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Research Article

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Development and validation of stability indicating RP-HPLC method for simultaneous determination of S (-) Pantoprazole and Mosapride Citrate in capsule dosage form

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ABSTRACT

S (-) Pantoprazole and Mosapride Citrate are used in combined dosage form for treating various gastrointestinal disorders, particularly for hyperacidity which is frequently associated with gastrointestinal dysmotility. Present research work was undertaken to develop and validate a novel, rapid, accurate, sensitive, precise and stability-indicating reverse-phase High-performance liquid chromatographic method (RP-HPLC) for simultaneous determination of S(-)Pantoprazole and Mosapride Citrate in capsule formulation. The chromatographic separation was achieved with the use of ACE3, C8, 100x4.6mm, 3µ analytical column at 35°C employing a gradient elution. Mobile phase consisting of Solution–A (20mM Ammonium acetate buffer of pH 5.5± 0.05) and Solution-B (Acetonitrile) used at a flow rate of 1.0mL/min with injection volume of 10µL and the detection was done at 278nm using UV detector. The retention times of S (-) Pantoprazole and Mosapride were found to be 3.5 min and 6.7 min respectively. The method was validated for System suitability criteria, specificity, accuracy, precision, linearity, filter validation and solution stability. The results obtained are well within limit as per ICH guideline. In addition, the proposed method was effectively applied for the routine analysis of QC samples.

Keywords: S(-)Pantoprazole, Mosapride, Method Development, Validation

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INTRODUCTION

S(-)Pantoprazole sodium, chemically 5-(difluoromethoxy)-2-[[(3,4-dimethoxy-2-pyridinyl)methyl]sulfinyl]-1H benzimidazole sodium salt hydrate[Fig-1] is a first generation proton pump inhibitor that decreases gastric acid secretion by irreversibly inhibiting the H+/K+-ATPase located within gastric parietal cells. S (-) Pantoprazole is used for hyperacidity and treatment of erosive esophagitis associated with Gastroesophageal reflux disease[1]. Mosapride citrate, chemically 4-Amino-5-chloro-2-ethoxy-N-[[4-[(4-fluorophenyl) methyl]-2-morpholinyl] methyl] benzamide citrate dihydrate [Fig-2] is a potent gastroprokinetic agent with selectivity for 5-HT4 receptors and is used in the treatment of gastrointestinal motility dysfunction associated with nonulcer dyspepsia[2]**.**Thus, the complementary pharmacological actions of Mosapride and S(-)Pantoprazole make their use in combined dosage form which is useful for treating various gastrointestinal disorders, in particular for hyperacidity which is frequently associated with gastrointestinal dysmotility. Combination drug products of Mosapride and S (-) Pantoprazole are widely marketed and successfully used in the treatment of gastro esophagealreflux disease and non-ulcer dyspepsia.

Each S (-) Pantoprazole and Mosapride citrate capsule contain S (-) Pantoprazole 10 mg as delayed release tablet and Mosapride citrate 5mg as immediate release tablet.

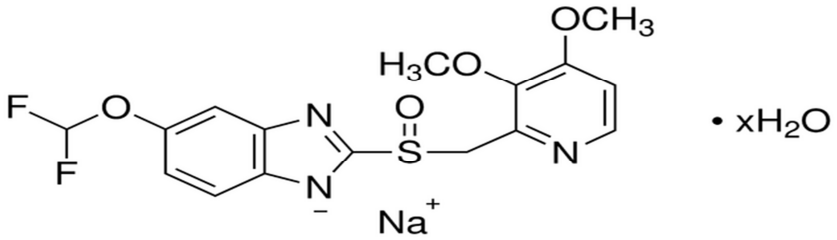

Fig. 1. Chemical structure of S (-) Pantoprazole Sodium

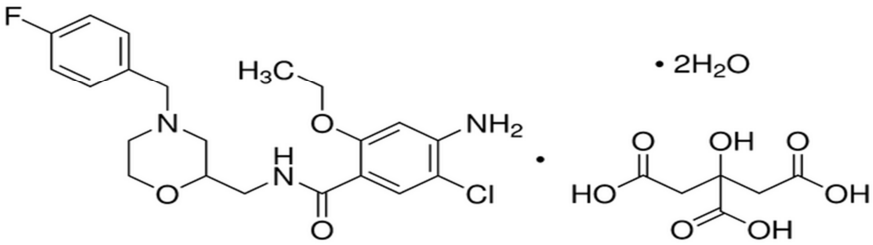

Fig.2. Chemical structure of Mosapride citrate dihydrate

From the literature review, we found that there are some spectrophotometric methods available for estimation of Pantoprazole and Mosapride in tablet formulation separately and in combination with of different drugs like Ondancetran, Cinitapride [3-6]. A few high performance liquid chromatographic analytical methods are published for determination of Pantoprazole and Mosapride in different dosage form and in combination with different drugs like Esmoprazole, Itopride[7-11].Thorough literature survey reveals that a single HPLC analysis method is available for estimation of related substances of both drugs that is S (-) Pantoprazole and Mosapride in fixed dose pharmaceutical dosage form [12]**.**No chromatographic method has been reported for estimation of S (-) Pantoprazole and Mosapride in fixed dose pharmaceutical dosage form. Therefore, the present study aimed to develop and subsequently validate a stability-indicating high-performance liquid chromatography (HPLC) method for the simultaneous determination of related substances of S (-) Pantoprazole and Mosapride in fixed dose combination dosage form. The scope of the present work isto quantify the content of S (-) Pantoprazole and Mosapride citrate in capsule formulation. Moreover, the current method has been validated for accuracy, precision, specificity, linearity, ruggedness, robustness and system suitability as per the recommendations of ICH guidelines [13-15]**.**

EXPERIMENTAL SECTION

Chemicalsand Reagents:

Working standards of S (-) Pantoprazole Sodium, Mosapride Citrate and capsule formulation (Each capsule contains10mg of S (-) Pantoprazole and 5mg of Mosapride Citrate) were provided by Emcure Pharmaceuticals Ltd Pune, India. All the solvents used were of HPLC grade. HPLC Water was generated in-house by using Merck Millipore, Milli-Q water purification system .Ammonium acetate, Glacial acetic acid, Sodium hydroxide pellets and Acetonitrile were procured from Merck, India Ltd. Hydrochloric acid and 50% Hydrogen peroxide were obtained from Rankem Ltd.

Instrumentation:

Simadzu HPLC system (Japan) was used consisting of quaternary pump, photodiode array detector, an auto injector and on- line degasser. The separation was achieved using ACE 3, C8, 100 mm x 4.6 mm, 3µm analytical column. Empower-2 software (Waters Corporation) was used for data acquisition. The analytical balance and pH meter used were manufactured by Mettler Toledo.

Chromatography Conditions:
The chromatographic separation was achieved using a analytical column as mentioned above by a step gradient programme presented in Table No-1. The mobile phase consists of two parts, Mobile phase-A (20mM Ammonium acetate buffer of pH 5.5± 0.05) and Mobile phase-B (Acetonitrile).20mM Ammonium acetate buffer was prepared by dissolving 1.54 gm of Ammonium acetate in 1000mL of mili-Q water, mixed well and pH was adjusted to 5.5±0.05 with diluted glacial acetic acid. The mobile phase was filtered through 0.45µm filter and degassed in an ultrasonication bath before use. The flow rate and column temperature was maintained at1.0mL/min and 35ºC respectively throughout the analysis. The sample cooler temperature was maintained at 8 ºC .The injection volume was kept at10µL and wavelength was optimized at 278 nm which was found suitable for detection and quantification of both S(-)Pantoprazole and Mosapride citrate. The stressed samples for forced degradation study were analyzed by using Photo Diode Array detector in the wavelength range of 200-400nm.

Table 1: Gradient Programme of Mobile Phase

Time in minutes	Flow rate (mL/minute)	% of Mobile phase A	% of Mobile phase B
0	1.0	65	35
3	1.0	60	40
6	1.0	25	75
7	1.0	65	35
10	1.0	65	35

Preparation of solutions:
Diluent used for preparation of standard and sample solution was prepared by mixing 0.02 N Sodium hydroxide and Acetonitrile with the ratio of 40:60 v/v.

Preparation of Standard Solution:
Weighed and transferred accurately about 60 mg of S (-) Pantoprazole sodium working standard and 26.5 mg of Mosapride citrate working standard into a 50 mL volumetric flask. Added about 30 mL of diluent, sonicated to dissolve and made up to the volume with diluent and mixed well.Pipetted out 4 mL from this solution in to 50 mL volumetric flask. Made up the volume with diluent and mixed well.

Preparation of Sample Solution:
Accurately weighed 20 capsules and calculated the average weight of capsules by subtracting weight of empty capsule shells from total weight of capsules. Transferred the content of 8 capsules without empty capsule shells into a 200 mL volumetric flask. Added about 180 mL of diluent and stirred on magnetic stirrer for about 15 minutes. Sonicated the solution for another 20 minutes with intermittent shaking at bath temperature not exceeding 25°C. Removed the flask from bath and kept to attain room temperature and volume made up to mark with diluent, mixed well. Filtered the solution through 0.45µ nylon syringe filter by discarding initial 3 mL of filtrate. Further diluted 5 mL of the filtrate to 25 mL with diluent and mixed well.

Forced degradation study of S (-) Pantoprazole and Mosapride citrate Capsules:
Forced degradation study was performed to determine the ability of the proposed method for determination of S (-) Pantoprazole, Mosapride Citrate in presence of degradation products that are generated during forced degradation. Sample solution was intentionally subjected to various stress conditions and chromatographed along with non treated samples (Control sample). Excipient blend was treated in the same manner as that of sample for respective stress conditions. Conditions of force degradation and results are given in Table No-2. Blank solutions (without sample) were prepared in similar manner.

RESULTS AND DISCUSSION

Optimization of the chromatographic conditions:
The main objective of this chromatographic method was to separate the peaks due to S (-) Pantoprazole and Mosapride citrate within a short run time. For optimizing the chromatographic conditions, analytical column, mobile phase compositions including pH of the buffer and flow rate were finalized and validated as per ICH guideline (Q_2R_1).

Based on the pKa values and pH dependent selectivity study of S(-)Pantoprazole and Mosapride over the range of pH 3.0 to 7.5, it was observed that pH 5.0 to 5.5 and pH 6.8 to 7.5 is the most suitable range for the chromatographic separation. Various trials were taken by using different columns and organic modifiers composition (Acetonitrile and methanol). Inertsil ODS, C18 (150mmx4.6mm, 5µ), Sunfire C18 (150mmx4.6mm, 3.5µ), Inertsil ODS 3V (150mmx4.6mm, 5µ) and ACE3, C8 100X4.6mm 3µ were tried. ACE3, C8 100X4.6mm 3µ column was found with good chromatographic separation. Effect of column temperature on chromatographic separation was studied over the range of 20°C to 45°C, It was observed that at 35°C the chromatographic separation was optimum. Thus it was kept as 35°C. S (-) Pantoprazole is highly susceptible to acid hydrolysis thus to increase the solution stability, mixture of 0.02 N sodium hydroxide and acetonitrile in the ratio of 40:60v/v was used.

Method Validation:

The analytical method validation was carried out as per ICH method validation guidelines. The parameters include System suitability criteria, specificity, accuracy, precision, linearity, filter validation and solution stability.

System suitability Test:

The system suitability test was performed by injecting 5 replicates of standard solution into the chromatograph and the chromatograms were recorded. Results obtained for Theoretical plates, USP tailing factor and %RSD of peak area were well within acceptable limits. Results obtained are summarized in Table No-3.

Specificity:

A photodiode array detector was used for analysis of stressed sample (Resulted from forced degradation) to determine the specificity of the method and to evaluate the homogeneity of the analyte peak. The peak purity obtained was found to be acceptable (purity angle < purity threshold) which confirmed that the peaks of the analytes are homogeneous and no additional peaks are co-eluting with the analytes. The results obtained from specificity test proved the ability of the method to access unequivocally the analyte of interest in the presence potential interference and its degradation products, indicating a high degree of specificity of the proposed method. The excipient blend preparations and diluent blank preparation didn't show any peak at the retention time of S (-) Pantoprazole, Mosapride citrate. Typical chromatograms of blank solution and standard solution are presented in **Fig-3 and 4.**

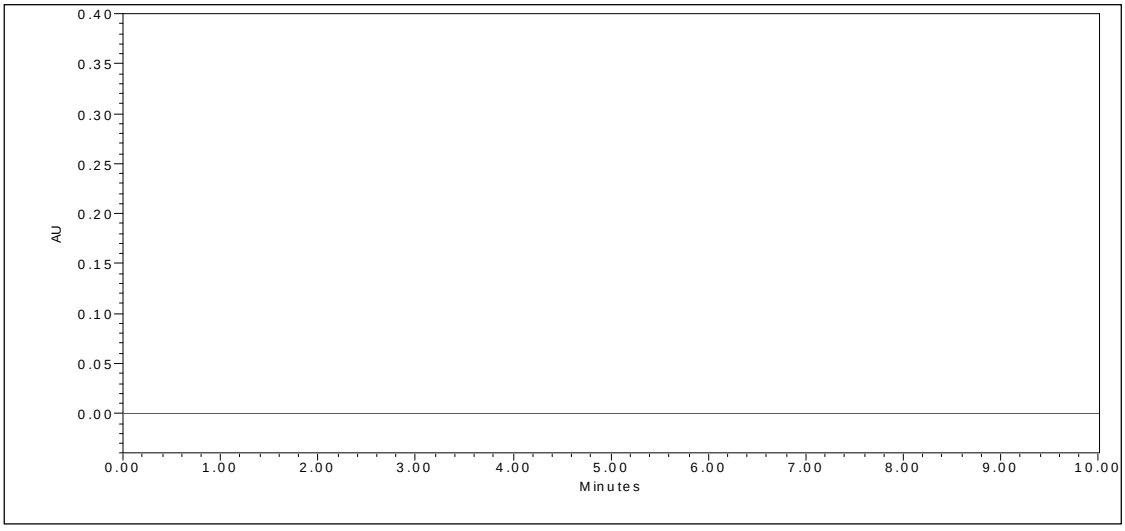

Fig. 3.Typical HPLC chromatogram of blank solution

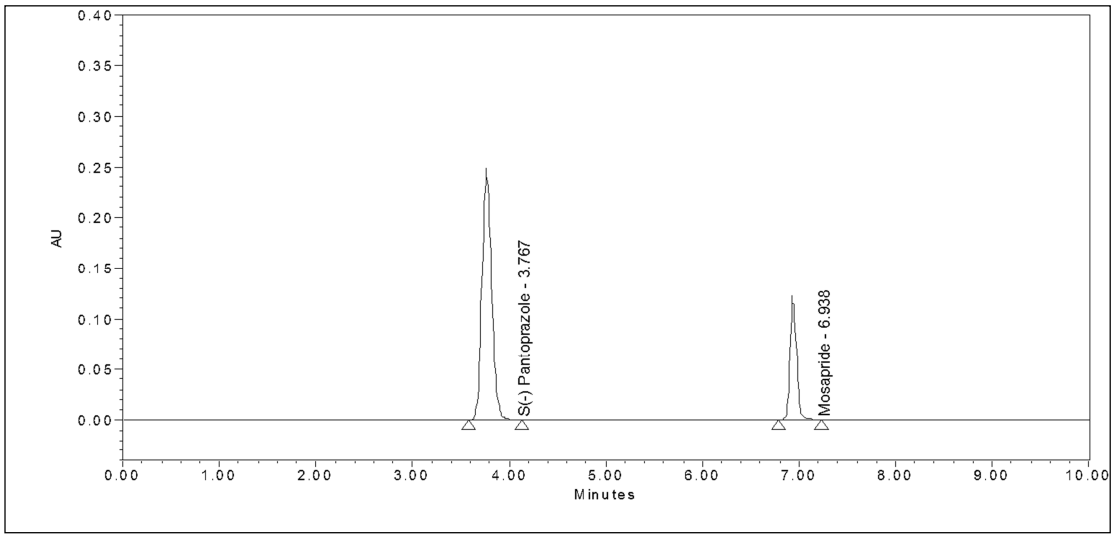

Fig. 4.Typical HPLC chromatogram of standard solution

Table 3: System Suitability Parameters

Peak name	Tailing factor		Theoretical plates		%RSD of 5 injections	
	Found	Acceptable limits	Found	Acceptable limits	Found	Acceptable limits
S(-)Pantoprazole	1.14	< 2.0	7213	>2000	0.08	< 2.0%
Mosapride	1.16	< 2.0	70616	>2000	0.10	< 2.0%

Table 2: Forced Degradation Conditions and Results

Condition	Degrading agents / Conditions	Exposure period	%Assay		%Degradation	
			S (-) Pantoprazole	Mosapride citrate	S (-) Pantoprazole	Mosapride citrate
Control Sample	Untreated	NA	99.18	100.59	NA	NA
Acid degradation	0.02 N HCL, 2 mL	For 1 hour at room temperature	98.38	100.97	0.80	0.38
Base degradation	0.02N NaOH, 2mL		98.44	101.64	0.74	1.05
Peroxide degradation	0.25 % H_2O_2, 2 mL		79.32	100.09	19.86	0.50
Thermal Degradation	60 °C, 2 Hr	60°C for 2 hours	95.12	96.53	4.06	4.06
Photolytic degradation	----	1.2 million lux hours and 200 watt hrs. / m^2	95.52	98.48	3.66	2.11

Linearity and range:

Linearity of the method was tested by preparing and injecting in triplicate a series of Standard preparations over a range of40µg/mL to 120µg/mL for S(-)Pantoprazole and over a range of 20µg/mL to 60µg/mL for Mosapride citrate. Average peak areas of each level were plotted against analyte concentration in µg/mL (ppm) and linear regression analysis was performed on the resulting plot. Results obtained are summarized in Table No-4.

Table 4: Results of Linearity (Correlation Coefficient)

Analyte	Correlation Coefficient
S(-)Pantoprazole	0.9999
Mosapride Citrate	0.9999

Accuracy:

Accuracy of the method was determined by standard addition method by spiking known concentration of S (-) Pantoprazole and Mosapride citrate in placebo solution. The % recovery of S (-) Pantoprazole and Mosapride citrate at different levels was evaluated. The samples for accuracy were prepared at 50%, 100%, and 150% level of sample

concentration spiking of API in triplicate with placebo. Results obtained for each level of recovery are summarized in Table No-5.

Table 5: Recovery of S (-) Pantoprazole and Mosapride Citrate

Spiking level	S (-) Pantoprazole		Mosapride citrate	
	%Mean Recovery	%RSD	%Mean Recovery	%RSD
Level-1(50%)	100.03	1.60	101.32	0.18
Level-2(100%)	100.27	0.45	101.13	0.45
Level-3(150%)	100.17	0.27	101.39	0.24

Method Precision:

During method precision six samples were prepared as per analytical method. The assay result of six samples was calculated and the % RSD for six samples was calculated. Results of method precision are summarized in Table No-6.

Table 6: Results of Method Precision

Parameter	Assay of S(-)Pantoprazole	Assay of Mosapride Citrate
Precision	99.35	102.02
	99.94	102.19
	99.70	101.93
	98.65	101.54
	98.57	100.41
	98.84	101.42
Mean	**99.18**	**101.59**
SD	**0.57**	**0.65**
%RSD	**0.58**	**0.64**

Robustness:

The robustness of an analytical method is the ability to remain unaffected by small changes in chromatographic parameters. For robustness study one standard solution was prepared and injected in five replicate to determine the system suitability parameters. The parameters altered for robustness study are change in flow rate (±0.1mL/minute), change in wavelength (±2 nm) and change in column oven temperature (±5°C). Results obtained for each parameter changed for robustness are summarized in Table No-7.

Table 7: Results of Robustness

Parameters	Values	Tailing Factor		USP Plates		%RSD of STD	
		PAN	MOS	PAN	MOS	PAN	MOS
Control sample	As per method	1.14	1.16	7213	70616	0.08	0.10
Flow (±0.1mL/min)	0.9mL/min	1.12	1.16	8052	73439	0.07	0.11
	1.1mL/min	1.14	1.13	6374	61246	0.05	0.05
wavelength (± 2 nm)	276nm	1.12	1.11	6661	53415	0.03	0.04
	280nm	1.12	1.11	6665	54791	0.04	0.07
Column oven temperature (±5°C)	30°C	1.14	1.16	7131	64418	0.03	0.03
	40°C	1.14	1.15	6866	70256	0.04	0.06

**PAN-: S (-) Pantoprazole, MOS-: Mosapride Citrate*

Filter validation:

For filtration study sample solution was filtered using different filters and results compared with centrifuged sample as unfiltered. Results obtained are summarized in Table No-8.

Stability of Analytical Solution:

To determine the stability of solutions, sample solution of S (-) Pantoprazole and Mosapride citrate was prepared as per method of analysis and Stored at 2°C to 8°C for a period of 24 hours. Sample and Standard preparations were analyzed initially and after 24 hours. From the results obtained solution was found to be stable up to 24 hours at Room temperature as well as at 2°C to 8°C.

Table 8: Results of Filter Validation

Sample No.	Assay of S(-)Pantoprazole	Assay of Mosapride Citrate
Unfiltered Sample	98.23	100.73
Filtered Sample -1	99.93	101.31
Filtered Sample -2	97.79	100.97
Filtered Sample -3	99.11	102.35
Filtered Sample -4	98.65	101.61
Filtered Sample -5	97.84	100.76
Absolute Difference between Unfiltered and Filtered Sample preparation		
Filtered Sample -1	1.70	0.58
Filtered Sample -2	-0.44	0.24
Filtered Sample -3	0.88	1.62
Filtered Sample -4	0.42	0.88
Filtered Sample -5	-0.39	0.03

CONCLUSION

In pharmaceutical formulations, the impurities and degradation products can change the pharmacological and toxicological potency of the active pharmaceutical ingredient which has adverse effect on quality, safety and efficacy of the drug. In this study, a rapid, precise, specific and stability indicating RP- HPLC method was developed for accurate quantification of S (-) Pantoprazole and Mosapride Citrate simultaneously. The analytical method was validated and the data reflects satisfactory results for the validation parameters according to ICH guidelines. It could be applied for the routine analysis of S (-) Pantoprazole and Mosapride Citrate in quality control laboratories, research institutions, industries, approved testing laboratories, bio-pharmaceutics, bio-equivalence studies and in clinical pharmacokinetic studies in near future.

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