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Research Article

"METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF PANTOPRAZOLE SODIUM AND ITOPRIDE HYDROCHLORIDE IN ITS BULK DOSAGE FORMS BY RP-HPLC"

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ABSTRACT

A rapid high performance liquid chromatographic method has been developed and validated for the estimation of pantoprazole sodium and Itopride hydrochloride simultaneously in combined dosage form drug was resolved on a c18 column (LENGTH 150mm*DIAMETER 4.6mm i.d, Particle size 5mm) in isocratic mode. The mobile phase used was acetonitrile and phosphate buffer in the ratio 40:60 mobile phase was delivered at the flow rate of 1ml/min. Ultraviolet detection was carried out at 207nm. Calibration curve was linear with correlation coefficient (r2) =0.999. The selected chromatographic conditions were found to separate pantoprazole sodium (rt= 3.52) and itopride Hydrochloride (rt= 2.51) having a resolution of 5.314min. The proposed method can be used for the analysis of commercially available dosage form (pantoprazole sodium and Itopride hydrochloride) in combined dosage form..

Keywords: Pantoprazole sodium, Itopride hydrochloride, High performance liquid chromatography, C18 column, RP-HPLC

INTRODUCTION

Pantoprazole sodium is chemically Sodium-[5-(Difluoromethoxy)-2-[[(3,4-dimethoxy-2pyri-dinyl)-methyl]-sulfinyl]-1H-benzimidazolidsesquihydrate **(figure1)** .Pantoprazole inhibits H^+/K^+ ATPase pump function thereby reducing gastric acid secretion.[1]. It also has a role in the eradication of H.pylori. The proton pump inhibitors are given in an inactive form. The inactive form is neutrally charged (lipophilic) and readily crosses cell membranes into intracellular compartments (like the parietal cell canaliculus) that have acidic environments. In an acid environment, the inactive drug is protonated and rearranges into its active form. The active form will covalently and irreversibly bind to the gastric proton pump, deactivating it[2].

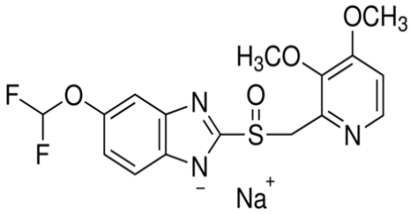

Fig. 1: Structure of pantoprazole sodium

Itopride is also known chemically as N-[[4-(2-dimethylaminoethoxy) phenyl] methyl]3,4- dimethoxy- benzamide hydrochloride **(figure2)**. Itopride increases acetylcholine concentrations by inhibiting dopamine D2 receptors and acetyl cholinesterase. Higher acetylcholine increases GI peristalsis, increases the lower esophageal sphincter pressure, stimulates gastric motility, accelerates gastric emptying, and improves gastro-duodenal coordination.

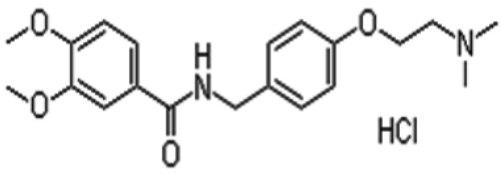

Fig. 2: structure of Itopride hydrochloride

Pantoprazole sodium and itopride hydrochloride in combination capsule dosage forms are available in capsule dosage forms in the ratio of 40:150. Literature survey reveals spectrophotometric [3], HPLC [4,5] and HPTLC [6] methods for the estimation of Itopride hydrochloride in bulk drugs, pharmaceutical formulation and biological samples whereas HPLC[7], RP-HPLC[8-10] methods for the estimation of Pantoprazole Sodium alone or in combination with other drugs in pharmaceutical formulation and biological samples. This paper describes a fast, sensitive, rapid and accurate method for developed and validated the analysis of Amlodipine in bulk dosage forms by using Reverse phase-High performance liquid chromatography (RP-HPLC). The proposed method is optimized and validated as per the International Conference on Harmonization (ICH) guidelines [11-13].

MATERIALS AND METHODS

Instrument

The HPLC system used was Shimadzu with model prominence equipped with UV detector source of deuterium lamp. The chromatogram was recorded at and peaks quantified by means of PC based Spinchrome software.

Reagents and Chemicals

Acetonitrile (AR Grade) and phosphate buffer were used as solvent. Hplc grade water and Pure Standard gift sample of pantoprazole and itopride provided by Chandra labs, Hyderabad. Capsules of pantolac caps (pantoprazole 40mg and Itopride 150mg) were purchased from local market.

Preparation of Solutions

Preparation of pantoprazole sodium and Itopride hydrochloride Standard Stock Solutions

Accurately weighed quantity of 26 mg pantoprazole and 100mg itopride was transferred to a 100mL volumetric flask, dissolved in 50 mL of mobile phase, sonicated for 15 min and the volume was made up to 100mL with mobile phase so that the concentration of stock solutions is 0.26 mg/ml of pantoprazole and 1mg/ml for itopride

Preparation of Working Standard Solutions

From the standard stock solutions prepared above the Working standards solutions of pantoprazole sodium and itopride were prepared by diluting the 1 mL, 2 mL, 3mL, 4 mL, 5 mL, and 6 mL of the stock solution in six different 100 mL volumetric flasks and adjusted to the mark with the mobile phase to the give the following concentrations. The concentrations obtained for pantoprazole sodium was: 2.6 µg \mL, 5.2 µg \mL, 7.8 µg \mL, 10.4 µg \mL, 13 µg \mL and 15.6 µg \Ml. The concentrations obtained for Itopride was: 10 µg\mL, 20 µg \mL, and 30µg \mL, 40 µg \mL,50 µg \mL and 60µg \ml

Preparation of Buffer

Accurately weighed quantity of 1.625 gm of potassium dihydrogen phosphate and 0.300 gm of dipotassium hydrogen phosphate were dissolved in 1000 mL of water.

Selection of Mobile Phase

The solution of pantoprazole sodium and itopride hydrochloride was injected into the hplc system and run in different solvent systems. Different mobile phases containing methanol, water, acetonitrile and phosphate buffer in different proportions were tried and finally acetonitrile and phosphate buffer (40:60v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable peak parameters for pantoprazole sodium and Itopride hydrochloride

Preparation of mobile phase

The mobile phase consisted of acetonitrile and phosphate buffer in the ratio of 40:60(v/v). The ph of the mobile phase was adjusted with of ortho phosphoric acid in the double distilled water. Mobile phase was filtered through a 0.45-µ m membrane filter, degassed with a helium spurge for 20 min and pumped from the respective solvent reservoir to the column (flow rate, 1 ml/min)

Selection of analytical wavelength

From the standard stock solution, further dilutions were prepared using mobile phase and scanned over the range of 200 – 400 nm and the spectrum was overlain. However the detection was carried at 207nm for pantoprazole and itopride was preferred on the basis of higher response. Hplc run at 207 nm has been found to be better with respect to resolution of the peaks and balanced area acquisition of both drugs. Hence, wavelength of 207 nm was finalized for the data acquisition in HPLC for the simultaneous estimation of both the drugs.

Analysis of capsule Formulation

The validated high performance liquid chromatography method was applied to simultaneous determination of Pantoprazole sodium and Itopride. Locally available capsule dosage form (Pantolac CAP) form pulse manufacturers contain Pantoprazole sodium 40mg and 150mg Itopride. Accurately weighed 20 capsules, average weight is taken and powdered. Amount equivalent to100mg of itopride and 26 mg of pantoprazole sodium was accurately weighed and taken in a 100 ml volumetric flask and 50 ml of mobile phase was added. The mixture was subjected to sonication for 20 min with intermediate shaking for complete extraction of drugs, Filtered through a whatmann filter paper and cooled to room temperature and solution was made up to mark with mobile phase From the above filtrate pipette out 5.0 ml into a 100 ml volumetric flask and diluted with mobile phase and 20µLof this solution was injected for HPLC analysis. The analyte peaks were identified by comparisons with those of respective standard for their retention time. The peak areas were used to calculate the drugs. The assay results, expressed as % of the label claim, are in table1 .This indicates that the amount of each drug in the product meets the requirements.

Table 1: Analysis of pantoprazole and Itopride

Drug % Assay Amount in mg	Label recovered in mg	Sample Peakarea	Standard Purity	Assay %
Pantoprazole	40mg	221.0605	99.85%	99.14%
Itopride	150mg	2860.764	99.85%	99.48%

Method Validation

Linearity

Linearity of the method was determined by mean of calibration graph using an increasing amount of each analyst. Linearity was evaluated by visual inspection of a calibration graph.The calibration curves were plotted over a concentration range of 2.6-13 mg/ml for Pantoprazole sodium and 10-60 mg/ml Itopride. Accurately measured standard stock solutions of each pantoprazole and itopride were prepared by diluting the(1 , 2 , 3, 4 , 5 , and 6) mL of the stock solution in six different 100 mL volumetric flasks and diluted up to the mark with distill water with the mobile phase to the give the following concentrations. The absorbance of solution was then measured at 207 nm. The calibration curves **(fig.4&5)** were constructed by plotting absorbance versus concentration and the regression equations were calculated.

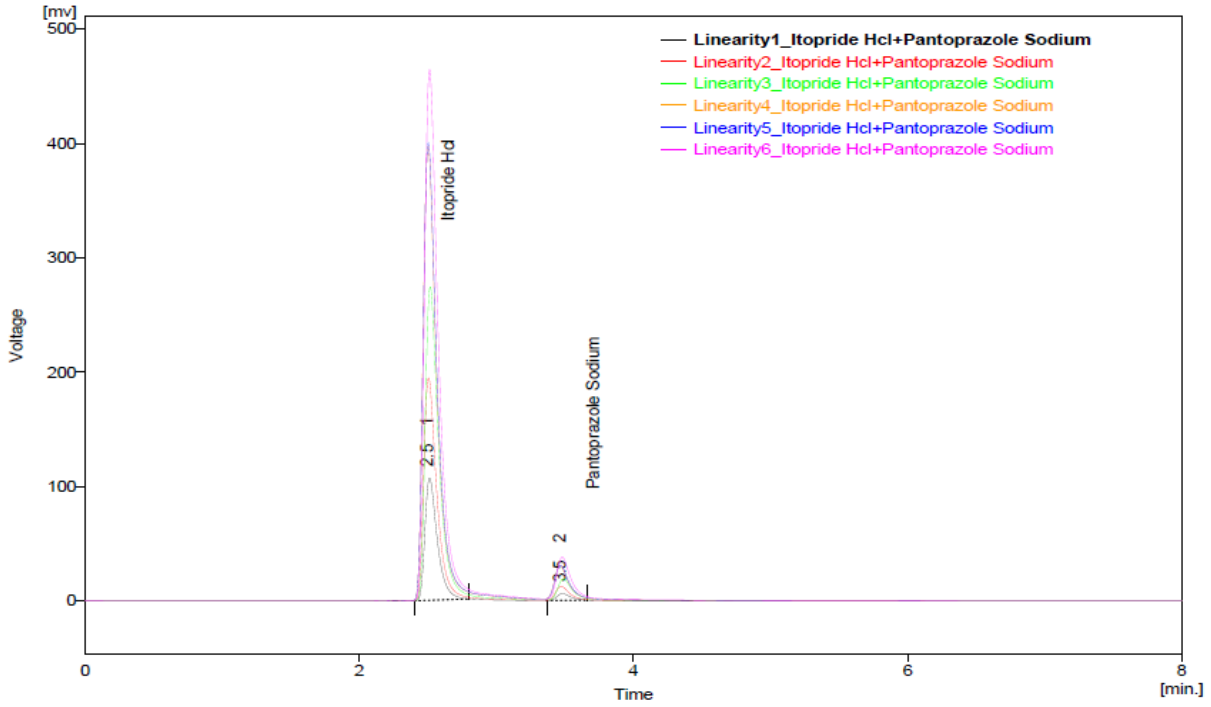

Fig. 3: Linearity chromatogram of pantoprazole and Itopride

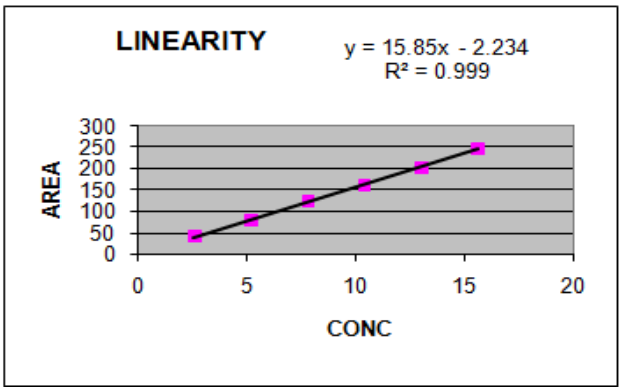

Fig. 4: Calibration curve for Pantoprazole sodium

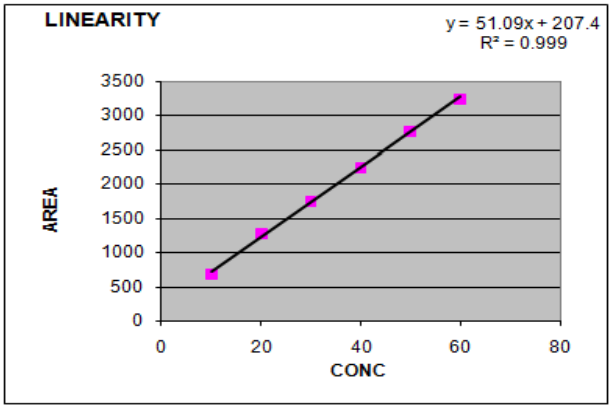

Fig. 5: Calibration curve for Itopride hydrochloride

Table 2: Linearity Range of pantoprazole and Itopride

Parameters	Pantoprazole	Itopride
Concentration µg/ml	2.6 to 13	10 to60
Correlation coefficient	0.999	0.999
Slope	15.85	51.09
Intercept	2.234	207.4

Precision

Repeatability involves analysis of replicates by the analyst using the same equipment and method and conducting the precision study over short period of time while reproducibility involves precision study at different occasions, different laboratories, and different batch of reagent, different analysts, and different equipments.

a) System Precision

The system precision was carried out to ensure that the analytical system is working properly. Injected Standard preparation five times into the HPLC. Calculated the RSD for pantoprazole sodium and Itopride Hydrochloride peaks in Standard preparation. The results obtained are tabulated as (table 3). The retention time and area of five determinations is measured and % RSD should be calculated.

Method Precision

In method precision, a homogenous sample of a single batch should be analyzed five times and was checked whether the method is giving consistent results for a single batch. The samples of pantoprazole sodium and itopride were analysed five times. The % RSD was calculated for the sample and results are tabulated as (table 4).

Table 3: System Precision of Pantoprazole sodium and Itopride

Pantoprazole sodium			Itopride	
S. No.	Rt	Area	Rt	Area
1	3.497	205.017	2.523	2730.397
2	3.473	205.703	2.51	2756.611
3	3.5	206.817	2.527	2730.272
4	3.487	205.009	2.517	2739.253
5	3.503	207.598	2.527	2773.934
Avg	3.492	206.029	2.52	2746.093
STDEV	0.01221	1.14603	0.00729	18.90241
%RSD	0.34956	0.55625	0.28935	0.688338

Table 4: Method precision of Pantoprazole Sodium and Itopride

Pantoprazole sodium			Itopride	
S. No.	**Rt**	**Area**	**Rt**	**Area**
1	3.497	205.017	2.523	2730.397
2	3.473	205.703	2.51	2756.611
3	3.5	206.817	2.527	2730.272
4	3.487	205.009	2.517	2739.253
5	3.503	207.598	2.527	2773.934
Avg	3.492	206.029	2.52	2746.093
STDEV	0.01221	1.14603	0.00729	18.90241
%RSD	0.34956	0.55625	0.28935	0.688338

Accuracy

Accuracy of the method was determined by applying the proposed method to sample capsule powder containing known amount of each drug to accuracy was then calculated as the percentage of analyze recovered by the assay. The results of the recovery analysis are shown in (Table 5)

Table 5: Accuracy and % Recovery of each analyte

Accuracy Level	Mean recovery of Pantoprazole (%)	Mean recovery of Itopride (%)
80	99.21	99.74
100	99.14	99.47
120	101.54	99.45

System Suitability

System Performance parameters of developed HPLC method were determined by injecting standard solutions. Parameters such as number of theoretical plates (N), tailing factor, resolution(R), retention time (RT) were determined. The results are shown in(Table 6); it indicates good performance.

Limit of Detection (LOD)

The limit of detection (LOD) is the smallest concentration that can be detected but not necessarily quantified as an exact value. LOD is calculated from the formula;

$$LOD = 3.3\sigma/S$$

Where, σ= standard deviation of the response, S = slope of calibration curve

Limit of Quantitation (LOQ)

The limit of quantitation is the lowest amount of analyte in the sample that can be quantitatively determined with precision and accuracy. LOQ is calculated from formula;

$$LOQ = 10\sigma/S$$

Where, σ= standard deviation of the response, S = slope of calibration curve

The values of Theoretical plates, Lod ,Loq and Resolution are shown in the table 6.

Robustness

The robustness of the method was determined to check the reliability of an analysis with respect to deliberate variations in method parameters. The typical variations are given below: Variation in flow rate by ± 0.1ml/min. Variation in wavelength by ± 2nm.The results are shown in table 7.

Ruggedness

The ruggedness of an analytical method is determined by analysis of aliquots from homogenous lots by different analysts using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The assay was performed in different condition, different analyst, and different dates. The results are given in Table 8.

Table 6: System Suitability parameters of pantoprazole and Itopride

Drug	Theoretical plates	LOD	LOQ	Resolution
Pantoprazole	5852	0.238	0.72	5.314
Itopride	3051	1.2209	3.6	

Table 7: Robustness parameters of pantoprazole and Itopride

S. No.	Parameter	Pantoprazole Sodium			Itopride hydrochloride		
		Rt	Area	Tailing Factor	Rt	Area	Tailing Factor
1	Initial sample	3.467	201.166	2.11	2.503	2779.224	1,783
2	Flow (+0.1ml/min)	3.234	205.957	1.952	2.323	2664.225	1.153
3	Flow (0.1ml/min)	3.907	237.293	1.708	2.817	3174.063	1.474
4	wave length(-2nm)	3.517	218.815	1,739	2.533	3205.603	1.100
5	Wavelength (+2nm)	3.537	211.589	2.000	2.547	2404.517	1.15

Table 8: Ruggedness of Pantoprazole sodium and Itopride

S. No.	Drug	Analyst 1 (area)	Analyst 2 (area)	SD	%RSD (Limit NMT 2.0%)
1	Pantoprazole sodium	213.602	221.828	1.77	0.88
2	Itopride	2888.207	2821.819	0.405	0.06

RESULT AND DISCUSSION

The objective of the proposed work was to develop methods for the Determination of pantoprazole and itopride and to validate the methods according to USP and ICH guidelines and applying the same for its estimation in pharmaceutical formulations. There is no official method for the estimation of above combination. The present developed HPLC method developed was found to be rapid, simple, precise, accurate and economic for routine estimation of pantoprazole and itopride in commercial dosage forms. In RP-HPLC method, HPLC conditions were optimized to obtain, an adequate separation of eluted compounds. Initially, various mobile phase

compositions were tried to elute title ingredient. Mobile phase and flow rate selection was based on peak parameters (height, capacity, theoretical plates, tailing or symmetry factor, run time, resolution). The instruments used for method development was the HPLC system shimadzu with model prominence and the software Spinchrome 21CFR, equipped with Uv detector source of deuterium lamp,C-18 (150MM*4.6MM*5µ)column and mobile phase comprising of acetonitrile

phosphate buffer(40:60).Different mobile phase was tried and mobile phase used was acetonitrile: phosphate buffer which satisfactorily gives symmetrical and well resolved peak for pantoprazole and itopride. The retention time for pantoprazole and itopride were 3.52 and 2.51 respectively flow rate kept at 1ml/min and UV detection performed at max 207 nm. The method

was validated as per ICH guidelines linearity for detector was observed in 2.6- 13µg/ml for pantoprazole and10-50µg/ml for itopride respectively percentage recovery of both drug was found in range 99.94-101.5 % indicating accuracy of proposed method the intra-day and inter day coefficient for pantoprazole and itopride were found to be 0.38-0.48%, 0.52 -0.47% and 0.04-0.43%, 0.00.78% the percentage RSD for both the capsule analysis and recovery studies is less than 2% indicating high degree of precision . The result of robustness study also indicate that the method is robust and is unaffected by small variation in chromatographic condition. It was observed that excipient present in formulation did not interfere with peaks of pantoprazole and itopride. For routine analytical purpose it is desirable to establish methods capable of analyzing huge number of samples in a short time period with good robust, accuracy, linearity and precision without any prior separation step. HPLC method generates large amounts of quality data which serve as highly powerful and convenient analytical tool.

CONCLUSION

From all results it can be conclude that the developed RP-HPLC method is simple, sensitive, accurate, precise, and selective. Percentage recovery shows that the method is free from interference of excipients used in the formulation

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