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Research

A Method Development for *Rabeprazole Sodium* And *Itopride Hydrochloride* Simultaneously By Hplc

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Abstract

The retention time of RP, PP, and IP were 5.35, 7.92, and 11.16 minutes, respectively. Validation of the proposed method was carried out according to International Conference on Harmonisation (ICH) guidelines.. The calculated limit of detection (LOD) values were 1, 0.3, and 1 µg/mL and limit of quantitation (LOQ) values were 2.5, 1, and 3 µg/mL for RP, PP, and IP correspondingly. Thus, the current study showed that the developed reverse-phase liquid chromatography method is sensitive and selective for the estimation of RP, PP, and IP in combined dosage form.

Keywords: Itopride, Rabeprazole, RP-HPLC

INTRODUCTION

Solvent delivery system

The mobile phase is pumped under pressure from one or several reservoirs and flows through the column at a constant rate. With micro particulate packing, there is a high- pressure drop across a chromatography column. Eluting power of the mobile phase is determined by its overall polarity, the polarity of the stationary phase and the nature of the sample components. For normal phase separations, eluting power increases with increasing polarity of the solvent but for reversed phase separations, eluting power decreases with increasing solvent polarity. Optimum separating conditions can be achieved by making use of mixture of two solvents. Some other properties of the solvents, which need to be considered for a successful separation, are boiling point, viscosity, detector compatibility, flammability and toxicity. [Sharma.B.K].

The most important component of solvent delivery system in HPLC is the pump, because its performance

directly effects the retention time, reproducibility and detector sensitivity. Among the several solvent delivery systems, (direct gas pressure, pneumatic intensifier, reciprocating etc.) reciprocating pump with twin or triple pistons is widely used, as this system gives less baseline noise, good flow rate reproducibility etc.

Liquid chromatographic detectors

Optical detectors are most frequently used. These detectors pass a beam of light through the flowing column effluent as it passes through a low volume (~ 10 ml) flow cell. The most commonly used detector in LC is the ultraviolet absorption detector. A variable wavelength detector of this type, capable of monitoring from 190 to 460-600 nm, will be found suitable for the detection of the majority samples. The function of the detector in HPLC is to monitor the mobile phase as it emerges from the column. Generally, there are two types of HPLC detectors, bulk property detectors and solute property detectors.

HPLC - method development

Guidelines

Analytical method development and validation plays an important role in the discovery, development and manufacture of pharmaceuticals. The official test methods that result from these processes are used by quality control laboratories to ensure the identity, purity and performance of drug products.

Basic criteria for new method development of drug analysis:

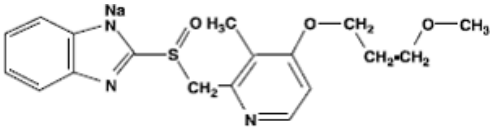
- The drug or drug combination may not be official in any pharmacopoeias.
- A proper analytical procedure for the drug may not be available in the literature due to patent regulations.
- Analytical methods may not be available for the drug in the form of a formulation due to the interference caused by the formulation excipients.
- Analytical methods for the quantitation of the drug in biological fluids may not be available.
- Analytical methods for a drug in combination with other drugs may not be available.
- The existing analytical procedures may require expensive reagents and solvents. It may also involve cumbersome extraction and separation procedures and these may not be reliable.

A good method development strategy should require only as many experimental runs as are necessary to achieve the desired final result. Finally method development should be as simple as possible, and it should allow the use of sophisticated tools such as computer modeling.

Drug profile

Rabeprazole sodium

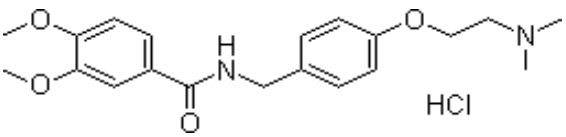
Structure



1. **Chemical name:** 2-[[[4-(3methoxypropoxy)-3-methyl-2-pyridinyl]-methyl]sulfinyl]-1H– benzimidazole sodium salt.
2. **Molecular formula: $C_{18}H_{20}N_3NaO_3S$**
3. **Molecular weight: 381.43.**
4. **Melting point: 140-141^{0}C**
5. **Description:** White to slightly yellowish-white solid.
6. **Solubility:** Very soluble in water and methanol, freely soluble in ethanol, chloroform and ethyl acetate and insoluble in ether and n-hexane.
7. **Category:** Proton-pump inhibitor
8. **Boiling point: 603.9 °C at 760 mmHg**

Itopride hydrochloride

Structure



1. **Chemical name:** N-[[4-(2-dimethylaminoethoxy)phenyl]methyl]-3,4-dimethoxy-benzamide hydrochloride.
2. **Molecular formula:** $C_{20}H_{26}N_2O_4 \cdot HCl$
3. **Molecular weight:** 394.90

4. **Description:** Crystalline Solid
5. **Solubility:** Freely soluble in methanol and acetonitrile
6. **Melting point:** 194-195° C
7. **Category:** Dopamine D2-receptor antagonist with anti-cholinesterase activity. Gastroprokinetic.
8. **Mode of Action**

Itopride increases acetylcholine concentrations by inhibiting dopamine D2 receptors and acetylcholinesterase. Higher acetylcholine increases GI peristalsis, increases the lower esophageal sphincter pressure, stimulates gastric motility, accelerates gastric emptying, and improves gastro-duodenal coordination.

AIM

From the extensive literature survey conducted, it was found that very few analytical methods were reported for the simultaneous estimation of *Rabeprazole Sodium* and *Itopride Hydrochloride* by reverse phase HPLC method. Aim of my work lies in developing a new precise, accurate and validated method for this drug combination. So it was felt that there is a need to develop a rapid, validated analytical method for the determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* simultaneously by RP-HPLC. So, my present work is aimed at the development of a new faster, economical, validated, accurate and reliable reverse phase HPLC method for the simultaneous determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* in capsule dosage form and the validation of the developed method.

MATERIALS AND METHODS

Instruments used

I. System : HPLC Shimadzu LC 10AT (Isocratic system)
   1. Pump : I80 (LC – 10 AT Vp series)
   2. Detector : UV-visible SPD 10 Avp
   3. Column : C-18 Phenomenex Luna (250mm x 4.6 mm internal diameter.)
   4. Injector : Rheodyne(U.S.A)

II. Elico pH meter
III. LABINDIA 3000 – Double beam UV-VISIBLE spectrophotometer.
IV. Gelman science vaccum pump.
V. A & D – Digital analytical balance.

Reagents and Chemicals

5. Sodium dihydrogen orthophosphate dehydrate A. R : Merck
6. Acetonitrile. : HPLC Grade, (Merck)
7. Methanol. : HPLC Grade, (Merck)
8. Water. : HPLC Grade

Reference Standards

1. ***Rabeprazole Sodium*** % purity - 99.6
2. ***Itopride Hydrochloride*** % purity –99.53

Preparation of phosphate buffer

15.6 gm of Sodium dihydrogen orthophosphate dehydrate AR is dissolved in 1000 ml of water to get a concentration of 0.1 M. Then the pH of the buffer was found to be 4.30.

Tablet Brand Used

RABEE-ISR (RPG LIFE SCIENCES**)**

Label claim:

Rabeprazole Sodium : 20mg (enteric coated granules)
Itopride Hydrochloride : 150mg

METHOD DEVELOPMENT AND OPTIMIZATION BY HPLC

Solubility

According to literature studies, it was found that *Rabeprazole Sodium* is Water soluble and *Itopride Hydrochloride* is soluble in methanol. The solubility of both the drugs in methanol, acetonitrile and water was checked and it was found that both the drugs were soluble in the above mentioned solvents. Finally methanol and acetonitrile were chosen as a component of mobile phase for the present work.

Selection of chromatographic condition

Proper selection of the method depends upon the nature of the sample (ionic / ionisable / neutral molecule), its molecular weight and solubility. The drugs selected in the present study are polar in nature and hence reversed phase HPLC method may be the most suitable method. The reversed phase HPLC was selected for the separation because of its simplicity and suitability.

Preparation of Standard Stock solutions

About 50 mg of *Rabeprazole Sodium* and *Itopride Hydrochloride* were accurately weighed and transferred into two separate 50 ml volumetric flasks and dissolved in minimum quantity of distilled water and made up to 50 ml with distilled water. The solutions were observed to contain 1000 µg/ml concentration.

Selection of maximum wavelength

The standard stock solutions were further diluted with distilled water to get 100 µg/ml concentration. These solutions were scanned separately between 200-400 nm range using distilled water as blank. From UV spectra, 258 nm and 286 nm were found to be the maximum absorption wavelengths of *Itopride Hydrochloride* and *Rabeprazole Sodium* respectively. Absorbance of combined stock solutions was also determined and the maximum wavelength for the analysis of both the drugs was determined at 285nm.

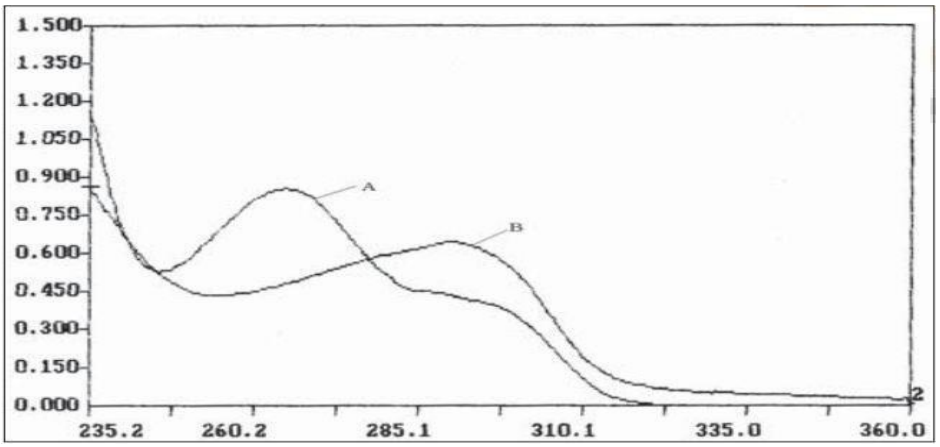


Fig 1: Combined U.V.Spectra of *Rabeprazole Sodium* and *Itopride Hydrochloride*.

Optimization of Chromatographic conditions

Different initial chromatographic conditions like the type of the column, the detector used, injection volume, column temperature, flow rate were selected and tried with different mobile phase compositions depending upon the type of the analyte. Further the most suitable chromatographic conditions for the analysis of *Rabeprazole Sodium* and *Itopride Hydrochloride* were optimized for carrying out further work.

Method development trials

Trial-I

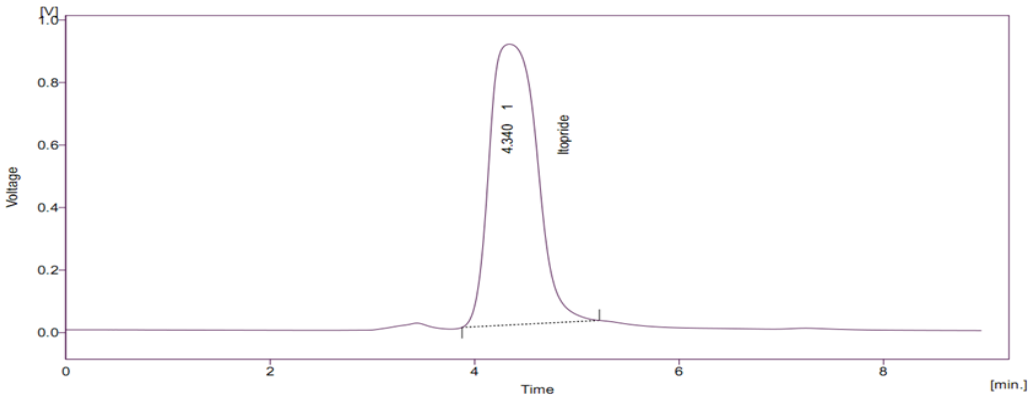
Objective: To develop a method for the determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* by HPLC.

Chromatographic conditions:

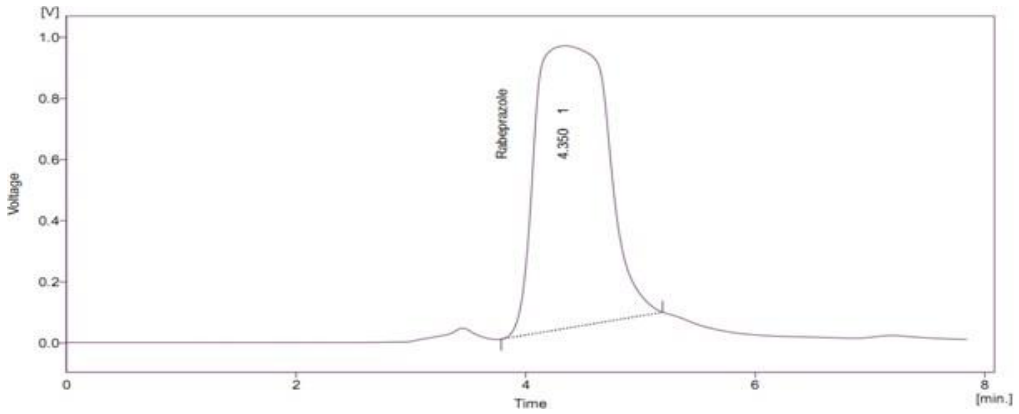
Phenomenex-Luna C-18 4.6 X 250 mm column containing packing of ODS chemically bounded to porous silica or ceramic micro particles, 5 µm.

Column temperature	:	Room temperature.
Flow rate	:	1.0 ml/min.
Injection volume	:	20 µl.
Wavelength	:	285 nm
Mobile phase composition :		Acetonitrile : Phosphate buffer (80:20 %v/v)

Chromatogram no. 1 Trial 1-*Itopride*



Chromatogram no. 2 Trial 1-*Rabeprazole*



Observation

In this trail, peak broadening was observed which is not good. Hence gone for further trail as it was not satisfactory.

Trial-II

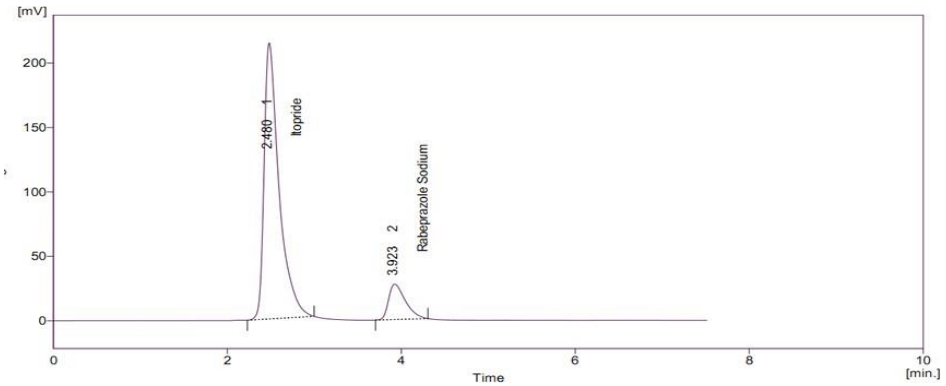
Objective: To develop a method for the determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* by HPLC.

Chromatographic conditions

Phenomenex-Luna C-18 4.6 X 250 mm column containing packing of ODS chemically bounded to porous silica or ceramic micro particles, 5 µm.

Column temperature : Room temperature.
Flow rate : 1.0 ml/min.
Injection volume : 20 µl.
Wavelength : 285 nm
Mobile phase composition : Methanol : Phosphoric acid solution (0.2%)[65:35 %v/v]

Chromatogram no. 3 Trial 2



Observation

In this trail, *Itopride Hydrochloride* and *Rabeprazole* peaks showed tailing. Eventhough the peaks are eluted earlier, tailing effect is not desirable and also the *Rabeprazole* peak is broad. Hence underwent for further trial.

Trial-III

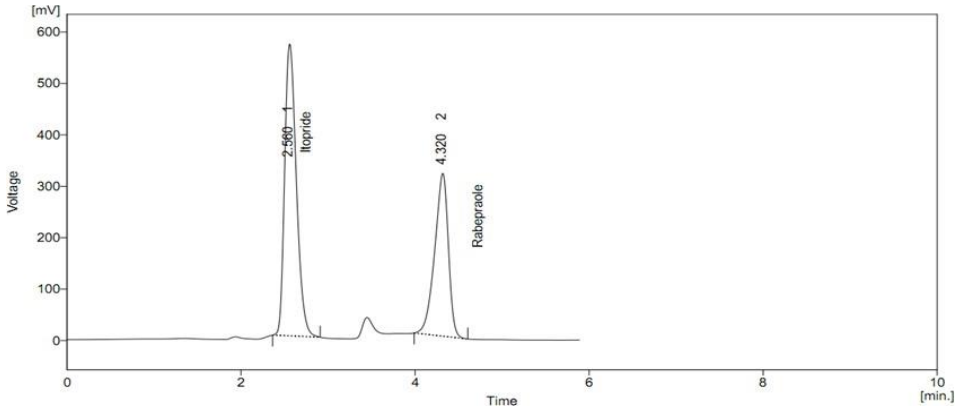
Objective: To develop a method for the determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* by HPLC.

Chromatographic conditions:

Phenomenex-Luna C-18 4.6 X 250 mm column containing packing of ODS chemicallybounded to porous silica, 5 µm.

Column temperature : Room temperature.
Flow rate : 1.0 ml/min.
Injection volume : 20 µl.
Wavelength :285nm
Mobile phase composition : Acetonitrile : 0.05M Phosphate Buffer pH-3.5 [40:60]

Chromatogram no. 4 Trial 3



Observation

In this trail, peak resolution was not satisfactory and also baseline linearity was not good. Hence another trial was carried out.

Trial-IV

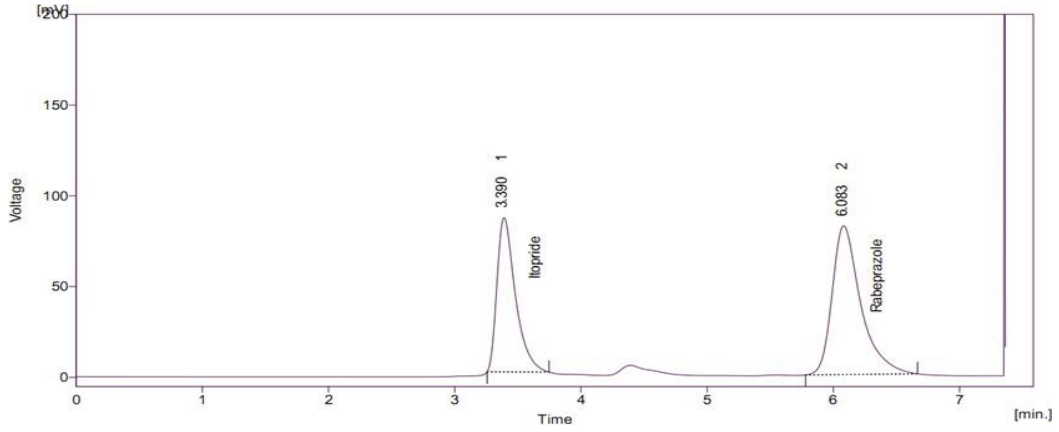
Objective: To develop a method for the determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* by HPLC.

Chromatographic conditions

Phenomenex-Luna C-18 4.6 X 250 mm column containing packing of ODS chemically bounded to porous silica, 5 µm.

Column temperature : Room temperature.
Flow rate : 1.0 ml/min.
Injection volume : 20 µl.
Wavelength : 285nm
Mobile phase composition : Methanol : 0.1M Sodium Phosphate Buffer [60:40]

Chromatogram no. 5 Trial 4



Observation

In this trial, peak asymmetry for *Itopride* was observed and also peak broadening for both peaks was observed. Hence another trial was undertaken.

Trial-V (OPTIMISED and PROPOSED METHOD)

Objective: To develop a method for the determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* by HPLC.

Chromatographic conditions:

Phenomenex-Luna C-18 4.6 X 250 mm column containing packing of ODS chemically bounded to porous silica, 5 µm.

Column temperature : Room temperature.

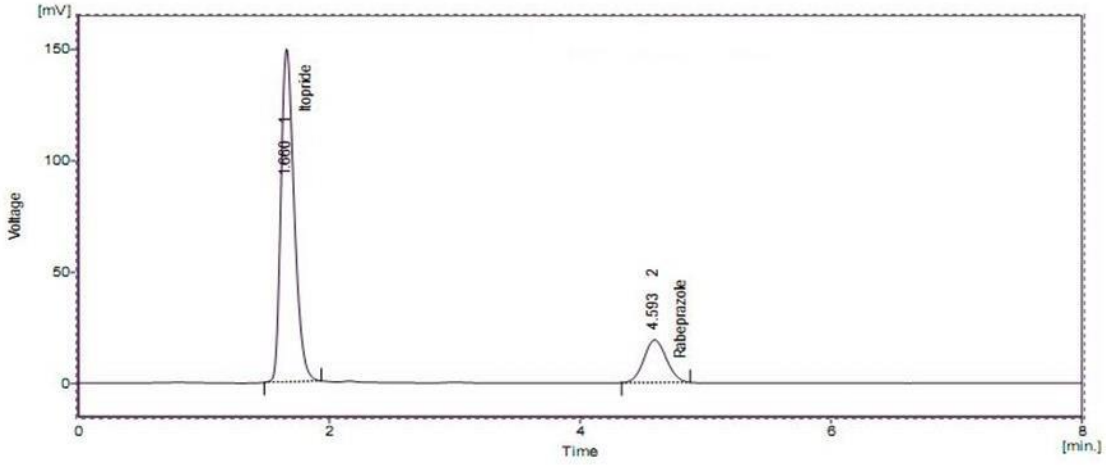
Flow rate : 1.0 ml/min.

Injection volume : 20 µl.

Wavelength : 285nm

Mobile phase composition : Methanol : 0.1M Sodium Phosphate Buffer [65:35]

Chromatogram no. 6 Trial 5



Observation

In this trial, both peaks are very good and less tailing effect is observed. Peaks are with good resolution and the retention times are also satisfactory. Hence this method is taken for validation.

Preparation of mobile phase

HPLC grade methanol and the prepared phosphate buffer (0.1M) were taken in 65:35 (%v/v) ratio. The mobile phase was then degassed first in vaccum filter and then in sonicator for about 30 minutes.

Preparation of phosphate buffer

15.6 gm of $NaH_2PO_4.2H_2O$ (sodium dihydrogen orthophosphate dihydrate) was dissolved in 1000ml of HPLC grade water and the buffer was degassed first in vaccum filter and then in sonicator for about 30 minutes.

Standard preparation

Weighed accurately about 6.66mg *Rabeprazole Sodium* working standard and 50mg *Itopride Hydrochloride* working standard into a clean and dry 50ml volumetric flask and 30 ml of mobile phase was added. Shaken well and the volume was then made up to 50ml with mobile phase. The standard preparation was filtered through vaccum filter and sonicated for 15 minutes.

Sample preparation

20 capsules were taken, weighed, opened and the granules were powdered. From the powdered mass, weighed accurately about 118.6 mg of powder and transferred to a 100 ml volumetric standard flask and the volume was made up to 100 ml with mobile phase. The solution was shaken to ensure the solubility of the powder and then filtered and sonicated.

Assay results obtained

Separately injected both the standard (3 injections) and sample preparations (2 injections) into the chromatograph and the peak area responses were recorded.

Calculation

The amount of *Rabeprazole Sodium* and *Itopride Hydrochloride* was calculated by using the following formula,

$$\frac{\text{Sample area} \times \text{purity of working standard}}{\text{Standard area}}$$

Table 1: Assay result for Replicate injections of Standards

Inj . No	Area of *Rabeprazole Sodium*	Area of *Itopride HCl*	% of *Rabeprazole Sodium* recovered	% of *Itopride HCl* (mg)
1	237.909	1098.915	101.125	99.8177
2	236.731	1095.815	100.624	99.5361
3	232.714	1092.996	98.916	99.280
Mean	235.7847	1095.909	100.2216	99.5446
S.D	2.723723	2.417329	1.15816	0.268951
%R.S.D	1.15517	0.220577	1.15559	1.155594

The mean recovery for assay result was found to be 100.2% and 99.54% for *Rabeprazole Sodium* and *Itopride Hydrochloride* respectively.

RESULTS AND DISCUSSION

A simple and rapid reverse phase liquid chromatography method has been developed and validated for simultaneous determination of *Rabeprazole Sodium* and *Itopride Hydrochloride* in capsule dosage form and it was carried out by using a mobile phase consisting of 0.1 M Phosphate buffer(P^H 4.30) and Methanol in the ratio of 35: 65 v/v. The column used was C_{18} Intersil ODS (250mm × 4.6mm) with flow rate of 1.0 ml/min and UV Detection was carried out.

The chromatograms of *Rabeprazole Sodium* and *Itopride Hydrochloride* reference standards were presented in chromatogram -11 and chromatogram -12 respectively. The individual peaks of *Rabeprazole Sodium* and *Itopride Hydrochloride* were identified by knowing the retention time 4.615 and 1.660 minutes respectively.

The results of analysis shows that the amount of drugs was in good agreement with the label claim of the formulation. The capsule shows percentage purity values ranging from 98.916% - 100.624% for *Rabeprazole Sodium* and 99.280% – 99.817% for *Itopride Hydrochloride* respectively.

The data regarding linearity for both the drugs were given in the table 10 and 11 and corresponding calibration graphs were shown in figure 4 and 5. The linearity of the method was determined at the concentration levels ranging from 2.5- 15 µg/ml for *Rabeprazole Sodium* and 20-120 µg/ml for *Itopride Hydrochloride*. The correlation coefficient of *Rabeprazole Sodium* and *Itopride Hydrochloride* was found to be 0.998 and 0.996 and

these are within limit. System suitability parameters such as resolution, tailing factor and number of theoretical plates are presented in Table.1. System precision was carried out, the RSD for peak area of *Rabeprazole Sodium* and *Itopride Hydrochloride* for six replicated injections was not more than 1.0% and the data was present in table .6. The acceptance criteria of method precision was found to be RSD NMT 1.0% and the method shows precision of 0.4805 and 0.5573 for *Rabeprazole Sodium* and *Itopride Hydrochloride* respectively.

CONCLUSION

High percentage of recovery shows that the method was free from the interferences of the excipients used in the formulations. The proposed method was highly accurate which showed good recovery of drug samples. The low standard deviation value and % RSD value indicate good precision. Mean percentage recovery above 95% indicates the reproducibility and accuracy of the newly developed method compared. The result of study include the proposed method is highly accurate, simple, precise and specific. Hence the developed chromatographic method for *Rabeprazole Sodium* and *Itopride Hydrochloride* is said to be rapid, simple, accurate, precise and cost effective that can be effectively applied for the routine analysis in research institution, quality control departments in industries, approved drug testing laboratories, biopharmaceutical studies and in clinical pharmacokinetic studies.

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