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Original Article

STABILITY INDICATING RP-HPLC METHOD FOR ESTIMATION OF RABEPRAZOLE SODIUM AND MOSAPRIDE CITRATE IN BULK AND FORMULATION

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ABSTRACT

Objective: Development and validation of reversed phase liquid chromatographic method for the quantitative determination of Rabeprazole sodium and Mosapride citrate in bulk and combined dosage form.

Methods: A thermo Inert sil, C_{18} (250 x 4.6 mm i. d., 5 µ) column with mobile phase containing methanol: buffer (ammonium acetate pH 6.5): acetonitrile in the ratio of (50:20:30 %) was used. The flow rate was 1.0 ml/min, column temperature was 25 °C and effluents were monitored at 245 nm.

Results: The retention times of Rabeprazole sodium and Mosapride citrate were 2.951 min and 4.195 min, respectively. Correlation co-efficient for Rabeprazole sodium and Mosapride citrate was found to be 0.9999 and 0.9999, respectively. The proposed method was validated with respect to linearity, accuracy, precision, specificity, and robustness. Recovery of Rabeprazole sodium and Mosapride citrate in formulations was found to be in the range of 97-103 % and 98-102 %, respectively confirms the non-interferences of the excipients in the formulation.

Conclusion: The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Rabeprazole sodium and Mosapride citrate in pharmaceutical dosage forms. Due to its simplicity, rapidness and high precision, the method was successfully applied to the estimation of Rabeprazole sodium and Mosapride citrate in combined dosage form.

Keywords: RP-HPLC, Rabeprazole sodium, Mosapride citrate, Methanol, Acetonitrile, Validation.

INTRODUCTION

Rabeprazole sodium is chemically (RS)-2-[(4-(3-methoxy propaxy)-3-methylpyridin-2-yl] methyl sulphonyl)-1*H*-benzo(*d*)imidazole (fig. 1). Rabeprazole sodium is an antiulcer drug in the class of proton pump inhibitors. As anti ulcer drug, it is used in short-term treatment in healing and symptomatic relief of duodenal ulcers and erosive or ulcerative gastro esophageal reflux disease (GORD); maintaining healing and reducing relapse rates of heartburn symptoms in patients with GORD; treatment of daytime and nighttime heartburn and other symptoms associated with GORD; long-term treatment of pathological hypersecretory conditions, including Zollinger-Ellison syndrome and in combination with Amoxicillin and Clarithromycin to eradicate *Helicobacter pylori* [1].

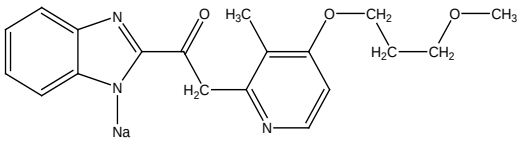

Fig. 1: Chemical structure of Rabeprazole sodium

Mosapride citrate is chemically (RS)-4-amino-5-chloro-2-ethoxy-*N*-[(4-(4-fluorobenzyl) morpholin-2-yl)methyl]bezamide citrate (fig. 2). Mosapride is a gastro pro-kinetic agent that acts as a selective $5HT_4$ agonist. The major active metabolite of Mosapride is known as M1, additionally acts as a $5HT_3$ antagonist. In addition to its pro-kinetic properties, Mosapride also exerts anti-inflammatory effects on the gastrointestinal tract which may contribute to some of its therapeutic effects.

Mosapride also promotes neurogenesis in the gastrointestinal tract which may prove useful in certain bowel disorders. The neurogenesis is due to Mosapride's effect on the 5-HT_4 receptor where it acts as an agonist [2].

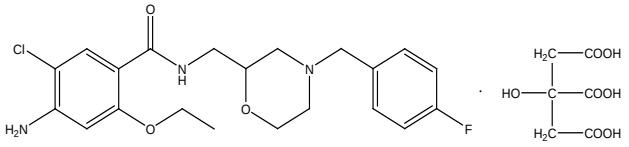

Fig. 2: Chemical structure of Mosapride citrate

The drug analysis plays an important role in the development of drugs, manufacturing and therapeutic use. Pharmaceutical industries rely upon quantitative chemical analysis to ensure that the raw material used and the final product obtained meets the required specification. The literature review indicates there are several analytical methods have been reported for estimation of these drugs as individual or in combination with other drugs, and also several analytical methods for the determination of simultaneous estimation of Rabeprazole sodium and Mosapride citrate by HPTLC, UPLC and UV in dosage formulation and its bio-analytical applications. Some of the reported RP-HPLC methods were not economical in terms of mobile phase composition, column dimensions and run times. Hence there is need for the development of newer method for estimation of Rabeprazole sodium and Mosapride citrate present in tablet to overcome above discussed hurdles. In addition, stability indicating RP-HPLC method for the simultaneous estimation of Rabeprazole sodium and Mosapride citrate in pharmaceutical dosage form are very scanty. Hence the main objective of this study is to develop a stability indicating RP-HPLC method for estimation of Rabeprazole sodium and Mosapride citrate & validate the developed method according to ICH guidelines by using various parameters [3-15].

MATERIALS AND METHODS

Chemicals and reagents

Rabeprazole sodium and Mosapride citrate were obtained as a gift sample from Aurobindo Pharma Ltd., Hyderabad. Milli-Q water,

HPLC grade methanol, acetonitrile and analytical grade ammonium acetate buffer were purchased from E. Merck (India) Ltd., Mumbai.

Instrumentation

The separation was carried out on HPLC system with Waters 2695 alliance with binary HPLC pump, Waters 2998 PDA detector, Waters Empower2 software and thermo Inert sil, C_{18} (250 x 4.6 mm i. d., 5 µ).

HPLC conditions

The mobile phase consisting of methanol, ammonium acetate buffer (pH 6.5) and acetonitrile (HPLC grade) were filtered through 0.45 µ membrane filter before use, degassed and were pumped from the solvent reservoir in the ratio of 50:20:30 % into the column at a flow rate of 1.0 ml/min.

The column temperature was maintained as 25 °C. The detection was monitored at 245 nm and the run time was 8 min. The volume of injection loop was 10 µl; prior to injection of the drug solution, the column was equilibrated for at least 30 min with the mobile phase flowing through the system.

Preparation of standard solution

20 mg of Rabeprazole sodium and 15 mg of Mosapride citrate were accurately weighed and transferred into a 100 ml volumetric flask. Dissolved with 25 ml of methanol and sonicated for 20 min.

Finally, the solution was diluted to the required volume using methanol. (200 µg/ml of Mosapride citrate and 150 µg/ml of Rabeprazole sodium). From the standard stock solution 2 ml was pipetted out into 10 ml volumetric flask and made up the volume with mobile phase. (40 µg/ml of Rabeprazole sodium and 30 µg/ml of Mosapride citrate).

Preparation of sample solution

Twenty capsules were accurately weighed and grinded to a fine powder. An amount of powder equivalent to 15 mg of Mosapride citrate and 20 mg of Rabeprazole sodium were weighed accurately and transferred into a 100 ml volumetric flask containing 25 ml of methanol and sonicated for 30 min and diluted to 100 ml with methanol, then the solution was filtered through 0.45 µm membrane filter and 2 ml of filtrate was taken into 10 ml volumetric flask and made up to the volume with methanol.

Procedure

10 µl of the filtered portion of the sample and standard preparations were injected into the chromatograph. The responses for the major peaks were recorded and the content of Rabeprazole sodium and Mosapride citrate was calculated.

Validation parameters

All the analytical validation parameters were determined according to ICH guidelines for the proposed analytical method [16-20]. The obtained validation parameters are presented in table 2.

System suitability

Standard solution was injected six times into system and chromatograms were recorded, % RSD (relative standard deviation) of retention time & peak area, theoretical plates and tailing factor were calculated.

Accuracy

Accuracy was determined in terms of % recovery. Sample solutions were prepared at three different concentration levels 50 %, 100 % and 150 %. Predetermined amount of standard was added to these solutions by spiking standard drug solution to the sample. % recovery was calculated by assaying these solutions.

System precision, method precision and intermediate precision

The system, method and intermediate precision of the proposed method are ascertained by injecting 6 replicates of test and standard sample, % RSD were calculated.

Specificity

Standard solution, sample solution, blank solution and placebo solution were injected simultaneously into the system and chromatograms were recorded.

Linearity

A linear relationship was evaluated across the range of the analytical procedure. A series of standard dilutions were prepared from the working standard solution in the concentration range of 20-80 µg/ml of Rabeprazole sodium and 15-60 µg/ml of Mosapride citrate, respectively. 10 µl of each solution was injected into HPLC system. Linearity is evaluated by plotting the peak area as a function of analyte concentrations.

Robustness

Robustness was carried out by changing small variations in method parameters like flow rate (± 0.2 ml), wavelength (± 2 nm) and temperature (± 5° C). Ruggedness wad done by studying changes with variation of analyst**.**

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) were determined for Rabeprazole sodium and Mosapride citrate.

Procedure for forced degradation studies

In order to demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 24 h at room temperature. Further forced degradation studies were conducted for indicating the stability of the method developed. The results of the degradation studies are presented in table 2.

Acid degradation

481.6 mg of sample equivalent to 20 mg Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask and added 10 ml of 0.1N HCl. Then sonicated for 30 min & added 10 ml of 0.1N NaOH for neutralisation & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

Base degradation

481.6 mg of sample equivalent to 20 mg of Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of 0.1N NaOH. Then sonicated for 30 min & added 10 ml of 0.1N HCl for neutralization & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

Peroxide degradation

481.6 mg of sample equivalent to 20 mg Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of 1 % peroxide. Then sonicated for 30 min & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

Thermal degradation

Sample was kept in oven for 1 h at 60 ºC. Above sample 481.6 mg equivalent to 20 mg Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of methanol. Then sonicated for 30 min & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

UV light degradation

Sample was kept in sun light for 1 day. Above sample 481.6 mg equivalent to 20 mg Rabeprazole sodium & 15 mg of Mosapride citrate was taken into 100 ml volumetric flask & added 10 ml of methanol. Then sonicated for 30 min & diluted volume with methanol. 2 ml of the above solution was transferred into 10 ml volumetric flask & made up volume with methanol in order to get a solution containing 40 µg/ml of Rabeprazole sodium & 30 µg/ml Mosapride citrate.

RESULTS AND DISCUSSION

The preliminary studies indicated that the desired system suitability parameters were obtained with the mobile phase containing methanol: buffer (ammonium acetate pH 6.5): acetonitrile in the ratio of (50:20:30 %). The mobile phase eluted the drug at lower retention times (2.951 and 4.195 min for Rabeprazole sodium and Mosapride citrate, respectively). The corresponding chromatograms were shown in the fig. 3 & 4 and the data are presented in table 1.

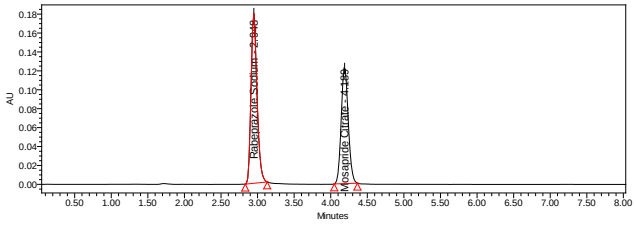

Fig. 3: Standard chromatogram of Rabeprazole sodium and Mosapride citrate

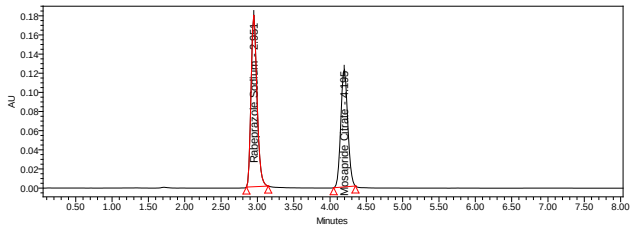

Fig. 4: Sample chromatogram of Rabeprazole sodium and Mosapride citrate

Table1: System Suitability Parameters

Parameters	Rabeprazole sodium	Mosapride citrate
Retention time	2.951	4.195
USP resolution	7.198194	
Theoretical plates	8102.312593	9879.942054
Tailing	1.127759	1.080256

The % RSD in precision, accuracy and robustness studies were found to be less than 2.0 %, indicating that the method is precise, accurate and robust. Accuracy data as shown in table 2.

The retention time of standard and sample solution of Rabeprazole sodium and Mosapride citrate were found to be almost same. Moreover, the blank solution and placebo solution doesn't produce any peak. Hence the proposed analytical method is specific for the simultaneous estimation of Rabeprazole sodium and Mosapride citrate. The linearity for HPLC method was determined at six concentration levels ranging from 20-80 µg/ml for Rabeprazole sodium and 15-60 µg/ml for Mosapride citrate. The calibration curve was constructed by plotting response factor against respective concentration of Rabeprazole sodium and Mosapride citrate. The plots of peak area Vs respective concentration of Rabeprazole sodium and Mosapride citrate were found to be linear in the range of 20-80 µg/ml and 15-60 µg/ml with coefficient of correlation (r^2) 0.9999. The linearity of this method was evaluated by linear regression analysis. The slope and intercept calculated for Rabeprazole sodium and Mosapride citrate were given in fig. 5 and fig. 6.

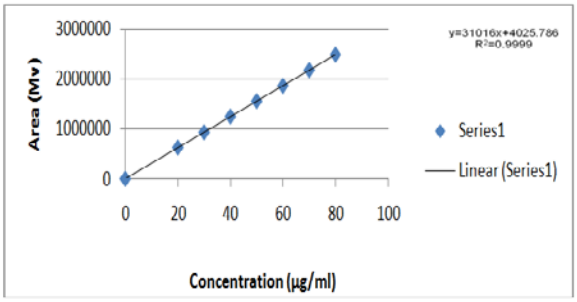

Fig. 5: Linearity curve for Rabeprazole sodium

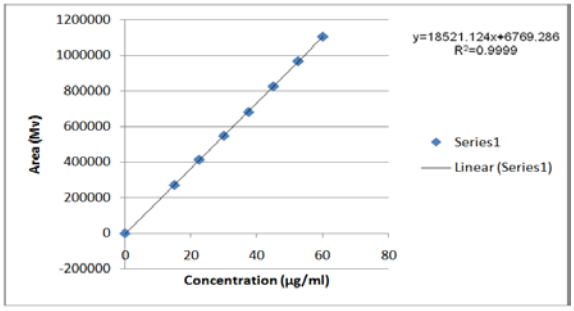

Fig. 6: Linearity curve for Mosapride citrate

Robustness of the method was determined by small deliberate changes in flow rate, temperature and wavelength. The low value of relative standard deviation indicates that the content of the drug was not adversely affected by these changes. Hence, the proposed method was robust. The LOD and LOQ were found to be 0.435 µg/ml and 1.319 µg/ml for Rabeprazole sodium and 0.594 µg/ml and 1.799 µg/ml for Mosapride citrate, respectively. The obtained data in validation studies are summarized in table 2. From the validation study it was cleared that all the observed values were within the acceptable range. Therefore, the method attempted to evaluate the stability of the drug under various stress conditions with different rates of decomposition. The developed method was able to detect decomposition. The chromatograms observed from samples, subjected to various stress conditions, are shown in fig. 7a to 7e. The amount of drug decomposed at various stress conditions are shown in table 2.

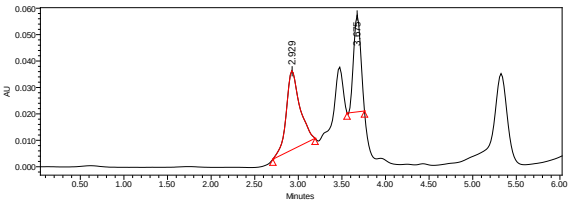

Fig. 7a: Chromatogram of acid degradation

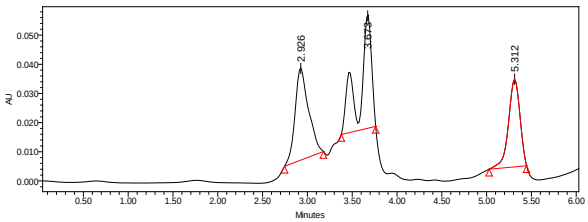

Fig. 7b: Chromatogram of peroxide degradation

Table 2: Validation parameters

S. No.	Parameter	Limits	Observation	
			Rabeprazole sodium	Mosapride citrate
1	Specificity	No interference	No interference	No interference
2	System precision		0.32788	0.61058
	Method precision	RSD NMT 2.0 %	0.28087	0.79084
	Intraday precision		0.12861	0.53061
3		Correlation coefficient NLT–		
	Linearity range	0.999	0.9999	0.9999
4		% Recovery range		
	Accuracy	98 –102 %	99.82-100 %	99.57-100.01 %
5		Signal noise ratio should be		
	Limit of Detection	more than 3:1	0.4354 µg/ml	0.59382 µg/ml
6				
	Limit of Quantitation	Signal noise ratio should be more than 10:1	1.319 µg/ml	1.799 µg/ml
7	Asymmetry factor			
		NMT 2 %	1.13	1.08
8	Number of Theoretical Plates			
		NLT 2500	7809	9726
9	Robustness	No effect on system suitability parameters	No effect on system suitability parameters	No effect on system suitability parameters
	Change in column temperature, Change in flow rate, change in wavelength			
10	Degradation	% net degradation – 1-50 %	-	-
	Acid		41.91 %	43.45 %
	Base		34 %	25.79 %
	Peroxide		41.11 %	33.68 %
	Heat		42.89 %	36.50 %
	Sun light		40 %	25.35 %

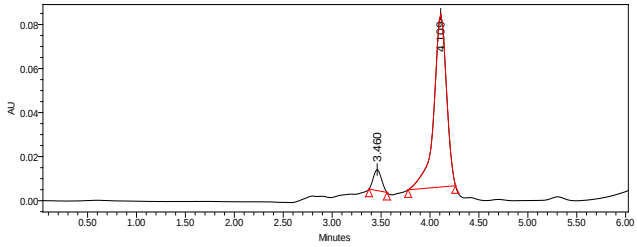

Fig. 7c: Chromatogram of thermal degradation

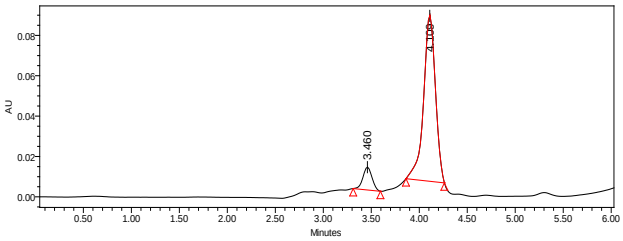

Fig. 7d: Chromatogram of UV degradation

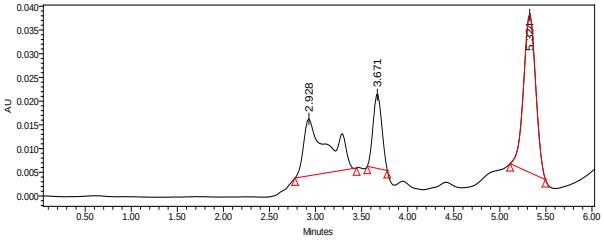

Fig. 7e: Chromatogram of base degradation

CONCLUSION

The proposed stability indicating RP-HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Rabeprazole sodium and Mosapride citrate in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Rabeprazole sodium and Mosapride citrate in pure and its pharmaceutical dosage forms.

CONFLICT OF INTEREST

None to declare

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