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Validated HPTLC method for simultaneous estimation of Rabeprazole Sodium, Paraetamol and Aceclofenac in bulk drug and formulation

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ABSTRACT

This paper describes a new, simple, precise, and accurate HPTLC method for simultaneous estimation of Rabeprazole sodium, Paracetamol and Aceclofenac as the bulk drug and in tablet dosage forms. Chromatographic separation of the drugs was performed on aluminum plates precoated with silica gel 60 F_{254} as the stationary phase and the solvent system consisted of toluene: ethyl acetate: methanol: acetic acid 6: 4: 0.8: 0.4 (v/v/v/v). Densitometric evaluation of the separated zones was performed at 256 nm. The three drugs were satisfactorily resolved with R_F values 0.35 ± 0.5, 0.42 ± 0.3 and 0.57 ± 0.42 for Rabeprazole sodium, Paracetamol and Aceclofenac respectively. The accuracy and reliability of the method was assessed by evaluation of linearity 100–200 ng/spot for Rabeprazole sodium, 15–90 ng/spot for Paracetamol and 12-72 ng/spot for Aceclofenac., precision (intra-day RSD 1.66–1.87 % and inter-day RSD 1.48–1.60 % for Rabeprazole sodium , and intra-day RSD 1.03–1.74 % and inter-day RSD 0.53–1.59 % for Paracetamol similarly intra -day RSD 1.12–1.73 % and inter-day RSD 0.62–1.73 % for Aceclofenac), accuracy (100.25 ± 0.12 % for Rabeprazole Sodium, 99.67 ±0.06 % for Paracetamol and 99.63 ± 0.18 % for Aceclofenac), and specificity, in accordance with ICH guidelines.

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

INTRODUCTION

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

Paracetamol, chemically 4-hydroxy acetanilide[fig 1(b)],is a centrally and peripherally acting nonopioid analgesic and antipyretic Literature survey reveals, there are UV, HPLC and HPTLC methods reported for the estimation of Paracetamol in Pharmaceutical formulations [3].

Aceclofenac, is chemically, 2-[(2,6-dichlorophenyl)amino] phenylacetoxyacectic acid[4] [Fig.1(c)] is a phenyl acetic 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[5]. Literature review reveals that methods have been reported for analysis of Rabeprazole by high performance liquid chromatography (HPLC) [6-7] and high performance thin layer chromatography (HPTLC) [8] and for estimation of Paracetamol by HPLC [9] either alone or in combination with other drugs and high performance thin layer chromatography (HPTLC) [10-12] either alone or in combination with other drug, similarly for analysis of Acelofenac by high performance liquid chromatography (HPLC) [13-16] and either alone or in combination with other drugs. The proposed method is validated as per ICH guidelines [17-19].

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.

MATERIALS AND METHODS

Working standards of pharmaceutical grade Rabeprazole sodium and Aceclofenac and Paracetamol were obtained as generous gifts from Jain Pharmaceutical Pvt. Limited, (Maharashtra, India). It was used without further purification and certified to contain 99.09 % and 99.10 % (w/w) on dry weight basis, Rabeprazole sodium and Aceclofenac respectively. Fixed dose combination tablets (ACE-PROXYVON) containing 10 mg Rabeprazole sodium, 500mg Paracetamol and 100 mg Aceclofenac were procured from Wockhardt 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 6 mm with a Camag 100 microlitre sample (Hamilton, Bonaduz, Switzerland) syringe on silica gel precoated aluminum plate 60 F_{254} plates, [20 cm × 10 cm 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 ^{o}C for 5 min 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 5 mm × 0.45 mm 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: 0.8: 0.4 (v/v/v/v) and 11.2 mL of mobile phase was used per chromatography run. Linear ascending development was carried out

in a 20 cm × 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile phase was 30 min at room temperature (25 ^{o}C ± 2) at relative humidity of 60 % ± 5. The length of each chromatogram run was 8 cm. Following the development the TLC plates were dried in a current of air with the help of an air dryer in a wooden chamber with adequate ventilation. The flow rate in laboratory was maintained unidirectional (laminar flow, towards the exhaust). Densitometric scanning was performed using a Camag TLC scanner III in the reflectance-absorbance mode at 256 nm and operated by CATS software (V 3.15, Camag).

Preparation of Standard Stock Solutions

Standard stock solutions of each concentration 1000µg/ml of Rabeprazole sodium, Paracetamol and Aceclofenac were prepared separately using methanol. From the standard stock solution, the mixed standard solution was prepared using the methanol to contain 100µg/ml of Rabeprazole sodium, Paracetamol and Aceclofenac.

Optimization of the HPTLC method

The TLC procedure was optimized with a view to develop a simultaneous assay method for Rabeprazole Sodium, Paracetamol and Aceclofenac respectively. The mixed standard stock solution (100µg/ml of Rabeprazole Sodium, 100 µg/ml of Paracetamol and 100µg/ml of Aceclofenac) was taken and 10µl band spotted on to TLC plates and run in different solvent systems. Initially, toluene, ethyl acetate and methanol were tried in different ratios. Toluene was used to impart the necessary non-polarity to mobile phase to obtain a suitable R_F value. Initially, toluene, ethyl acetate and methanol in the ratio of 5: 5: 2 v/v/v was selected but tailing and fronting with peaks was observed. Acetic acid 0.1 mL added to improve tailing and fronting effects of peaks. Finally, the mobile phase consisting of toluene: ethyl acetate: methanol: acetic acid in the ratio of 6: 4: 0.8: 0.4 v/v/v/v was found optimum (Figure 2). In order to reduce the neckless effect TLC chamber was saturated for 20 min using saturation pads. The mobile phase was run upto a distance of 8 cm; which takes approximately 20 min for complete development of the TLC plate.

Validation of the method

Validation of the optimized TLC method was carried out with respect to the following parameters.

Linearity and range

From the mixed standard stock solution 100µg/ml of Rabeprazole Sodium, 15µg/ml Paracetamol and 12µg/ml of Aceclofenac, 1 to 6 µl solution spotted on TLC plate to obtain final concentration 100-600 ng/spot for Rabeprazole Sodium, 15-90 ng/spot Paracetamol and 12-72 ng/spot for Aceclofenac. Each concentration was applied six times to the TLC plate. The plate was then developed as per procedure described above.

Precision

The precision of the method was verified by repeatability and intermediate precision studies. Repeatability studies were performed by analysis of three different concentrations (100, 300, 500 ng/spot for Rabeprazole Sodium 15, 45, 75 ng/spot, Paracetamol and 12, 36, 60 ng/ spot for

Aceclofenac) of the drugs in three times on the same day. The intermediate precision of the method was checked by repeating studies on three different days.

Limit of detection and limit of quantitaiton

Limits of detection (LOD) and quantification (LOQ) represent the concentration of the analyte that would yield signal-to-noise ratios of 3 for LOD and 10 for LOQ, respectively. LOD and LOQ were determined by measuring the magnitude of analytical background by spotting a blank and calculating the signal-to-noise ratio for Rabeprazole Sodium, Paracetamol and Aceclofenac by spotting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ.

Robustness of the method

Following the introduction of small changes in the mobile phase composition (± 0.1 mL for each component), the effects on the results were examined. Mobile phases having different compositions, e.g. toluene: ethyl acetate: methanol: acetic acid (6: 4: 0.8: 0.3 v/ v/v/v), (6: 4: 0.9: 0.4 v/v/v/v), (6: 4.1: 0.8: 0.4 v/v/v/v), (5.9: 4: 0.8: 0.4 v/v/v/v), were tried and chromatograms were run. The amount of mobile phase was varied over the range of ± 5 %. The plates were prewashed with methanol and activated at 60 °C for 2, 5, and 7 min respectively prior to chromatography. The time from spotting to chromatography and from chromatography to scanning was varied from ± 10 min.

Specificity

The specificity of the method was determined by analyzing standard drug and test samples. The spot for Rabeprazole sodium, Paracetamol and Aceclofenac in the samples was confirmed by comparing the R_F and spectrum of the spot with that of a standard. The peak purity of Rabeprazole sodium, Paracetamol and Aceclofenac was determined by comparing the spectrum at three different regions of the spot i.e. peak start (S), peak apex (M) and peak end (E).

Accuracy

Accuracy of the method was carried out by applying the method to drug sample (Rabeprazole sodium, Paracetamol and Aceclofenac combination Tablet) to which know amount of Rabeprazole sodium, Paracetamol and Aceclofenac standard powder corresponding to 80, 100 and 120% of label claim had been added (Standard addition method), mixed and the powder was extracted and analyzed by running chromatogram in optimized mobile phase.

Analysis of a marketed formulation

To determine the content of Rabeprazole sodium, Paracetamol and Aceclofenac in conventional tablet (Brand name: ACE-PROXYVON containing 10 mg Rabeprazole Sodium, 500mg Paracetamol and 100 mg Aceclofenac), twenty tablet were weighed, their mean weight determined and finely powdered. The weight of the tablet triturate equivalent to 10 mg of Rabeprazole sodium, 500mg Paracetamol and 100 mg Aceclofenac was transferred into a 50 mL volumetric flask containing 30 mL methanol, sonicated for 30 min and diluted to 50 mL with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (200µg/ml for Rabeprazole sodium, 10000µg/ml Paracetamol and 2000µg/ml Aceclofenac respectively). Then 5 mL of the above filtered solution was diluted to produce a concentration of 100 µg/mL, 5000 µg/mL and 1000 µg/mL for Rabeprazole Sodium, Paracetamol and Aceclofenac respectively and 1 µL of this solution (100, 5000 and

1000 ng/spot for Rabeprazole Sodium, Paracetamol and Aceclofenac respectively) was applied to a TLC plate which was developed in optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

The results of validation studies on simultaneous estimation method developed for Rabeprazole sodium, Paracetamol and Aceclofenac in the current study involving toluene: ethyl acetate: methanol: acetic acid (6: 4: 0.8: 0.4, v/v/v/v) as the mobile phase for TLC are given below.

Linearity

The drug response was linear (r^2 = 0.999 for Rabeprazole sodium ,r^2=0.999 Paracetamol and 0.999 for Aceclofenac) over the concentration range between 100-600 ng/spot for Rabeprazole sodium, 15-90 ng/spot Paracetamol and 12-72 ng/spot for Aceclofenac. The mean (± RSD) values of the slope, intercept and correlation coefficient for Rabeprazole sodium, Paracetamol and Aceclofenac were 2.166(± 0.96), 33.43 (± 1.24), 0.999 (± 1.42), and 9.872(± 0.16), 6.822 (± 1.62) ,0.999 (± 1.12) and 20.29(± 0.73), 131.5.43 (± 0.67) and 0.999 (± 0.93) respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

LOD and LOQ

Signal-to-noise ratios of 3: 1 and 10: 1 were obtained for the LOD and LOQ respectively. The LOD and LOQ of Rabeprazole sodium, Paracetamol and Aceclofenac were found to be 80 ng/spot and 100 ng/spot, 12 ng spot and 14 ng/spot and 10 ng/spot and 12 ng/spot, respectively.

Robustness of the method

The standard deviation of peak the areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in Table 2 indicated robustness of the method.

Specificity

The peak purity of Rabeprazole sodium, Paracetamol and Aceclofenac was assessed by comparing their respective spectra at the peak start, apex and peak end positions of the spot i.e., r (S, M) = 0.9990 and r (M, E) = 0.9991. A good correlation (r = 0.9995) was also obtained between the standard and sample spectra of Rabeprazole sodium, Paracetamol and Aceclofenac respectively.

Recovery Studies

As shown from the data in Table 3 good recoveries of the Rabeprazole sodium, Paracetamol and Aceclofenac in the range from 99.14 to 101.25 % were obtained at various added concentrations. The average recovery of three levels (nine determinations) for Rabeprazole sodium, Paracetamol and Aceclofenac were 100.25% (± 1.06), 99.67% (± 1.12) and 99.63% (± 0.40) respectively.

Analysis of a formulation

Experimental results of the amount of Rabeprazole sodium, Paracetamol and Aceclofenac in tablet, expressed as a percentage of label claim were in good agreement with the label claims thereby suggesting that there is no interference from any of the excipients which are normally present in tablet. The drug content was found to be 98.40% ± 0.42, 99.95±0.16 and 99.73 ±0.28% for Rabeprazole sodium, Paracetamol and Aceclofenac respectively (Table 4).

Table 1 Precision studies

Concentration (ng/spot)	Repeatability (n=6)			Intermediate precision (n=6)		
	Measured conc.	(%) RSD	Recovery (%)	Measured conc.	(%) RSD	Recovery (%)
Rabeprazole Sodium						
100	99.22	1.80	99.22	99.61	1.50	99.61
300	298.71	1.87	99.57	299.51	1.60	99.83
500	498.92	1.66	99.78	498.21	1.48	99.64
Paracetamol						
15	14.95	1.03	99.66	14.76	1.18	98.40
45	44.21	1.47	98.24	44.63	1.59	99.17
75	74.73	1.74	99.64	74.84	0.53	99.78
Aceclofenac						
12	11.91	1.12	99.25	11.93	1.09	98.41
36	35.41	1.62	98.36	35.73	1.73	99.25
60	59.89	1.73	99.81	59.54	0.62	99.23

Table 2 Robustness testing

Parameter	SD of peak area for **Rabeprazole Sodium**	% RSD	SD of peak area for **Paracetamol**	% RSD	SD of peak area for **Aceclofenac**	% RSD
Mobile phase composition (± 0.1 ml)	4.96	0.72	7.05	0.97	19.75	0.74
Amount of mobile phase (± 5%)	8.31	1.64	18.18	1.57	21.65	1.32
Time from spotting to chromatography (± 10 min.)	8.85	1.79	14.37	1.47	11.06	1.09
Time from chromatography to scanning (± 10 min.)	5.62	1.42	9.17	1.21	16.87	1.31

(n = 6)

Table 3 Recovery studies

Label claim (mg/tablet)	Amount added (mg)	Total amount (mg)	Amount Recovered (mg) ± % RSD	% Recovery
Rabeprazole Sodium				
10	8 (80%)	18	18.22 ± 0.87	101.25
10	10 (100%)	20	19.93 ± 1.04	99.66
10	12 (120%)	22	21.96 ± 0.68	99.84
Paracetamol				
500	400 (80%)	900	897.43± 1.03	99.71
500	500 (100%)	1000	999.99± 1.35	99.99
500	600 (120%)	1100	1092.56 ± 0.97	99.32
Aceclofenac				
100	80 (80%)	180	178.45 ± 0.85	99.14
100	100 (100%)	200	199.93 ± 1.06	99.96
100	120 (120%)	220	219.57 ± 1.69	99.80

(n = 6)

Table 4 Analysis of commercial formulation

Drug	Label claim (mg/tablet)	Amount found* (mg)	% of drug content*
Rabeprazole sodium	10	9.84	98.40
Paracetamol	500	499.75	99.95
Aceclofenac	100	99.73	99.73

** Each value is a mean of six determinations*

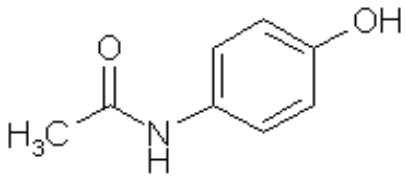

Fig.1(a). Paracetamol

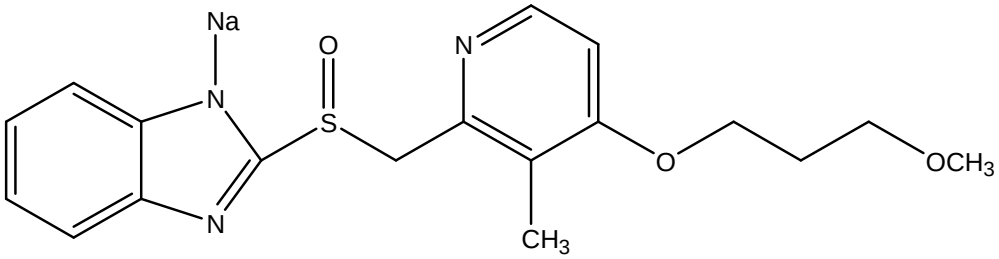

Fig.1(b).Rabeprazole Sodium

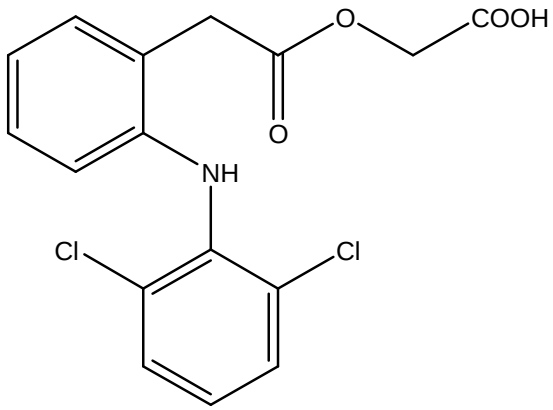

Fig.1(c). Aceclofenac

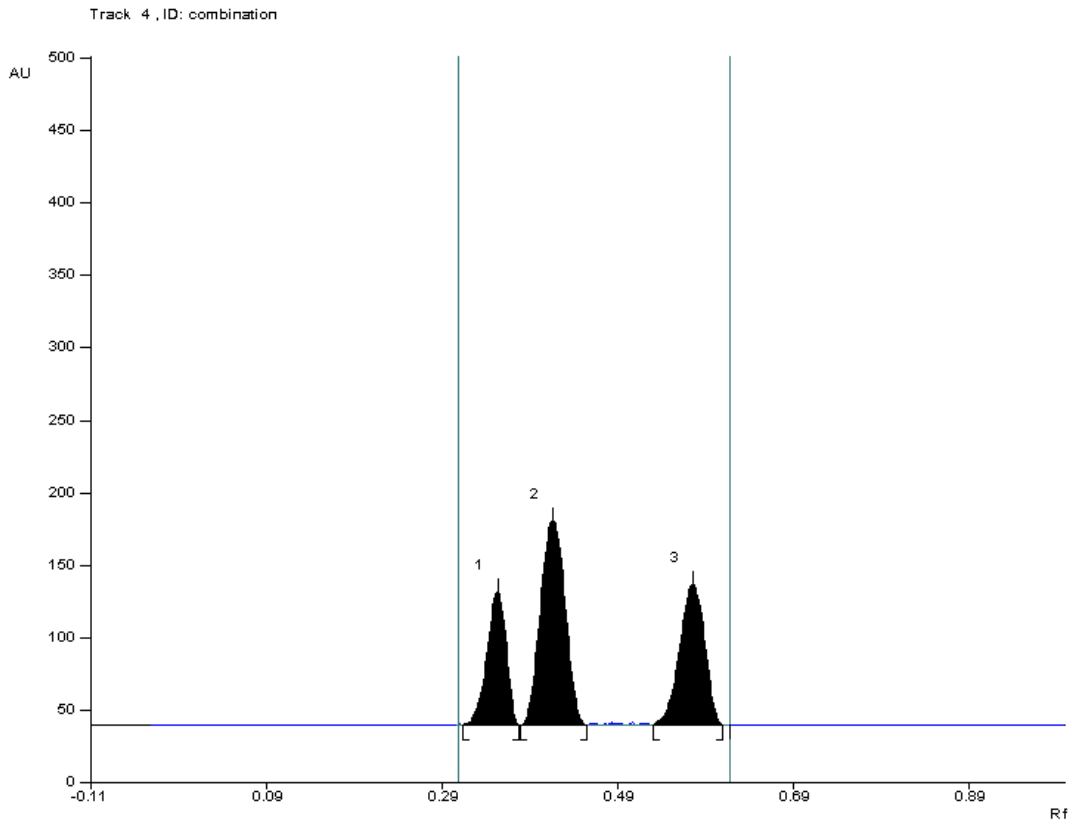

Fig.2. Chromatogarm of standard Rabeprazole Sodium Rf (0.35), Paracetamol R_f (0.42) and Aceclofenac R_f (0.57)

CONCLUSION

Introducing TLC into pharmaceutical analysis represents a major step in terms of quality assurance. The developed TLC technique is precise, specific and accurate. Statistical analysis proves that the method is suitable for the analysis of Rabeprazole sodium, Paracetamol and Aceclofenac as bulk drug and in pharmaceutical formulation without any interference from the excipients. It may be extended to study the degradation kinetics of Rabeprazole sodium, Paracetamol and Aceclofenac and also for its estimation in plasma and other biological fluids. The proposed TLC method is less expensive, simpler, rapid, and more flexible than HPLC.

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