RP-HPLC Method development and validation for the simultaneous estimation of Aceclofenac and Rabeprazole Sodium in the bulk and marketed formulation

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Abstract

A simple, accurate and precise RP-HPLC method was developed for the estimation of Aceclofenac and Rabeprazole Sodium in the bulk and marketed formulation. The method was performed by using the mobile phase containing Methnol, Water and Acetonitrile (60:30:10 v/v), pH- 6.14 was found to be most suitable for RP-HPLC as it showed sharp peak with symmetry and significant reproducible retention time for Aceclofenac (4.549 min) along with Rabeprazole Sodium (6.048 min). From the results of system suitability parameters, it was observed that the peak was sharp and symmetrical with satisfactory capacity factor and column efficiency. From the developed methods, percent content of Aceclofenac and Rabeprazole Sodium was found to be 99.97%, 99.91% and 99.94% 99.6%, 99.8% and 94.45% respectively by RP-HPLC. The method was validated by parameters like accuracy, precision, specificity, linearity, ruggedness and robustness. The results indicated that, the method is simple, accurate, precise, rugged. The method described enables to the quantification of Aceclofenac and Rabeprazole Sodium. The advantages lie in the simplicity of sample preparation and the low costs of reagents used. The proposed HPLC conditions ensure sufficient resolution and the precise quantification of the compounds. Results from statistical analysis of the experimental results were indicative of satisfactory precision and reproducibility. Hence, this HPLC method can be used for routine drug analysis.

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Introduction

Rabeprazole sodium Is chemically known as 2 -[[[4 -(3-methoxyprop oxy)-3methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole sodium salt[1] Rabeprazole Sodium (RBP) is proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the gastric H+, K+-ATPase enzyme system at the secretory surface of the gastric parietal cell and used in the treatment of GERD and duodenal ulcers. It has a faster onset of action and lower potential drug interactioncompared to omeprazole. It is official in Indian Pharmacopoeia[2]. Aceclofenac (ACE) chemically, 2-[(2,6-dichlorophenyl)amino] phenylacetoxyacectic acid[3] is a phenylacetic acid derivative with potentanalgesic and anti-inflammatory propreties. It is largely used in the symptomatic treatment of pain and of inflammatory or degenerative art hropathies like osteoarthritis, rheumatoid arthritis and ankylosingspondylities[4]. To our knowledge, based on the literature survey, HPLC[5-6] and HPTLC[7] methods for rabeprazole sodium and HPLC[8-12] methods for aceclofenac are reported either alone or in combination with other drugs. So far, no method has been reported for about the simultaneous quantitation of rabeprazole sodium and aceclofenac by HPLCin bulk drug and in capsule dosage form. This present study reports for the first time simultaneous estimation of rabeprazole sodium and aceclofenac by HPLC in bulk drug and in tablet dosage form. The proposed method is validated as per ICH guidelines[13-15].

Materials & Methods

Chemicals & Reagents: The drugs used for the present investigation were available at RANBAXY, Mumbai. **Aceclofenac Purity** 99.81%, **Rabeprazole Sodium Purity (Assay)** 99.79%. The marketed preparations were purchased from the local market Brand Name ALTRADAY RANBAXY, Mumbai Composition Aceclofenac (200 mg) Rabeprazole Sodium (20 mg) all chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India

Apparatus & Chromatographic Conditions: An isocratic elution HPLC system of Shimadzu with LC20AD pump and SPD-20A Photo Diode Array detector was used working via LC-Solution software. The separation was carried on Hypersil BDS column (Thermo scientific) with C18 packaging and 250 x 4.6mm dimensions (internal diameter) 5µm particle size. The analysis of elution was completed at 283 nm on ambient temperature. The run time was set at 15 minutes for this analysis at flow rate of 1.0 ml/minute.

Preparation of Mobile Phase: Methanol (60ml) was mixed with water (30 ml) & Acetonitrile (10), and then pH was adjusted to 6.14 by addition of glacial acetic acid. The final mobile phase was then filtered by passing through 0.22µm membrane filter and degassed before use.

Preparation of Standard Solution: 50 mg of Aceclofenac and 50 mg of Rabeprazole Sodium were

weighed and dissolved in 50ml of mobile phase with shaking. Then it was sonicated and the volume was made upto 100 mL by using mobile phase. From standard stock solution of drug, appropriate dilutions were made using the mobile phase. This solution was filtered through 0.2µm membrane filter and 20 µL of this solution was injected for HPLC analysis and quantified based on the AUC of the above standard.

Results & Discussion

System Suitability: Before performing the main analysis, the system suitability was evaluated. For this purpose, various parameters were calculated as per their standard procedure e.g. retention time (for bulk and capsule of Aceclofenac and Rabeprazole Sodium), theoretical plates number of the column (for column efficiency), tailing factor, relative standard deviation of peak area and retention time. The Table 1 shows the result for these parameters. The column efficiency was much better for analysis i.e. ≥2000. The tailing factor was also within range. Moreover, the calculated relative standard deviation for the retention time and peak area (mean of 6 replicates) also within acceptance criteria. Depending on all these information, it reflects that the proposed method will be suitable for routine analysis.

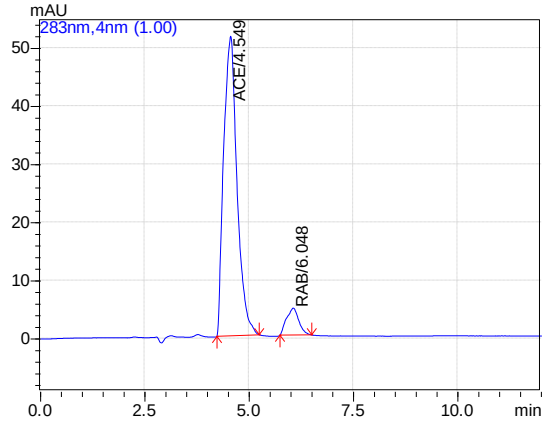


Fig. 1: Chromatogram of working standard for Aceclofenac and Rabeprazole Sodium

Table 1: Details of chromatogram of working standard containing Aceclofenac and Rabeprazole Sodium

Sr. No	Name	RT(min)	Area	Theoritical plates	Resolution	Asymmetry
1	ACE	4.549	1146314	734.466	-	1.256
2	RAB	6.048	93582	1914.856	2.449	1.094

Analysis of Capsule formulation: Brand: ALTRADAY Each Tablet contains: Aceclofenac: 200mg Rabeprazole Sodium: 20mg

Procedure

Take ten capsule, each containing 200 mg of Aceclofenac and Rabeprazole Sodium 20 mg .The capsule were crushed to fine powder and amount of powder equivalent 200 mg of Aceclofenac and Rabeprazole Sodium 20 mg were weighed and transferred to100 mL dried volumetric flask. Sufficient amount of mobile phase was added to dissolve the content and shaken for 20 min. The volume was made up to 100 ml with mobile phase. Then solution was filtered by using membrane filter and digassed. From this solution appropriate dilutions of Aceclofenac and Rabeprazole Sodium were made to get the final concentrations and injected into the system to get the chromatogram. The chromatogram obtained is shown in fig no.2 and the area obtained in each chromatogram of five replicate was correlated with regression equation and the amount found is calculated which was within the limit of label claim as mentioned in Table 11.

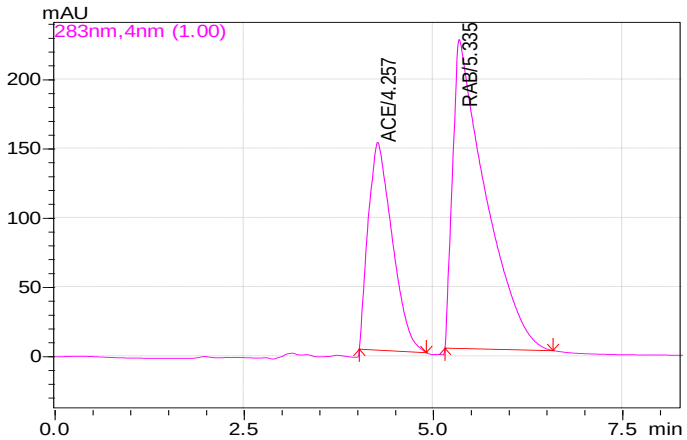


Fig. 2: Chromatogram of Aceclofenac and Rabeprazole Sodium in Capsule Formulation

Table 2: Details of chromatogram of Aceclofenac and Rabeprazole Sodium

Sr. No	Name	RT(min)	Area	Therotical plates	Resolution	Asymmetry
1	ACE	4.257	3312480	730.849	-	1.581
2	RAB	5.335	6721977	707.471	1.505	3.219

Table 3: Analysis of ALTRADAY Capsule

Sr. No.	Amount present in(µg)		Amount found in(µg)		% Label claim	
	ACE	RAB	ACE	RAB	ACE	RAB
1	200	20	199.95	19.92	99.97	99.6
2	200	20	199.82	19.96	99.91	99.8
3	200	20	199.88	19.89	99.94	99.45
4	200	20	199.91	19.91	99.95	99.55
5	200	20	199.93	19.86	99.96	99.3

Linearity: The linearity of Aceclofenac was observed in the range of 50-250 µg/ml and that of Rabeprazole Sodium was in the range of 10-50 µg/ml. Detection wavelength used was 283 nm with correlation coefficient 0.9958 and 0.9956 for Aceclofenac and Rabeprazole Sodium respectively.

Table 4: Linearity of Aceclofenac

Standard conc.→	50 µg/ml	100µg/ml	150 µg/ml	200µg/ml	250µg/ml
Replicates ↓	Peak area				
1	198031	576650	879550	1217915	1503894
2	198036	576653	879556	1217918	1503898
3	198034	576658	879562	1217923	1503889
Mean	198033.7	576653.7	879556	1217919	1503894
±SD	2.516611	4.041452	6	4.041452	4.50925
%RSD	0.001271	0.000701	0.000682	0.000332	0.0003

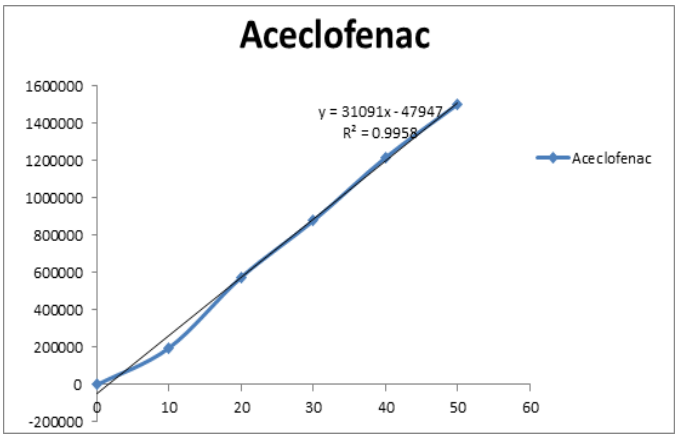
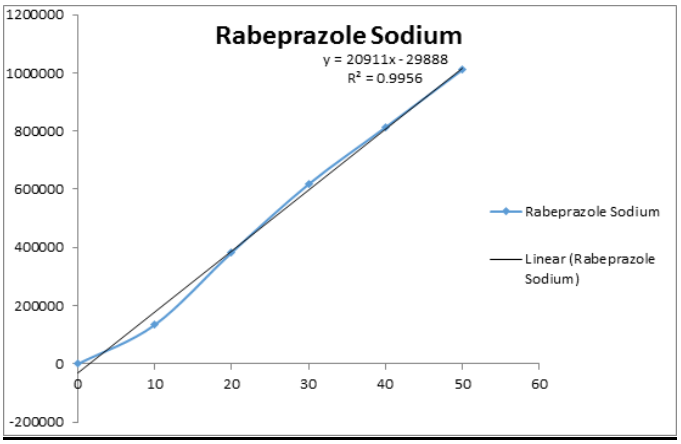


Table 5: Linearity of Rabeprazole Sodium

Standard conc.→	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
Replicates ↓	Peak area				
1	134592	382816	617234	810510	1010106
2	134598	382821	617236	810513	1010109
3	134589	382818	617230	810518	1010113
Mean	134593	382818.3	617233.3	810512.7	1010109
±SD	4.582576	2.516611	3.05505	4.618802	3.511885
%RSD	0.003405	0.000657	0.000495	0.00057	0.000348



Precision: The result obtained for intra-day and inter-day variation are shown in following Table0

Table 6: Intra-day variability of Aceclofenac

Conc. (µg/ml)	Peak area			Mean area	± SD	% RSD
	Trial 1	Trial 2	Trial 3			
10	198031	198036	198034	198033.7	2.516611	0.001271
20	576650	576653	576658	576653.7	4.041452	0.000701
30	879550	879556	879562	879556	6	0.000682

Table 7: Intra-day variability of Rabeprazole Sodium

Conc. (µg/ml)	Peak area			Mean area	± SD	% RSD
	Trial 1	Trial 2	Trial 3			
10	134592	134598	134589	134593	4.582576	0.003405
20	382816	382821	382818	382818.3	2.516611	0.000657
30	617234	617236	617230	617233.3	3.05505	0.000495

Table 8: Inter-day variability of Aceclofenac

Conc. (µg/ml)	Peak area			Mean area	± SD	% RSD
	Day 1	Day 2	Day 3			
10	198033	198031	198039	198034.3	4.163332	0.002102
20	576652	576656	576657	576655	2.645751	0.000459
30	879553	879551	879566	879556.7	8.144528	0.000926

Table 9: Inter-day variability of Rabeprazole Sodium

Conc. (µg/ml)	Peak area			Mean area	± SD	% RSD
	Day 1	Day 2	Day 3			
10	134591	134596	134585	134590.7	5.507571	0.004092
20	382818	382826	382819	382821	4.358899	0.001139
30	617235	617234	617238	617235.7	2.081666	0.000337

Accuracy: To check the accuracy of proposed method, level of recovery carried out at 80, 100 and 120% of the concentration as per standard addition method.

Table 10: Recovery Studies of Capsule Sample

Capsule sample	Level of recovery %	Amount of sample drug taken (µg/ml)		Amount of standard drug added (µg/ml)		Total amount recovered (µg/ml)		% Recovery	
	Drug	Aceclofenac	Rabeprazole Sodium	Aceclofenac	Rabeprazole Sodium	Aceclofenac	Rabeprazole Sodium	Aceclofenac	Rabeprazole Sodium
Altra Day	80	200	20	160	18	359.22	37.46	99.78	98.57
	80	200	20	160	18	359.48	37.62	99.85	99
	80	200	20	160	18	359.82	37.85	99.95	99.60
	100	200	20	200	20	399.09	39.11	99.77	97.77
	100	200	20	200	20	399.36	39.59	99.84	98.97
	100	200	20	200	20	399.79	39.79	99.94	99.47
	120	200	20	240	22	439.25	41.33	99.82	98.40
	120	200	20	240	22	439.69	41.51	99.93	98.83
	120	200	20	240	22	439.83	41.86	99.92	99.66

Limit of detection (LOD)

- The Limit of detection of Rabeprazole Sodium was found to be 0.0007072 µg/ml
- The Limit of detection of Aceclofenac was found to be 0.0002591 µg/ml

Limit of Quantitation (LOQ)

- The Limit of quantitation of Rabeprazole Sodium was found to be 0.002143 µg/ml
- The Limit of quantitation of Aceclofenac was found to be 0.000785 µg/ml

Robustness: Robustness of the method was determined by carrying out the analysis under conditions during which mobile phase ratio and pH was altered. Variation of mobile phase pH and ratio were seemed to have greater impact on resolution and hence it should be meticulously controlled.

Table 11: Analysis of ALTRADAY

Sr. No.	Amount present in(mg)		Amount found in(mg)		% Label claim	
	Aceclofenac	Rabeprazole Sodium	Aceclofenac	Rabeprazole Sodium	Aceclofenac	Rabeprazole Sodium
1	200	20	199.5	19.92	99.97	99.6
2	200	20	199.82	19.96	99.98	99.8
3	200	20	199.88	19.89	99.94	99.45
4	200	20	199.91	19.91	99.95	99.55
5	200	20	199.93	19.86	99.96	99.3

Table 12: Statistical Validation of ALTRADAY Capsule Sample

Level of % recover	%Mean		± SD		Standard error of mean	
	Aceclofenac	Rabeprazole Sodium	Aceclofenac	Rabeprazole Sodium	Aceclofenac	Rabeprazole Sodium
80	99.86	99.05	0.07	0.42	0.05	0.30
100	99.85	98.40	0.07	0.71	0.05	0.50
120	99.89	99.27	0.05	0.52	0.04	0.37

Conclusion

A simple isocratic RP-HPLC method has been developed for the determination of Aceclofenac and Rabeprazole Sodium in bulk form and marketed preparation, using a PDA detector. The method was validated for accuracy, precision, specificity and linearity. The method has a relatively short run time that allows quantifying a large number of samples in routine and quality control analysis of capsules. In order to reduce cost of analysis and to increase sample throughput during routine analysis, the method is being further optimized, employing statistical experimental design.

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