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Development and validation of RP-HPLC method for the estimation of Rabeprazole and Mosapride in raw and capsule formulation

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ABSTRACT

A sensitive feasible RP-HPLC method has developed and validated for the analysis of Rabeprazole and Mosapride in capsule. Successful separation of drugs products is developed on a C (18) column reversed-phase using and using mobile phase composition of Methanol: Phosphate buffer (55:45 v/v). Linearity ranges for Rabeprazole is 8 - 40 µg/ml and 6 - 30 µg/ml for Mosapride respectively. The absorption maxima were observed at 280nm. The HPLC, capsule formulation assay shows percentage purity ranging from 99.20 to 100.50% for Rabeprazole and 99.53 to 100.60% for Mosapride. The mean percentage purity is 100.20% and 100.10% for Rabeprazole and Mosapride respectively. The chromatographic retention time of Rabeprazole and Mosapride was found to be 3.2 and 6.5 minutes respectively. The tailing factor was 0.877 and 0.840 for Rabeprazole and Mosapride respectively. The developed method validated according to the ICH guidelines. The method was found to be applicable for determination and validation of Rabeprazole and Mosapride in combined capsule form.

Keywords: Rabeprazole (RPZ), Mosapride (MSP), HPLC and UV.

INTRODUCTION

Rabeprazole (RPZ), 2-[[4-(3-methoxypropoxy)-3-methyl-2- pyridinyl]-methylsulfinyl]-1H-benzimidazole, used a proton-pump inhibitor. The chemical structure of Rabeprazole is shown in figure 1a.The predominant use of Rabeprazole is to prevent to treat and gastroesophageal reflux, occurred backward flow of acid from the stomach and injury of the esophagus possibly. Rabeprazole controls the gastric acid secretion by inhibition of the gastric H+, K+ ATPase enzyme system [1-2]. Mosapride (MSP), 4-amino-5-chloro-2-ethoxy-N-[[4-[(4-fluorophenyl) methyl]-2-morpholinyl] methyl]-benzamide, gastroprokinetic agent used to enhance gastric motility and esophagitis reflux. The chemical structure of Rabeprazole is shown in figure 1b. The gastroprokinetic agent that acts as a $5HT_4$ selective agonist and major active metabolite of mosapride, known as M1 [3-4].

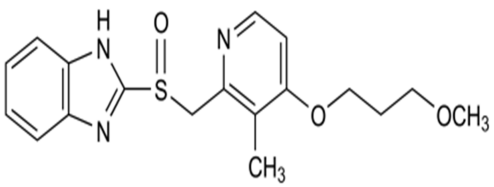

Figure 1a: chemical structure of Rabeprazole

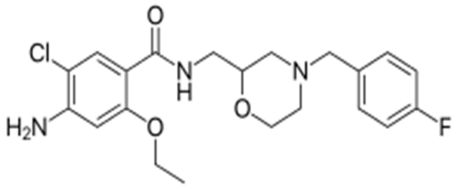

Figure 1b: chemical structure of Mosapride

Literature review shows several methods has been developed and reported for Rabeprazole and Mosapride estimation in biological fluids and there are some methods reported by [7], spectroscopy [8], HPTLC HPLC, UPLC and capillary electrophoresis [9-11]. Two methods were reported for estimation of this combination first is UV spectroscopy [12] and the other is HPTLC method [13-19]. Method development of HPLC estimation for this combination is new method will fulfil all requirements of validation according to ICH guidelines.

MATERIALS AND METHODS

Chemicals and reagents: The working standard of Rabeprazole and Mosapride was purchased from Sigma, UK. The Marketed sample of VELOZ-M Strength Rabeprazole 20 mg and Mosapride Citrate 15 mg manufactured and marketed by Torrent Pharma purchased from the local Pharmacy, Chennai, India. Methanol HPLC grade was purchased from Merck, Darmstadt, Germany, Orthophosphoric acids purchased from Fisher Scientific (UK).

Instrumentation

HPLC instrumentation and chromatographic condition:

HPLC system of Shimadzu LC-20 AT, with an auto sampler (SIL-20AC HT, Shimadzu, Japan) and SPD-10 detector (SPD- M20A, Japan) was used. For data recording the LC-solution software used. A Zorbax Eclipse Plus, Agilent Technology column (150mm x 4.6mm, 5µm) was used Pore size of the column 95Å. For degassing mobile phase, power sonic 505 ultrasonic baths (Hwashin technology, Seoul, Korea) was used. By using oven (CTO-20AC) column was maintained at a temperature of 39ºC and 1 ml/min was the flow rate. Analysis was carried over with 20µl injection volume using SPD-10 detection at 280nm. 10 minutes was set as run time.

Preparation of Standard solution for HPLC

Preparation of Mobile phase: Phosphate buffer was prepared using 0.25g of KH_2PO_4 in 1000 ml of HPLC grade water by using phosphoric acid pH adjusted to a 6 (±0.5). It was filtered with 0.45µ membrane filters and degassed in an ultrasonic bath for 10 minutes. The ratio of Methanol: phosphate buffer (55: 45) v/v.

Preparation of Rabeprazole (RPZ) and Mosapride (MPZ) Stock solution: Accurately 20 mg of RPZ (RS) and 15 mg of MSP (RS) was taken separately in 100 ml volumetric flasks and mixed with 25 ml of mobile phase solution and sonicated for 10 minutes and 75ml of mobile phase was added to the mark and cooled to room temperature. To get the concentration of 8-40 µg/ml of RPZ 6-30 µg/ml of MSP varying quantities of standard stock solution was diluted with mobile phase. Both RPZ and RPZ powder freely soluble in methanol and does not have any interference in the absorption peaks.

Preparation of sample solution: 15 capsules of marketed sample of VELOZ-M weighed accurately and powder equivalent of 20.00 mg of RPZ and made up to 50 ml with mobile phase and the resulting solution was filtered through Whatman 1 filter paper. 6 ml filtrate made up to 100 ml of mobile phase to get effective concentration of 24 µg/ml of RPZ and 18 µg/ml of MSP.

Method validation: The present method was proceeded to obtain new, sensitive and easy method for simultaneous estimation by HPLC from capsule formulation. According to the ICH guidelines recommendations the experimental was validated and USP-30 for parameters such as, system suitability, accuracy, precision, linearity and specificity.

System suitability: System suitability parameters like resolution, retention time, tailing factor and column theoretical plates was performed by injecting six replicates of standards and two replicates of sample preparation at a 100% level to cross verify the accuracy and precision of the chromatographic system.

Linearity: The chromatographic method linearity was established by plotting a graph to concentration vs peak area of RPZ and MSP standard and determining the correlation coefficients (R2) of the two compounds. For the linearity studies of RPZ and MSP the specific range was determined at 8-40 µg/ml of RPZ 6-30 µg/ml of MSP for RPZ and MSP respectively were injected into the HPLC system. For 60 minutes column was equilibrated with the mobile phase before injection of the solutions.

Accuracy: The recovery experiments show the accuracy of the method. The recovery was performed by adding RPZ and MSP working standards to placebo (excipients mixture) in the range of test concentration (60%, 80% and 100 %) and expressed as percent (%) recovered. Three samples were prepared for each recovery level. The recovery statistical results are within the acceptance range (S.D. < 2.0) value for RPZ and MSP. The percentage recovery of the drug was calculated by the formula given below.

Precision: In the proposed method **t**he intraday and interday precision was determined by analyzing the sample responses 4 repeats on the same day and 4 different days of a week for 4 different concentrations of standard solutions of RPZ and MSP. 24-40 µg/ml of RPZ 18-30 µg/ml of MSP for RPZ and MSP respectively and results are represented in terms of % RSD.

Specificity: The analytical method specificity is to measure the compound accurately in presence of interferences like excipients, degradants and matrix components. The HPLC of standard mixture and formulation shows specificity of method. The HPLC method is able to access the analyte in presence of excipients.

Statistical Parameters: The results of assay obtained are subjected to the following statistical analysis, standard deviation, relative standard deviation, coefficient of variation and standard error.

RESULT AND DISCUSSION

The HPLC chromatogram of RPZ and MSP are presented in figure 2 and 3. Wavelength 280nm was selected by scanning all standard drugs over a wide range of wavelength 200-400nm. Linearity was evaluated by plotting peak area as a functional of analyte concentration for both RPZ and MSP. The graphical representation was given in figure 4 and 5; data is presented in table 1 and 2.

The specific range was determined from linearity studies, for both drugs and found to be 8-40 µg/ml for RPZ and 6-30 µg/ml for MSP. The data was analyzed by linear regression least square fit method. The slop, intercept, correlation coefficient and regression equation were also determined and the data presented in table 3.

The system suitability parameters like resolution, tailing factor, retention time and theoretical plates for the developed RP-HPLC method are presented in figure 6; the data are presented in table 4.

The RPZ and MSP chromatographic retention time found to be 3.2 and 6.5 minutes respectively. This is well within the specific limits of 10 minutes. The high – resolution value of 11 RPZ and MSP indicates complete separation of the drugs. The tailing factor was found to be 0.877 and 0.840 for RPZ and MSP respectively. The peaks are symmetrical and theoretical plates for RPZ and MSP were 8097 and 9795 respectively which shows the column efficient performance. The limit of detection and limit of quantification for RPZ and MSP are presented in table 5. The quantitative estimation of RPZ and MSP capsule formulation was carried out by RP-HPLC method using Methanol: Phosphate buffer (55:45 v/v) using C18 column as the stationary phase. Chromatogram RPZ and MSP in capsule formulation shown in the figure 6. The quantitative estimation of the capsule formulation is presented in table 6 and graphically presented in figure7. Recovery studies of RPZ and MSP from capsule formulation shown in table 7

The capsule formulation shows percentage purity ranging from 99.20 to 100.50% for RPZ and 99.53 to 100.60% for MSP. The mean percentage purity is 100.20% and 100.10% for Rabeprazole and Mosapride respectively. The percentage deviation was found to be -0.2 to +0.1% and -0.5 to +0.1 for RPZ and MSP respectively. The RSD values are below 2% indicating the method precision and the accuracy of the method shown by the low standard error values. This shows a good index of accuracy and reproducibility of the developed method. All the parameters including flow rate, detection wavelength sensitivity was maintained constant.

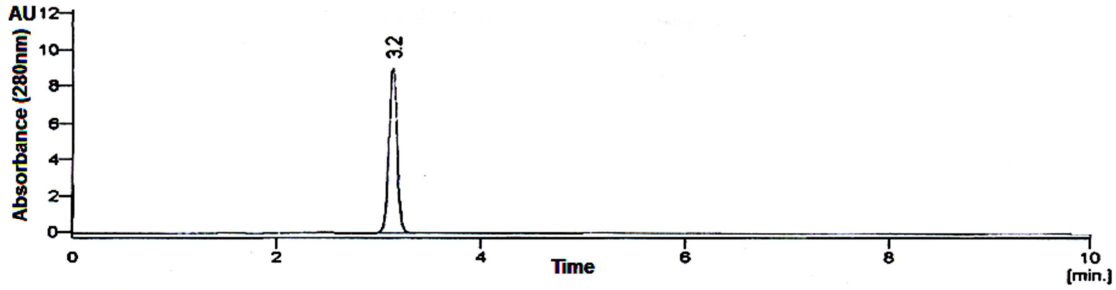

Figure 2: A Typical Chromatogram of Rabeprazole Standard

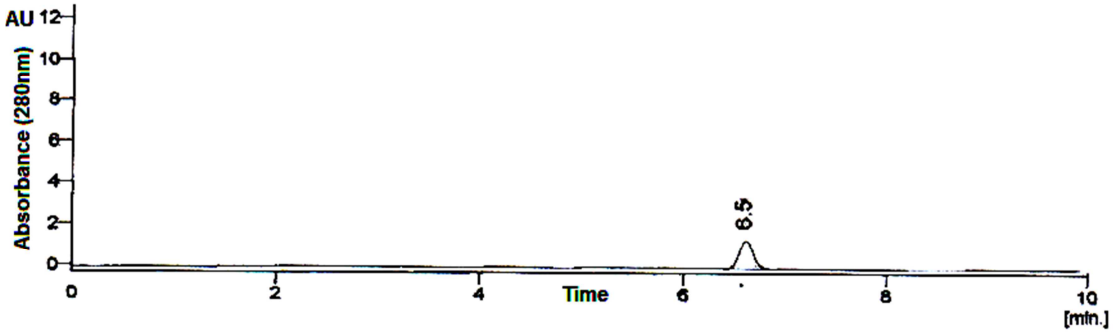

Figure 3: A Typical Chromatogram of Mosapride Standard

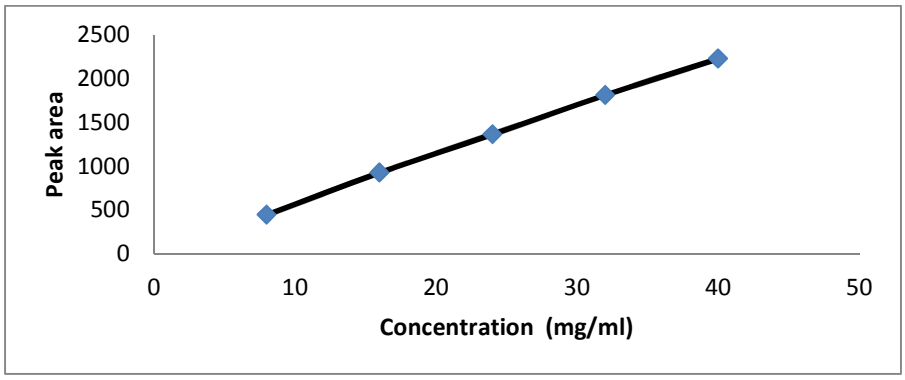

Figure 4: Calibration graph of Rabeprazole 8-40 µg/ml precision

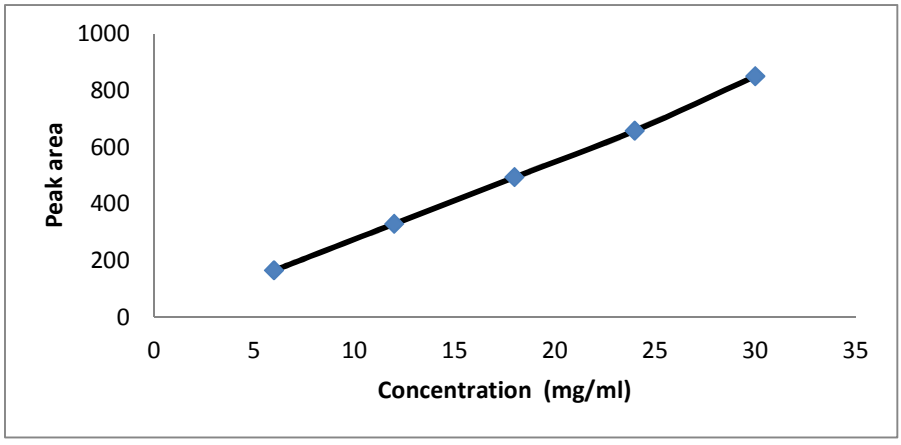

Figure 5: Calibration graph of Mosapride 6-30 µg/ml precision

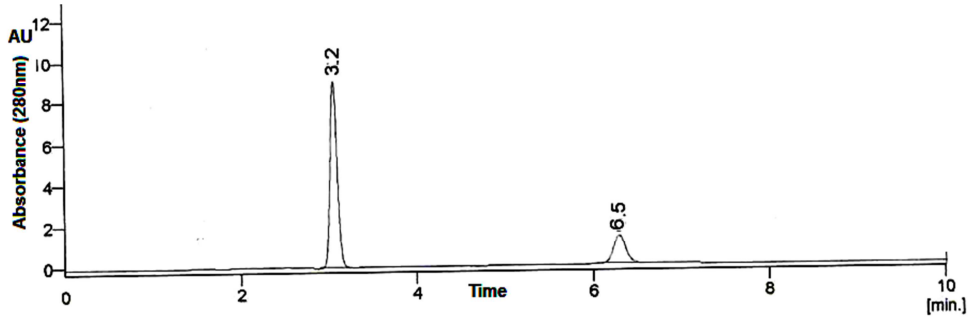

Figure 6: Chromatogram of Rabeprazole and Mosapride in capsule formulation

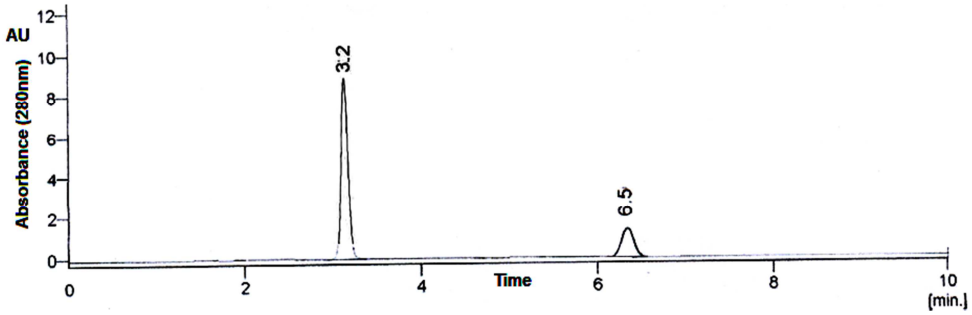

Figure 7: Quantitative estimation (Assay) of Rabeprazole and Mosapride in capsule formulation

Table 1: HPLC linearity data for Rabeprazole

SNo	Concentration (µg/ml)	Peak area
1	8	444.29
2	16	924.58
3	24	1362.29
4	32	1810.16
5	40	2225.15

Table 2: HPLC linearity data for Mosapride

SNo	Concentration (µg/ml)	Peak area
1	6	164.25
2	12	328.95
3	18	493.20
4	24	657.45
5	30	849.69

Table 3: Results of statistical parameters Statistical parameters

SNo	Parameters	Rabeprazole	Mosapride
1	Standard deviation (SD)	9.03	5.18
2	Relative standard deviation (RSD)	0.00716	0.0112
3	% RSD	0.716	1.121
4	Standard error (SE)	0.03286	0.01205
5	Correlation Coefficient (r)	0.9997	0.9994
6	Slope (a)	55.591	28.323
7	Intercept (b)	19.106	11.114
8	Regression equation Y = (a X + b)	**Y = 55.591 X + 19.106**	Y = 28.323 X -11.114

Table 4: Results of system suitability parameters

SNo	Parameters	Rabeprazole	Mosapride
1	Theoretical plates	8097	9795
2	Tailing factor	0.877	0.840
3	Resolution factor	11	11
4	Retention time	3.2	6.5
5	Calibration range or Linear dynamic range	8-40	6-30

Table 5: Results of Limit of detection (LOD) & limit of quantification LOQ

Parameters	Rabeprazole	Mosapride
LOD (ng/ml)	0.530	0.600
LOQ (ng/ml)	**1.580**	**1.600**

Table 6: Quantitative estimation (Assay) data of Rabeprazole and Mosapride

S No	Drug	Label claim (mg/cap)	Amount found (mg/cap)	Mean amount found (mg/cap)	Percentage purity (% w/w)	Mean percentage purity (% w/w)	% Deviation
1	RPZ	20	20.07	20.04	100.05	100.20	+ 0.7
			20.10		100.20		+1.0
			20.01		100.50		+0.1
			19.98		99.90		-0.2
			20.04		100.35		+0.4
2	MSP	15	15.02	15.02	100.13	100.10	+0.1
			14.97		99.80		-0.2
			14.93		99.53		-0.5
			15.10		100.66		+0.6
			15.06		100.40		+0.1

Table 7: Recovery studies of Rabeprazole and Mosapride from capsule formulation

S No	Drug	Amount of Drug present in preanalyzed Sample	Amount of Standard drug (RS) added (µg/ml)	Amount of drug recovered (µg/ml)	% Recovery	Mean recovery in Percentage
1	Rabeprazole	32	24.00	56.93	101.23	100.67
			32.00	64.28	100.88	
			40.00	71.62	99.05	
2	Mosapride	18	18.00	36.92	101.22	100.78
			24.00	42.08	100.33	
			30.00	48.24	100.80	

CONCLUSION

The proposed and developed RP-HPLC method is precise, accurate, and sensitive. The method is rapid, reproducible, and economical and does not have any interference due to the excipients in the pharmaceutical preparations.

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