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Research Article

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VALIDATED HPTLC METHOD FOR SIMULTANEOUS ESTIMATION OF RABEPRAZOLE SODIUM AND ACECLOFENAC IN BULK DRUG AND FORMULATION

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ABSTRACT

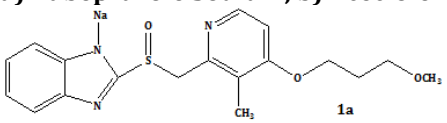
A simple, precise and robust high performance thin layer chromatographic method was developed and validated for the determination of rabeprazole sodium and aceclofenac in the combined pharmaceutical capsule dosage form. The precoated silica gel 60F254 aluminum plate was selected as the stationary phase and solvent system consisted of toluene: ethyl acetate: methanol: acetic acid 6: 4: 1: 0.2 (v/v/v/v) used as developing solvents. The detection of rabeprazole sodium and aceclofenac was carried out at 279nm. The developed method was validated for linearity, accuracy, precision, limit of detection, limit of quantitation and robustness parameters. The correlation coefficient of rabeprazole sodium and aceclofenac were 0.997 and 0.998 observed respectively. The average percentage recovery of rabeprazole sodium and aceclofenac were found to be 99.12 % and 99.99 % respectively. Intra and inter day precision measured as coefficient of variation were less than 2% for both analytes. The proposed HPTLC method has potential applications for determination of rabeprazole sodium and aceclofenac in combined capsule dosage form.

Keywords: Thin layer Chromatography, Densitometry, Validation and Quantification, Rabeprazole Sodium and Aceclofenac.

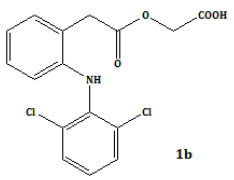
INTRODUCTION

Rabeprazole sodium is chemically known as 2-[[[4-(3-methoxypropoxy) -3methyl -2-pyridinyl] -methyl] sulfinyl] -1H-benzimidazole sodium salt[1] (Figure **1a**). Rabeprazole Sodium (RBP) is proton pump inhibitor that suppress 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.[2]

Figure 1. a) Rabeprazole Sodium, b) Aceclofenac



1a



1b

Aceclofenac (ACE) chemically, 2-[(2,6-dichlorophenyl) amino] phenylacetoxyacectic acid (Figure **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. It is official in Indian Pharmacopoeia.[3,4] Literature review reveals that methods have been reported for analysis of rabeprazole sodium by high performance liquid chromatography (HPLC)[5,6] and high performance thin layer chromatography (HPTLC)[7] and for estimation of aceclofenac by HPLC[8-12] either alone or in combination with other drugs.

Today TLC is rapidly becoming a routine analytical technique due to its advantages of low operating costs, high sample throughput and the need for minimum sample preparation. The major advantage of TLC is that several samples can be run simultaneously using a small quantity of mobile phase-unlike HPLC - thus reducing the analysis time and cost per analysis.

To date, there have been no published reports about the simultaneous estimation of rabeprazole sodium and aceclofenac by HPTLC in bulk drug and in pharmaceutical dosage forms. This present study reports for the first time simultaneous estimation of rabeprazole sodium and aceclofenac by TLC in bulk drug and in pharmaceutical dosage forms. The proposed method is validated as per ICH guidelines.[13-15]

MATERIALS AND METHODS

Materials

Working standards of pharmaceutical grade rabeprazole sodium (batch no. AF01/10/034) and aceclofenac (batch no. 290682) were obtained as generous gifts from Jain Pharmaceutical Pvt Ltd, (Maharashtra, India). It was used without further purification and certified to contain 99.09

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% and 99.10 % (w/w) on dry weight basis, rabeprazole sodium and aceclofenac respectively. Fixed dose combination capsule (Altraday) containing 20mg rabeprazole sodium and 200mg aceclofenac were procured from Ranbaxy Pvt. Ltd. India. All chemicals and reagents of analytical grade were purchased from Merck Chemicals, Mumbai, India.

Instrumentation

The samples were spotted in the form of bands of width 6mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60_{F-254} plates, [20cm × 10cm with 250 µm thickness; E. Merck, Darmstadt, Germany)] using a Camag Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110˚C for 5min prior to chromatography. A constant application rate of 0.1µL/s was used and the space between two bands was 5 mm. The slit dimension was kept at 5mm × 0.45mm and the scanning speed was 10 mm/s. The monochromator bandwidth was set at 20 nm, each track was scanned three times and baseline correction was used. The mobile phase consisted of toluene: ethyl acetate: methanol: acetic acid (6: 4: 1: 0.2 (v/v/v/v). Linear ascending development was carried out in a 20cm × 10cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30min at room temperature (25˚C ±2) at relative humidity of 60% ±5. The length of each chromatogram run was 8cm. Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 279nm and operated by CATS software (V 3.15, Camag).

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 10 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 100µg/ml of each drug. 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 range between 100 to 200 ng/spot, whereas aceclofenac had a range between 1000 to 2000 ng/spot.

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 the samples were spotted on the TLC 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. Analysis of commercial formulation (n=6)

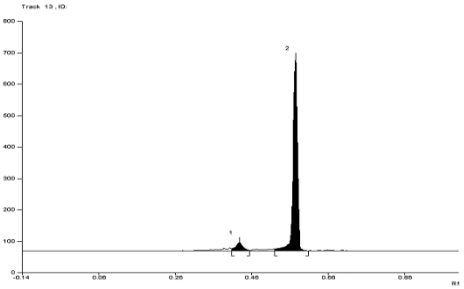
S No	Drug	Label claim (mg/capsule)	Amount found (mg/capsule±SD)	Label claim (%)
1	Rabeprazole sodium	20 mg	19.74 ± 0.43	98.70
2	Aceclofenac	200 mg	199.05 ± 0.89	99.52

RESULTS AND DISCUSSION

HPTLC Method Development and Optimization

Initially, ethyl acetate and toluene in the ratio of 4:4(v/v) was tried for both drugs. Then, toluene ethyl acetate and methanol in the ratio of 6:4:1 (v/v/v) was tried. The developed spots were diffused. To the above mobile phase, 0.1mL glacial acetic acid was added. Both the peaks were symmetrical in nature and tailing was observed. To improve resolution, the volume of glacial acetic acid was increased to 0.2 mL. The optimized mobile phase contained toluene: ethyl acetate: methanol: acetic acid in the ratio of 6: 4: 1: 0.2 v/v/v/v. The analytes were monitored at 279 nm and the R_f were found to be 0.43 and 0.57 for rabeprazole sodium and aceclofenac, respectively (Figure **2**).

Figure 2. Chromatogrm of standard Rabeprazole Sodium R_f (0.43) and Aceclofenac R_f (0.57)



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 100 to 200 ng/spot, whereas aceclofenac had a range between 1000 to 2000 ng/spot. The linear regression equation for two drugs was

Rabeprazole sodium

$$y = 5.517x - 308.2 \quad (r^2 = 0.997)$$

Aceclofenac

$$y = 3.390x - 275.5 \quad (r^2 = 0.998)$$

Where **y** is peak area and **x** is concentration.

Accuracy: Accuracy of the developed method was confirmed by doing a recovery study as per ICH guidelines 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).

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).

Table 2. Recovery studies (n =3)

Label claim (mg/capsule)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Rabeprazole Sodium				
20	16 (80%)	36	35.82 ± 0.71	99.52
20	20 (100%)	40	39.32 ± 1.07	98.32
20	24 (120%)	44	43.79 ± 0.91	99.53
Aceclofenac				
200	160 (80%)	360	360.60 ± 11.03	100.16
200	200 (100%)	400	398.44 ± 10.57	99.87
200	240 (120%)	440	439.77 ±15.69	99.94

Table 3. Precision studies

Concentration (ng/spot)	Repeatability (n=3)			Intermediate precision (n=3)		
	Measured conc. ±SD	(%) RSD	Recovery (%)	Measured conc. ±SD	(%)RSD	Recovery (%)
Rabeprazole Sodium						
100	98.22 ± 1.17	1.19	98.22	98.51 ± 1.32	1.33	98.51
140	139.71 ± 2.6	1.8	99.79	139.12 ± 2.4	1.72	99.37
180	178.92 ± 2.9	1.62	99.40	179.21 ± 2.2	1.20	99.56
Aceclofenac						
1000	998.89 ± 10.7	1.07	99.88	997.45 ± 11.0	1.10	99.74
1400	1381.41 ± 16.0	1.15	98.64	1391.73± 14.7	1.05	99.40
1800	1795.24 ± 37.3	1.40	99.73	1773.91 ± 19.4	1.09	98.55

LOD and LOQ: The LOD and LOQ were found to be 80 and 100 ng/spot for rabeprazole sodium and 10 and 12 ng/spot for aceclofenac, respectively.

Robustness: Robustness is checked by making slight deliberate change in the experimental procedures. The result obtained is shown in Table **4**.

Table 4. Robustness testing (n = 3)

Parameter	SD of peak area for Rabeprazole Sodium	% RSD	SD of peak area for Aceclofenac	% RSD
Mobile phase composition (± 0.1 ml)	3.46	0.93	14.12	0.84
Amount of mobile phase (± 5%)	6.12	1.38	15.02	1.01
Time from spotting to chromatography (± 10 min.)	4.63	0.79	11.17	0.97
Time from chromatography to scanning (± 10 min.)	3.28	1.42	10.27	0.71

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 factor of standard drugs and the retention factor 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.

CONCLUSION

The developed HPTLC method is simple, precise, accurate and reproducible and can be used for simultaneous determination of rabeprazole sodium and aceclofenac in capsules. The method was validated as per ICH guidelines. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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