypropoxy]-3methyl-2pyridinyl]-methyl] sulfinyl]-1H-benzimidazole sodium salt (**Figure 2**) Rabeprazole sodium (RBP) is proton pump inhibitor that suppress gastric H^+,K^+-Atpase enzyme system at the secretory surface of the gastric parietal cell and used in the treatment of GERO and Duodenal ulcers.It ha a faster onset of action and lower potential drug interaction compared to Omeprazole.

Literature survey reveals, that spectrophotometric methods [1, 2] have been proposed for the estimation of Aceclofenac. Other UV methods [3, 4] have also been proposed for the estimation of Aceclofenac with other drugs. Derivative spectroscopic method [5] has been reported for the analysis of Aceclofenac. A single RP-HPLC method [6] was reported for the analysis of Aceclofenac and few RP-HPCL methods [7-9] were also proposed for the Simultaneous estimation of Aceclofenac with other drugs. UV method [10] has been proposed for the analysis of Rabeprazole individually. Few spectrophotometric methods [11, 12] have been reported for the estimation of Rabeprazole with other drugs. A single spectrophotometric method [13] was proposed for the Simultaneous estimation of Aceclofenac and Rabeprazole and a UV method [14] was proposed for the estimation of Aceclofenac and Rabeprazole with other drugs. Few HPTLC methods [15-17] of

Rabeprazole were reported with other drugs. A Single RP-HPLC method [18] was proposed for the analysis of Rabeprazole sodium and other RP-HPLC method [19] was proposed for the estimation of Rabeprazole sodium with other drugs. However, no method has been developed for estimation of these drugs in combined dosage form. This paper presents simple, rapid, reproducible and economical method for RP-HPLC simultaneous estimation of Aceclofenac and Rabeprazole sodium in capsules.

MATERIALS AND METHODS

Aceclofenac and Rabeprazole sodium were obtained as a gift sample by Itros pharmaceutical Ltd. Pune. All chemicals used of HPLC grade (MERCK.chem.Ltd.,Mumbai) double distilled water was used throughout the study.Fixed dose combination capsule (ALTRADAY) containing 200mg of Aceclofenac IP (as sustained release pellets) and 20mg of Rabeprazole sodium IP (as enteric coated pellets) was procured from local market.

INSTRUMENTS:

HPLC (WATERS E 2695) separation module equipped with SUNFIRE C_{18} column (250×4.6mm×5µ) UV-Detector (Waters), Ultrasonic bath sonicator (BIOTECHNICS), Analytical balance (SATORIUS) and Vaccum pump (BIOTECHNICS) were used.

SELECTION OF CHROMATOGRAPHIC PARAMETERS

1. Chromatographic mode:

The reverse phase HPLC was selected for separation, as it was convenient than any other forms of the liquid Chromatography and was more likely to give good peak shapes at a reasonable retention times.

2. Stationary Phase:

The stationary phase with C_{18} column bonded phase i.e SUNFIRE C_{18} column (250×4.6mm×5µ) was selected on the basis of reverse phase HPLC mode and number of carbon present in molecule i.e analyte. The stationary phase is selected finally due to its efficiency in separating compounds with good peak shapes and less tailing.

3. Preparation of Mobile Phase:

For the preparation of mobile phase suitable for the present determination Ammonium acetate buffer and Acetonitrile of HPLC grade were mixed, filtered and degassed in such a way that the final volume consisted of the ratio (50:50%v/v) respectively.

Ammonium acetate buffer is prepared by mixing 0.5gm of Ammonium acetate in 100ml of double distilled water. It is filtered through 0.45µ membrane filter.

4. Preparation of Standard Solution:

Weigh accurately and transfer about 20mg of Aceclofenac and 2mg of Rabeprazole of working standard into a 50ml clean, dry volumetric, dissolved and diluted with the mobile phase. The Aliquots of the standard stock solution of 0.25, 0.5, 0.75, 1, 1.25, 1.5ml were transferred into 10ml volumetric flasks and the required volume was diluted with mobile phase.

5. Preparation of sample solution:

20 tablets, each containing 200mg of aceclofenac and 20mg of Rabeprazole sodium were weighed and powdered. A quantity of tablet powder equivalent to 200mg of Aceclofenac and 20mg of Rabeprazole was accurately weighed and transferred to the 50ml volumetric flask, about 25ml of mobile phase was added and shaked thoroughly for 10min and then sonicated for 5min.The volume was adjusted to the mark with mobile phase. The filterate was diluted to obtain the concentration of 40µg/ml of Aceclofenac and 4µg/ml of Rabeprazole sodium.

6. Procedure for Assay:

A steady base line was recorded with the optimized chromatographic conditions and standard and sample solutions of 20ml was separately injected into the HPLC and the chromatogram was recorded from the peak area of Aceclofenac and Rabeprazol sodium the amount of the drug in the sample can be calculated.

METHOD VALIDATION

The optimized chromatographic method was completely validated according to the procedures described in ICH Q2 (R1) for the validation of analytical methods.

Linearity:

A series of dilutions were prepared from the working standard solution in the concentration range of 10-60µg/ml for Aceclofenac and 1-6µg/ml for Rabeprazole sodium. 20µl of each sample was injected into HPLC. Calibration curve was constructed by plotting the peak area versus the drug concentration (**Figure 3, 4**) (**Table 1, 2**)

Accuracy:

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method.Accuracy was determined at three levels.50%, 100%, 150% of the target concentrations. The data shows excellent recoveries at all levels (**Table 3, 4**). The average recoveries for replicate determination at 50%, 100%, 150% levels were within the acceptable criteria.

Precision:

Method precision:

Six assay samples of drug product at 100% of working sample concentration were prepared and injected into the chromatographic system. The percent assay and % RSD are calculated (**Table 5**)

System precision:

Six injections of the standard solution were injected into the chromatographic system.The percent assay and %RSD are calculated.(**Table 5**)

Robustness and Ruggedness:

Robustness was evaluated by small deliberate variation in the chromatographic conditions at three different levels -1,0,+1. The factors selected were flow rate (±0.1ml/mm) and the organic modifier in the mobile phase ±1%. The results obtained were unaffected by small variations in these parameters.

Ruggedness of the method was evaluated by using different results obtained from different analysts and instruments was <2.0%.

Specificity:

Specificity was performed to exclude the possibilities of interference with excipients in the region of elution of Aceclofenac and Rabeprazole sodium.The specificity and selectivity of the method was tested under normal conditions and the results of the tests proved that the components other than the drug did not produce a detectable signal at the retention place of Aceclofenac and Rabeprazole sodium.

Sensitivity:

Sensitivity of the proposed method was estimated in terms of limit of Detection (LOD) and limit of Quantitation(LOQ).

LOD=3.3 SD/s

LOQ=10SD/s

Where SD is the residual standard deviation and‘s’ is the slope of the line.

System stability test:

System stability testing is essential for the assurance of the quality performance of the chromatographic system.The earlier prepared solutions for chromatographic conditions were tested for system suitability testing.(**Table 5**)

RESULTS AND DISSCUSSIONS

A satisfactory separation and good peak symmetry was obtained with stationary phase SUNFIRE C_{18} column [250×4.6mm×5µ] mobile phase comprising of Ammonium acetate buffer : Acetonitrile in ratio of (50:50%v\r) at a flow rate of 1ml/1min to get better reproducibility and repeatability. Quantification was achieved with UV detection at 300nm ,based on peak area.The retention time was found to be 2.72 and 3.95 min.for Aceclofenac and Rabeprazole sodium respectively.(figure 5)

Method validation

The proposed method was validated as per ICH guidelines. The solutions of the drugs were prepared as per the earlier adapted procedure given in the experiment.

1. Linearity:

The calibration curves obtained showed. Good linear relationship over the concentration range of 10-60µg/ml for Aceclofenac and 1-6µg/ml for Rabeprazole sodium. Linear regression equation was found to be Y=14921x+44968 for Aceclofenac and y=74134x+25809 for Rabeprazole sodium. R^2 was found to be 0.999 for both the drugs.The results are expressed in the table.(**Figure 3,4**)(**Table 1,2**)

2. Accuracy:

The difference between theoretical added amount and practically achieved amount is called accuracy of analytical method. %Recovery was found to be 99.62% for aceclofenac and 99.2% for Rabeprazole sodium and the results were expressed in table.(**Table 3,4**)

3. Precision:

The %RSD of method precision for Aceclofenac and Rabeprazole sodium was found to be 0.08 and 0.22 respectively. %RSD of system precision for Aceclofenac and Rabeprazole sodium was found to be 0.15 and 0.33 respectively.

The %RSD below 2.0 shows high precision of proposed method.(**Table 5**)

4. Robustness:

The robustness of the method is used to determine the capacity of the intended method to remain unaffected by changing flow rate and % of Acetonitrile. The developed method was found to be robust

5. Specificity:

There was no other interfering peak around the retention time of Aceclofenac and Rabeprazole sodium; also the base line did not show any significant noise.

6. Sensitivity:

The LOD & LOQ values for each analyte were established by signal to noise ratio method.Calculated LOQ values allowed confident determination of analytes by the proposed methods for the assay. (**Table 5**)

System suitability test:

The number of high theoretical plate=2367 , less tailing and less run time(1ml/min) indicates that proposed method was suitable for selective determination of Aceclofenac and Rabeprazole sodium.(**Table 5**)

Table 1. Linearity range of Aceclofenac

S.No.	Concentration (µg/ml)	Peak Area
1	10	1507164
2	20	3031261
3	30	4559817
4	40	6029731
5	50	7501050
6	60	8976569

Table 2. Linearity range of Rabeprazole

S.No.	Concentration (µg/ml)	Peak Area
1	1	748121
2	2	1511834
3	3	2269019
4	4	3005290
5	5	3728671
6	6	4460186

Table 3. Accuracy (Recovery Studies) for Aceclofenac

S.No	Actual Concentration Of Standard Added(µg/ml)	Amount Of Standard Recovered(µg /ml)	% Recovery
1	20 (50%)	49.64	99.29
2	40 (100%)	100.42	100.42
3	60 (150%)	148.75	99.17
	Average Recovery		99.62

Table 4. Accuracy (Recovery studies) for Rabeprazole

S.No.	Actual Concentration Of Standard Added(µg/ml)	Amount Of Standard Recovered(µg/ml)	% Recovery
1	20 (50%)	49.69	99.37
2	40 (100%)	99.10	99.10
3	60 (150%)	148.71	99.14
	Average Recovery		99.2

Table 5. Validation Parameters

S.No.	Validation Parameters	Aceclofenac	Rabeprazole
1	Linearity Range(µg/ml)	10-60	1-6
2	Correlation Coefficient	0.999	0.999
3	Precision(%RSD) (i) Method Precision	0.08	0.22
	(ii)System Precision	0.15	0.33
4	Sensitivity (i) LOD	0.98480	0.44260
	(ii) LOQ	3.07380	1.38160
5	Tailing factor	1.22	1.42
6	Formulation Assay(%)	99.62	99.65

Figure 1:Structure of Aceclofenac

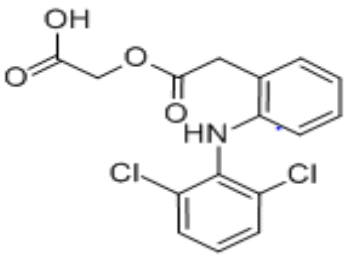


Figure 2: Structure of Rabeprazole Sodium

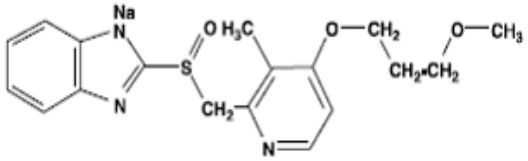


Figure 3. Calibration Curve of Aceclofenac

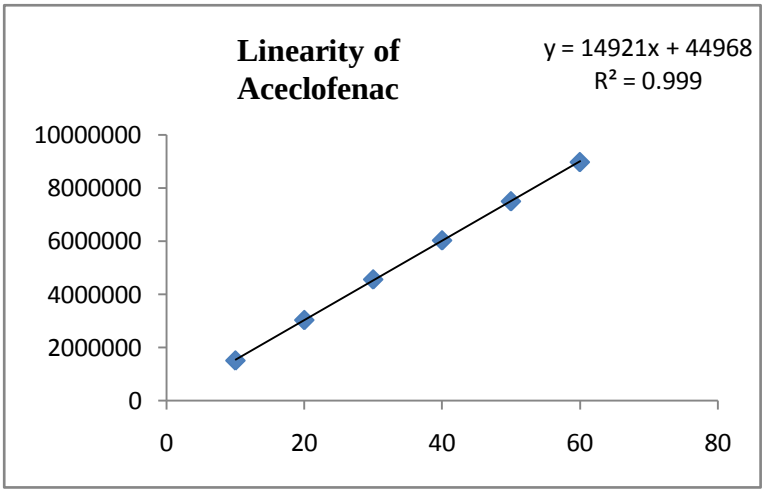


Figure 4. Calibration Curve of Rabeprazole

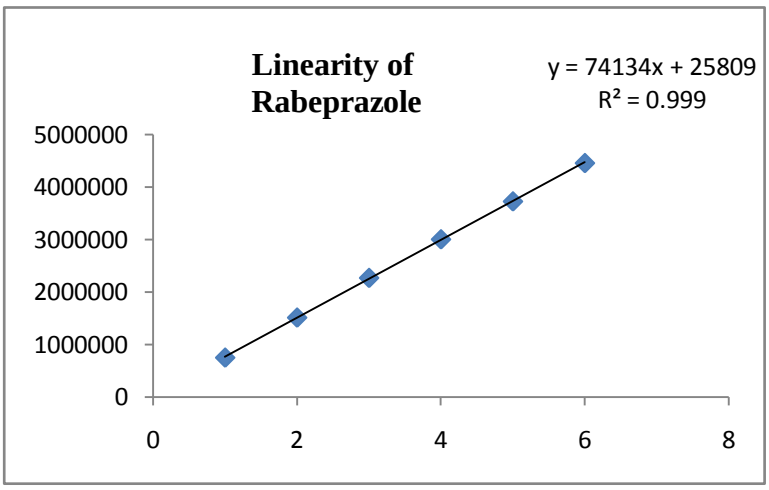
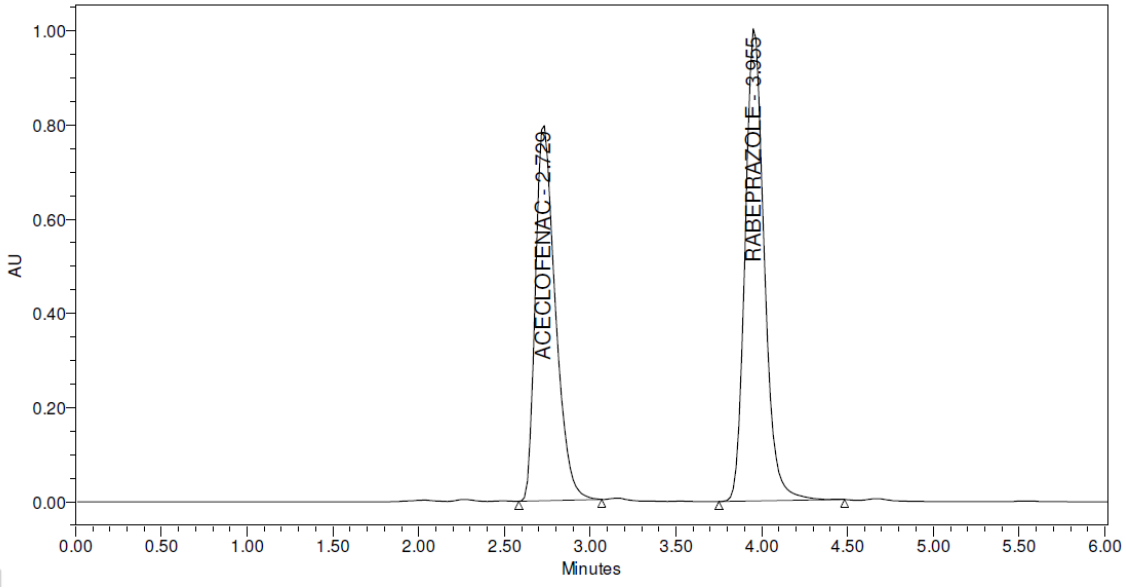


Figure 5.Chromotogram of Aceclofenac and Rabeprazole



CONCLUSION

The proposal study describes a new RP-HPLC method using mobile phase for the estimation of Aceclofenac and Rabeprazole sodium in combined pharmaceutical dosage formulation. The method was validated and found to be simple, sensitive, accurate and precise. It was also proved to be convenient and effective for the determination of Aceclofenac and Rabeprazole sodium in the bulk and pharmaceutical dosage form. The % Recovery shows that the method is free from interference of the excipients used in formulation. Moreover, the lower solvent consumption along with the short analytical run time leads to cost effective chromatographic method.

ACKNOWLEDGEMENT

The author would like to thank richer health care, Hyderabad for providing necessary facilities to carry out the work.

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