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Method Development and Analysis on High Performance Liquid Chromatograph for the Simultaneous Estimation of Pantoprazole and Itopride in Oral Dosage Form (Tablet)

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ABSTRACT: Attempts were made to develop RP-HPLC method for simultaneous estimation of Itopride HCl & Pantoprazole for the RP - HPLC method, Waters Gradient System with UV Detector and C18 column with 250mm x 4.6 mm i.d and 5µm particle size. After different trial run injections with different solvent mixture ratio and pH of the mobile phase is also alter during one trail condition, From all the traial injection separation of peaks are observed in the mobile phase of the Acetonitrile: 0.05 % OPA (60:40) ration hence this mobile phase ration and pH was used as the mobile phase for the method. The detection wavelength was 252 nm and flow rate was 0.7 ml/min. In the developed method, the retention time of Itopride HCl and Pantoprazole were found to be 4.42 min and 8.59 min. The developed method can be used for the routine quality control analysis of Itopride HCl and Pantoprazole in bulk drug as well as in formulations after complete method Validation.
Keywords: UV Detector, RP - HPLC, quality control, Pantoprazole, Itopride

1.INTRODUCTION

The term 'Chromatography' covers those processes aimed at the separation of the various species of a mixture on the basis of their distribution characteristics between a stationary and a mobile phase.

MODES OF CHROMATOGRAPHY[2]

Different Modes of chromatography are defined basically according to the nature of the interactions between the mobile phase and the stationary phase, which may starts from hydrogen bonding, Vander walls forces, electrostatic forces or hydrophillic and hydrophobic forces are based on the size of the particles (e.g. Size exclusion chromatography)

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Different modes of chromatography are as follows -

- Normal Phase Chromatography.
- Reverse Phase Chromatography.
- Reverse Phase – ion pair Chromatography.
- Ion Chromatography.
- Ion-Exchange Chromatography.
- Affinity Chromatography.
- Size Exclusion Chromatography.

In 1960's chromatographers, started modifying the polar nature of silanol group by chemically replying silica with organic silanes. The ideal was to make lower polar or non-polar so that polar detergents can be used to separate water-answerable polar composites. Since the ionic nature of the chemically modified silica is now reversed i.e. it's non-polar or the nature of the phase is reversed. The chromatographic separation carried out with similar silica is appertained to as rear- phase chromatography. A large number of chemically clicked stationary phases grounded on silica are available commercially. Silica grounded stationary phases are still most popular in retrograde phase chromatography still other absorbents grounded on polymer(styrene- divinyl benzene co polymer) are sluggishly gaining ground. Simple composites are better retained by the reversed phase face, the lower water-answerable(i.e. the more non-polar) they are.

The retention decreases in the following order aliphatic> convinced dipoles(i.e. CCl4)> endless dipoles(e.g.CHCl3)> weak Lewis bases(Ethers, Aldehydes, Ketones)> strong Lewis bases(amines)> weak Lewis acids(alcohols, phenols)> strong Lewis acids(carboxylic acids). Also the retention increases as the number of carbon tittles increases. As a general rule the retention increases with adding contact area between sample patch and stationary phase i.e. with adding number of water motes, which are released during the immersion of a emulsion, fanned chain composites are eluted more fleetly than their corresponding normal isomers. In rear phase systems the strong seductive forces between water motes arising from the 3-dimensional inter molecular hydrogen clicked network, from a structure of water that must be distorted or disintegrated when a solute is dissolved. Only advanced polar or ionic solutes can interact with the water structure.

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2. MATERIAL AND METHODS

MATERIALS

Pantoprazole was obtained from Dr. Reddy's Laboratories and Itopride HCl was obtained from D.K Phrmachem Pvt. Ltd. Span 40 ,Eudragit L- 100, Carbopol 934P ,HPMC K15M, Sodium Bicarbonate ,Magnesium Stearate and Citric Acid was obtained from Merch Laboratories and Renkem.

FOR HPLC METHOD:

The HPLC system consisted of a Waters Gradient System UV detector. Model no. 2695 The software used was Empower-3.

FORMULATION METHOD:

Pantoprazole microspheres:

Microspheres were formulated by spray drying method. Drug loaded enteric coated microspheres were prepared by dissolving Eudragit L-100 in two different Drug to polymer ratios. Feed solutions were prepared by dissolving the polymer and dispersing drug in the same solvent i.e, Ethanol with the help of Ultrasonic Homogeniser (Biologics, Inc. Model 150 V/T).

Pantoprazole-loaded microspheres were obtained by spraying the feed-solution with a spray dryer using spray nozzle. The solution was fed to the nozzle with a peristaltic pump, automized by the force of compressed air and blown together with heated air to the chamber where the solvent in the droplets was evaporated. The dried microparticles were harvested from cyclone collector pot. Batches formulated by using Factorial design method are-

Itopride Hydrochloride Sustained Release Floating Tablets:

Itopride HCl floating tablets were prepared by direct compression method. All the ingredients i.e, Itopride HCl, Carbopol 934P, HPMC K15M, Sodium Bicarbonate, Citric acid and magnesium stearate were weighed and powder mixture were prepared by dry blending for 20 minutes. 190 mg of resultant powder was manually compressed in ten station mini rotary tablet punching machine. The Batches formulated by using factorial design are-

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For HPLC method:

The HPLC system consisted of a Waters Gradient System UV detector. Model no. 2695 The software used was Empower-3.

3. EXPERIMENTAL WORK

A) Selection of stationary phase:

The column used in this method C_{18} Hypersil BDS. The configuration of the column is 4.6 x 250 mm, particle size 5 µm.

B) Selection analytical wavelength:

The sensitivity of HPLC method depends upon proper selection of detection wavelength. An ideal wavelength is one that gives good response for the drug that is to be detected. Appropriate dilution of each stock solution with mobile phase, various concentrations of Pantoprazole and Itopride HCl were prepared separately. Each solution was scanned in the spectrum mode between the range 200 to 400 nm were overlaid. The wavelength selected for the analysis was 252 nm at which both drugs showed significant absorbance. The overlain UV spectra of Pantoprazole and Itopride HCl in the mobile phase

Fig.1 UV Spectra of Itopride HCl

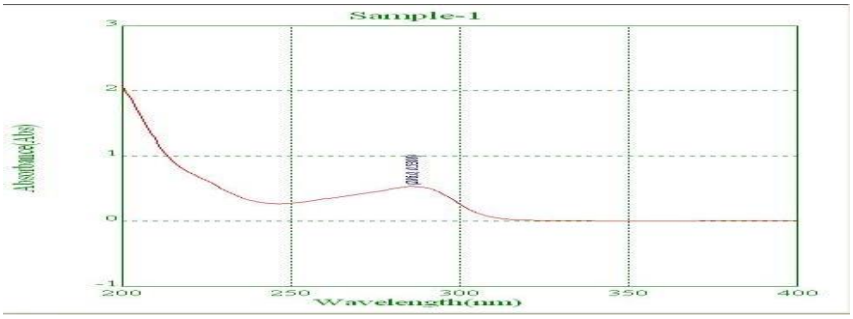

Fig.2 : Uv Spectra of Pantoprazole

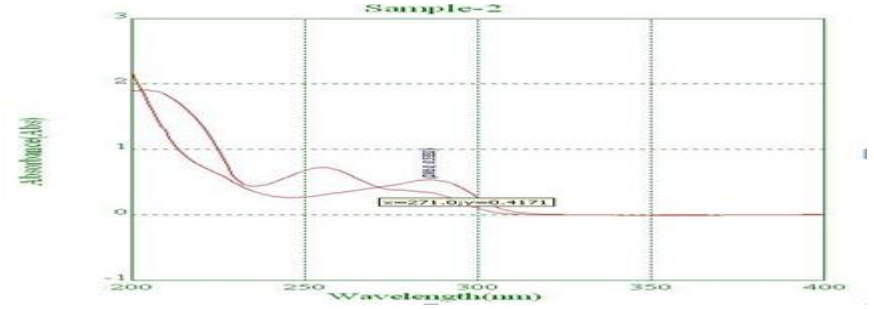

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Table 1-A: Chromatographic Condition during Different Trials Run

Fig. No.	Column used	Mobile phase, Flow Rate and Wavelength	Inj. Vol.
1	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH 3.0 (80:20) , 252nm, Flow rate 0.7ml	20 µl
2	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH 3.0 (70:30), 252nm,Flow rate 0.7ml	20 µl
3	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH 7.0 (60:40), 252 nm, Flow rate 0.7mL	20 µl
4	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH 3.0, (60:40) 252nm,Flow rate 0.7ml	20 µl
5	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Acetonitrile: OPA 0.05% in Water (60:40) 252nm, Flow rate 0.7ml	µl

4. Optimization of HPLC Method/Selection of mobile phase

The optimization of HPLC method were done for the selection of proper mobile phase for method development Pure drug products were injected and run in different solvent systems. In this, different trials are taken with different ratio of mobile phase. For trials methanol and water at different flow rate and pH were used. Different combinations of mobile phases were tried for selections of proper mobile phase are given below table.

5. Preparation of standard stock solutions

30 mg of Itopride HCl and 40 mg of Pantoprazole was weighed accurately and transferredto separate 100 ml volumetric flask, dissolved in sufficient quantity of methanol: water and diluted to 100 ml with the same solvent (Methanol**:** Water, 60:40v/v) to give a solution of 300 ppm of Itopride and 400 ppm of pantoprazole.

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Table no. 2 Observation table of Different Trials Run of Chromatographic Condition

Fig.No.	Column used	Mobile phase, Flow Rate and Wavelength	Inj.Vol.	Observation	Conclusion
1	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH3.0 (80:20) , 252nm, Flow rate 0.7ml	20 µl	Well resolved peaks were not obtained.	Hence rejected
2	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH3.0 (70:30), 252nm, Flow rate 0.7ml	20 µl	Well resolved peaks were not obtained.	Hence rejected
3	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH7.0 (60:40), 252 nm, Flow rate 0.7mL	20 µl	Well resolved peaks were not obtained.	Hence rejected
4	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Methanol : water pH3.0, (60:40) 252nm, Flow rate 0.7ml	20 µl	Sharp and Well resolved peaks were not obtained.	Hence rejected
5	Hypersil BDS C18 (250 ×4.6mm, 5.0µ)	Acetonitrile: OPA0.05% in Water (60:40) 252nm, Flow rate 0.7ml	20 µl	Sharp well resolved peaks were obtained	Hence selected

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Table No 3: Optimized parameters for HPLC method.

S No.	Parameter	Description
1	Stationary Phase	C18 column with 250 mm x 4.6 mm i.d. and 5 µm particle size
2	Mobile Phase	Acetonitrile: 0.05% OPA inwater (60:40)
3	Flow Rate	0.7 ml/min
4	Detection wavelength	252 nm
5	Detector	UV detector
6	Injector	Auto Injection
7	Injection volume	20µl
8	Column Temperature	Ambient
9	Run Time	10 min

A) Preparation of Itopride Standard Solution.

30 mg of Itopride HCl was weighed accurately and transferred to 100 ml volumetric flask, dissolved in sufficient quantity of methanol: water and diluted to 100 ml with the same solvent (Methanol: Water, 60:40v/v) to give a solution of 300 ppm of Itopride.

B) Preparation of Itopride Standard Solution.

40 mg of Pantoprazole was weighed accurately and transferred to 100 ml volumetric flask, dissolved in sufficient quantity of methanol: water and diluted to 100 ml with the same solvent (Methanol**:** Water, 60:40v/v) to give a solution of 400 ppm of pantoprazole.

C) Preparation of mix standard solutions:

30 mg of Itopride HCl and 40 mg of Pantoprazole was weighed accurately and transferred to separate 100 ml volumetric flask, dissolved in sufficient quantity of methanol: water and diluted to 100 ml with the same solvent (Methanol**:** Water, 60:40v/v) to give a solution of 300 ppm of Itopride and 400 ppm of pantoprazole.

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Fig. 20. Chromatogram of Blank

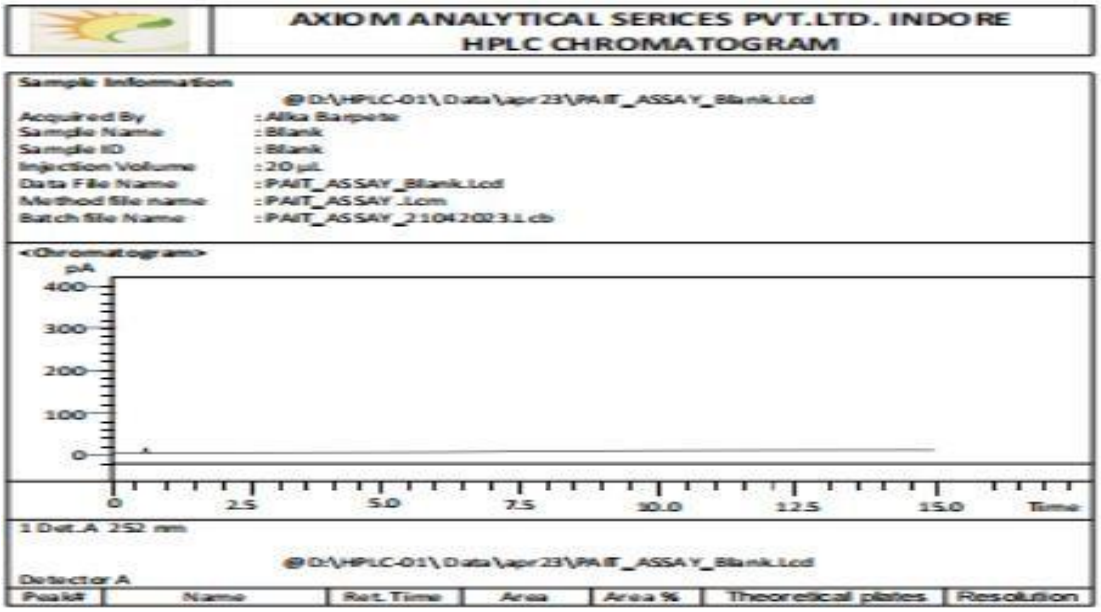
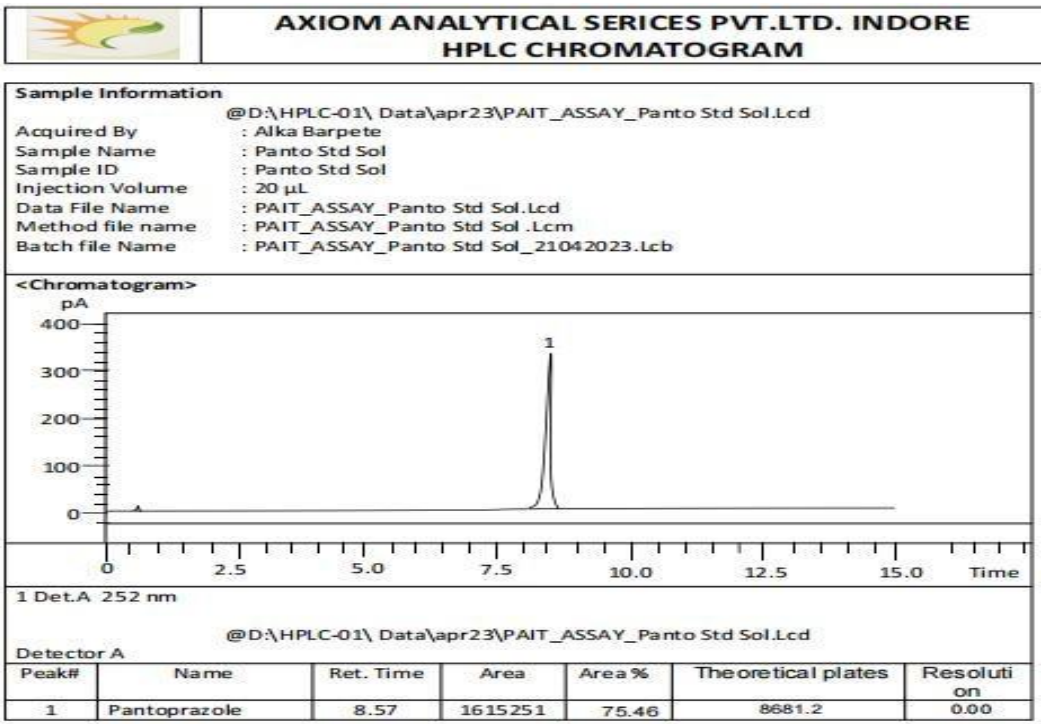


Fig .22. Chromatogram of standard Pantoprazole



Peak#	Name	Ret. Time	Area	Area %	Theoretical plates	Resolution
1	Pantoprazole	8.57	1615251	75.46	8681.2	0.00

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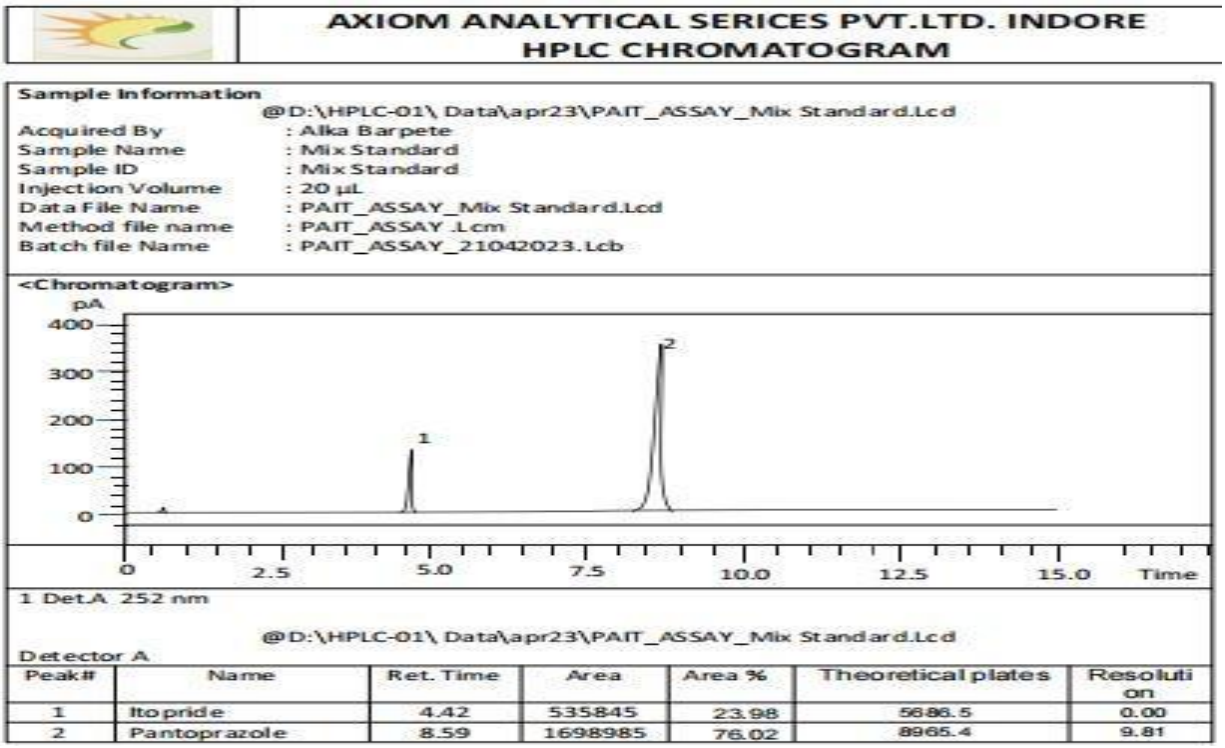
Fig. 23. Chromatogram of standard mixture of Pantoprazole and Itopride HCl



Peak#	Name	Ret. Time	Area	Area %	Theoretical plates	Resolution
1	Itopride	4.42	535845	23.98	5686.5	0.00
2	Pantoprazole	8.59	1698985	76.02	8965.4	9.81

Table No 4 Details of chromatogram of standard mixture Pantoprazole & ItoprideHCl

Sr. No	Name of drug	RT (min)	Area	T.Plates	Resolution
1	**Itopride HCl**	4.4200	535845	4038.3	0.0
2	**Pantoprazole**	8.5900	1698985	8681.2	9.81

C) Analysis of formulation:

Each capsule content: Itopride HCl: 30 mg

Pantoprazole : 40 mg

Procedure:

For analysis of the tablet dosage form, 20 tablets were weighed individually and their average weight was determined after that they were crushed to fine powders and power equivalent to 30 mg of Itopride and 40 mg of Pantoprazole was taken and transferred to100 ml volumetric

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flask and diluted with diluent (Methanol**:** Water, 60:40v/v). Sonicate, then volume was made up to mark with (Methanol**:** Water, 60:40v/v).The amounts of Itopride and Pantoprazole per tablet were calculated byextrapolating the value of area from the calibration curve. Analysis procedure was repeated two times with tablet formulation. Result is shown in (Table No.12)

Fig. 24. Chromatogram of Itopride and Pantoprazole in tablet formulation Run-1.

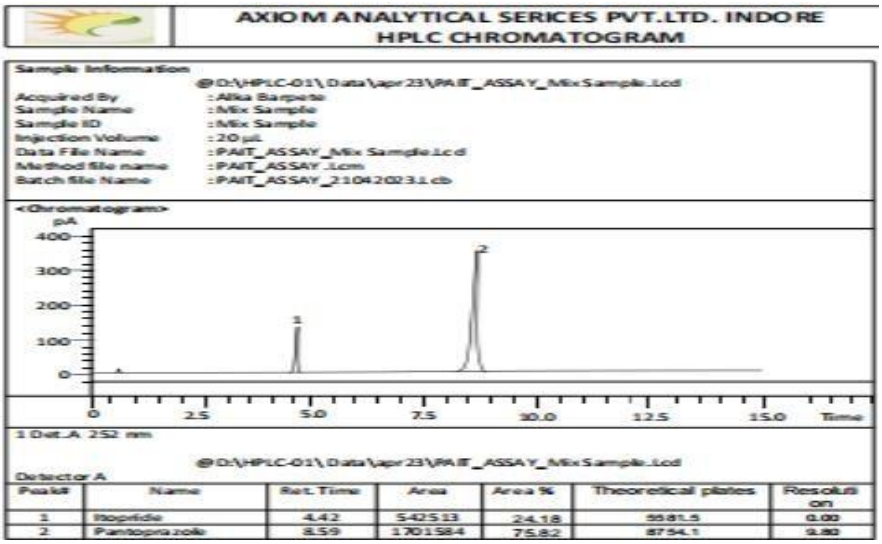

Fig. 25. Chromatogram of Itopride and Pantoprazole in tablet formulation Run-2.

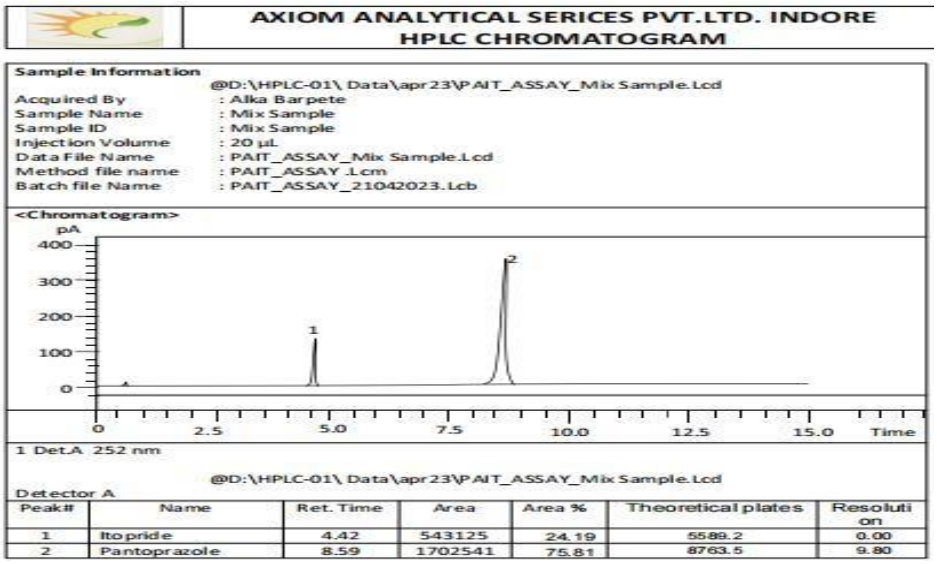

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Table 5 : Details of chromatogram of Itopirde & Pantoprazole in tablet formulation Run-1

Sr.no	Name of drug	RT (min)	Area	Theoretical Plates	Resolution
1	Itopride HCl	4.42	542513	5581.5	0.00
2	Pantoprazole	8.59	1702541	8754.1	9.80

Table 6: Details of chromatogram of Itopirde & Pantoprazole in tablet formulation Run-2

Sr.no	Name of drug	RT (min)	Area	Theoretical Plates	Resolution
1	Itopride HCl	4.42	543125	5589.2	0.00
2	Pantoprazole	8.59	1701584	8763.5	9.80

Table:10 Analysis of marketed formulation.

Sr.no	Amount present in mg		Amount found in mg		% Label claim	
	Itopride	Pantoprazole	Itopride	Pantoprazole	Itopride	Pantoprazole
1	30	80	29.8234	39.9807	99.41	99.95
2	30	80	29.8571	39.9253	99.52	99.81
Mean	_	_	_	_	99.57	99.88
SD	_	_	_	_	0.08	0.10
%RSD	_	_	_	_	0.08	0.10

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4. Summary and conclusion: Attempts were made to develop RP-HPLC method for simultaneous estimation of Itopride HCl & Pantoprazole For the RP - HPLC method, Waters Gradient System with UV Detector and C_{18} column with 250mm x 4.6 mm i.d and 5µm particle sizeThe objective of the proposed work was to develop methods for the Determination of pantoprazole and Itopride 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, 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 Waters with model 2695 and the software Empower-3 with 21CFR compliance, equipped with UV detector source of deuterium lamp model 2487,C-18 BDS (250MM*4.6MM*5µ) column and mobile phase comprising of Acetonitrile: Water (OPA 0.05%) (60:40).Different mobile phase was tried and mobile phase used was acetonitrile: Water (OPA 0.05%) which satisfactorily gives symmetrical and well resolved peak for pantoprazole and Itopride. The retention time for pantoprazole and Itopride were 8.59 and 4.42 respectively flow rate kept at 1ml/min and UV detection performed at max 252 nm. HPLC method generates large amounts of quality data which serve as highly powerful and convenient analytical tool.

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