Journal of Innovations in Pharmaceuticals and Biological Sciences
www.jipbs.com
JIPBS
ISSN: 2349-2759

Research Article

Development and Validation of Reverse Phase-HPLC Method for Estimation of Aceclofenac and Rabeprazole Sodium in Bulk and Combined Dosage Form

Rajani N. Jinde*[1], Pathan Azhar Khan[2], Manadar Abhyankar[3], Megha Jadhav[4]

[1]Student, Department of Clinical Research, CRB Tech, Pune. India
[2]Trainer, Department of Clinical Research, CRB Tech, Pune. India
[3]Director, CRB Tech, Pune. India
[4]Department of Quality Assurance, P. Wadhwani College of Pharmacy, Yavatmal

Abstract

The present work describes a validated reverse phase high performance liquid chromatographic method involved with diode array detection for simultaneous estimation of Rabeprazole sodium (RAB) and Aceclofenac (ACL) in combined dosage form. Chromatographic separation was performed on ProntosilC18(4.6 id x 250 mm 5µm particle size) Column with mobile phase consisting methanol: water pH 2.5 (75:25 v/v).The flow rate was 1.0 ml/min and eluents were monitored at 273nm by using Photo Diode Array(PDA) detector at ambient temperature. The separation of both drug were found effectively on 3.23min of RAB and 7.78min of ACL with total run time upto10 minutes. The proposed method was found to be simple as was involved with water as mobile phase component it was fast, accurate, precise, reproducible, linear, robust and rugged which have been further used for simultaneous analysis of RAB and ACL in combined pharmaceutical formulations.

Key words: Rabeprazole sodium, Aceclofenac, Reversed phase- HPLC, PDA.

***Corresponding Author: Rajani N. Jinde,** Department of Clinical Research, CRB Tech, Pune, India. Mobile No. 9561595487 Email : rajani.jinde@gmail.com, Website: www.crbtech.in

1. Introduction

Rabeprazole sodium is chemically 2-[[4-(3-methoxypropoxy)-3-methyl- pyridine yl]methylsulfinyl]-1H-Benzoimidazole Sodium salt. It is a substituted benzimidazole that inhibits gastric acid secretion and primarily used in thetreatment of Ulcerative Gastroesophageal Reflux Disease (GERD). It is Rabeprazole belongs to a class of antisecretary compounds that suppress gastric acid.[1] Figure 1 shows the structure of Rabeprazole sodium

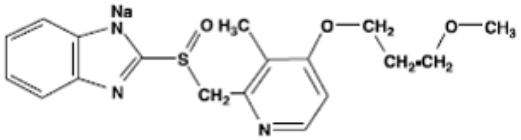

Figure 1 : Chemical structure of Rabeprazole Sodium

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Aceclofenac is chemically [2-[(2,6 dichlorophenyl)amino] phenyl acetoxy acetic acid a non-steroidal anti-inflammatory drug. It has potent analgesic and anti-inflammatory properties and an improved gastrointestinal tolerance. It is used for the relief of pain and inflammation rheumatoid arthritis.[2] Figure 2 shows the structure of Aceclofenac.

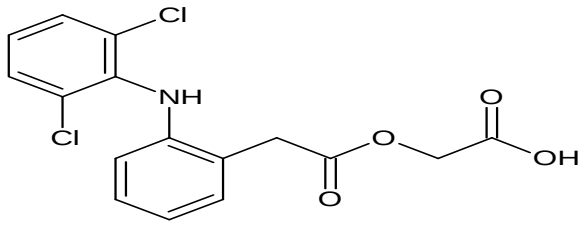

Figure 2 : Chemical structure of Aceclofenac

Simple and accurate reverse phase liquid chromatographic method for the estimation of RAB and ACL was developed. The developed method was validated to ensure the compliance in accordance with ICH guidelines.

2. Experimental

Instrumentation

The HPLC system consisted of Waters ™ 600 Controller system, Waters 996 PDA Detector and Water In-line Degasser AF. Data acquisition was performed by the Empower software. Analysis was carried out at 273 nm with Prontosil C18 (4.6(id) x 250mm, 5 µm partical size,) or equivalent column, using a mixture of Methanol:Water pH 2.5 (75:25 v/v) maintained by Orthophosphoric acid (OPA) and Triethylamine (TEA) (0.02%) as the mobile phase using a low pressure gradient mode with flow rate at 1.0 ml/min. The mobile phase was degassed and filtered before pumping into HPLC system.

Reagents and Chemicals

Methanol HPLC grade was procured from Spectrochem Pvt. Limited, Mumbai. Water of HPLC grade by LOBA CHEMIE Pvt. Ltd. Mumbai. Reference standards of RAB and ACL were provided by RANBAXY Pharmaceuticals Ltd, Nagpur. OPA was procured from RANBAXY Fine chemicals ltd. New Delhi. TEA procured from LOBA CHEMIE Pvt. Ltd. Mumbai. Handy pH meter was used to modify pH belonging to HANNA.

Chromatographic conditions

Prontosil C18 column (250 mm x 4.6 mm (i.d.), 5 µm particle size) was used at ambient temperature. The mobile phase consisted of Methanol:Water (pH 2.5) (75:25 v/v) at a flow rate of 1.0 ml/min. The mobile phase was filtered through nylon 0.45 µm membrane filter (47mm diameter) and degassed before use. The elution was monitored at 273 nm, and the injection volume was 20 µl.

Preparation of solutions

Preparation of mobile phase

Methanol and water in the ratio of 75:25 v/v was employed as a mobile phase. Water was sonicated after maintaining the pH by OPA and TEA upto 2.5.

Preparation of Rabeprazole sodium and Aceclofenac standard stock solution

Accurately weighed RAB (10 mg) and ACL (10 mg) were transferred to two separate 10 ml volumetric flask. 10 ml methanol was added to the flask. The drug was dissolved with sonication and the final volume was adjusted with methanol up to the mark to prepare a 1000µg/ml stock solution of both drugs.

Preparation of Rabeprazole sodium and Aceclofenac working standard solution (100µg/ml)

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From the above stock solutions (1000µg/ml) of both drugs, an accurately measured 1.0 ml volume of the stock solutions were transferred into separate 10 ml volumetric flasks and the final volume were adjusted with methanol to prepare 100 µg/ml working solutions.

Determination of wavelength of maximum absorbance

The standard solutions of RAB and ACL were scanned in the range of 200 -400 nm against mobile phase as a blank. RAB and ACL showed maximum absorbance at 273 nm. So the wavelength selected for the determination of RAB and ACL was 273 nm.

Method Validation

Construction of Linearity plot

Calibration curves were plotted over a concentration range of 50-150µg /ml for RAB and ACL. Accurately measured standard stock solutions of RAB and ACL (0.5, 0.75, 1.0, 1.25 and 1.5ml) were transferred to a series of 10 ml volumetric flasks and the volume in each flask was adjusted to 10 ml with methanol. The resulting solutions were injected into the column and the peak area obtained at flow rate of 1.0 ml/min for RAB and ACL respectively at 273 nm.

Estimation of Rabeprazole sodium and Aceclofenac in capsule dosage forms

20 Capsule granules were weighed to obtain the average Capsule granules weight. A sample of the claimed granules to contain 20 mg of RAB and 200mg of ACL active ingredients was mixed with 20 ml of methanol. The mixture was allowed to stand with intermittent sonication to ensure complete solubility of drug. Further the resulting solution was passed through 0.45 µm membrane filter. An aliquot of this solution (1 ml) was transferred to a 10ml volumetric flask and made up to a sufficient volume with methanol to get 100µg/ml of RAB and 1000µg/ml of ACL concentration.

3. Results and Discussions

Method development

Methanol and water in the ratio of 75:25 v/v were employed as a mobile phase. The present RP – HPLC method for the quantification of RAB and ACL in bulk and pharmaceutical dosage forms, revealed as simple, accurate and precise method with significant retention time of 3.233min of RAB and 7.78 min of ACL. The typical chromatograms of RAB and ACL were shown in figure 3.

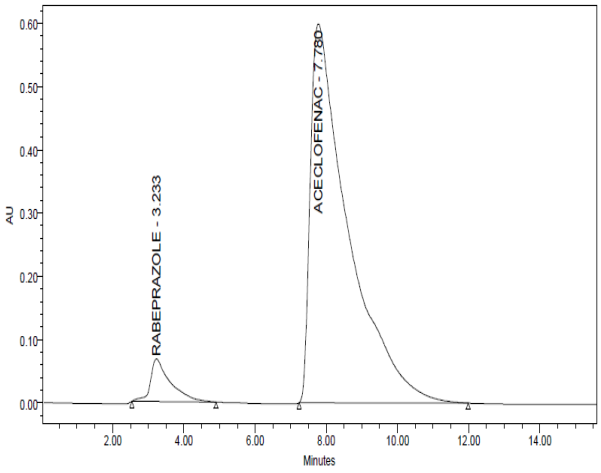

Figure 3: Typical Chromatogram of Rabeprazole sodium and Aceclofenac

Method Validation

Method precision

The intraday and inter-day variations of the method were determined using five replicate injections of three concentrations and analysed on the same day and three different days over a period of two weeks. Results were shown in Table 1.

Accuracy (% Recovery)

Accuracy of the method was assured by use of the standard addition technique, involving analysis of formulation samples. The results obtained for both drugs were compared to those expected. The recovery

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Drugs	Observed Concentration*			
	System precision Area Response	%RSD	Method precision Area Response	%RSD
Rabeprazole sodium	90572	0.97318	90108	0.080
Aceclofenac	18735739	2.098061	1845607	1.187

*Mean of five values
Results of method precision study showed ±

Table 1 : system and method precision of Rabeprazole sodium and Aceclofenac

experiments were carried out in triplicate by spiking previously analyzed samples of the tablets (50%, 100%, 150%) with three different concentrations of standards (RAB 100, 150, 200µg/ml and ACL 1000, 1500, 2000µg/ml). Relative standard deviation of all the parameters was less than 2%, which indicates that the proposed method is repeatable. The results were shown in table 2.

Drug	% recovery	% RSD
Rabeprazole sodium	100.43	1.039
Aceclofenac	99.83	0.67

Table 2 : accuracy of Rabeprazole sodium and Aceclofenac

System suitability

To know reproducibility of the method system suitability test was employed to establish the parameters such as tailing factor, theoretical plates, LOQ and LOD. The results were shown in table 3.

Ruggedness

Ruggedness of the method (intermediate precision) was estimated by preparing five dilutions of the RAB and ACL. The results were shown in table 4.

Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters (± 10% flow rate, ± 0.1 unit pH, ± 10% organic mobile phase) and provides an indication of its reliability during normal usage. The results were shown in table 5.

Specificity

The ICH document defines specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. For the current method the blank chromatogram do not exhibited any interference.

Analysis RAB and ACLin combined dosage forms

Pharmaceutical formulation (Altraday capsule) of RAB and ACL was purchased from local pharmacy. The responses of formulations were measured at 273 nm for quantification of RAB and ACL by using RP-HPLC. The amounts of RAB and ACL present in sample solution were determined. Results are given in Table 6.

Suitability Parameters	**Observation**	
	Rabeprazole sodium	**Aceclofenac**
Retention time (min.)	3.231	7.707
Theoretical Plates	2187.13	2241.6
Tailing factor	1.59	2.0
Linearity Range (µg/ml)	10-50	10-50
Relative standard deviation (%RSD)	1.63	0.6385

Table 3 : System suitability of Rabeprazole sodium and Aceclofenac

Drugs	**% Label Claim**	
	Rabeprazole sodium	**Aceclofenac**
Mean	99.89	100.49
%RSD	2.0	0.788

Table 4 : Ruggedness of Rabeprazole sodium and Aceclofenac

Sr. No	**System sutability parameter**		**observation**							
			As such	**flow**		**Organic phase**		**pH**		**Limit**
				+10%	**-10%**	**+10%**	**-10%**	**+0.1unit**	**-0.1unit**	
1.	The % RSD of peak area response for five replicate injections	RAB	1.6	0.06	0.97	1.3	1.56	0.97	0.72	NMT 2.0
		ACL	0.6	0.21	0.04	1.8	1.7	1.42	1.57	
2.	Theoretical plates	RAB	2184	2231	2168	3206	2193	3187	3174	NLT 2000
		ACL	2241	2218	3177	2236	3191	2264	2245	
3.	Tailing factor	RAB	1.5	1.135	1.3	1.11	1.37	1.43	1.59	NMT 2.0
		ACL	2	1.65	2	1.38	1.9	1.7	1.95	

Table 5 : Robustness of Rabeprazole sodium and Aceclofenac

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Sr.no.	Rabeprazole sodium and		Aceclofenac	
	Assay (mg)	Assay %	Assay (mg)	Assay %
Mean	197.93	98.69	198.65	99.32
%RSD	0.086	0.089	0.257	0.259

Table 6: Analysis RAB and ACL in combined dosage forms

4. Conclusion

The results of the study reveal that the proposed RP-HPLC method for the estimation of RAB and ACL is simple as it involves water as component of mobile phase and not involving any buffer which is considered to be disadvantageous for column life. The method was accurate, precise and linear and was successfully applied for estimation of RAB and ACL in bulk and combined dosage form.

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