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Application of Stability-Indicating RP-TLC Densitometric Determination of Rabeprazole Sodium in Bulk and Pharmaceutical Formulation

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Abstract

A simple, sensitive, selective, precise and stability- indicating reversed-phase thin layer chromatography (RP-TLC) densitometric determination of rabeprazole sodium as bulk and from pharmaceutical formulation have been developed and validated as per International Conference on Harmonisation (ICH) guidelines. The method employed RP-TLC aluminium plates precoated with silica gel 60 RP-18 F-254 S as the stationary phase. The mobile phase consisted of acetone: water (3.5:1.5 *v/v*). The system was found to give compact spot for rabeprazole sodium (R_f value of 0.45 ± 0.02). Densitometric detection was carried out at λ = 284 nm. The linear regression analysis data for the calibration plots showed good linear relationship with r = 0.9998 in the working concentration range of 400 – 2400 ng spot $^{-1}$. The method was validated for precision, accuracy, ruggedness, robustness, specificity, recovery, limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were found to be19.89 and 60.29 ng per spot, respectively. The drug was subjected to acid and alkali hydrolysis, oxidation, dry heat and photodegradation, all the peaks of the degradation products were well resolved from the standard drug with significantly different R_f values. Statistical analysis proves that the developed RP- TLC densitometry method is reproducible and selective. The developed RP-TLC densitometric method can be applied for identification and quantitative determination of rabeprazole sodium in bulk drug and tablet formulation.

Keywords:

Rabeprazole sodium; Reverse-phase thin-layer chromatography; Validation; Stability-indicating; Degradation

1. Introduction

Rabeprazole sodium is chemically designated as (±)-sodium 2-[[4-(3-methoxy-propoxy)-3-methylpyridine-2-yl] methylsulfinyl]-1*H*-benzimidazole [1] (Fig.1). It is proton pump inhibitor that covalently binds and inactivates the gastric parietal cell proton pump (H+/K+ ATPase) and used in the treatment of peptic ulcer [2]. Rabeprazole, a prodrug is metabolized by cytochrome P450 or CYP450 in the acid environment [3]. In the preclinical trial it has been proved that rabeprazole is six times more active than omeprazole in inhibiting the enzyme activity of isolated gastric vesicles [4].

In literature, several analytical methods; LC – tandem mass spectrometry [5], HPLC using solid phase extraction [6] have been published for estimation of rabeprazole in human plasma. Several HPLC methods [7 - 9] have been studied for estimation of rabeprazole in pharmaceutical formulations. Stability-indicating HPLC [10], structural elucidation of rabeprazole sodium photodegradation products [11], identification and characterization of

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potential impurities of rabeprazole sodium [12], capillary electrophoresis [13] have been studied. HPTLC methods [14, 15] for determination of rabeprazole sodium in combinations with other drugs have been reported. Many spectrophotometric methods [16-18] have been reported for determination of rabeprazole sodium in bulk and pharmaceutical formulations. Supercritical fluids and chiral chromatographic columns for the separation of rabeprazole enantiomers are also published [19, 20].

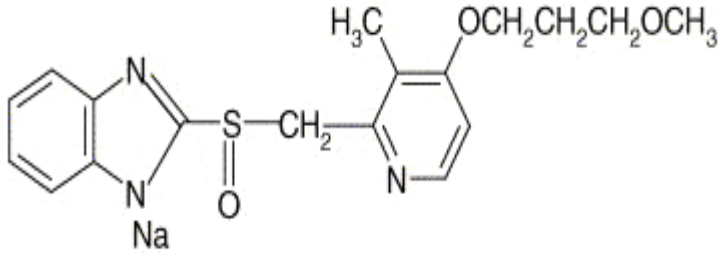

Fig.1. Chemical structure of rabeprazole sodium

According to International Conference on Harmonization (ICH), the objective of validation of an analytical procedure is to demonstrate that it is suitable for its intended purpose. The extend of drug product testing should be established by assessing whether or not acceptable changes has occurred at the end of forced degradation studies [21 - 25].

The aim of present work is to develop a simple, accurate rapid and sensitive, stability indicting reverse- phase TLC-Densitometric method for determination of rabeprazole sodium in bulk and pharmaceutical formulation.

2. Experimental

2.1. Materials

Rabeprazole sodium was supplied by Torrent Pharmaceutical Ltd. Ahmedabad, India as a gift sample. All chemical and reagents used were of analytical grade and purchased from Merck ltd. (Mumbai, India).

2.2. HPTLC Instrumentation

The samples were spotted in the form of bands of width 6 mm with Camag microlitre syringe on silica gel 60 RP-18 F-254 S (20 cm x 10 cm with 0.2 mm thickness E. Merck, Germany) using Camag Linomat 5 (Switzerland). A constant application rate of 150 nL sec $^{-1}$ was employed and space between two bands was 15.4 mm. The slit dimension was kept 6 mm x 0.45 mm. The mobile phase consisted of acetone: water (3.5:1.5 *v/v*) and 8.0 mL mobile phase was used per chromatography. Linear ascending development was carried out in 20 cm x 10 cm twin trough glass chamber saturated with mobile phase. The optimized chamber saturation time for mobile phase was 30 min at room temperature (25^0C ± 2) and relative humidity 60% ± 5. The length of chromatogram run was approximately 8 cm. Subsequent to the development; RP- TLC plates were dried in the current of air with the help of an air dryer. Densitometric scanning was performed on Camag TLC scanner 3 in reflectance –absorbance mode at 284 nm. The source of radiation used deuterium lamp emitting radiation in the range 190 nm to 400 nm.

2.3. Preparation of Standard solution and calibration graph of rabeprazole sodium

Standard stock solution was prepared by dissolving 10 mg of rabeprazole sodium in 10 mL methanol to obtain concentration (1.0 mg mL^{-1})

Different volumes of stock solution 0.4 – 2.4 µL were spotted on RP- TLC plate in three replicate to obtain concentration 400, 800, 1200, 1600, 2000 and 2400 ng $spot^{-1}$ of rabeprazole sodium , respectively. The plate was developed using previously described mobile phase. The data of peak area versus drug concentration were treated by linear least square regression.

2.4. Method validation

2.4.1. Precision

Repeatability of sample application and measurement of peak area were carried out using six replicate of same spot (1000 ng $spot^{-1}$ of rabeprazole sodium). The intra-day and inter-day variation for the rabeprazole sodium was carried out at three different concentration levels of about 800, 1200 and 1600 ng $spot^{-1}$.

2.4.2. Robustness of the method

By introducing various changes in the previous chromatographic conditions the effects on the results were examined.

2.4.3. Limit of detection and limit of quantification

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantitated as an exact value. The quantitation limit of individual analytical procedure is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. In order to determine detection and quantification limit, concentrations in the lower part of the linear range of the calibration curve were used. Stock solution of rabeprazole sodium (0.1 mg mL^{-1}) was prepared and different volume of stock solution in the range 400 to 800 ng were spotted in triplicate. The amount of rabeprazole sodium by spot versus average response (peak area) was graphed and the equation for this was determined. The standard deviations (S.D.) of responses were calculated. The average of standard deviations was calculated (A.S.D.). Detection limit was calculated by (3.3 × A.S.D.)/b and quantification limit was calculated by (10×A.S.D.)/b, where “b” corresponds to the slope obtained in the linearity study of method.

2.4.4. Specificity

The specificity of the method was ascertained by analyzing standard drug and sample. The spot for rabeprazole sodium in sample was confirmed by comparing the Rf values and spectra of the spot with that of standard. The peak purity of rabeprazole sodium was accessed by comparing the spectra at three different levels, i.e., peak- start (S), peak- apex (M) and peak- end (E) positions of the spot.

2.5. Recovery studies

Recovery studies was carried out by applying the method to drug sample to which a known amount of rabeprazole sodium corresponding to 80 %, 100 % and 120 % of standard drug rabeprazole sodium has been over spotted. At each level of the amount three determinations were performed and results obtained were compared with expected results.

2.6. Analysis of the marketed formulation

To determine the content of rabeprazole sodium in conventional tablets (label claim: 10 mg of rabeprazole); the twenty tablets were weighed, mean weight determined and ground into fine powdered in a glass mortar. An amount of powder equivalent to 10 mg rabeprazole sodium was transferred to 100 mL volumetric flask, extracted with methanol, sonicated for 30 min and diluted to mark with the same solvent. The resulting solution was filtered, using 0.45 µm filter (Millifilter, Milford, MA). The solution (10 µL, 1000 ng of rabeprazole sodium) was spotted for assay of rabeprazole sodium. The spots at Rf 0.45 for rabeprazole sodium were observed in the densitogram extracted from tablets. There were no interferences from the excipients commonly present in the tablets.

2.7. Forced degradation of standard rabeprazole sodium

In all degradation studies the average peak area of rabeprazole sodium after application (1000 ng spot $^{-1}$) of seven replicates was obtained. The plate was developed and scanned in above established chromatographic conditions. Peak area was recorded for each concentration of degraded drug.

2.8. Preparation of acid and base induced degradation product

The 10 mg of rabeprazole sodium was separately dissolved in 10 mL of methanolic solution of 0.1 M HCl and 0.1 M NaOH. These solutions were kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1 mL of above solutions was taken and neutralized, then diluted up to 10 mL with methanol. The resultant solutions were applied on RP-TLC plate in triplicate (10 µL each, i.e. 1000 ng spot $^{-1}$).

2.9. Hydrogen peroxide-induced degradation

The 10 mg of rabeprazole sodium was dissolved in 10 mL of methanolic solution of hydrogen peroxide (10% v/v). The solution was kept for 8 h at room temperature in the dark in order to exclude the possible degradative effect of light. The 1 mL of above solution was taken and diluted up to 10 mL with methanol. The resultant solution was applied on RP-TLC plate in triplicate (10 µL each, i.e. 1000 ng spot $^{-1}$).

2.10. Dry heat degradation product

The powdered drug was stored at 55^0C for 2 h under dry heat condition showed significant degradation. The degraded product was resolved from the standard. In all degradation studies, the average peak areas of rabeprazole sodium after application (1000 ng spot $^{-1}$) of three replicates were obtained.

2.11. Photochemical degradation product

The 10 mg rabeprazole sodium was dissolved in 10 mL of methanol. The solution was kept in the sun light for 8 h. The resultant solution was applied on RP-TLC plate in triplicate (1 µL each, i.e. 1000 ng spot $^{-1}$).

3. Results and discussion

3.1. Optimization of chromatogram

Initially, trial experiments were performed in a view to select a suitable mobile phase for the accurate estimation of the drug. The mobile phase consisting of acetone: water (3.5:1.5 v/v) gave compact spot, sharp and symmetrical peak with R_f value of 0.45 for rabeprazole sodium (**Fig.2).** It was observed that pre-saturation of TLC chamber with mobile phase for 30 min ensure good reproducibility and peak shape of rabeprazole sodium.

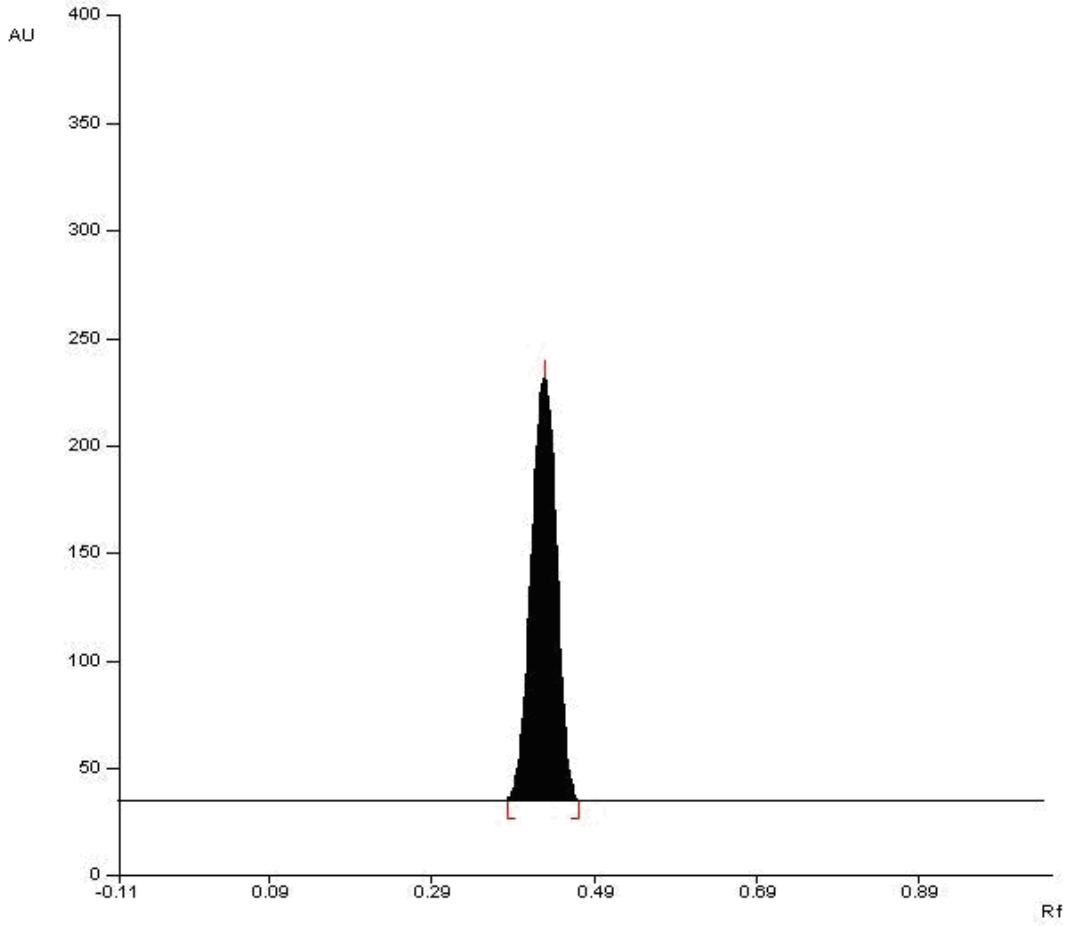

Fig. 2. Chromatogram of rabeprazole sodium in mobile phase acetone: water (3.5: 1.5 *v/v* with R_f = 0.45)

3.2.Method Validation

3.2.1. Linearity

The linear regression data for the calibration curves showed good linear relationship over the concentration range 400 - 2400 ng (co-relation co-efficient, $r^2 = 0.9998$) with slope 4.83 and intercept 31.38. No significant difference was observed in the slopes of standard curves.

3.2.2. Precision

The precision of the developed RP-TLC method was expressed in terms of % relative standard deviation (% R.S.D.). The results depicted in Table 1 revealed high precision of the method.

Table 1: Intra-day and inter-day precision of RP-TLC method

Amount ng spot $^{-1}$	Amount found*	S. D.	%R. S. D.
Intra-day			
800	797.26	7.69	0.96
1200	1189.55	5.21	0.44
1600	1582.72	4.62	0.29
Inter-day			
800	798.96	8.27	1.04
1200	1188.87	0.52	0.04
1600	1584.97	3.42	0.22

* mean of three determinations

3.2.3. Robustness of the method

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

3.2.4. Recovery

The proposed method when used for extraction and subsequent estimation of rabeprazole sodium from pharmaceutical dosage forms after over spotting with 80 %, 100% and 120% of additional drug afforded recovery of 99.56- 101.04 as listed in Table 3.

3.3. Ruggedness of the method

Ruggedness of the method was performed by applying 1000 ng spot $^{-1}$ for rabeprazole sodium, by two different analyst keeping same experimental and environmental conditions.

Summary of validation parameters are shown in Table 4.

Table 2: Results of Robustness Studies *****

Parameters	S.D. of peak area	% R.S.D.
Mobile phase composition		
(Acetone: water , 4.0:1.0)	44.38	0.92
(Acetone : water , 3.0: 2.0)	13.63	0.28
Mobile phase volume		
8.0 mL	20.18	0.42
10.0 mL	45.90	0.95
Development distance		
7 cm	41.50	0.86
7.5 cm	49.14	1.02
8 cm	69.73	1. 45
Relative humidity		
55	32.41	0.67
65	38.71	0.80
Duration of saturation		
20 min	35.43	0.73
25 min	49.84	1.04
30 min	51.36	1.07
Activation of prewashed RP-TLC plates		
8 min	36.77	0.76
10 min	44.97	0.93
12 min	58.29	1.21
Time from spotting to chromatography	38.31	0.79
Time from chromatography to scanning	42.69	0.88

* mean of three estimations for each parameter

Table 3: Recovery Studies*

Initial amount (ng)	Excess drug added to the analyte (%)	Amount recovered (ng)	%Recovery	%R.S.D.
1000	0	1010.36	101.03	0.51
1000	80	800.97	100.11	0.71
1000	100	1010.5	101.04	0.03
1000	120	1194.72	99.56	0.53

*mean of three determinations at each level

Table 4: Summary of Validation Parameter

Parameters	Data
Linearity range (ng spot $^{-1}$)	400 – 2400
Correlation coefficient	0.9998
Limit of detection (ng spot $^{-1}$)	19.89
Limit of quantitation (ng spot $^{-1}$)	60.29
Recovery (n = 9) (% R.S.D.)	0.75
Ruggedness (% R.S.D.)	
Analyst I (n = 3)	0.74
Analyst II (n = 3)	0.64
Precision (%R.S.D.)	
Repeatability of application (n = 6)	0.30
Intra-day (n = 3)	0.29 - 0.96
Inter-day (n = 3)	0.04 – 1.04
Robustness	Robust
Specificity	Specific

3.4. Limit of detection and limit of quantitation

Detection limit and quantification limit was calculated by the method as described in chromatographic conditions and found to be 19.89 ng and 60.29 ng, respectively. This indicates adequate sensitivity of the method.

3.5. Assay of marketed formulation

A single spot at Rf of 0.45 was observed in the chromatogram of the drug samples extracted from conventional tablets. There was no interference from the excipients commonly present in the conventional tablets. The drug content was found to be 100.13 with a R.S.D. % of 0.29. The low values of R.S.D. % indicated the suitability of this method for routine analysis of rabeprazole sodium in pharmaceutical dosage forms.

3.6. Forced degradation

The chromatograms for rabeprazole sodium showed additional peaks at R_f value 0.26, 0.31 in the acid degraded samples and 0.32 in base degraded samples The concentration of the drug was found to be changing from the initial concentration indicating that rabeprazole sodium undergoes degradation under acidic and basic conditions. The samples degraded with hydrogen peroxide showed additional peak at R_f value 0.36. The spot of degraded product was well resolved from the drug spot. The photo degraded sample showed additional three peak at R_f value 0.22, 0.29 and 0.38. Significant degradation was observed in standard that were left in day light for 8 h. The samples degraded under dry heat conditions showed additional peak at Rf value 0.27, 0.31 and 0.41. The spot of degraded product was well resolved from the drug spot. This indicates that the drug is susceptible to acid/base hydrolysis,

oxidation and dry heat degradation. The results of accelerated degradation studies are listed in Table 5. Schematic representation of degradation product is shown in Fig.3.

Table 5: Forced Degradation of Rabeprazole Sodium

Sample exposure condition	Number of degradation products (R_f values)	Rabeprazole sodium remained (ng/1000 ng)	Recovery (%)
0.1 M HCl, 8h,RT[a]	2 (0.26,0.31)	925.56	92.56
0.1 M NaOH, 8h, RT[a]	1(0.32)	956.23	95.62
10 % H_2O_2, 8h, RT[a]	1(0.36)	960.81	96.08
Dry heat, 2H , 55^0C	3(0.27, 0.31, 0.41)	910.23	91.02
Photostability, 8H	3(0.22,0.29, 0.38)	885.35	88.53

[a] RT = Room temperature

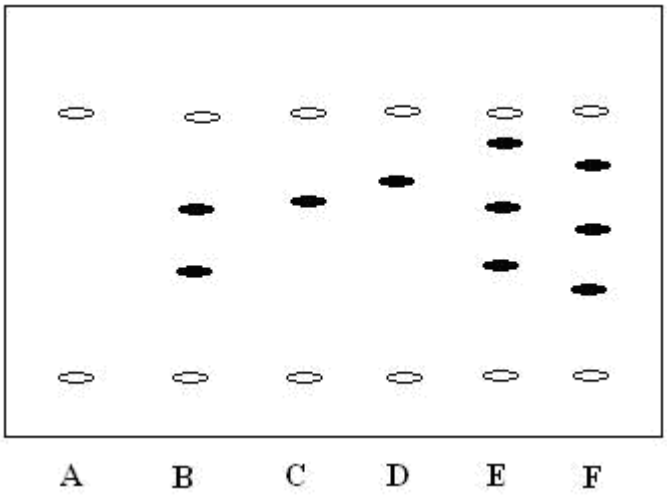

Fig.3: Degradation products (DP) of rabeprazole sodium from acid, base, oxidation, dry heat and photo degradation.
A) standard rabeprazole sodium; B) rabeprazole sodium + 0.1N HCl; C) rabeprazole sodium + 0.1N NaOH; D) rabeprazole sodium + 10% H_2O_2; E) rabeprazole sodium exposed to dry heat; F) rabeprazole sodium exposed to light heat

4. Conclusion

The developed and validated RP- TLC technique is precise, specific, accurate and stability-indicating for the determination of drug. Statistical analysis proves that the method is reproducible and selective for the analysis of rabeprazole sodium as bulk drug and in tablet formulations. Method can be used to determine the purity of the drug available from various sources by detecting the related impurities. As the method could effectively separate the drugs from their degradation products; therefore, it can be employed as a stability indicating one.

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