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Simultaneous Estimation Of Rabeprazole Sodium And Aceclofenac In Pharmaceutical Dosage Form By HPLC

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Abstract: A simple, high performance liquid chromatographic method has been developed for the simultaneous determination of rabeprazole sodium and aceclofenac in pharmaceutical dosage form. The elution was performed using a mobile phase mixture of acetonitrile: water in the ratio of 50:50 at a flow rate of 1 ml/min on a thermo hypersil ODS C18 column (250 × 4.6 mm, i.d., 5 µm) at ambient temperature. The drugs were monitored at a wavelength of 279 nm and were separated within 10 min. The linear range is between 1 to 6 µg/ml for rabeprazole sodium and 0.5 to 5 µg/ml for aceclofenac with limits of quantitation and detection values was 0.8 µg/ml and 0.5 µg/ml for rabeprazole sodium and 0.4 and 0.2 µg/ml for aceclofenac, respectively. % RSD for intra- and inter-day studies was found to be less than 2 for all the selected concentrations. Moreover, the method was validated as per ICH guidelines and the results were found to be within the acceptable range. Hence, the proposed method can be used for the routine quality control of the drugs and can also be applied to pharmacokinetic studies.
Keywords: Rabeprazole sodium, Aceclofenac, HPLC, Validation.

Introduction

Rabeprazole sodium is chemically known as 2-[[[4-(3-methoxypropoxy)-3methyl-2-pyridinyl]-methyl] sulfinyl]-1H-benzimidazole sodium salt[1] (Fig. 1a). 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 interaction compared to omeprazole. It is official in Indian Pharmacopoeia[2].

Aceclofenac (ACE) chemically, 2-[(2,6-dichloro phenyl)amino] phenylacetoxyacectic acid[3] (Fig. 1b) is a phenylacetic acid derivative with potent analgesic and anti-inflammatory propreties. It is largely used in the symptomatic treatment of pain and of inflammatory or degenerative arthropathies like osteoarthritis, rheumatoid arthritis and ankylosing spondylities[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 HPLC in 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].

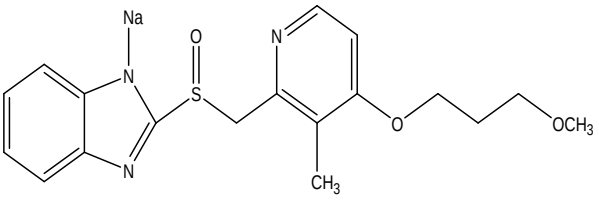

Figure 1a Rabeprazole Sodium

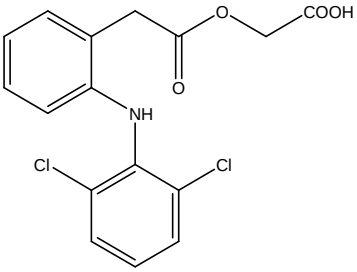

Figure 1b Aceclofenac

Experimental

Materials

Jain Pharmaceuticals Ltd. Pune, India, kindly supplied pure drug sample of rabeprazole sodium as a gift sample of Batch No. 290682 and aceclofenac of Batch No. AF01/10/034. It was used without further purification and certified to contain and 99.09 % (w/w) for rabeprazole sodium and for 99.10 % (w/w) for aceclofenac on dried basis. All chemicals and reagents used were of HPLC grade and were purchased from Merck Chemicals, India.

Chromatographic System and Conditions

The HPLC system consisted of a Pump (model Jasco PU 2080), Intelligent LC pump with sampler programmed at 20 µL capacity per injection was used. The detector consisted of UV/ VIS (Jasco UV 2075) model operated at a wavelength of 279 nm. The data was integrated using Jasco Borwin version 1.5, LC-Net II/ADC system. The column used was thermo hypersil ODS–C_{18} (250 × 4.6 mm, i.d., 5 µm) at ambient temperature. The mobile phase consisted of acetonitrile: water in the ratio of 50:50 v/v and was set at a flow rate of 1 ml/min. The total run time was 10 min. Before analysis, both the mobile phase and sample solutions were degassed by the use of a sonicator and filtered through 0.2-mm filter paper. The identities of two compounds were established by comparing retention time of the sample solution with those of standard solutions.

Preparation of Standard Solution and Construction of Calibration Plots

The standard stock solutions of rabeprazole sodium and aceclofenac were prepared by dissolving 10 mg of each drug in 100 ml of methanol. From this solution, 1 ml of solution were taken and diluted to 10 ml with the same to get a solution containing 10 µg/ml of each drug. From the stock solutions, further dilutions were prepared by diluting required volume of solution with methanol, and their area was noted by injecting 20 µl into the system. After that, a calibration curve was plotted between concentration against their respective area for rabeprazole sodium and aceclofenac separately. From the calibration curve, it was found that rabeprazole sodium had linearity ranges between 1 to 6 µg/ml, whereas aceclofenac had a range between 0.5 to 5 µg/ml.

Assay of marketed formulation

For the analysis of pharmaceutical formulation (Brand name: Altraday, Label claim: 20 mg rabeprazole sodium and 200 mg aceclofenac per capsule), twenty capsule of each drug were weighed and powdered individually. The mixture of formulation was prepared by weighing amount equivalent to labeled claim from the powdered formulation. To this, a suitable amount of methanol was added. The mixture was subjected to sonication for 30 min for a complete extraction of the drugs, and then filtered and diluted with methanol at a suitable concentration range and injected into HPLC system for the analysis. The amounts of rabeprazole sodium and aceclofenac per capsule were calculated by extrapolating the value of area from the calibration curve. Analysis procedure was repeated six times with capsule formulation. The result of analysis of capsule formulation is reported in Table 1.

Table 1. Result of assay of tablet formulation (n=6)

Drug	Label claim (mg/capsule)	Amount found (mg/capsule ± SD)	Label claim (%)
Rabeprazole sodium	20 mg	19.89 ± 1.26	99.45
Aceclofenac	200 mg	199.54 ± 1.21	99.50

Results And Discussion

HPLC Method Development and Optimization

The drugs were soluble in solvents like water and acetonitrile. During the development phase, the mobile phase containing methanol-water and methanol-buffer solutions, resulted in peaks with poor resolution and the acetonitrile-methanol resulted in asymmetric peaks with a greater tailing factor (>2) and high run time. The successful use of both acetonitrile and water reduced tailing and resulted in good peak symmetry and resolution. The optimized mobile phase contained acetonitrile: water in the ratio of 50:50 at a flow rate of 1 ml/min. The analytes were monitored at 279 nm and the retention times were found to be 4.7 and 6.3 min for rabeprazole sodium and aceclofenac, respectively (Fig. 2).

Validation of the Developed Method

The method was validated for linearity, accuracy, precision, LOD and LOQ, robustness and specificity study. All the validation study was carried out by replicate injection of the sample and standard solutions.

Linearity

The linearity was determined for two drugs, rabeprazole sodium and aceclofenac, separately by plotting a calibration graph of peak area against their respective concentration. From the calibration curve, it was clear that rabeprazole sodium had linearity between 1 to 6 µg/ml, whereas aceclofenac had a range between 0.5 to 5 µg/ml. The linear regression equation for two drugs was

Rabeprazole sodium:y =25520x-15942 (r^2= 0.999)

Aceclofenac: y =44542x-10982 (r^2= 0.998)

Where y is peak area and x is concentration.

Accuracy

Accuracy of the developed method was conformed by doing a recovery study as per ICH norms at three different concentration levels (80%, 100% and 120%) by replicate analysis (n=3). Standard drug solutions were added to a preanalyzed sample solution, and then percentage of drug content was calculated. The results of the accuracy study are reported in **Table 2**. From the recovery study, it was clear that the method is very accurate for quantitative estimation of rabeprazole sodium and aceclofenac in capsule dosage form because all the statistical results were within the acceptance range (i.e., % RSD <2.0).

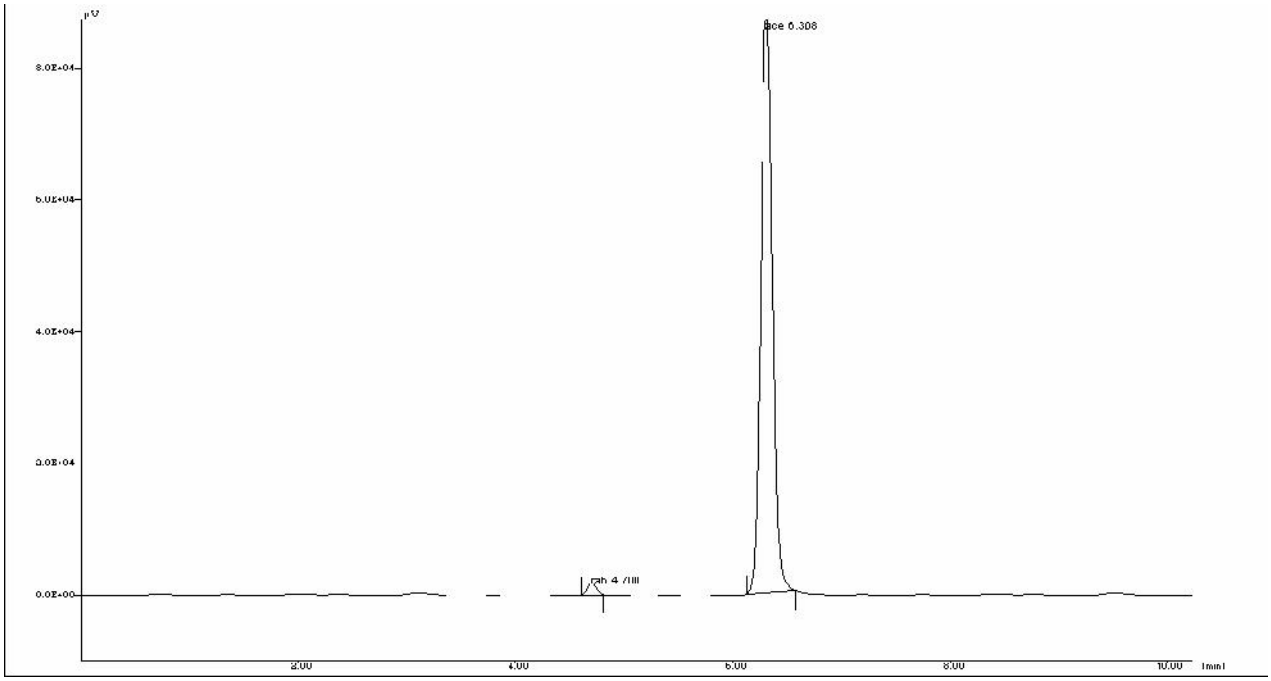

Figure 2 HPLC chromatogram of standard Rabeprazole sodium (T_R=4.7) and Aceclofenac (T_R =6.3)

Table 2. Recovery studies (n = 6)

Label claim (mg/capsule)	Amount added (mg/ capsule)	Total amount (mg)	Amount recovered (mg ± % RSD)	% Recovery
Rabeprazole Sodium				
20	16 (80%)	36	36.00 ± 0.96	100.00
20	20(100%)	40	39.85 ± 1.01	99.62
20	24 (120%)	44	43.50 ± 0.78	98.86
Aceclofenac				
200	160 (80%)	360	360.46 ± 1.16	100.13
200	200 (100%)	400	396.84 ± 1.40	99.21
200	240 (120%)	440	438.76 ± 0.98	99.72

Precision

Precision was determined by studying the repeatability and intermediate precision. Repeatability result indicates the precision under the same operating conditions over a short interval time and interassay precision. The standard deviation and relative standard deviation were calculated for two drugs. Intermediate precision was carried out by doing intra- and interday precision studies. In the intraday study, the concentrations of two drugs were calculated on the same day at an interval of 1 h. In the interday study, the concentrations of drug contents were calculated on three different days, and the study expresses within-laboratory variation in different days (Table 3). The developed method was precise for quantitative study because the precision study was found statistically significant (% RSD <2.0 for intra- and interday studies).

LOD and LOQ

LOD and LOQ studies were carried out to evaluate the detection and quantization limits of the method to determine the presence of any impurities by using following equation:

LOD = 3:3 σ/S

LOQ = 10 σ/S

Where σ is the standard deviation and S is the slope of the curve.

The LOD and LOQ were found to be 0.5 µg/ml and 0.8 µg/ml rabeprazole sodium and 0.2 µg/ml and 0.4 µg/ml for aceclofenac, respectively.

Robustness

The robustness of the proposed method was found after altering the parameters deliberately: variation of flow rate, percentage of acetonitrile in the mobile phase and solvents from different lot were taken. The retention time of the compound was evaluated, and the resolution had no significant changes when the parameters were changed. The relative standard deviation (%RSD) was found to be less than 2 (Table 4).

Specificity

Specificity of the method was assessed by comparing the chromatograms obtained from standard drugs with the chromatogram obtained from capsule solutions. Because the retention time of standard drugs and the retention time of two drugs in sample solutions were the same, the method was specific. The developed method was specific as no interference of excipients was found.

Table 3. Precision studies

Conc. (µg/ml)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured Conc.	(%) RSD	Recovery (%)	Measured Conc.	(%)RSD	Recovery (%)
Rabeprazole Sodium						
1	0.985	0.01	98.50	0.995	1.61	99.50
3	2.945	0.83	98.16	2.961	0.98	98.70
5	4.921	1.62	98.42	4.978	1.16	99.56
Aceclofenac						
0.5	0.493	1.16	98.60	0.497	1.08	99.40
2	1.985	0.73	99.25	1.961	0.79	98.05
4	3.956	1.09	98.50	3.931	1.58	98.27

Table 4 Robustness testing[a] (n = 3)

Factor[a]	Level	Retention time	Retention factor	Asymmetry
Rabeprazole Sodium				
A: Flow rate (ml/min)				
0.9	-1	4.94	0.97	1.052
1.0	0	4.70	0.88	1.048
1.1	+1	4.52	0.80	1.041
Mean ± SD (n = 3)		4.72 ± 0.05	0.88 ± 0.04	1.047 ± 0.04
B: % of acetonitrile in the mobile phase (v/v)				
49	-1	4.96	0.98	1.051
50	0	4.70	0.88	1.048
51	+1	4.62	0.84	1.042
Mean ± SD (n = 3)		4.76 ± 0.04	0.90 ± 0.05	1.047 ± 0.03
C: Solvents of different lots				
First lot		4.70	0.88	1.048
Second lot		4.62	0.84	1.051
Mean ± SD (n = 3)		4.66 ± 0.01	0.86 ± 0.01	1.049 ± 0.01
Aceclofenac				
A: Flow rate (ml/min)				
0.9	-1	6.40	1.56	1.059
1.0	0	6.30	1.52	1.055
1.1	+1	6.28	1.51	1.049
Mean ± SD (n = 3)		6.32 ± 0.04	1.53 ± 0.01	1.054 ± 0.04
B: % of acetonitrile in the mobile phase (v/v)				
49	-1	6.42	1.56	1.058
50	0	6.30	1.52	1.055
51	+1	6.22	1.48	1.051
Mean ± SD (n = 3)		6.31 ± 0.06	1.52 ± 0.02	1.054 ± 0.05
C: Solvents of different lots				
First lot		6.3	1.52	1.055
Second lot		6.2	1.48	1.053
Mean ± SD (n = 3)		6.25 ± 0.04	1.5 ± 0.03	1.054 ± 0.01

[a] Three factors were slightly changed at three levels (-1, 0, 1)

Conclusion

A new, reversed-phase HPLC method has been developed for simultaneous quantization of rabeprazole sodium and aceclofenac in capsule formulation. It has been shown that the developed method achieved accuracy, repeatability, linearity, precision, and specific, which prove the reliability of the method. The run time is relatively short, 10 min, which enables rapid quantization of many samples in routine and quality-control analysis of capsule formulation. The same solvent was used throughout the experimental work, and no interference of any excipients matrices was found. The result shows that the method could find practical application as a quality-control tool for the simultaneous estimation of two drugs from their combined dosage form in a quality-control laboratory.

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