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Method develpopment and validation of Itopride Hydrochloride and Rabeprazple Sodium in pharmaceutical dosage form by Reversed Phase High Performance Liquid Chromatography

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ABSTRACT

Itopride hydrochloride is a benzamide derivative in the class of prokinetic drugs . it has been approved for the symptomatic treatment of disorders like non-ulcer dyspepsia. chronic gastritis , diadetic gastro paresis or functional dyspepsia. the present study was designed to study the method of development in pharmaceutical dosage forms by reversed hplc . through developed validation method we can analyse the drug in reversed phase pharmaceutical dosage form by hplc. validation is a constant evolving process that starts before an instrument is placed on line and continuous long after method development and transferred. here a new hplc method for itopride hydrochloride and rabeprazole sodium was developed and validated the same. the best chromatographic seperation was performed on phenomenex c_{18} column and the uv detection wave length was set at 268nm . the mobile phase used was methanol : water : acetonitrile (50:40:10) . the retention time of itopride hydrochloride and rabeprazole sodium was found to be 2100 and 6700 . The final results of retention time was compared with the marketed product.

key words: Validation, HPLC, Phenomenex c_{18} column, Reversed phase.

INTRODUCTION

The high performance liquid chromatography is a method of separation in which the stationery phase is contained in a column , one end of which is attached to a source of pressurized liquid eluent (mobile phase).

Today the development of a method of analysis is usually based on prior art or existing literature, using the same or quite similar instrumentation. It is rare today that an HPLC – based method is developed that does not in some way relate or compare to existing, literature-based approaches .The development of any new or improved method usually tailors existing approaches and instrumentation to the current analyte, as well as to the final needs or requirements of the method. Method development usually requires selecting the method requirements and deciding on what type of instrumentation to utilize and why.

Development of method by HPLC:

Selection of solvent to be used as mobile phase:

Choosing of suitable solvent in which both the drugs are soluble and stable. The solvent must be easily available economical and of the HPLC grade**.**

Selection of mobile phase:

For the mobile phase, the first variable to be decided is whether an organic or aqueous eluent should be used. With RP-HPLC analysis, either an aqueous eluent or a very polar organic solvent such as methanol or acetonitrile should be fixed first. If the K' values are too large with an aqueous solvent, organic solvent should be tried. If the K' values are too low with the organic solvent the separation should be attempted using a mixture of two solvents in various properties.

K'- Capacity factor is a measurement of the degree where the peak of interest is located with respect to the void volume, i.e. Elution time of non retained components. Generally the value of K' if >2.

If a buffer is used,the pH as well as ionic strength of the buffer can be tried.

In order to select the wavelength for carrying out the analysis critical examination of the Ultraviolet absorption spectra of the drug should be done.

The main aim and objective of the present study was To develop simple,rapid,specific and sensitive spectroscopic methods for determination of Itopride hydrochloride and Rabeprazole sodium in combination dosage form for routine quality control analysis. No method was found for simultaneous estimation of Itopride hydrochloride and Rabeprazole sodium in combination dosage form.

EXPERIMENTAL SECTION

Materials

Table: 1

S.No	Name	Model	Manufacturer/Supplier
1	Weighing Balance	K-ROY	Shimadzu Corporation Japan
2	Sonicator	2510	Branson
3	pH Meter	7007	Digisun/Electronics
4	HPLC	LC-10AT	Shimadzu Corporation Japan
5	Oven	TIC-C95	Serwell Instruments

Table :2 Chemicals Used

S.No	Name	Grade	Manufacturer/Supplier
1	Acetonitrile	HPLC	Merck
2	Methanol	HPLC	Merk
3	Pottassium dihydrogen phosphate	G.R	Merck
4	Water	-	In house production

S.No	Name	Specification
1	Itopride Hydrochloride	As Reference Std
2	Rabeprazole Sodium	As Reference Std

Methods

Absorprtion maxima of itopride hydrochloride

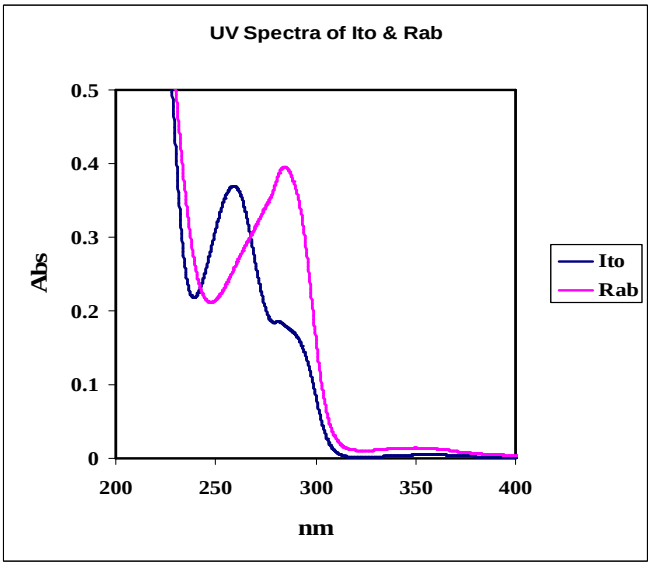
Absorption maxima

Weighed 100mg of itopride hydrochloride and transferred to 100ml of volumetric flask. Added 20ml of mobile phase (50:40:10) to dissolve and made up the volume to 100ml with mobile phase. Pipetted 1ml of the solution to of volumetric flask made up the volume with 100ml mobile phase (10 microgram /mm). Measured the absorbance of the solution at 268 nm.

Absorption maxima of Rabeprazole sodium

Absorption maxima

Weighed 100mg of Rabeprazole sodium and transferred to 100ml, volumetric flask. Added 20ml of mobile phase (50:40:10) to dissolve and made up the volume to 100ml with mobile phase. Pepetted 1ml of the solution to 100ml of the volumetric flask . Made up the volume with 100ml mobile phase (10 microgram /mm) Measured the absorption of the solution at 285 nm. Overlay spectrum for itopride Hcl and Rabeprazole Na was shown in figure 1



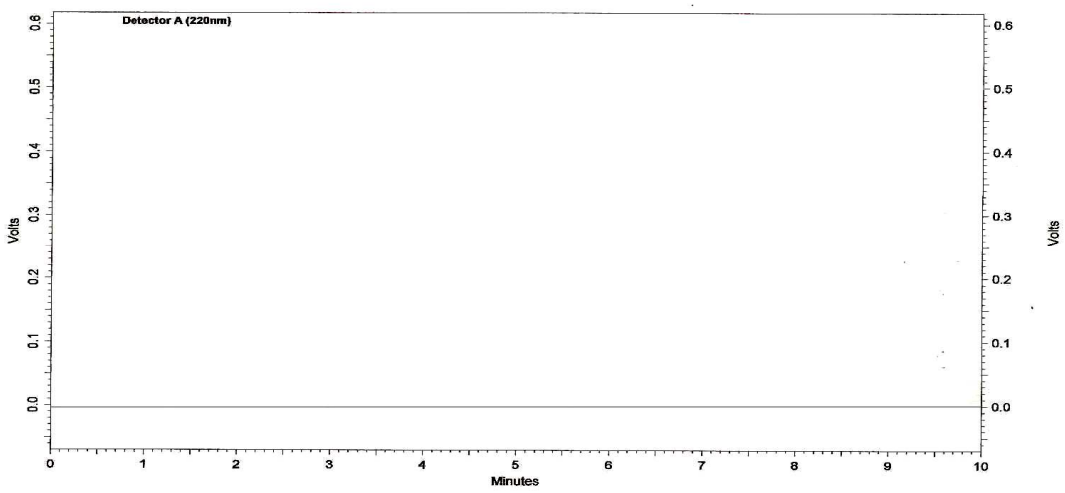
Method development for the assay of itopride hydrochloride and rabeprazole sodium by Reversed- Phase HPLC

- **Initialization of the instrument**
  The column was placed on the instrument and switch on the instrument and washed with filtered water for 30 mts. Then run the mobile phase for 30 mts for column saturation. Take the desired amount of Itopride Hydrochloride and Rabeprazole Sodium mixture and found out the result.

HPLC Graph for Mobile Phase

Take the desired amount of mobile phase in the syringe and set the chromatographic conditions as per Table 1and found out the results.

HPLC figure for mobile Phase



No peak was found in Mobile Phase

Chromatographic Condition 1

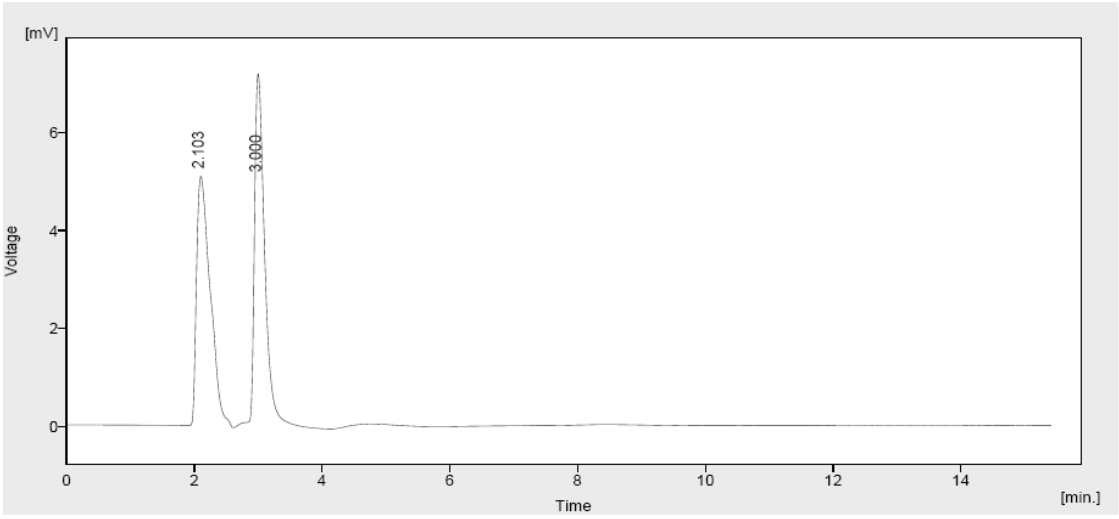
- **Preparation of Mobile Phase**
  Prepared the mobile phase ie methanol water ratio (80:20) filtered and degassed mixture of the solution *Methanol : water (80:20)* .
- **Standard Preparation of Itopride Hydrochloride**
  Weighed 44.12mg of Itopride Hydrochloride and made up to 50ml with mobile phase.
- **Standard Solution of Rabeprazole Sodium**
  Weighed 5.88 mg of Rabeprazole Sodium and made up to 50ml with mobile phase
- **Standard Solution of Itopride Hydrochloride and Rabeprazole Sodium Mixture**

Taken 25ml of Itopride Hydrochloride from the above solution and 25ml of Rabeprazole Sodium and made up to 100ml with mobile phase.

Table:3

Parameters	Description
Column Name	**Phenomenex Luna(C18(2), 250*4.6mm, 5µ**
Flow Rate	**1.0 ml/ min**
Mobile Phase	**Methanol:Water (80:20)**
Injection Volume	**20µl**
Detector	**268nm**
Run Time	**15 Mts**

Filled the standard solution of Itopride Hydrochloride and Rabeprazole Sodium in the syringe, set the chromatographic condition as per Table1, saved the chromatographic condition and made the sequence to run the standard solution of Itopride Hydrochloride and Rabeprazole Sodium for 15mts.



In this mobile phase two peaks were obtained with in 3 mins RT_{ito} 2.1 and RT_{rab} 3.00 the peaks shape for Itopride was not good also the resolution was poor.

Chromatographic Condition 2
Here the mobile phase was changed.

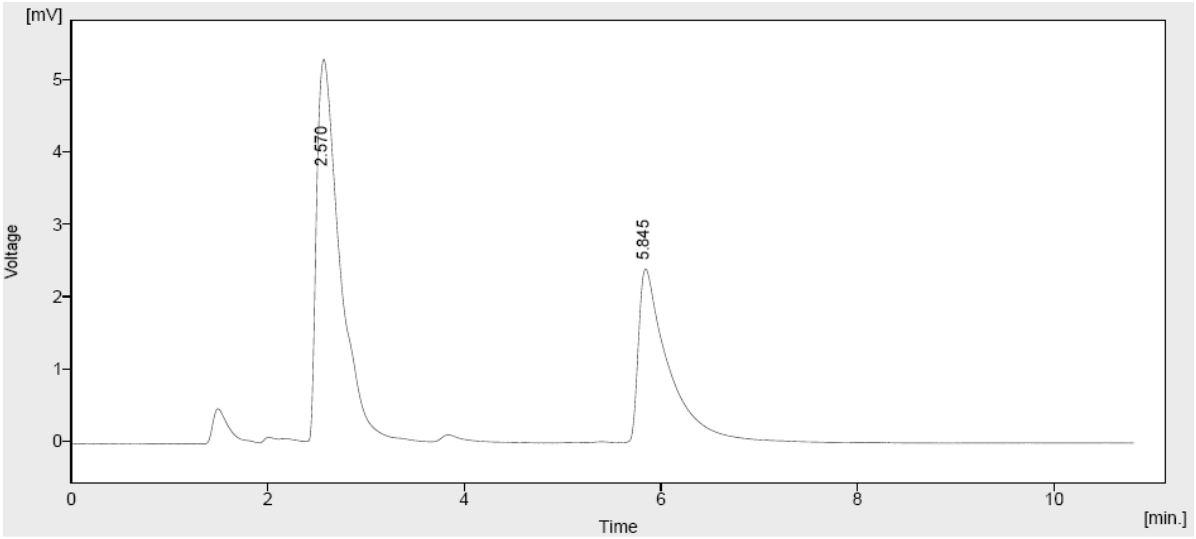
Preparation of mobile Phase
Prepared the mobile phase ie methanol water ratio (50:50) filtered and degassed mixture of the solution.

- **Standard Preparation of Itopride Hydrochloride**
  Weighed 20mg of Itopride Hydrochloride and made up to 100ml with mobile phase.
- **Standard Solution of Rabeprazole Sodium**
  Weighed 20 mg of Rabeprazole Sodium and dissolved in mobile phase and made up to 100ml with mobile phase
- **Standard solution of Itopride Hydrochloride and Rabeprazole Sodium Mixture**

Taken 50ml of Itopride Hydrochloride from the above solution and 25ml of Rabeprazole Sodium and made up to 100ml with mobile phase.

Parameters	Description
Column Name	**Phenomenex Luna (C18(2), 250*4.6mm) 5µ**
Flow Rate	**1.0ml/min**
Mobile Phase	**Methanol:water (50:50)**
Injection Volume	**20µl**
Detector	**268nm**
Run Time	**12 Mts**

Filled the standard solution of Itopride Hydrochloride and Rabeprazole Sodium in the syringe,set the chromatographic condition as per Table2. Saved the chromatographic conditions and run the standard solution of Itopride Hydrochloride and Rabeprazole Sodium for 10mts.



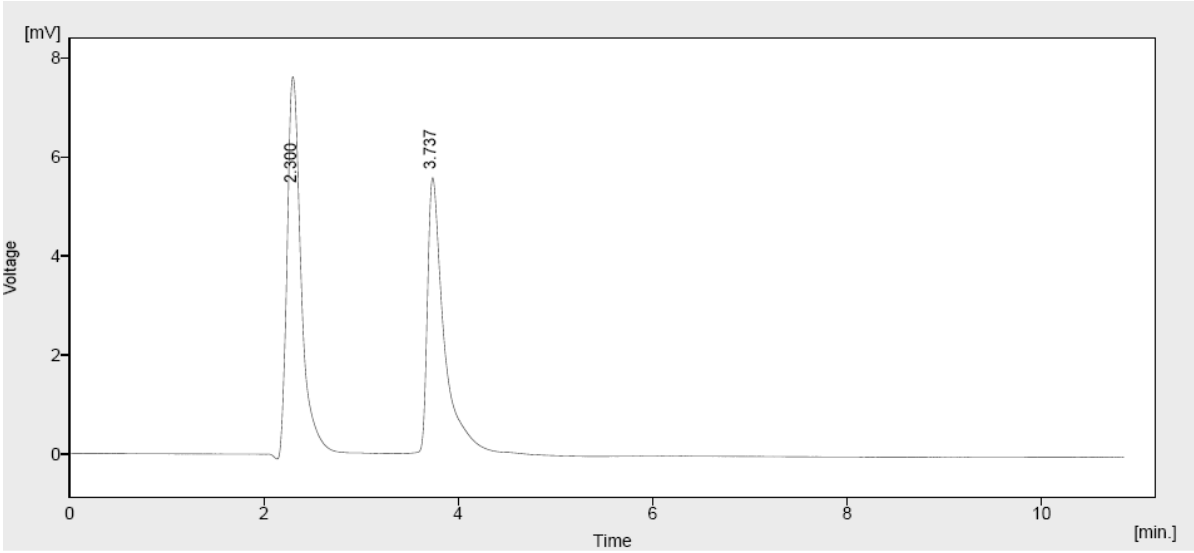
In this mobile phase two peaks were obtained with in 6 mins RT_{ito} 2.5 and RT_{rab} 5.8 though the resolution was good the peaks shape for Itopride and Rab were not good because of tailing effect.

Chromatographic Condition 3

The chromatographic conditions were changed with respect to detector. Prepared the standard solution of Itopride Hydrochloride and Rabeprazole Sodium as per the above steps.

Parameters	Description
Column Name	**Phenomenex Luna (C18(2),250*4.6mm) 5µ**
Flow Rate	**1.0ml/min**
Mobile Phase	**Methanol :water (60:40)**
Injection Volume	**20µl**
Detector	**268nm**
Run Time	**10 Mts**

Filled the standard solution of Itopride Hydrochloride and Rabeprazole Sodium in the syringe and set the chromatographic conditions as per Table 3.Run the standard solutions of Itopride Hydrochloride and Rabeprazole Sodium for 10 mts.



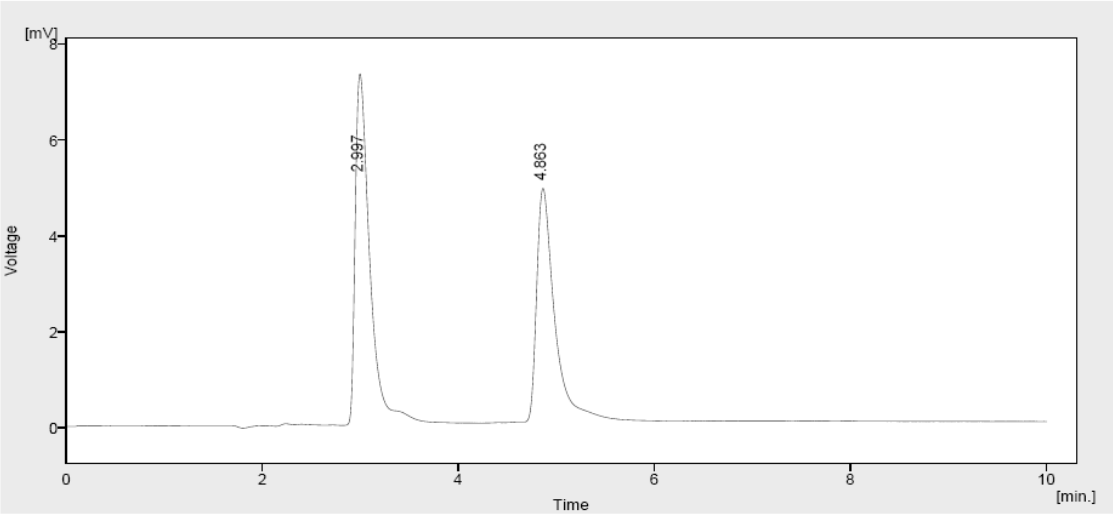
In this mobile phase two peaks were obtained with in 4 mins RT_{ito} 2.3 and RT_{rab} 3.7 though the resolution was improved the peaks shape for Itopride and Rabeprazole were not good because of tailing effect.

Chromatographic Condition 4

Here the mobile phase ratio was changed.. Run the standard solution as per the above steps.

Parameters	Description
Column Name	**Phenomenex Luna(C18(2),250*4.6mm) 5µ**
Flow Rate	**1.0ml**
Mobile Phase	**Methanol :water (55:45)**
Injection Volume	**20µl**
Detector	**268nm**
Run Time	**10 Mts**

Filled the standard solution of Itopride Hydrochloride and Rabeprazole Sodium in the syringe and set the chromatographic conditions as per Table 4.Run the standard solutions of Itopride Hydrochloride and Rabeprazole Sodium for 10 mts.



In this mobile phase two peaks were obtained with in 5 mins RT_{ito} 2.9 and RT_{rab} 4.8 though the resolution was improved the peaks shape for Itopride and Rab were not

Developed Method

Mobile Phase Preparation

Prepared a filtered and degassed mixture of the solution methanol : water acetonitrile (50:40:10)

Table no: 10 Chromatographic Conditions for the Developed method

Parameters	Description
Column Name	**Phenomenex Luna (C18(2),250*4.6mm) 5µ**
Flow Rate	**1.5 ml**
Mobile Phase	**Methanol :water:ACN (50:40:10)**
Injection Volume	**20µl**
Detector	**268nm**
Run Time	**10 Min**

Standard Preparation of Itopride Hydrochloride

Weighed 20mg of Itopride Hydrochloride and made up to 100ml with mobile phase.

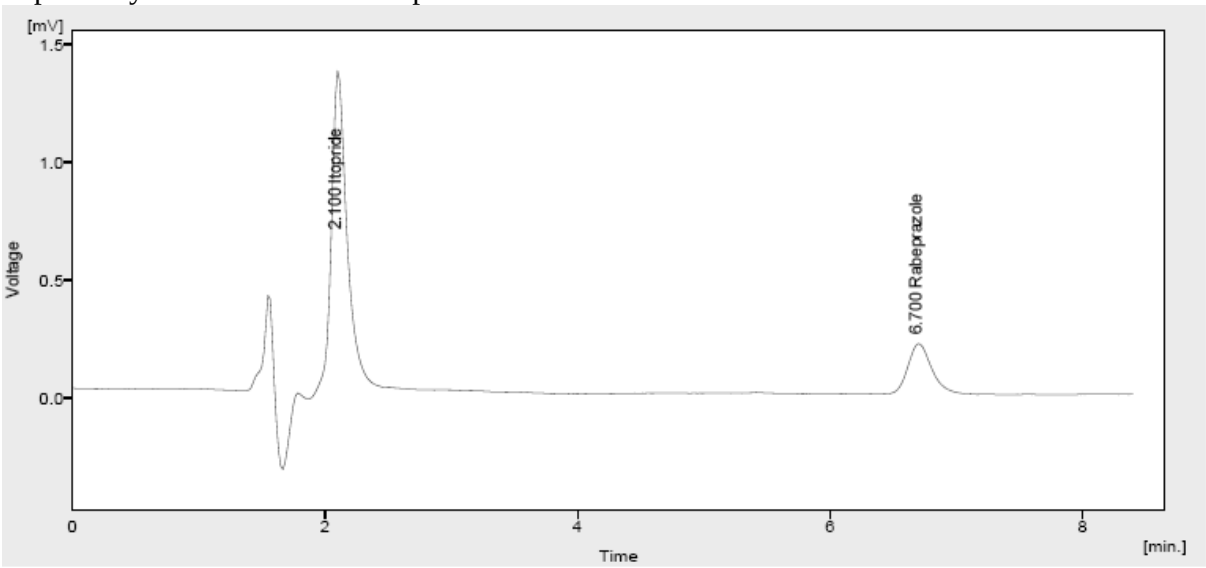
Standard Solution of Rabeprazole Sodium

Weighed 20 mg of Rabeprazone Sodium in mobile phase and made up to 100ml with mobile phase

Standard solution Itopride Hydrochloride and Rabeprazole Sodium Mixture

Taken 50ml of Itopride Hydrochloride from the above solution and 25ml of Rabeprazole Sodium and made up to 100ml with mobile phase.

Filled the standard solutions of Itopride Hydrochloride and Rabeprazole Sodium in the syringe and set the chromatographic conditions as per the above table and run the standard solutions of Itopride Hydrochloride and Rabeprazole Sodium for 10mts.



Result Table (ESTD - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\CHROMATOGRAM\CONDITIONS\SAMPLE 100UG MET-WAT-ACN 29-1-09-CAL)

	Compound Name	Reten. Time [min]	Area [mV.s]	Area [%]
1	Itopride	2.100	11.422	82.0
2	Rabeprazole	6.700	2.515	18.0
		Total	13.938	100.0

Validation of developed method

Developed method was validated according to ICH guidelines.

Method validation of Itopride Hydrochloride and Rabeprazole Sodium Injection

Prepare the mobile phase and arrange the chromatographic conditions as per the above developed method.

Validation Parameters

9.1. Precision

- Reproducibility

Five solutions of the test were prepared as per the developed method filled the standard solutions of Itopride Hydrochloride and Rabeprazole Sodium in the syringe and set the chromatographic conditions as per Table I f.Results are shown in the Table 12.

- Intermediate Precision(Ruggedness)

Intermediate precision study was carried out by preparing the six replicate in the above concentration. It was carried out by two different analysts on two different dates as per the following matrics.

Preparation of Itopride Hydrochloride and Rabeprazole Sodium

- **Standard Preparation of Itopride Hydrochloride**

Weighed 20mg of Itopride Hydrochloride made up to 100ml with mobile phase.

- **Standard Solution of Rabeprazole Sodium**
  Weighed 20 mg of Rabeprazole Sodium and dissolved in mobile phase and made up to 100ml with mobile phase

Standard solution of Itopride Hydrochloride and Rabeprazole Sodium Mixture
Taken 50ml of Itopride Hydrochloride from the above solution and 25ml of Rabeprazone Sodium and made up to 100ml with mobile phase.

Filled the standard solutions of Itopride Hydrochloride and Rabeprazole Sodium in the syringe and set the chromatographic conditions as per the table. If the results are shown in the table.

Day-1	**Analyst-1**
Day-2	**Analyst-2**

9.2. Accuracy
The accuracy of the test method was carried out by preparing the samples at a level of 60%, 80% and 100% of target concentration. The samples were prepared in triplicate in each level.

Preparation of solution of Itopride Hydrochloride and Rabeprazole Sodium for accuracy (100%)
Weighed 44.12mg of Itopride Hydrochloride and 5.88 mg of Rabeprazole Sodium in a standard flask and made up the volume to 50 ml with mobile phase. Filtered the solution and sonicated it

Preparation of solution of Itopride Hydrochloride and Rabeprazole Sodium for accuracy (80%)
Taken 40ml from the above solution and made up to 50 ml with mobile phase in a volumetric flask. Then filtered the solution and sonicated it.

Preparation of Itopride Hydrochloride and Rabeprazole Sodium for accuracy (60%)
Taken 20 ml of from the 100% solution and made up to 50 ml with mobile phase in a volumetric flask. Then filtered the solution and sonicated it.

Results are shown in Table 11 (11.2, 11.3, 11.4, 11.5, 11.6, 11.7).
The chromatograms obtained are shown in figure 4 (a,b,c)

Validation of method for assay of tablets
9.3. Linearity and Range
Solutions of the concentration levels of about 100ppm, 80ppm, 60ppm, 40ppm,20ppm of Itopride Hydrochloride and Rabeprazole Sodium was prepared.

Preparation of standard solution of Itopride Hydrochloride and Rabeprazole Sodium for Linearity (100ppm)
Weighed 44.12g of Itopride Hydrochloride and 5.88g of Rabeprozole in a 50ml standard flask and made up the volume with mobile phase to 50 ml, sonicated the solution.

Preparation of standard solution of Itopride Hydrochloride and Rabeprazole Sodium for Linearity (80ppm)
Taken 4ml from the above solution and made up to 50ml with mobile phase and sonicated it.

Preparation of standard solution of Itopride Hydrochloride and Rabeprazole Sodium for Linearity (60ppm)
Taken 3 ml from the 1000ppm solution and made up to 50ml with mobile phase in a standard flask and sonicated it.

Preparation of standard solution of Itopride Hydrochloride and Rabeprazole Sodium for Linearity (40ppm)
Taken 2ml from the 100ppm solution and made up to 50ml with mobile phase in a standard flask and sonicated it.

Preparation of standard solution of Itopride Hydrochloride and Rabeprazole Sodium for Linearity (20ppm)
Taken 2.5 ml from 40ppm solution and made up to 50ml with mobile phase in a standard flask and sonicated it.

Run the solutions as described above.
Results are shown in table 10.1.
Linearity Graphs are shown in figure 2 (a,b,c).

Range : 20ppm-100ppm

9.4. Specificity.
Preparation of solution of Itopride Hydrochloride for specificity
Weighed 44.12mg of **Itopride Hydrochloride** in a volumetric flask and made up the solution to 50 ml with mobile phase.

Preparation of solution of Rabeprazole Sodium for specificity
Weighed 5.88mg of Rabeprazone Sodium in a volumetric flask and made up the solution to 50 ml with mobile phase.

Preparation of solution of Itopride Hydrochloride and Rabeprazole Sodium for specificity
Taken 40ml from the Itopride Hydrochloride standard solution and added 10 ml from the Rabeprazole Sodium standard solution and made up to 100ml with mobile phase in a standard flask. Filtered the standard solutions of **Itopride Hydrochloride and Rabeprazole Sodium** and taken in the syringe .Set the chromatographic conditions as per table I f.Run the standard solutions of **Itopride Hydrochloride and Rabeprazole Sodium** for 10 mts.

The chromatograms obtained are shown in figure 3 (a,b,c)

9.5. Robustness
The following variations were used to validate the methods for robustness.

Parameters	Changed Value	
Flow Rate	**1.5ml**	**1.3ml**
Detector	**268nm**	**285nm**

- **Standard Preparation of Itopride Hydrochloride**
  Weighed 44.12mg of Itopride Hydrochloride and made up to 100ml with mobile phase.
- **Standard Solution of Rabeprazole Sodium**
  Weighed 5.88 mg of Rabeprazole Sodium and dissolved in mobile phase and made up to 100ml with mobile phase
- **Standard solution Itopride Hydrochloride and Rabeprazole Sodium Mixture**
  Taken 50ml of Itopride Hydrochloride from the above solution and 25ml of Rabeprazone Sodium and made up to 100ml with mobile phase.
  Run the chromatographic conditions as per the above table

RESULTS

10.1. Linearity Data

S.No	Concentration	Area of itopride hydrochloride	Area of Rabeprazole Sodium
1	20ppm	22572	4224
2	40ppm	41903	8841
3	60ppm	62060	14217
4	80ppm	92959	21047
5	100pm	11422	25152

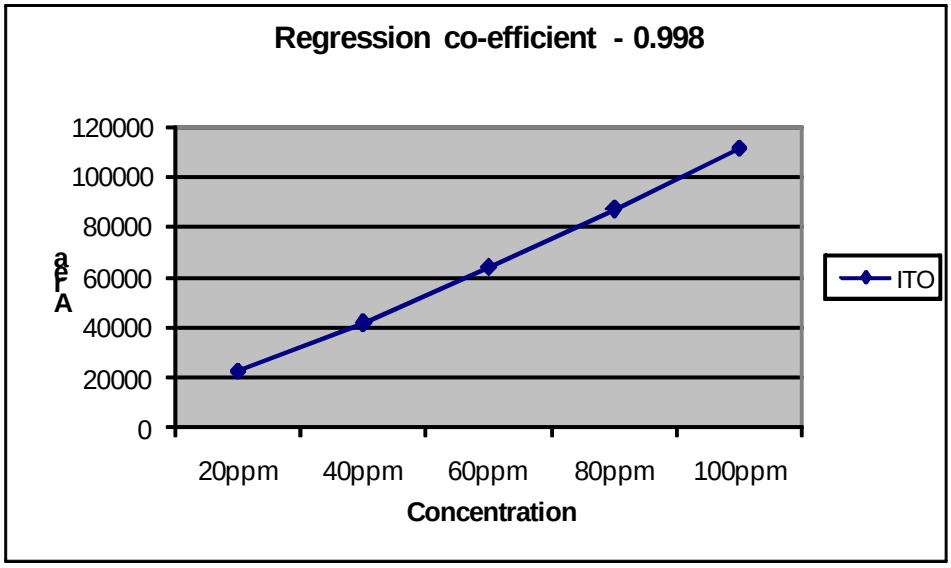
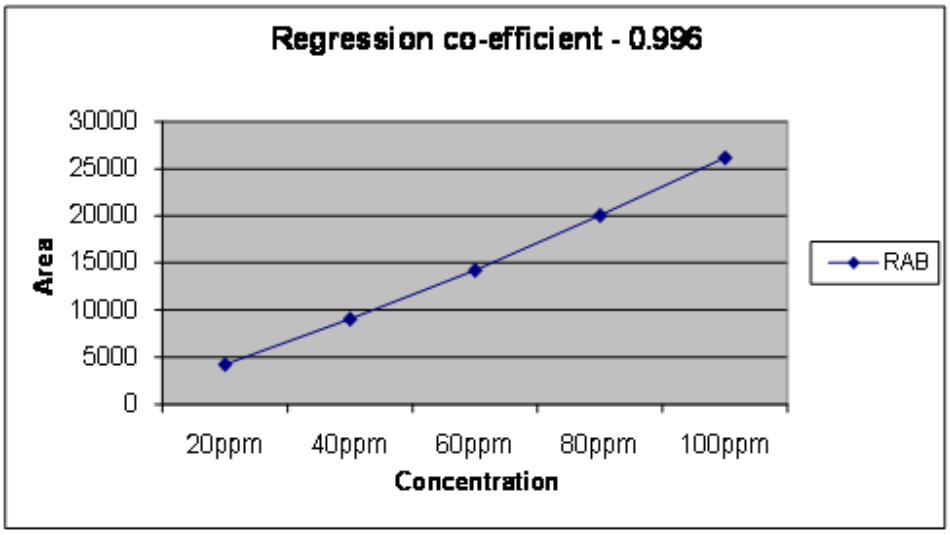


Figure: 4 Calibration curve between the area and concentration of Itopride Hydrochloride.

The regression coefficient was found to be 0.998.



The regression coefficient was found to be 0.996.

Figure:5 Calibration curve between the area and concentration of Rabeprazole Sodium.

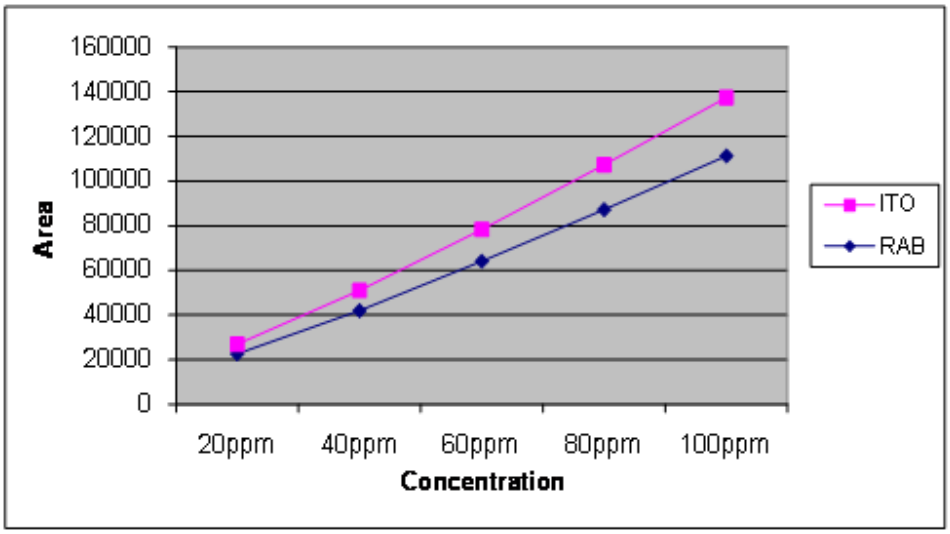
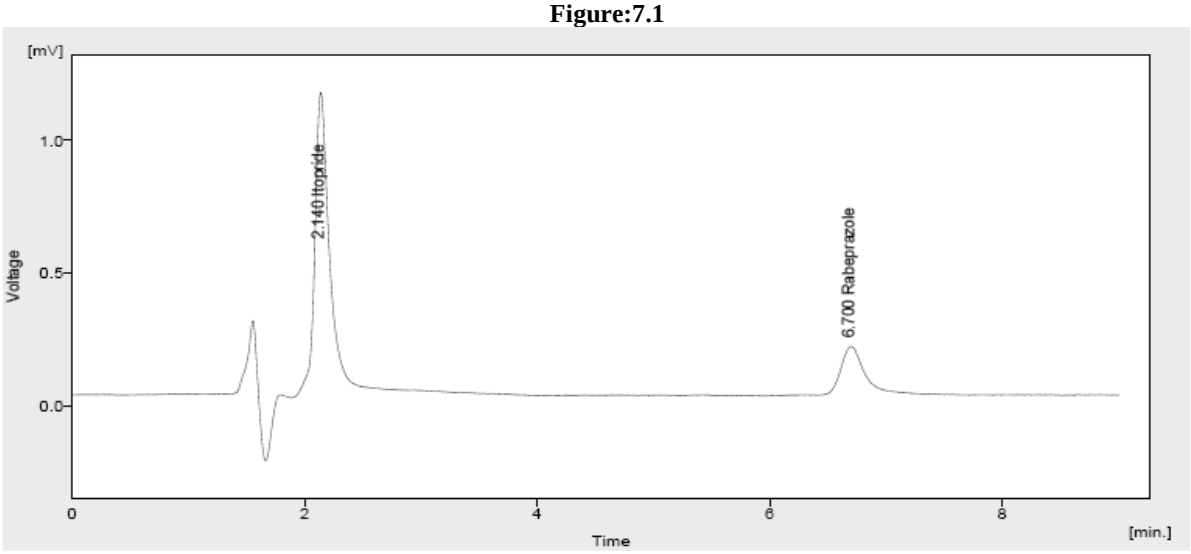


Figure:6 Calibration curve of Itopride hydrochloride and Rabeprazole Sodium

10.2. Specificity

Specificity of itopride hydrochloride and Rabeprazole Sodium

Figure:7.1



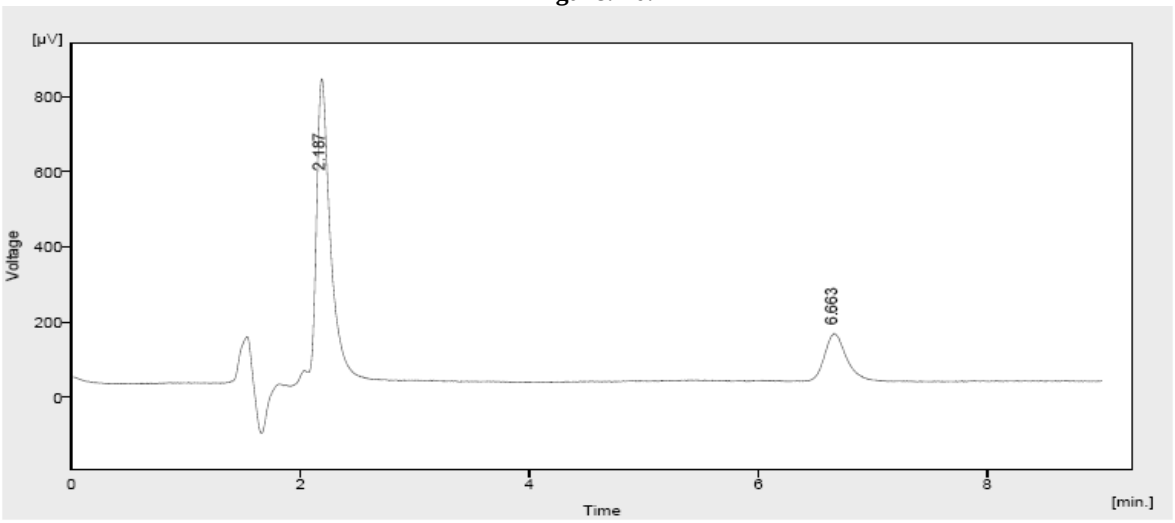
Result Table (ESTD - sample 80ug met-wat-acn 29-1-09-sst)

	Compound Name	Reten. Time [min]	Area [mV.s]	Area [%]
1	Itopride	2.140	8.982	81.0
2	Rabeprazole	6.700	2.105	19.0
		Total	11.086	100.0

10.3. Accuracy

Recovery for Itopride Hydrochloride and Rabeprazole Sodium at 60% level.

Figure: 10.1



Result Table (Uncal - sample 60ug met-wat-acn 29-1-09)

	Reten. Time [min]	Area [mV.s]	Area [%]
1	2.187	6.206	81.4
2	6.663	1.422	18.6
	Total	7.628	100.0

11.1. Accuracy

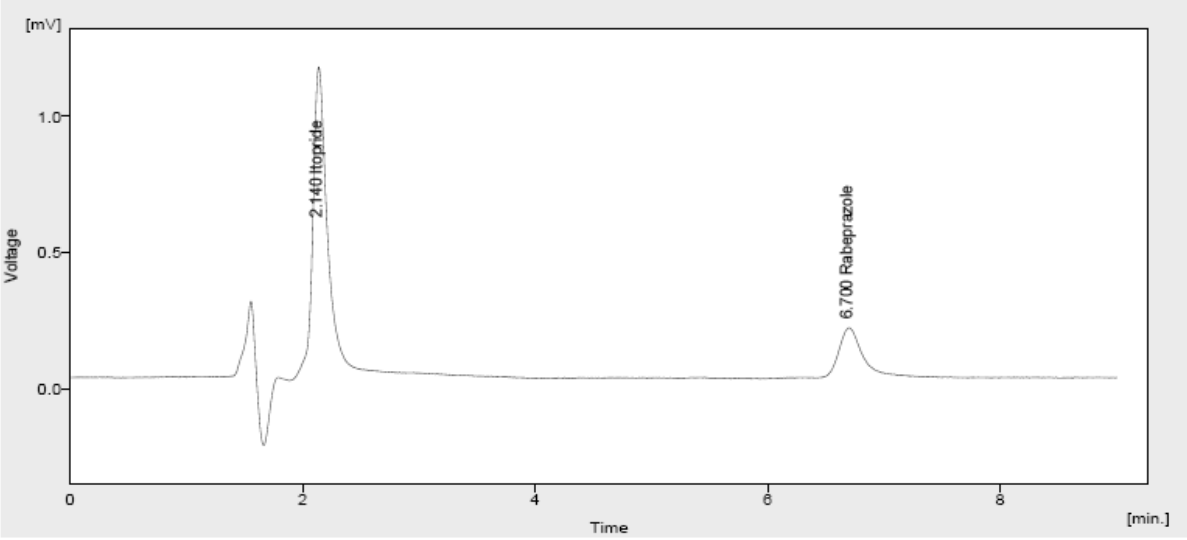
Table :11.2 Accuracy 60% for Rabeprazole sodium

Accuracy 60%				
Drug name	**S.No**	**Area of Rabeprazole**	**Total amount**	**% Recovery**
Rabeprazole	**1**	**2844**	**32.096**	**100.8**
	2	**2864**	**32.15**	**101.08**
	3	**2859**	**31.94**	**99.5**
Average				**100.46**
S.D				**± 0.808**
R.S.D				**± 0.803**

Table :11.3 Accuracy 60% for Itopride hydrochloride

Accuracy 60%				
Drug name	**S.No**	**Area of Itopride**	**Total amount**	**% Recovery**
Itopride	**1**	**124122**	**232.9**	**92.12**
	2	**124114**	**233.24**	**92.48**
	3	**124130**	**232.594**	**91.77**
Average				**92.1234**
S.D				**± 0.77**
R.S.D				**± 0.83**

*Here the S.D of the % amount of **Itopride Hydrochloride** was 0.77 and for **Rabeprazole Sodium** was 0.808%.The R.S.D of **Itopride Hydrochloride** was 0.83 and for **Rabeprazole Sodium** was 0.803.*



Result Table (ESTD - sample 80ug met-wat-acn 29-1-09-sst)

	Compound Name	Reten. Time [min]	Area [mV.s]	Area [%]
1	Itopride	2.140	8.982	81.0
2	Rabeprazole	6.700	2.105	19.0
		Total	11.086	100.0

Figure:9.2 Recovery for Itopride Hydrochloride and Rabeprazole Sodium at 80% Level

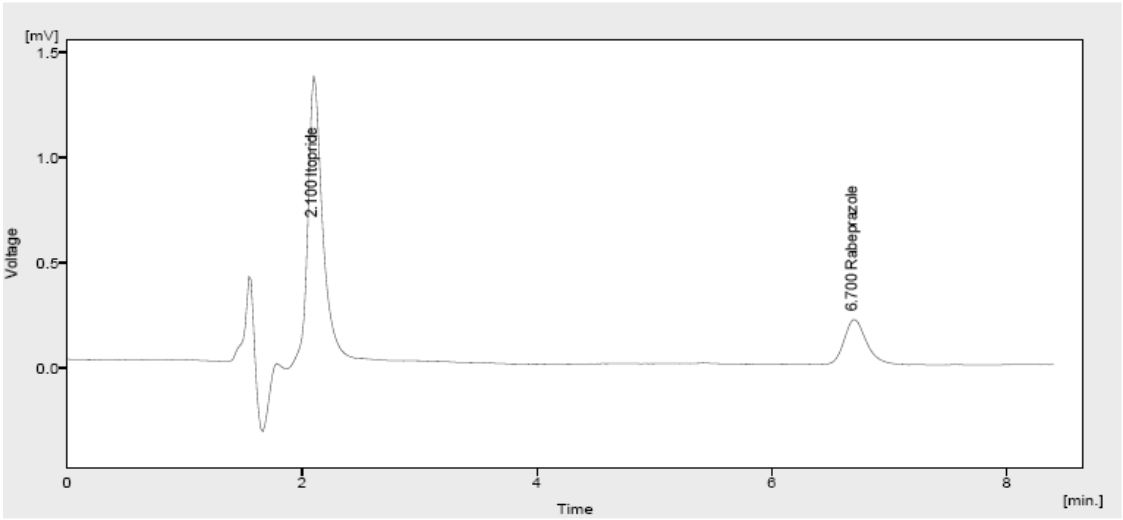
Table :11.4 Accuracy for 80% level Rabeprazole sodium

Accuracy 80%				
Drug name	**S.No**	**Area of Rabeprazole**	**Total amount**	**% Recovery**
Rabeprazole	1	4246	36.584	100.50
	2	4260	36.435	102.72
	3	4254	36.325	102.03
Average				**101.75**
S.D				**± 0.755**
R.S.D				**± 0.74**

Table no:11.5 Accuracy for 80% level in itopride hydrochloride

Accuracy 80%				
Drug name	**S.No**	**Area of Itopride**	**Total amount**	**% Recovery**
Itopride	1	18598	270.13	100.10
	2	18588	270.13	100.19
	3	18566	268.468	98.74
Average				**99.68**
S.D				**± 0.728**
R.S.D				**± 0.730**

Here the S.D of % amount of itopride hydrochloride was 0.728 and % amount of Rabeprazole sodium was 0.755 R.S.D of % amount of itopride hydrochloride was 0.730 and Rabeprazole sodium was 0.74.



Result Table (ESTD - C:\DOCUMENTS AND SETTINGS\ADMIN\DESKTOP\CHROMATOGRAM\STD CURVE\SAMPLE 100UG MET-WAT-ACN 29-1-09-CAL)

	Compound Name	Reten. Time [min]	Area [mV.s]	Area [%]
1	Itopride	2.100	11.422	82.0
2	Rabeprazole	6.700	2.515	18.0
		Total	13.938	100.0

Figure: 9.3 Recovery for itopride hydrochloride and Rabeprazole Sodium at 100% Level

Table no: 11.6 Accuracy for 100% level Rabeprazole sodium

Accuracy 100%				
Drug name	**S.No**	**Area of Rabeprazole**	**Total amount**	**% Recovery**
Rabeprazole	1	5030	40.09	100.45
	2	5040	39.99	99.95
	3	5034	40.05	100.25
Average				**100.2**
S.D				**± 0.18**
R.S.D				**± 0.18**

Table no:11.7 Accuracy for 100% level Itopride hydrochloride

Accuracy 100%				
Drug name	**S.No**	**Area of Itopride**	**Total amount**	**% Recovery**
Itopride	1	22844	301.48	100.98
	2	22820	301.7	101.14
	3	22840	301.46	100.97
Average				**100.7**
S.D				**± 0.369**
R.S.D				**± 0.367**

Here the S.D of % amount of Itopride hydrochloride 0.30 and % amount of Rabeprazole sodium was 0.62 .The R.S.D of the % amount of Itopride hydrochloride was 0.30 and % amount of Rabeprazole sodium was 0.67.

The calculated average % recovery ±SD at different concentrations were found to be 100.46, ± 0.808,101.75 ± 0.755, 100.2 ± 0.18 for Rabeprazole sodium and 92.1234 ± 0.77, 99.68 ± 0.728,100.7 ± 0.369 for Itopride hydrochloride which shows that this method has a good recovery. The RSD for Itopride hydrochloride and Rabeprazole sodium was found to be < 2 for different concentrations. According to ICH guidelines the method has good accuracy if RSD is <2

RESULTS AND DISCUSSION

Table no:16

RESULTS WITH ACCEPTANCE CRITERIA				
S. No	**Parameter**	**Acceptance Criteria**	**Results Obtained**	
1	**Specificity**	**Should not interfere with impurities**	**No interference was found**	
2	**Linearity and Range**	**Corelation co-efficient not less than 0.97**	**Itopride hydrochloride**	**Rabeprazole Sodium**
			0.998 200ppm-1000ppm	**0.996**

3	**Precision**	**R.S.D not more than 2%**	**Itopride hydrochloride**	**Rabeprazole Sodium**	
			0.6	**0.5**	
4	**Accuracy**	**R.S.D for % recovery at each accuracy level not more than 2%**	**Accuracy**	**Itopride hydrochloride**	**Rabeprazole Sodium**
			60%	**0.83**	**0.803**
			80%	**0.730**	**0.74**
			100%	**0.367**	**0.18**
		Recovery of Drug	**Avg% Recovery**	**Itopride hydrochloride**	**Rabeprazole Sodium**
			60%	**92.1234**	**100.46**
			80%	**99.68**	**101.75**
			100%	**100.7**	**100.2**
5	**Ruggedness**	**RSD not more than 2%**	**Itopride hydrochloride**	**Rabeprazole Sodium**	
			0.077	**0.506**	

CONCLUSION

Validation is a constant evolving process that starts before an instrument is placed online and continuous long after method development and transfer.

Here a new HPLC method for itopride hydrochloride and rabeprazole sodium was developed and validated the same. In this different chromatographic conditions were used to develop the method. The best chromatographic separation was performed on phenomenex C_{18} column and the UV detection wavelength was set at 285nm. The mobile phase used was methanol : water : acetonitrile (50 : 40 : 10). The retention time of itopride hydrochloride and rabeprazole sodium was found to be 2.100 and 6.700.

The developed method for itopride hydrochloride and rabeprazole sodium was validated as per ICH guidelines and USP. The results obtained were within the parameters prescribed in the ICH and USP guidelines. Hence this method can be used for the routine analysis of both the drugs simultaneously.

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