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ANALYTICAL METHOD DEVELOPMENT & VALIDATION FOR SIMULTANEOUS ESTIMATION OF PANTOPRAZOLE AND MOSAPRIDE IN PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

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ABSTRACT

A simple, specific and accurate reverse phase high performance liquid chromatographic method was developed for the simultaneous determination mosapride and pantoprazole in pharmaceutical dosage form. The column used was BDS in isocratic mode, with mobile phase containing phosphate buffer and acetonitrile (55:45 v/v) adjusted to pH 3.0 with dilute ortho phosphoric acid solution. The flow rate was 1.1 ml/ min and effluents were monitored at 260 nm. The retention times of mosapride and pantoprazole were found to be 2.399 min and 3.191 min, respectively. The linearity for mosapride and pantoprazole were in the range of 7.5-45 µg/ml and 20-120 µg/ml respectively. The recoveries of mosapride and pantoprazole were found to be 99.22 to 100.09% and 98.02 to 99.98%, respectively. The proposed method was validated and successfully applied to the estimation of mosapride and pantoprazole in combined tablet dosage forms.

Keywords: Validation, Mosapride, RP-HPLC, Dosage form.

INTRODUCTION

Chemically, Mosapride is (RS)-4-amino-5-chloro-2-ethoxy-N-{[4-(4-fluorobenzyl) morpholin-2-yl] methyl} benzamide. The chemical formula is C21H25ClFN3O3. The molecular formula is 421 g/mol. Mosapride is a gastroprokinetic agent that acts as a selective 5HT4 agonist. The major active metabolite of mosapride, known as M1, additionally acts as a 5HT3 antagonist, which accelerates gastric emptying throughout the whole of the gastrointestinal tract in humans, and is used for the treatment of gastritis, gastro-oesophageal reflux disease, functional dyspepsia and irritable bowel syndrome. It is recommended to be taken on an empty stomach (i.e. at least one hour before food or two hours after food) [1].

Pantoprazole is chemically, 6-(difluoromethoxy)-2-{[(3,4-dimethoxypyridin-2-yl)methane]sulfinyl}-1H-1,3, benzodiazole. It is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of oesophagus caused by gastro-oesophageal reflux disease. The chemical formula is $C_{16}H_{15}F_2N_3O_4S$. The molecular formula is 383.37g/mol. Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H^+, K^+) ATPase enzyme system at the secretory surface of gastric parietal cell. This effect is dose related and leads to both basal and stimulated gastric acid secretion irrespective of the stimulus [2].

Different analytical methods have been reported in the literature for the assay of mosapride and pantoprazole in pharmaceuticals and include spectrophotometry, TLC, HPLC, HPTLC, LC-MS [3-12]. The present study was to establish a simple, sensitive and low cost RP-HPLC method for simultaneous estimation of mosapride and pantoprazole in bulk as well as in other dosage forms. The developed method was validated as per ICH guidelines [13-14].

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MATERIALS AND METHODS

Reagents

Mosapride and Pantoprazole were kindly supplied by Zydus Cadila Healthcare Ltd and Dr Reddy Laboratories Ltd. Acetonitrile, water (HPLC grade, Merck) and all the other reagents of AR grade were purchased from M R Enterprisers. A capsule Pantec Plus (Intas Laboratories Pvt Ltd) containing 15mg of mosapride and 40mg of pantoprazole were used.

INSTRUMENTATION

The LC system consisted of a Waters model 515, PDA detector 2487 10µL sample loop. The output signals were monitored and integrated using Empower 2 software.

Chromatographic conditions

The elution was isocratic and the mobile phase consisted of a mixture of buffer (accurately weighed and transferred 1.41gm of Sodium dihydrogen Orthophosphate in a 1000ml of volumetric flask add about 900ml of milli-Q water, add 1ml of triethylamine and degass to sonicate and finally make up the volume with water, then pH adjusted to 4.0 with dil. Ortho phosphoric acid solution) and acetonitrile (45:55 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A BDS Column 250mm x 4.6 mm, 5µ was used for determination. The flow rate was 1.0 ml/min and the column was operated at ambient temperature (30^0C). The volume of sample injected was 10 µL. Prior to injection of the solutions, column was equilibrated for at least 30min with mobile phase flowing through the system. The UV detector was set at wavelength of 260nm. A typical RP-HPLC chromatogram of mosapride and pantoprazole is shown in (Fig. 1).

Diluent

At first dissolved in methanol then diluted with Water and acetonitrile (50:50).

Standard Preparation (160µg/ml Pantaprazole and 60µg/ml Mosapride)

Accurately weighed and transferred 6mg of mosapride and 16mg of pantoprazole working Standards into a 100 ml clean dry volumetric flask, add 7ml of diluent, sonicated for 30 minutes and make up to the final volume with diluent. From the above stock solution, 1ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation

About 5 tablets were taken and their average weight was calculated. The tablets were crushed to a fine powder and drug equivalent to 15mg and 40mg were transferred to a 100ml volumetric flask, dissolved in diluent. Transfer 0.8ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter.

Method Validation

The developed method was validated as per ICH guidelines [13,14] for its accuracy, linearity, precision, specificity, robustness, limit of detection and limit of quantification by using the following procedures. The parameters are validated as shown in table 9.

System suitability

System suitability and chromatographic parameters were validated such as asymmetry factor, tailing factor and number of theoretical plates were calculated.

Linearity

Linearity of this method was evaluated by linear regression analysis and calculated by least square method and studied by preparing standard solutions of mosapride and pantoprazole at different concentration levels. Absorbance of resulting solutions was measured and the calibration curve was plotted between absorbance Vs concentration of the drug (Figure: 2 & 3). The response was found to be linear in the range 15-90ppm & 40-240ppm for mosapride and pantoprazole. The data was given in Table 1&2.

Accuracy

Accuracy was performed in triplicate for various concentrations of mosapride and pantoprazole equivalent to 50%, 100% and 150% of the standard amount were injected into the HPLC system as per the test procedure. The average % recovery was calculated. The data was given in table 2&3.

Precision

The solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits. Data was given in the table 5.

Limit of detection and Limit of Quantification

LOD and LOQ were calculated from the average slope and standard deviation from the calibration curve as per ICH guidelines. The LOD and LOQ of mosapride were found to be 0.15µg/ml and 0.155µg/ml respectively. The LOD and LOQ of pantoprazole were found to be 0.45µg/ml and 0.45µg/ml respectively.

Robustness

Robustness was done by small deliberate changes in the chromatographic conditions and retention time of mosapride and pantoprazole were noted. The factors selected were flow rate and variation in temperature. The

results remained unaffected by small variations in these parameters as shown in tables 6&7.

Assay

The assay and % purity were calculated for brands Pantec Plus (Intas Laboratories Pvt Ltd) with label claim 15mg and 40mg. The observed value was compared with that of standard value without interference from the excipients used in the tablet dosage form. The results are given in the table 8.

Table 1. Linearity data of Pantoprazole

S.No.	Linearity level	Concentration (ppm)	Area
1.	Level-1	40	1334437
2.	Level-2	80	2672345
3.	Level-3	120	3976630
4.	Level-4	160	5357864
5.	Level-5	200	6587233
6.	Level-6	240	8019846
	Correlation coefficient		**0.999**

Table 2. Linearity data of Mosapride

S.No.	Linearity level	Concentration (ppm)	Area
1.	Level-1	15	594804
2.	Level-2	30	1191504
3.	Level-3	45	1760033
4.	Level-4	60	2380491
5.	Level-5	75	2975231
6.	Level-6	90	3541825
	Correlation coefficient		**0.999**

Table 3. Accuracy data of Pantoprazole

Accuracy	Areas of samples	Avg.area of Standard	Amount added	Amount found	% Recovery	Mean Recovery
Accuracy 50%	2686832 2673155 2653798	5327798	80	79.8	100.46 99.95 99.92	99.88
Accuracy 100%	5351769 5356241 5341289	5327798	160	160.08	100.05 100.13 99.85	100.01
Accuracy 150%	8011878 8018405 8058582	5327798	240	238.1	99.85 99.93 100.43	100.01

Table 4. Accuracy data of Mosapride

Accuracy	Areas of samples	Avg.area of Standard	Amount added	Amount found	% Recovery	Mean Recovery
Accuracy 50%	1154163 1150185 1156225	2294449	3.0	2.9	100.20 99.86 100.38	100.15
Accuracy 100%	2308597 2301917 2307650	2294449	6.0	6.2	100.21 99.92 100.17	100.10
Accuracy 150%	3457505 3448320 3454095	2294449	12.0	11.01	100.06 99.79 99.96	99.94

Table 5. Precision data of Pantoprazole and Mosapride

Injection	Pantoprazole Area	%Assay	Mosapride Area	%Assay
Injection-1	5370525	100.40	2309945	100.27

Injection-2	5375739	100.50	230405	99.99
Injection-3	5341483	99.86	2310102	100.28
Injection-4	5344683	99.92	2307209	100.15
Injection-5	5366012	100.31	2302008	99.93
Injection-6	5374509	100.47	2307738	100.18
Average	5362159	100.24	2306735	100.13
Standard deviation	15196.6	0.2841	3355.7	0.15
%RSD	**0.3**	**0.3**	**0.1**	**0.1**

Table 6. Robustness data relating to change in flow rate

S.No		**Pantoprazole**			**Mosapride**		
	Flow rate	**Avg.Peak area**	**Std.dev**	**%RSD**	**Avg.peak area**	**Std.dev**	**%RSD**
01	1.0ml/min	673815	17490	2.6	261156	5965.2	2.3
02	1.2ml/min	5349433	7904.7	0.1	2302589	1368.1	0.1

Table 7. Robustness data relating to change in temperature

S.No		**Pantoprazole**			**Mosapride**		
	Temperature	**Avg.Peak area**	**Std.dev**	**%RSD**	**Avg.peak area**	**Std.dev**	**%RSD**
01	(-)1^0C	581433	8764.6	1.5	235446	2466.2	1.0
02	(+)1^0C	584595	810.8	0.1	235046	1430.9	0.6

Table 8. Assay data

S.No	**Sample**	**Label**	**Pantoprazole**		**Mosapride**	
			Amount found	%PurityRSD(±)	Amount found	% PurityRSD (±)
01	Pantec Plus	40mg/15mg	14.98	99.73±0.12	39.87	99.56±0.48

Table 9. System suitability parameters

Validation Parameters	**Results**	
	Pantoprazole	**Mosapride**
Linearity range (ppm)	40-240ppm	15-90ppm
Regression Equation	y = 33260x+1481.2	y = 39465x+1771.6
Correlation Coefficient (r)	0.999	0.999
Accuracy	98.02% to 99.98%	99.22% to 100.09%
Precision (%RSD)	0.3	0.1

Figure 1. HPLC Chromatogram of Pantoprazole and Mosapride in optimized chromatographic conditions

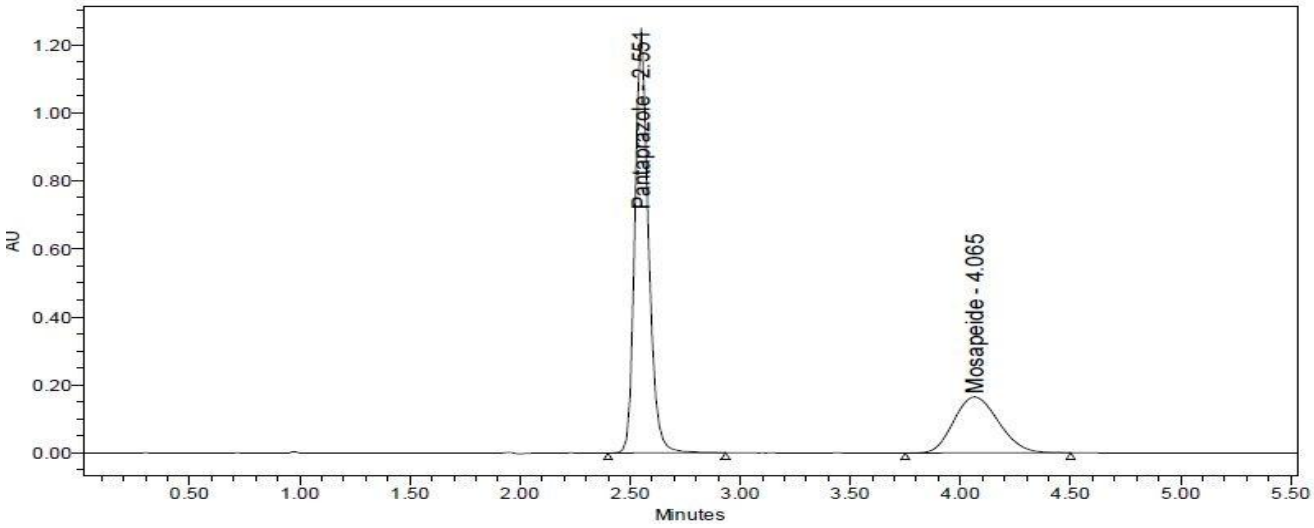


Figure 2. Linearity of Pantoprazole in the range 40-240ppm

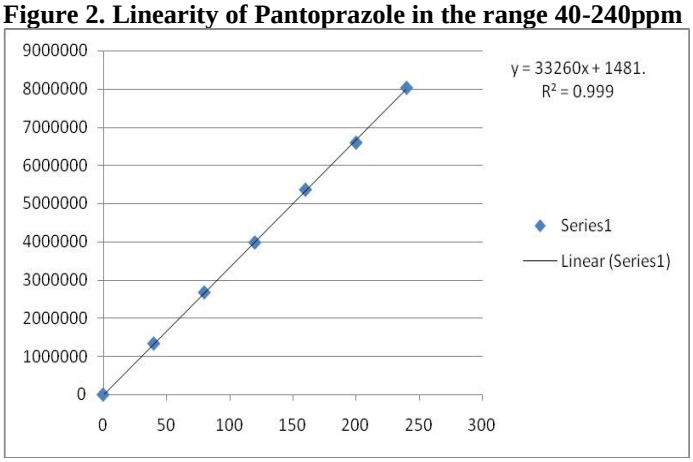
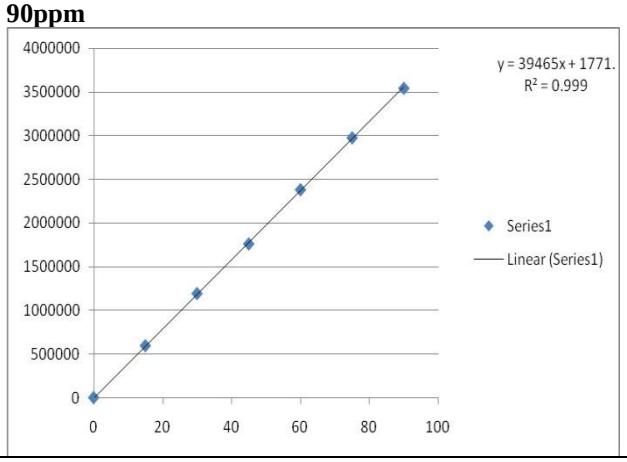


Figure 3. Linearity of Mosapride in the range 15-90ppm



RESULTS

A reverse-phase column procedure was proposed as a suitable method for the simultaneous estimation of mosapride and pantoprazole dosage form. The chromatographic conditions were optimized by changing the mobile phase composition. Different ratios were experimented to optimize the mobile phase. Finally, buffer and acetonitrile in the ratio 45:555v/v was used as mobile phase, which showed good resolution of mosapride and pantoprazole peak. The wavelength of detection selected was 260nm, as the drug showed optimized absorbance at this wavelength. By our proposed method the retention time of mosapride and pantoprazole were about 4.065mins and 2.551mins and none of the impurities were interfering in its assay.

DISCUSSION

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate.It canthereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in pharmaceutical formulation with virtually no interference of additives. Hence the proposed method can be successfully applied in simultaneous estimation of mosapride and pantoprazole in marketed formulation.

CONCLUSION

The proposed method is rapid, accurate and sensitive. It makes use of fewer amounts of solvents and change of set of conditions requires a short time. This method can be suitably analyzed for the routine analysis of mosapride and pantoprazole in bulk and its pharmaceutical dosage forms. It does not suffer from any interference due to common excipients present in pharmaceutical preparation and can be conveniently adopted for quality control analysis.

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