RESEARCH ARTICLE

Analytical method development and validation for simultaneous estimation of mosapride and pantoprazole in bulk & 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 Altima 150mm x 4.6 mm, 5µ 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.

Key words: 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 $C_{21}H_{25}ClFN_3O_3$. The molecular formula is 421 g/mol. Mosapride is a gastroprokinetic agent that acts as a selective $5HT_4$ agonist. The major active metabolite of mosapride, known as M1, additionally acts as a $5HT_3$ 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 the esophagus caused by gastroesophageal reflux disease. The chemical formula is $C_{16}H_{15}F_2N_3O_4S$. The molecular formula is 383.37 g/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 the gastric parietal cell. This effect is dose-related and leads to inhibition of 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 Moza 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 2998 with 20 µL 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 1.36gm of Potassium 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 3.0 with dil. Ortho phosphoric acid solution) and acetonitrile (55 : 45 v/v). The mobile phase was filtered through a 0.45-µm (HVLP, Germany) membrane filter prior to use. A Altima 150mm x 4.6 mm, 5µ was used for determination. The flow rate was 1.1 ml/min and the column was operated at ambient temperature (~30°C). 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

Accurately weighed and transferred 15mg of mosapride and 40mg 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, 2ml was pipetted out in to a 10ml volumetric flask and then make up to the final volume with diluent.

Sample Preparation

About

20 tablets were taken and their average weight was ca lculated. 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 2ml from the above solution into 10ml volumetric flask and filtered through 0.45µ membrane filter to get concentration of 30µg/ml and 80µg/ml.

Method Validation

The developed method was validated as per ICH guidelines [13-14] for its accuracy, linearity, precision, specificity, robustness, ruggedness, 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 w ere 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 7.5-45µg/ml & 20-120µg/ml for mosapride and pantoprazole. The data was given in table 1.

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 per the test procedure. The average % recovery was calculated. The data was given in table 2.

Precision

A) Method Repeatability

Six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 3.

B) Intermediate Precision (Day to Day variability)

Two days as per test method conducted the study. For Day-1 and Day-2, six sample solutions of the same concentration (100%) were prepared and injected into the HPLC system as per test procedure. The results were given in table 6.

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 0.292µg/ml and 0.885µg/ml respectively. The LOD and LOQ of pantoprazole were found to be 2.357µg/ml and 7.142µg/ml respectively.

Robustness and Ruggedness

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 the mobile phase composition. The results remained unaffected by small variations in these parameters as shown in table 4 and 5.

Ruggedness of the method was checked by using different days and instruments. The relative standard deviation of the results obtained from different days and instruments was <2.0%. The results were given in table 6 and 7.

Assay

The assay and % purity were calculated for brands Moza Plus(Intas Laboratories Pvt Ltd) and Pantop-M(Aristo Pharmaceuticals 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 were given in table 8.

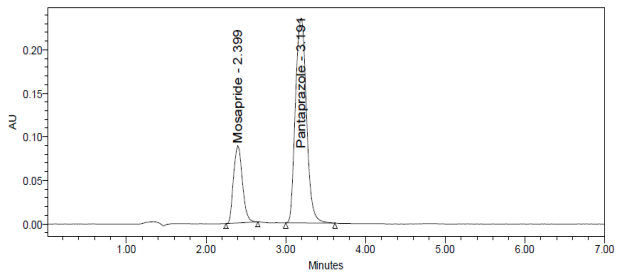

Fig 1: HPLC chromatogram of Mosapride and Pantoprazole in optimized chromatographic conditions

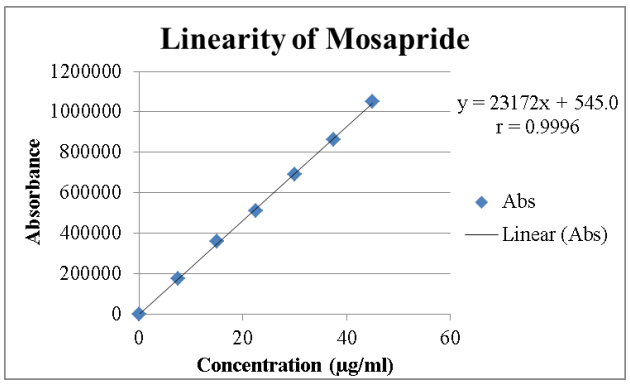

Fig 2: Linearity of Mosapride in the range 7.5 to 45µg/ml.

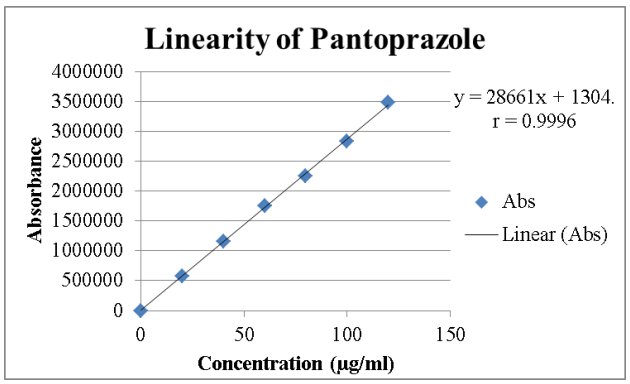

Fig 3: Linearity of Pantoprazole in the range 20 to 120µg/ml.

Table 1: Linearity data of Mosapride and Pantoprazole

S. No	Mosapride			Pantoprazole		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	7.5	2.427	175739	20	3.225	573452
2	15	2.431	358423	40	3.231	1158111
3	22.5	2.399	512205	60	3.191	1747546
4	30	2.426	690553	80	3.232	2255883
5	37.5	2.399	864055	100	3.184	2835958
6	45	2.426	1052470	120	3.225	3475785
	r = 0.9996 y = 23172x + 545			r = 0.9996 y = 28661x + 1304		

Table 2: Accuracy data

S. No		Mosapride			Pantoprazole		
	Spiked level	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD	Amount added (µg/ml)	Amount present (µg/ml)	Average %Recovery* ± %RSD
1(n=6)	50%	15.00	14.89	99.29 ± 1.05	40.00	39.89	99.74 ± 0.15
2(n=6)	100%	29.99	29.84	99.49 ± 0.24	79.96	79.77	99.77 ± 0.67
3(n=6)	150%	44.98	44.90	99.37 ± 0.76	119.94	119.72	99.82 ± 1.07

*n=6 (Average of 6 determinations)

Table 3: Precision data of Nebivolol and Valsartan

S. No	Mosapride			Pantoprazole		
	Conc(µg/ml)	Rt(mins)	Area	Conc(µg/ml)	Rt(mins)	Area
1	30	2.411	690990	80	3.212	2263191
2	30	2.399	685687	80	3.225	2298696
3	30	2.402	689721	80	3.197	2280396
4	30	2.435	693991	80	3.235	2254555
5	30	2.427	686081	80	3.193	2272845
6	30	2.344	687622	80	3.216	2305102
Mean			689015			2279131
Std.dev			3187			19781
%RSD			0.46			0.87

Table 4: Robustness data relating to change in flow rate (1.0ml/min)

S .No		Mosapride			Pantoprazole		
	Flow rate (ml/min)	Average Peak Area*	Std.dev	%RSD	Average Peak Area*	Std.dev	%RSD
1	1.0ml/min	691045	1834	0.27	2280986	15090	0.66
2	1.1ml/min	690636	973	0.14	2266273	10467	0.46
3	1.2ml/min	691959	2081	0.30	2277743	20415	0.90

*n=3 (Average of 3 determinations)

Table 5: Robustness data relating to change in mobile phase composition

S. No	Mobile phase variation (%)	Mosapride			Pantoprazole		
		Average peak area*	Std. dev	% RSD	Average peak area*	Std. dev	% RSD
1	M.P-1 (Buffer:ACN:: 56:44)	690124	2367	0.34	2291224	15770	0.69
2	M.P-2 (Buffer:ACN:: 55:45)	691658	1309	0.19	2266338	8150	0.36
3	M.P-3 (Buffer:ACN:: 54:46)	690728	2866	0.41	2286397	19729	0.86

*n=3 (Average of 3 determinations)

Table 6: Ruggedness data relating to change of day

S. No	Inter-day precision					
	Mosapride			Pantoprazole		
	Peak area			Peak area		
	Conc (µg/ml)	Day-1	Day-2	Conc (µg/ml)	Day-1	Day-2
1	30	689283	688272	80	2278484	2284747
2	30	690228	687333	80	2284747	2294847
3	30	692938	689374	80	2294847	2281163
4	30	693484	691837	80	2267484	2271726
5	30	688292	693838	80	2274847	2281711
6	30	687392	690387	80	2271821	2286483
Mean		690270	690174		2278705	2283446
SD		2475	2390		9852	7569
%RSD		0.36	0.35		0.43	0.33

Table 7: Ruggedness data relating to change of instrument

S. No	Instrument to Instrument					
	Mosapride			Pantoprazole		
		Peak area			Peak area	
	Conc (µg/ml)	Day-1	Day-2	Conc (µg/ml)	Day-1	Day-2
1	30	689737	687191	80	2264847	2285746
2	30	687828	689111	80	2285746	2294847
3	30	689227	693271	80	2294478	2284747
4	30	691273	692847	80	2271927	2271198
5	30	690282	690183	80	2271927	2294847
6	30	692847	691883	80	2281280	2289288
Mean		690199	690748		2278368	2286779
Std.dev		1729	2354		10847	8769
%RSD		0.25	0.34		0.48	0.38

Table 8: Results of analysis of laboratory samples (Assay)

S. No	Sample	Label	Mosapride		Pantoprazole	
			Amount found	%Purity ± RSD*	Amount found	%Purity ± RSD*
1	Brand-1 (MOZA PLUS)	15mg/40mg	14.98	99.73 ± 0.12	39.87	99.56 ± 0.48
2	Brand-2 (PANTOP-M)	15mg/40mg	14.96	99.63 ± 0.12	39.82	99.44 ± 0.34

*n=3 (Average of 3 determinations)

Table 9: System suitability parameters

Validation parameter	Results	
	Mosapride	Pantoprazole
Linearity range (µg/ml)	7.5 – 45	20 - 120
Regression equation	y = 23172x + 545	y = 28661x + 1304
Correlation Coefficient(r)	0.9996	0.9996
Accuracy	99.22% to 100.09%	98.02% to 99.98%
Precision (%RSD)	0.46	0.87
Robustness (%RSD)		
Flow rate: (1.0ml/min & 1.2ml/min)	NMT 0.30	NMT 0.90
Mobile phase: Buffer : ACN(56:44 & 54:46)	NMT 0.41	NMT 0.86
Ruggedness (%RSD)		
Interday – (Day 1 & Day 2)	NMT 0.43	NMT 0.35
Instrument to Instrument (Inst-1 & Inst-2)	NMT 0.48	NMT 0.38

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 55:45v/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 2.399mins and 3.191mins 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 can thereby 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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