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Research Article

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF PANTOPRAZOLE AND MOSAPRIDE IN CAPSULE DOSAGE FORM

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ABSTRACT

An estimation method based on RP-HPLC has been identified for determining the concentrations of pantoprazole sodium and mosapride citrate in combined pharmaceutical formulation. The experiment was performed on Hypersil BDS (4.6mm X 150nm), 5µm column in isocratic mode, with 1 mL/min flow of orthophosphoric acid adjusted, mobile phase consisting of a 40:60 mixture of mixed sodium phosphate buffer (0.007M) and acetonitrile of pH 4. A UV detector was used with the measurement of absorption at 278 nm. The method resulted in retention times of 2.803 minutes and 5.167 minutes for pantoprazole sodium and mosapride citrate respectively.

The method was validated according to ICH guidelines for system suitability parameters, linearity, precision, accuracy, specificity, ruggedness, robustness. Recoveries of pantoprazole and mosapride were found to be in the range of 99.77% ± 0.23% and 99.44% ± 0.50%. Linearity was observed in the range of 5-30 µg/mL and 1.9-11.4 µg/mL for pantoprazole and mosapride respectively. The LOD for pantoprazole sodium and were Mosapride citrate determined to be 0.1599 µg/mL and 0.1790µg/mL respectively, while LOQ for these were respectively 0.4894 µg/mL and 0.5480 µg/mL. As, all the parameters of ICH guidelines for validation are satisfied, this method is suitable for the simultaneous estimation of pantoprazole sodium and mosapride citrate in combined pharmaceutical formulations.

Keywords: Pantoprazole, Mosapride, RP-HPLC, BDS, Oesophagitis, Dyspepsia.

INTRODUCTION

Pantoprazole belongs to a category of drugs which acts an inhibitor to proton pumps [1]. It is widely prescribed for ulcers like peptic and esophagus, apart from being suggested for acid dyspeptic orders occurring in the upper region of GI tract [1, 2]. This finds use in the erosion treatment [2]. This is a prodrug that inactivates the enzyme irreversibly, through the formation of sulphonamide that reacts with ATP-ase to form disulphide bond by covalent linkage [3]. Mosapride is a drug that is widely known for its capability to facilitate and enhance gastric emptying [4]. It increases the motility of large intestine as well [4]. Mosapride acts by increasing the release of acetylcholine [4].

Bhatt et al. [5] have reported a first derivative spectroscopic method for estimating the concentrations of pantoprazole and mosapride in a solid dosage form. A method based on thin layer chromatography and high performance liquid chromatography has been reported for the simultaneous estimation of this combination [6]. Pantoprazole and Mosapride were eluted at the residence times of 4.4 min and 11.4 min respectively [6]. RP-HPLC, being a widely used method for estimation of drugs [7, 8, 9], has been attempted here for the combined formulation of pantoprazole and mosapride.

MATERIALS AND METHODS

Instrumentation

The HPLC system used for the method development is Shimadzu system with LC-20AT pump, Rheodyne injector, BDS hypersil C_{18} column (150 × 4.6 mm, 5µm) from Thermo Company, SPD-20A detector and with spin chrome software by using 60:40 mixture of acetonitrile: mixed sodium phosphate buffer. The pH was adjusted to 4.0 using orthophosphoric acid. After filtering the mobile phase under vacuum through a 0.45 µm membrane filter, it was degassed for 5 minutes. Experiments were carried out with injection volume of 20 µL for samples and standards, while supplying mobile phase at 1mL/min, with absorbance measured using a UV-detector at 278 nm.

Chemicals & Reagents

- Water (MilliQ HPLC grade water)
- Acetonitrile (HPLC grade)
- Methanol (HPLC grade)
- Disodium hydrogen phosphate
- Sodium dihydrogen phosphate
- Ortho phosphoric acid
- Pantoprazole and Mosapride standards

Preparation of pH 4 mixed phosphate buffer

About 132 mg of sodium dihydrogen phosphate and 664 mg of disodium hydrogen phosphate was dissolved in 400 mL of distilled water. pH was adjusted to 4 using Orthophosphoric acid.

Preparation of mobile phase

To the 400 mL of buffer solution, 600 mL of acetonitrile solution was added, mixed well and degassed. Mobile phase was used as the diluent.

Preparation of standard solution

About 25 mg of Pantoprazole sodium and 9.5 mg of Mosapride citrate dihydrate working standards were weighed accurately and each was transferred in to a separate 50mL volumetric flask. After adding 20 mL of diluent to each flask, the contents were subjected to sonication for 5 min. The rest of volume was made up with diluent. Standard solutions of pantoprazole and mosapride were diluted with diluent to obtain concentrations of 5-30 µg/mL and 1.9-11.4 µg/ mL respectively.

Preparation of sample

10 capsules were accurately weighed and ground to prepare a fine powder. Suitable quantity of this powder representing 40 mg of Pantoprazole and 15 mg of mosapride was accurately weighed and transferred to into a 50 mL volumetric flask. About 20 mL of diluent was added & sonicated for 20 minutes with occasional swirling to dissolve. It was cooled and made up using diluent.

RESULTS AND DISCUSSION

After several chromatographic experiments with different columns, composition and flow rate of mobile phase, the following optimum conditions were identified: (i) mobile phase: 60:40 ratio of acetonitrile: mixed sodium phosphate buffer with pH 4 (ii) flow rate: 1 mL/min. This resulted in elution of Pantoprazole and Mosapride at 2.110 and 4.170 minutes respectively. Hence a run could be completed within 6 minutes, a typical chromatogram for which is shown in figure 1.

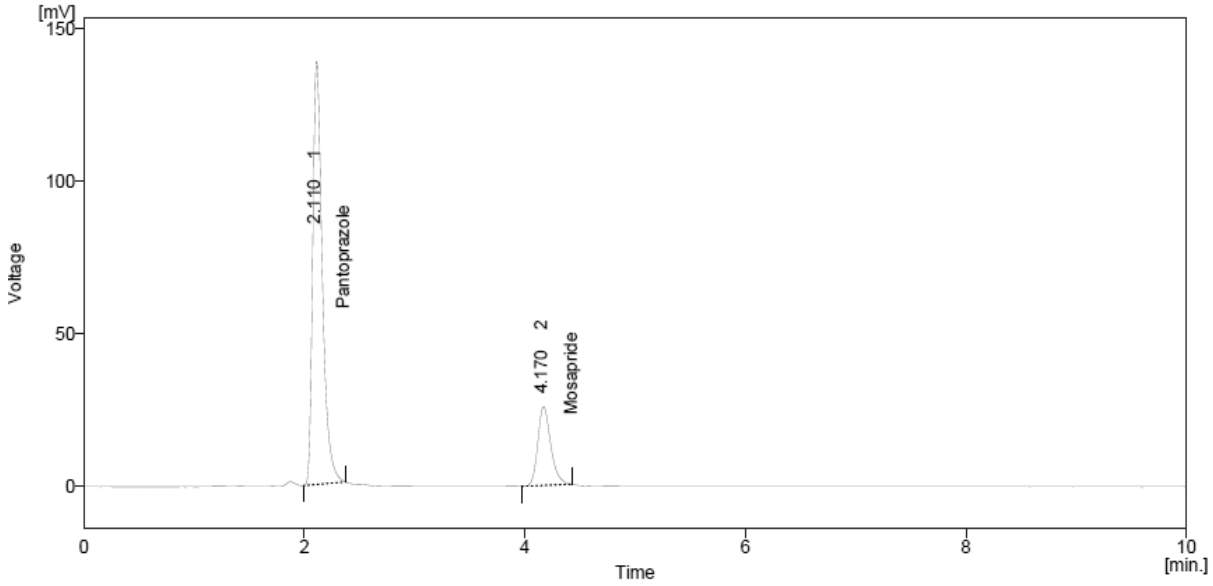

Fig. 1: Chromatogram of the standard solution, with retention times of 2.110 min and 4.170 min for pantoprazole sodium and mosapride citrate respectively

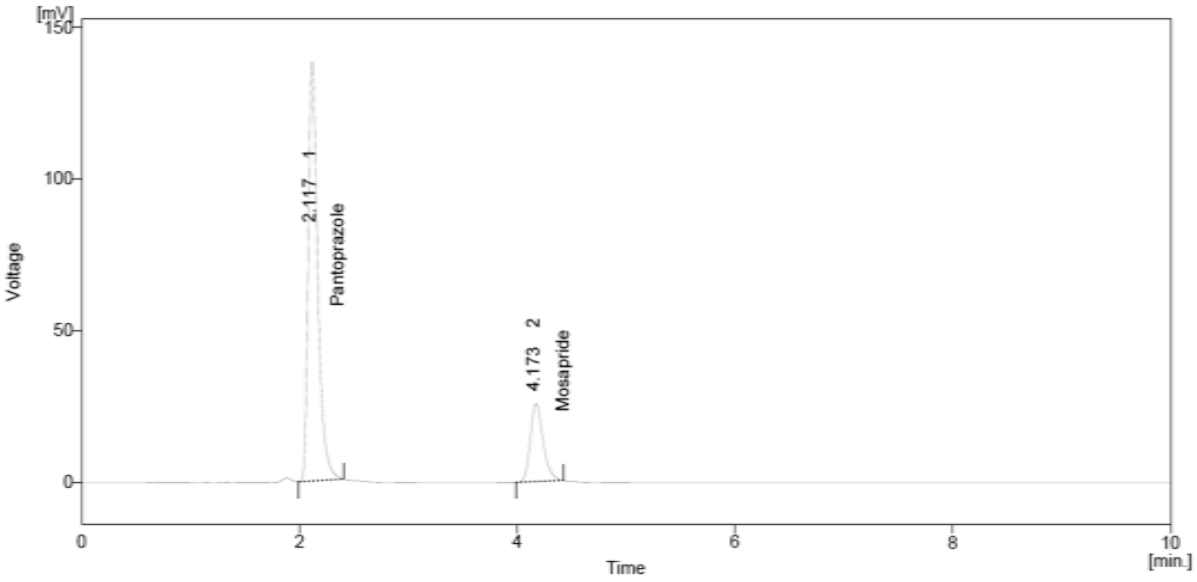

Fig. 2: Chromatogram showing elution of pantoprazole sodium at 2.117 min and mosapride citrate at 4.173 min.

From the Fig. 1, we can infer that the two peaks of pantoprazole sodium and mosapride citrate are well-resolved. The peak area in mV corresponding to 2.110 minutes is 838.370 and for 4.170 min, the peak area is 211.427.

After confirming that the method developed could give two well defined peaks which are also well resolved, sample solutions of capsules containing pantoprazole sodium and mosapride citrate were analysed by the developed method yielding a chromatogram as shown in Fig. 2. The percentage assays for pantoprazole sodium and mosapride citrate were calculated using standard procedures.

METHOD VALIDATION

System suitability parameters

About 20 µL of standard solution of pantoprazole sodium and mosapride citrate was injected six times in the HPLC system and the system suitability parameters were recorded as per procedure. The results are shown in Table 1.

Linearity

Various concentrations of standard solutions (containing 5-30 µg/mL of pantoprazole sodium and 1.9-11.4 µg/mL of mosapride citrate) were analyzed to ascertain the linearity of the method (Fig. 3). The results are shown in Fig. 4 and 5 where a linear relationship between peak area and analyte concentration is evident. Correlation coefficient close to 0.99 was obtained for both the drugs demonstrating linearity.

Accuracy

Accuracy was determined by standard addition method in triplicate for various concentrations of pantoprazole sodium and mosapride citrate dihydrate in the concentrations of 22.5, 27.5 & 32.5 µg/mL and 8.55, 10.45 & 12.35 µg/mL respectively and injected into the HPLC system as per the procedure. The percentage mean recoveries for pantoprazole and mosapride were found to be 99.87 and 99.64 respectively. The summary of accuracy results are expressed in table 2 and 3.

Table 1: System suitability parameters

Serial Number	Parameters	Measured values for pantoprazole sodium	Measured values for mosapride citrate
1.	Theoretical plates	2864	6333
2.	Tailing factor	0.9	1.01
3.	Assymmetry	1.3	1.1
4.	% RSD of peak retention time	0.16	0.058

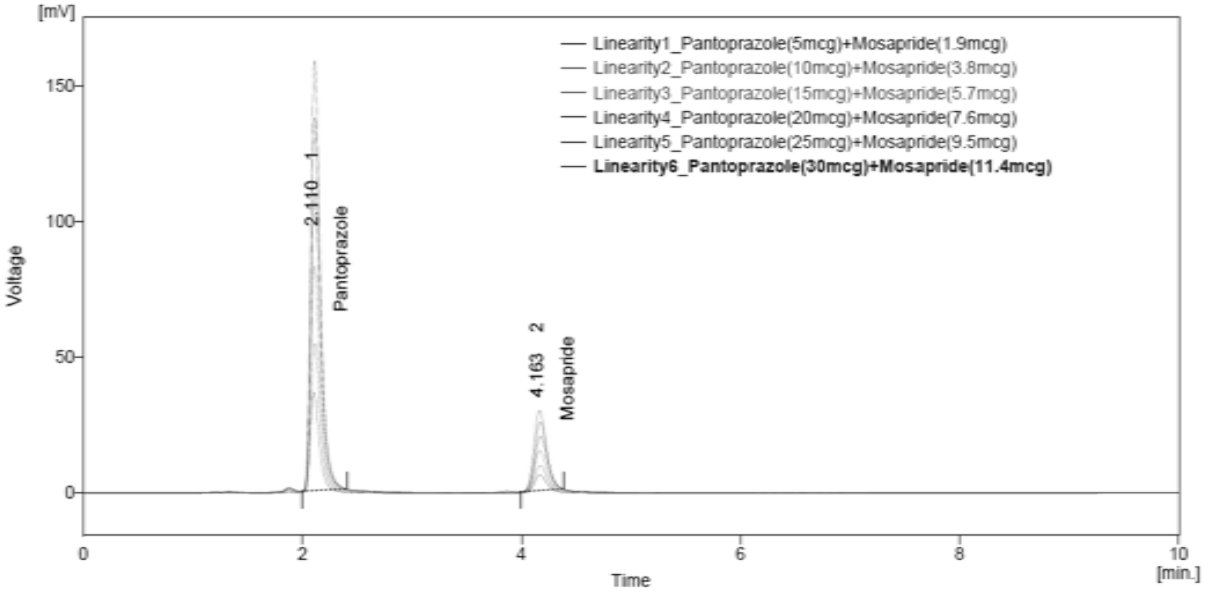

Fig. 3: Overlaid chromatograms of the standard solution showing the elution of pantoprazole sodium at 2.1 min and mosapride citrate at 4.1 min.

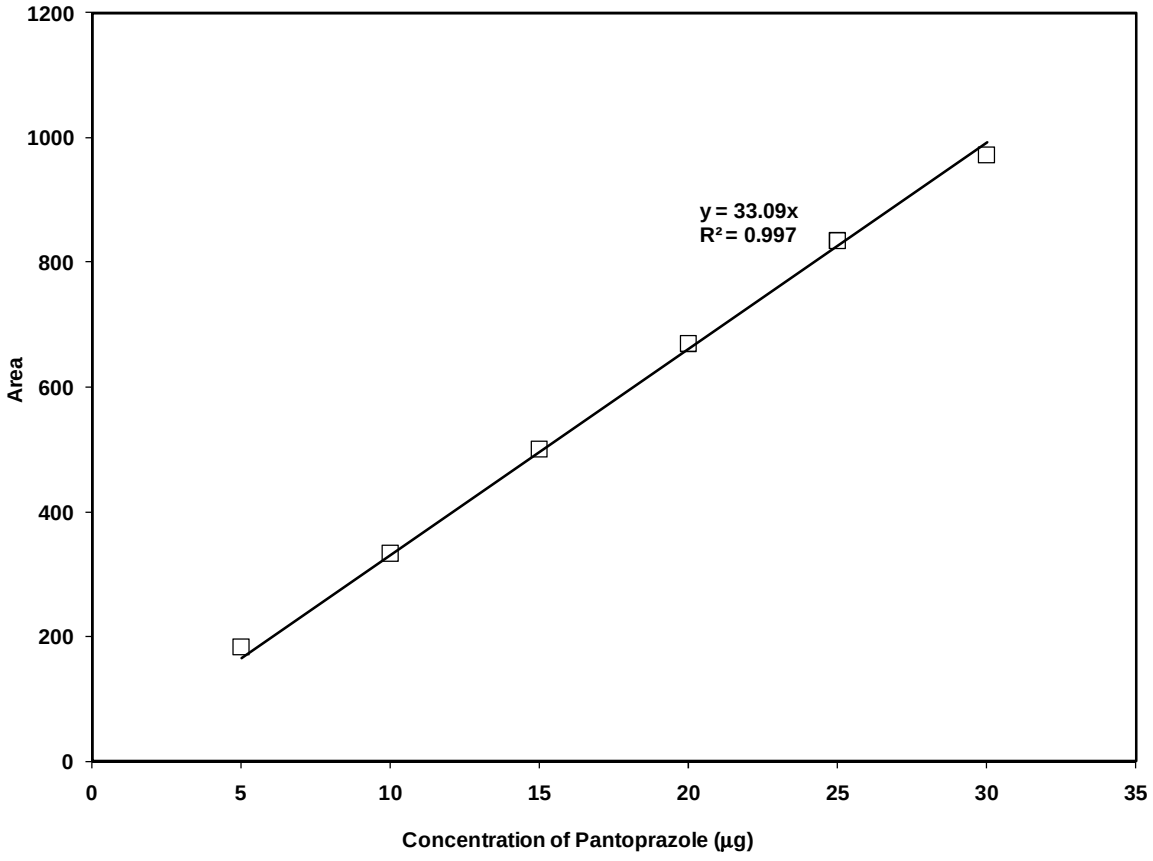

Fig. 4: Linearity graph of Pantoprazole sodium

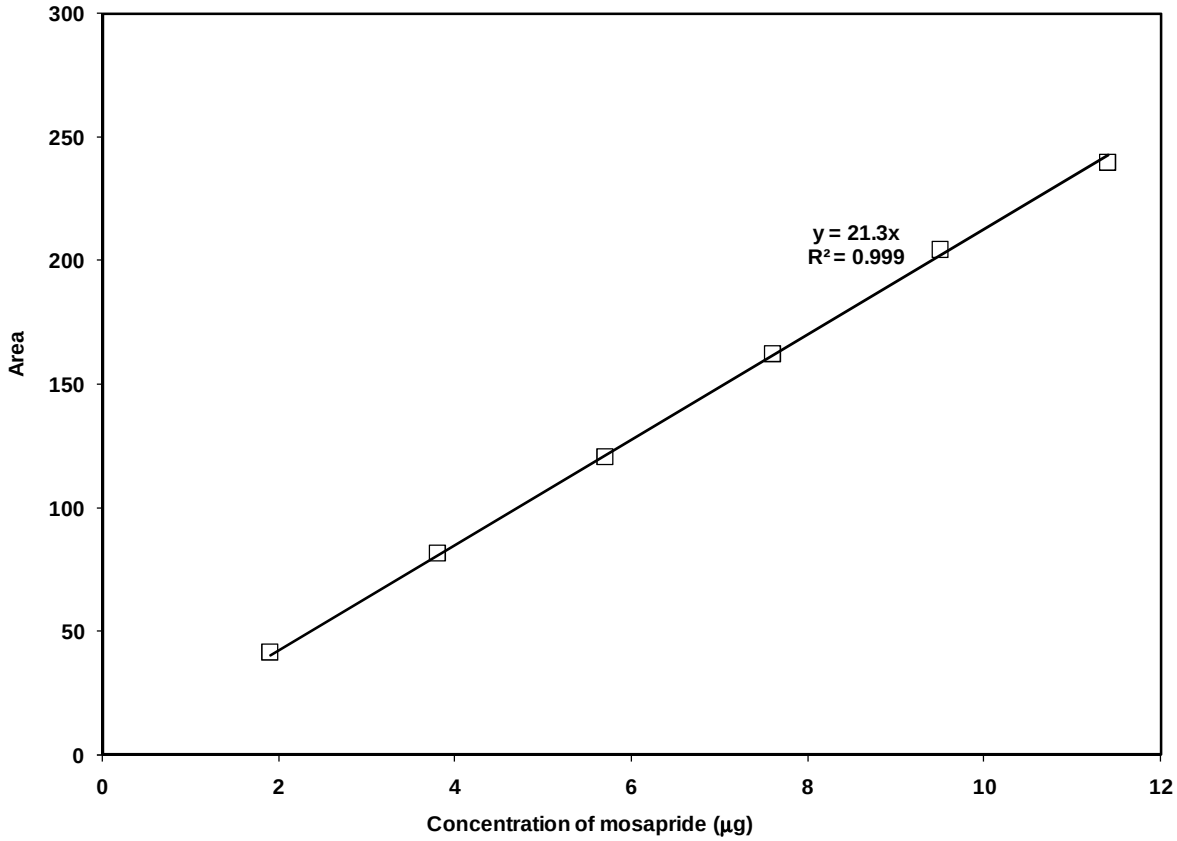

Fig. 5: Linearity graph of Mosapride citrate

Table 2: Recovery Results for Pantoprazole sodium

Sample no.	Spiked conc. (µg/mL)	Amt recovered	% Recovery	Mean % Recovery
1.	22.50	22.48	99.89	99.87
2.	27.50	27.44	99.77	
3.	32.50	32.49	99.96	

Table 3: Recovery Results for Mosapride citrate

Sample no.	Spiked conc. (µg/mL)	Amt recovered	% Recovery	Mean % Recovery
1.	8.55	8.54	99.90	
2.	10.45	10.41	99.59	99.64
3.	12.35	12.28	99.44	

Precision

The method's precision was assessed by injecting the replicate samples into the HPLC system and for repeatability (intra-day precision). The %RSD was found to be < 2 (Table 4), which is within the acceptable limit demonstrating the method's precision.

Table 4: Precision results for Pantoprazole and Mosapride

Pantoprazole		Mosapride	
Injection No	**Area**	**Injection No**	**Area**
1	838.37	1	211.427
2	837.524	2	210.662
3	837.314	3	209.677
4	837.410	4	208.992
5	837.823	5	211.089
Average	837.6882	Average	210.3694
SD	0.42644	SD	1.012251
%RSD	0.05	%RSD	0.48

Specificity

To check the specificity, the chromatogram of the sample solution of the capsule dosage form containing pantoprazole sodium and mosapride citrate with excipients was compared with the chromatogram of the standard solution of pantoprazole sodium and mosapride citrate. The chromatograms show that there are no interferences of the excipients for the chosen analytes.

To further ascertain the specificity, the sample solution of the capsule dosage form was prepared with 0.1M NaOH and the recorded chromatogram is shown in Fig. 6.

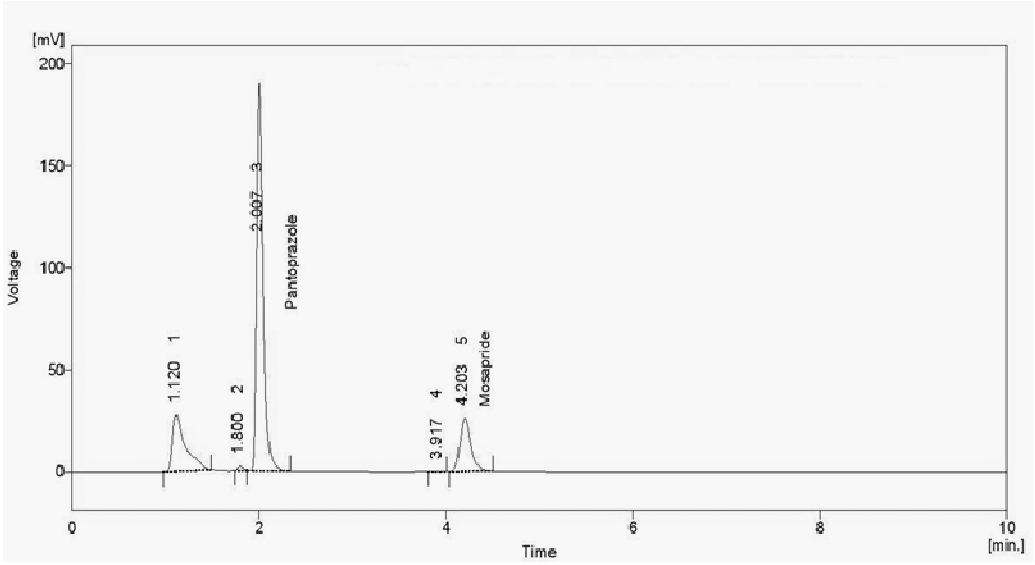

Fig. 6: Chromatogram of the sample solution prepared in 0.1M NaOH.

From Fig. 6, it can be inferred that, the preparation of sample in 0.1M NaOH did not affect the retention times and areas of pantoprazole sodium and mosapride citrate. Hence the developed method is specific.

Limit of Detection (LOD)

The Limit of Detection was determined from linearity curve method using slope and standard deviation of precision. Limit of Detections of 0.1599 µg/mL and 0.17907 µg/mL were obtained for pantoprazole sodium and mosapride citrate respectively.

Limit of Quantification (LOQ)

The Limit of Quantification was calculated from the linearity curve method using slope, and standard deviation of intercepts of calibration curve. Limit of Quantifications of 0.489 µg/mL and 0.548 µg /mL were obtained for pantoprazole and mosapride.

Ruggedness:

Ruggedness of a method is ascertained by repeating the analysis by different analysts at different time points (days) utilizing different instruments. Table 5 shows the summary of ruggedness tests.

Table 5: Results of "Test for ruggedness"

Concentration of PANT (µg/mL)	R_t	Area	Concentration of MOSP (µg/mL)	R_t	Area
25	2.123	835.047	9.6	4.19	209.916
25	2.123	835.956	9.6	4.187	210.938
25	2.12	837.987	9.6	4.18	210.845
25	2.123	834.225	9.6	4.187	207.862
25	2.123	839.131	9.6	4.19	210.26
Avg	2.1224	836.4692	**Avg**	4.1868	209.9642
St dev	0.001342	2.043976	**St dev**	0.004087	1.248315
%RSD	**0.06**	**0.244**	**%RSD**	**0.09**	**0.59**

Table 5 shows that the calculated percentage relative standard deviations were 0.244% and 0.59% respectively, for areas of pantoprazole sodium and mosapride citrate. Hence, as per the ICH guidelines, the method developed is rugged.

Robustness

Robustness is a measure to check the reliability of the method when small deliberate changes are made. In the present work, robustness was measured by changing mobile phase's pH (by ±0.3%) and the flow rate (0.9-1.1 mL/min). Insignificant changes in the retention time and assay values due to this variation confirm the method's robustness. Fig. 7 shows a typical chromatogram for a mobile phase flow rate of 0.9 mL/min while Fig. 8 shows for a flow rate of 1.1 mL/min.

From Fig. 7 and Fig. 8, it can be inferred that the minor change in flow rate of the mobile phase did not show significant difference in retention times of the pantoprazole sodium and mosapride citrate. Also, it was found that, there is no significant variation in the assay of the drugs pantoprazole sodium and mosapride citrate. Hence, the developed method is robust.

The method thus satisfies all the different parameters of validation.

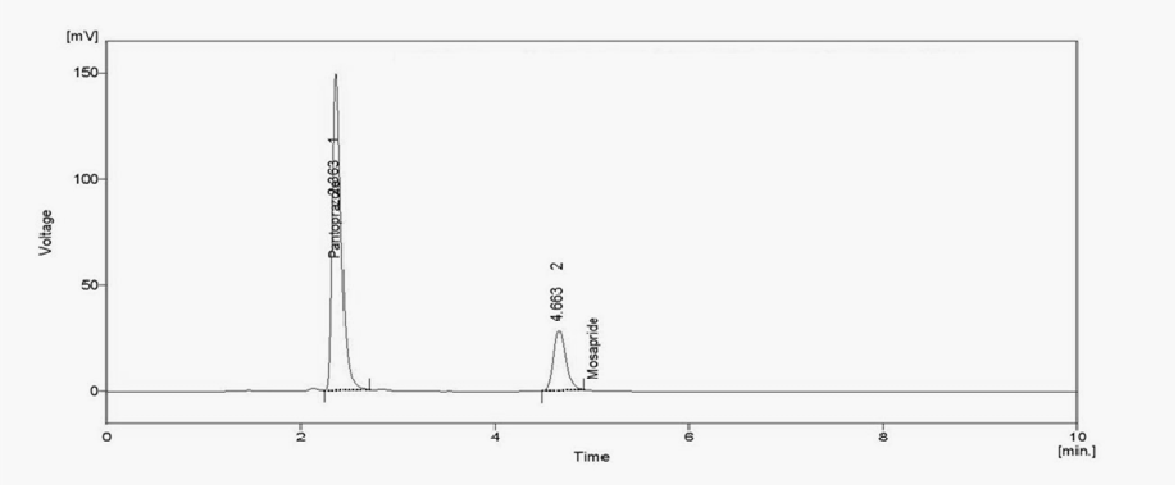

Fig. 7: The chromatogram obtained when the flow rate was changed to 0.9 mL/min

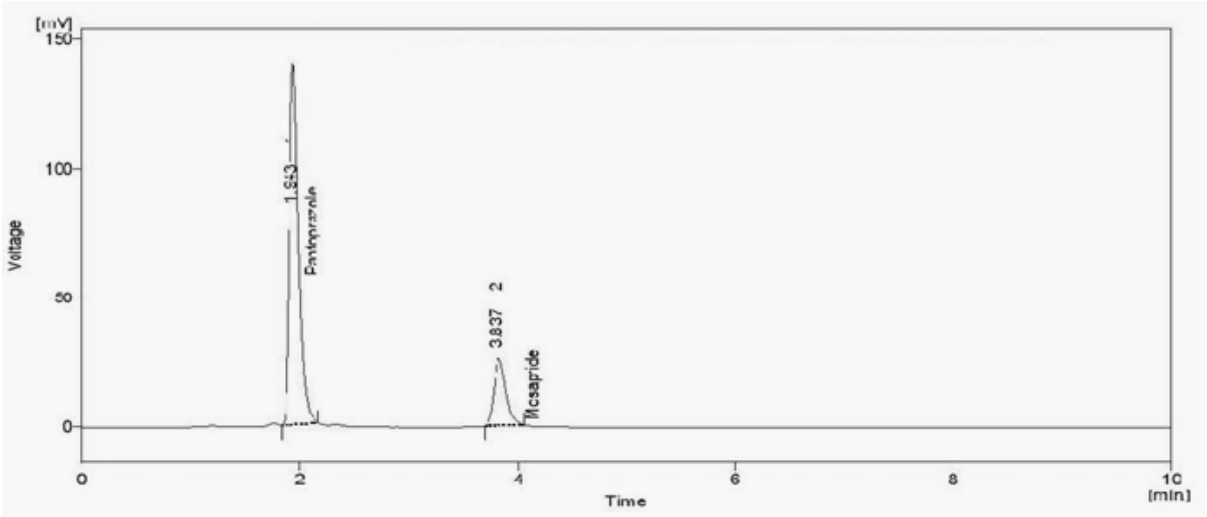

Fig. 8: Chromatogram obtained when the flow rate was changed to 1.1mL/min.

CONCLUSIONS

The HPLC method developed in the present study for the estimation of pantoprazole sodium and mosapride citrate is simple with run time than six minutes and yields sharp, well resolved peaks. The method satisfies the tests for accuracy, precision and ruggedness. Hence this method may be applied for the analysis of the dosage forms containing pantoprazole sodium and mosapride citrate in combination.

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