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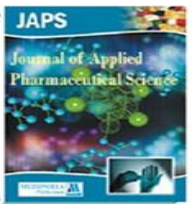

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Development and validation of RP-HPLC method for determination of content uniformity of rabeprazole sodium in its tablets dosage form

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ABSTRACT

The aim of the present work was to develop simple, shorter and effective HPLC method with UV detection (285nm) and subsequent validation for the content uniformity determination of Rabeprazole Sodium in marketed tablet samples. The method uses isocratic mobile phase of 0.1M sodium phosphate buffer (pH adjusted to 6.5 with sodium hydroxide solution) and acetonitrile 65:35 compositions on reverse phase Lichrosphere RP-100 C8 column. The RSD was observed to 0.21 percentage and linearity range of (LOQ) 0.025 – 150 percentage of label claim established with 0.9999 correlation, 8 different brands marketed samples were successfully analysed for content uniformity and compared the results with the USP and other guidelines for acceptance criteria. The developed method was found precise, linear, rugged and robust for validated parameters. The method can be used for assay and the content uniformity determination of Rabeprazole Sodium in its tablet dosage form.

Key words: Rabeprazole sodium; Content uniformity test; Tablet dosage form; Market sample analysis.

INTRODUCTION

Content uniformity or the Uniformity of Dosage unit is defined as the degree of uniformity in the amount of active substance among dosage units. The risk assessment strategy underlying content uniformity testing is the assumption that some pre-specified limits exist where safety and efficacy outcomes may change if content uniformity fails (Williams et al., 2002) moreover drug content and content uniformity depends on a number of processes associated with its manufacture, hence it is obviously unrealistic to presume every unit to be contained exactly the same amount of the active ingredient as of the label claim, and also due to increased awareness of physiological availability, pharmacopieal standards and specifications have been established to provide limits for allowable variations for the active ingredients in single dosage units (Martins et al., 1998) of all coated and uncoated tablets intended for oral administration where the range of size of the dosage form available include 25 mg or smaller sizes.

To study and compare the content uniformity test, a new class of substituted benzimidazole proton pump inhibitor (Dekkers et al., 1998) Rabeprazole (±)-sodium 2-[[4-(3-methoxypropoxy)-3-methylpyridine-2-yl] methylsulfinyl] -1 H-benzimidazole (Fig. 1) which covalently binds and inactivates the gastric parietal cell proton pump (H^+/K^+ ATPase) has been identified. Primary use of Rabeprazole Sodium is to treat heartburn and gastroesophageal reflux disease, ulcers, bacterial Infection due to Helicobacter pylori and Zollinger-Ellison Syndrome, Since Rabeprazole Sodium is unstable in acidic medium it is usually available in enteric coated tablets (Janssen et al., 1999), it offers the same mode of action as that of the other available proton pump inhibitors, but the differing pyridine and benzimidazole substituents result in small, but

potentially significant different physical and chemical properties, it undergoes activation over a wide pH range when compare to other proton pump inhibitors (Williams et al., 1999).

Several HPLC methods have been published in the literature to quantify Rabeprazole Sodium in plasma samples (Huang et al., 2005) enentiomeric separations (Del Nozal et al., 2004, Toribio et al., 2005, Miura et al., 2006, Rao et al., 2006) identification of degradation products (Dev et al., 2009) and the dissolution test (Cassia et al., 2006), though the drug is not part of any pharmacopoeial compendium and none of the literature method is available for the content uniformity test. The aim of the present work was to develop and validate a simple and rugged content uniformity method for estimation of Rabeprazole Sodium in its tablet dosage form and also to prove its application by comparing it with 8 different marketed samples.

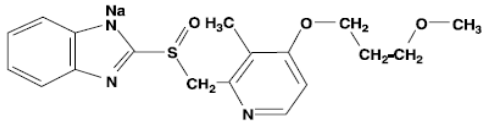


Fig. 1: Structure of Rabeprazole Sodium

MATERIALS AND METHODS

Materials

Rabeprazole Sodium enteric coated tablets of Ranbaxy, FDC Limited, Dr Reddys, Zydus Healthcare, Torrent Pharma, Alembic, Intas Pharma and Lupin ltd were purchased from the local market. Rabeprazole Sodium working standard used was purchased from LC-GC Promochem, Banglore. Sodium dihydrogen orthophosphate monohydrate, Sodium Hydroxide, Acetonitrile and Methanol of HPLC grade were purchased from Merck Laboratories, Mumbai. Millipore Membrane filter of 0.45µm was purchased from modern scientific lab mumbai. MilliQ water was used throughout the analysis and all other reagents used were AR grade.

Instrumentation

A liquid chromatograph (Agilent 1100 series) equipped with G1322A Degasser, G1311A Quaternary pump, G1313A Automatic Liquid Sampler, G1316A Column Compartment, G1314A Variable Wavelength Detector and Chemstation software was used for the content uniformity and assay test of all samples. Mettler Toledo pH meter (Model Multiseven) was used for pH determination of all solutions. Perkin Elmer UV Spectrophotometer was used for the wavelength selection.

HPLC method conditions

A Lichrosphere 100 RP C8 column (100 x 4.6mm,5µ) was eluted with a mobile phase containing Acetonitrile-Sodium Phosphate buffer (pH 6.5;0.1M) (35:65,v/v) at a flow rate of 1.2 mL min^{-1} Rabeprazole Sodium was determined by UV detection at 285 nm. The injection volume of 10 µl and the run time was 10 min.

Preparation of sample solution

For content uniformity testing, one tablet or equivalent amount of granule were placed into each of ten 50 mL volumetric flasks. Approximately 30 mL of 0.05M methanolic sodium hydroxide solution (diluent) was added to each volumetric flask and sonicate until the tablets were dispersed in the solution. The resultant solutions were cooled and volume was made up to the mark with the same diluents. The solutions were shaken well for uniform distribution. Filtered a portion of the solution using 0.45µm membrane filter and the filtrate was injected for analysis.

Preparation of standard solution

Approximately 20 mg of Rabeprazole Sodium working standard was accurately weighed into a 50 mL volumetric flask and dissolved in the diluent consisting of 0.05M methanolic sodium hydroxide solution, mixed well and was injected for analysis.

RESULTS AND DISCUSSION

Method development and optimization

Rabeprazole Sodium is white to slightly yellowish-white solid, very soluble in water and methanol, freely soluble in ethanol, chloroform, ethyl acetate and insoluble in ether and n-hexane. It is more stable in basic conditions, while degrading in acidic conditions. The Chromatographic conditions were optimized for the Rabeprazole Sodium content uniformity and assay method within a short analysis time (10 min) using simple mobile phase and an acceptable peak tailing (<1.5). In order to achieve these goals, the Chromatographic mobile phase and column were chosen first. The dissociation constant (pKa) of Rabeprazole Sodium is 4.53. Initially the mobile phase chosen with pH 8.0 where precipitation observed for standard preparation, hence the mobile phase pH was reduced and optimized as 6.5 with phosphate buffer. The 10 ppm Rabeprazole Sodium in 0.01M NaOH Solution was scanned between 200 – 400 nm in a UV spectrophotometer. The maximum absorbance (λmax) was obtained at 285 nm as shown in Fig. 2 and hence the wavelength 285nm was selected in further development.

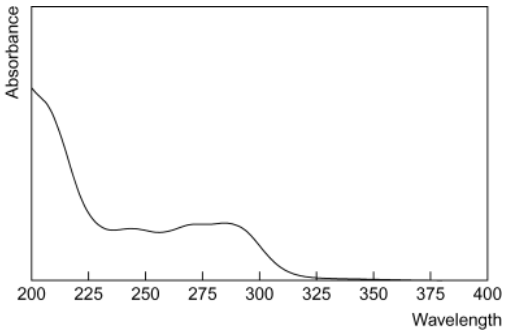


Fig. 2: Rabeprazole Sodium UV Spectrum

Columns of Luna C18, Waters Spherisorb C18 and Hypersil BDS were tried, no better peak shape and non reproducible retention time was observed (Fig. 3a, 3b, 3c).

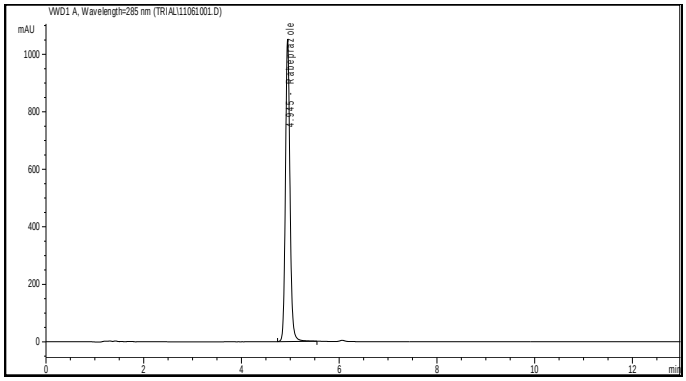
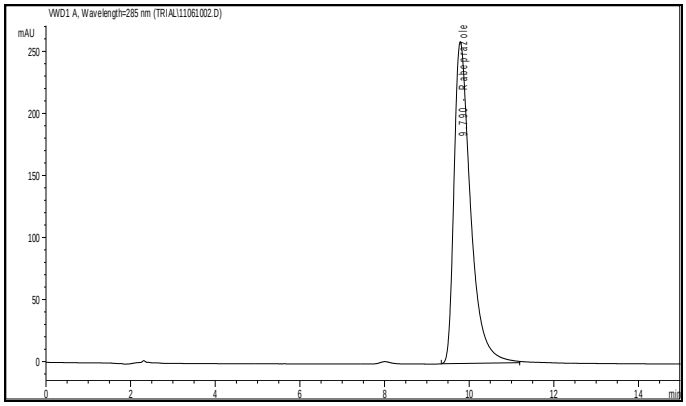
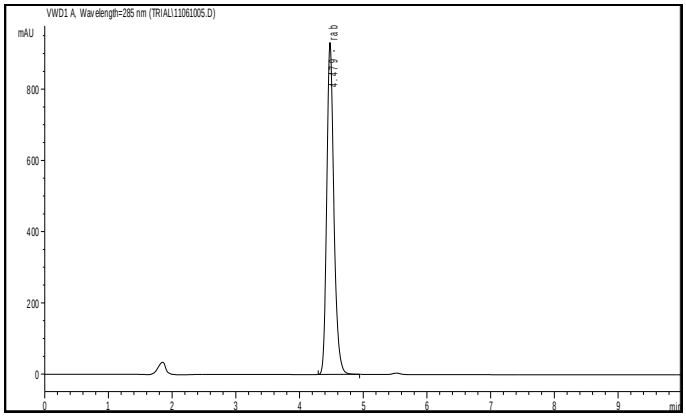


Fig. 3: Method development chromatograms of Rabeprazole Sodium on different columns. A) Luna C18 (150 X4.6, 5µ).



B) Spherisorb C18 (250 X4.6, 5µ)



C) Hypersil BDS C18 (4.6 X 200 mm, 5µ)

The good peak shape (Tailing about 1.3) and retention time (about 3.2 min) were observed with Lichrosphere 100 RP-C8 column with a flow rate of 1.2 mL min-1. Since the sample is stable in basic conditions, solubility is more in water and methanol, the diluent chosen to be 1:1 mixture of 0.01M methanolic sodium hydroxide and the solution found to be stable up to 72 hours. Typical chromatogram of standard and sample is shown in Fig. 4a and 4b.

Method validation

Sensitivity

The limit of detection (LOD) was estimated to be 0.03 µg mL^{-1} with a signal to noise ratio of greater than 3.The limit of quantitation (LOQ) was estimated to be 0.1 µg mL^{-1} with a signal to noise ratio equal to 10. The sensitivity of the method is adequate for the quantitation of Rabeprazole Sodium in Sample.

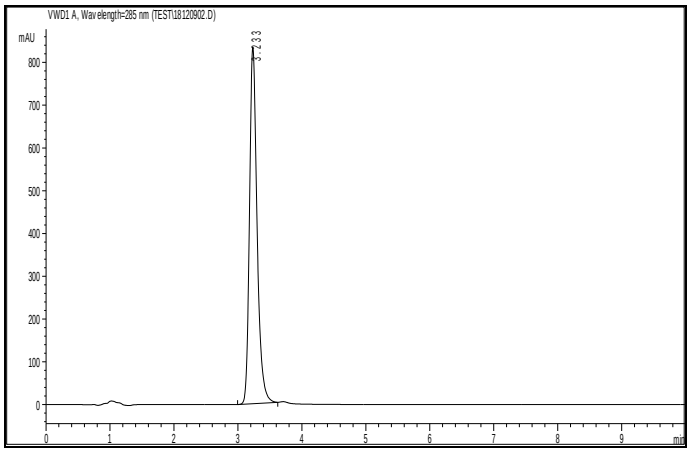
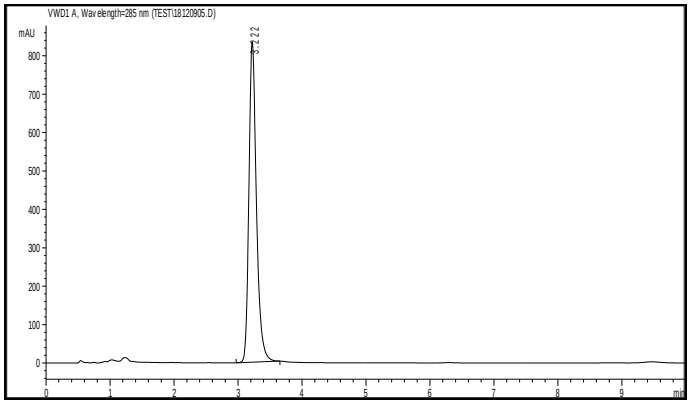


Fig. 4: Rabeprazole Sodium Chromatograms on Lichrosphere 100 RP C8 column (100 x 4.6mm, 5µ). A) Rabeprazole Sodium standard chromatogram



B) Rabeprazole Sodium sample solution chromatogram

Linearity

The linearity of Rabeprazole Sodium was established by preparing a series of placebo spiked with Rabeprazole Sodium standard solutions ranging from 0.1 µg mL^{-1} to 0.6 mg mL^{-1} which corresponds to 0.025 – 150% of the method concentration (0.4 mg/mL). The correlation coefficient (R) observed as 0.9999 with y = 1656.4x-817.14 (Fig. 5).

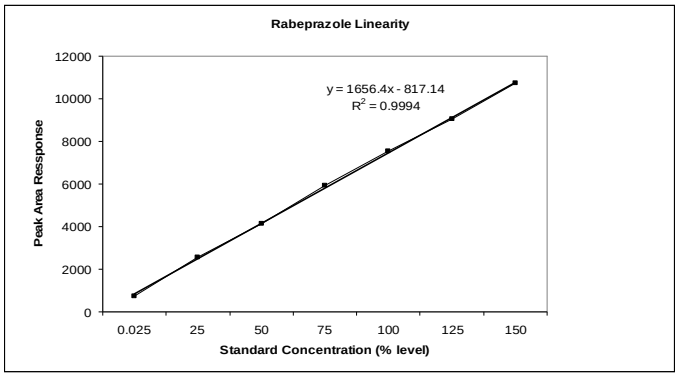


Fig. 5: Linearity plot

Accuracy

The Accuracy of the method was determined by spiking the weighed quantity of Rabeprazole Sodium equivalent to 50%, 100% and 150% of the method concentration into the sample preparation and calculated the % recovery as % label claim and amount recovered. The results are given in table 1.

Table.1: Data of recovery studies.

% Level Recovery	Amount added (mg)	Amount Recovered(mg)	% Recovery	Mean % Recovery	Overall % Recovery
	9.78	9.72	99.4		
50	9.74	9.56	98.2	98.9	
	9.32	9.25	99.2		
	20.52	20.31	99.0		
100	20.75	20.69	99.7	99.5	99.3
	20.88	20.85	99.9		
	29.01	28.8	99.3		
150	28.78	28.55	99.2	99.3	
	28.8	28.65	99.5		

System Precision and method precision

The system Precision was determined by performing ten replicate injections of standard solution at the method concentrations (0.4 mg mL^{-1}). The RSD was found to be 0.21 %. The method precision was performed by injecting ten replicate preparations of sample solution, the RSD was found to be 0.27 %. The results are given in table 2 and 3.

Table.2: Result of system precision

No. of Injections	Area (mAu)
1	6598.61572
2	6600.88184
3	6597.62695
4	6591.57178
5	6617.59424
6	6624.38623
7	6621.72461
8	6584.68359
9	6588.84521
10	6595.21924
Avg.	6602.11494
SD	14.11
% RSD	0.21

Table.3: Result of method precision.

Sample No.	Obtained Label claim (mg/tablet)	Obtained % Label Claim
1	20.12	100.6
2	20.13	100.7
3	20.11	100.6
4	20.13	100.7
5	20.14	100.7
6	20.19	101.0
7	20.1	100.5
8	20.15	100.8
9	20.29	101.5
10	20.15	100.8
Avg.	20.15	100.8
SD	0.05	0.27
% RSD	0.27	0.27

Method repeatability/intermediate precision

Method repeatability/intermediate precision was assessed from the assay of six samples by two different analysts using different chromatographic systems on different days. The chromatographic results are summerised on Table 3, the % RSD was found less than 2.0 %, and hence the Repeatability is well within the acceptance criteria. The results are shown in table.4.

Table.4: Result of intermediate precision.

Sample No.	Analyst 1		Analyst 2	
	Label claim obtained (mg/tablet)	% Assay	Label claim obtained (mg/tablet)	% Assay
1	21.58	107.9	21.36	106.8
2	21.61	108.1	21.49	107.5
3	21.65	108.3	21.48	107.4
4	21.97	109.9	21.47	107.4
5	21.66	108.3	21.72	108.6
6	21.82	109.1	21.12	105.6
Mean (6)	21.72	108.6	21.44	107.2
SD	0.15	0.75	0.20	0.98
% RSD	0.69	0.69	0.91	0.91

Application of the method

This method has been applied to investigate the samples of Rabeprazole Sodium in 8 different marketed brands. For comparison, the same samples were analyzed by this validated method and the results were compared as per the USP, EP, JP pharmacopoeial guidelines for uniformity of dosage forms determination. All the samples comply with the acceptance value and the specifications limit according to the pharmacopeias and as per Bergum method (Cholayudth 2004). Acceptance Value is calculated using the following formula.

$$| M - X | + kS$$

Where,

M = Reference value

X = Mean of individual contents

k = Acceptability constant, (If n = 10 then k = 2.4, If n = 30, then k = 2.0)

S = Sample standard deviation.

T is Target test sample amount at time of manufacture. For purposes of this pharmacopeia, unless otherwise specified in the individual monograph, T is 100.0%, and for manufacturing purposes, T is the manufacturer's approved target test amount value at the time of manufacture.

The sample acceptance value for Content Uniformity tests to pass was that it should be less than 15.0 for 10 units, the % RSD should be less than 6.0 and the Mean value should be between 85% to 115% of average content, with the condition of only one dose unit is outside 85 to 115 % and between 75 – 125% of average content.

The validated method was used to determine the assay of different marketed brands. The overall data of assay result is tabulated in table 5 and graphical data is shown in Fig. 6.

CONCLUSION

A simple, rapid and reliable HPLC method has been developed and successfully validated. The developed method was used for the content uniformity test of 8 different marketed tablet samples of Rabeprazole Sodium. The results were comparable and comply with different pharmacopeial limits. The method has significantly reduced runtime with better peak shape. The method

Table 5: Result of Content Uniformity (% label claim) of different marketed brands.

Dose Unit	% Label Claim obtained							
	Ranbaxy	FDC	Dr.Reddy's	Zydus	Torrent	Alembic	Intas	Lupin
1	100.6	97.1	107.9	95.4	99.3	103.1	102.5	99.8
2	100.8	98.9	108.1	100.5	100.5	104.0	104.6	98.7
3	99.2	104.0	108.3	95.6	98.0	103.9	102.3	100.0
4	101.8	96.9	109.8	95.4	98.1	104.2	103.7	98.8
5	99.9	100.2	108.3	100.6	98.2	102.3	103.7	98.5
6	102.3	100.9	109.1	98.5	99.0	103.7	103.7	98.8
7	100.8	99.0	106.3	100.5	100.8	103.1	103.5	99.0
8	102.3	103.7	109.2	100.7	98.7	105.1	102.0	99.8
9	101.4	104.1	109.1	95.7	100.7	105.5	103.6	97.2
10	101.0	104.5	107.4	98.0	98.9	102.4	103.9	95.6
Average	101.0	100.9	108.4	98.1	99.2	103.7	103.4	98.6
SD	1.0	3.0	1.0	2.4	1.1	1.1	0.8	1.3
% RSD	1.0	2.9	0.9	2.4	1.1	1.0	0.8	1.4
Min	99.2	96.9	106.3	95.4	98.0	102.3	102.0	95.6
Max	102.3	104.5	109.8	100.7	100.8	105.5	104.6	100.0
Reference Value, M	101.0	100.9	101.5	98.5	99.2	101.5	101.5	98.6
Acceptance Value, AV	2.4	7.1	9.3	6.1	2.6	4.8	3.8	3.2
Delta from M for Min (% of LC)	1.8	4.0	4.8	3.1	1.2	0.8	0.5	3.0
Delta from M for Max (% of LC)	1.3	3.6	8.3	2.2	1.6	4.0	3.1	1.4

*LC: Label claim

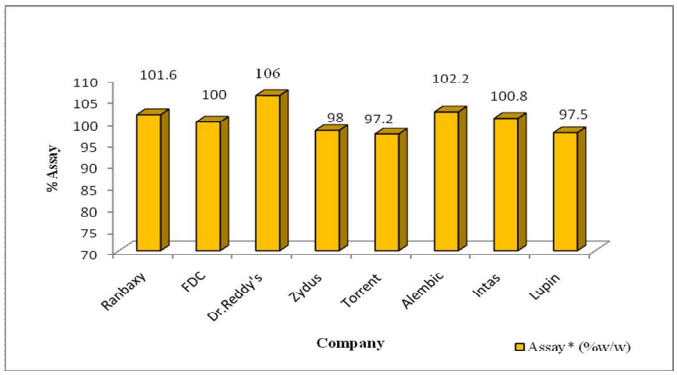


Fig. 6: Comparison of assay (%w/w) of different marketed brands by developed method.

found to be linear, accurate, rugged and robust for validated parameters. The solutions found to be stable up to 72 hours. This method offers better turnaround of analytical values. Using the same method, assay was performed for individual samples and found that values are between good agreements. Hence this will be an excellent method for the assay determination and content uniformity of Rabeprazole Sodium in oral solid dosage form.

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