https://doi.org/10.48047/AFJBS.6.15.2024.7673-7723

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

METHOD DEVELOPMENT AND VALIDATION OF COMBINATION PRODUCT OF ILAPRAZOLE AND ITOPRIDE BY HPLC Method

Arjun Saraf*, Santoshi Shah, Shivanand Patil

Student of M. Pharmacy (Quality Assurance)

College Name- Shree Dev Bhoomi Institute of Education Science and Technology, Dehradun

00977-9857049550/9807547116

Email : arjunsaraf16@gmail.com

Designation- Associate Professor

College Name- Shree Dev Bhoomi Institute of Education Science and Technology, Dehradun

Contact No- 9760329636

Mail Id-santoshi.shahmph@gmail.com

Director's Name- Dr.Shivanand Patil

Contact No-+91 73009 28999

Mail Id-drshivapatilg@gmail.com

Department of Pharmacy (Quality Assurance) Shree Dev Bhoomi Institute of Education Science and Technology Pondha Rd, Mahajun, Uttarakhanda 248007

*Corresponding Author: Arjun Saraf

Address: Department of Pharmacy (Quality Assurance) Shree Dev Bhoomi Institute of Education, Science and Technology, Pondha Rd, Mahajun, Uttarakhanda 248007

Email : arjunsaraf16@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.7673-7723

ABSTRACT

Itopride hydrochloride is a prokinetic drug derived from benzamide. It has been granted certificate for the treatment of symptoms associated with illnesses such as non-ulcer dyspepsia. Potential etiologies for the illness are chronic gastritis, diabetic gastroparesis, or functional dyspepsia. The main objective of this research was to investigate the process of developing pharmaceutical dosage forms using reversed high-performance liquid chromatography (HPLC). By utilizing a sophisticated validation technique, we can effectively analyze the medication in a pharmaceutical dose form using reversed-phase high-performance liquid chromatography (HPLC). Validation is an ongoing process that begins before the use of an instrument and extends much beyond the invention and transmission of a method. A novel HPLC technique was developed and validated for the analysis of itopride hydrochloride and ilaprazole sodium. The most effective chromatographic separation was accomplished by using a Phenomenex C18 column, with UV detection set at a wavelength of 268nm. The mobile phase used consisted of a mixture of ethanol, water, and acetonitrile at a ratio of 50:40:10. The retention time of itopride hydrochloride was determined as 2100, whereas the retention time of Ilaprazole sodium was reported as 6700. The retention time data of the commercially available product were compared to the most optimal ones.

Keywords: Validation, High Performance Liquid Chromatography (HPLC), Phenomenex C18 column, Reversed phase.

INTRODUCTION

1.1. GENERAL BACKGROUND:

In formulation of any new drug molecule, analysis is an important component. There are two types of analysis, one is qualitative analysis and other is quantitative analysis. Identification of components or analyte of mixture or sample is qualitative analysis whereas, in quantitative analysis, there is determination of amount of components or analyte of mixture or sample. There is no need for validation for compendial dosage forms because the procedure will already be established. However, a suitable and validated method must be provided for the analysis of the drug(s) in the bulk and tablet dosage forms for non-compendial products. To determine the amount of drug content in a dosage form when an appropriate method is not available, a simple, selective, rapid, sensitive, accurate, precise, and repeatable approach must be developed.

When it comes to the research, development, and production of new pharmaceutical products, the creation of appropriate analytical methods and their validation are crucial. Good manufacturing practices guidelines states different elements need to be fulfilled by the pharmaceutical industries. Among them validation is one of the key elements to fulfill the requirement of Current Good Manufacturing Practices (cGMP) and Good Laboratory Practices (GLP). GMP is the quality aspects for both production and quality control. It ensures that the processes necessary for production and laboratory testing are clearly defined, validated, reviewed, and documented. GMP has legal components, covering responsibilities for, manufacturing, for testing, for distribution and addressing to product defects and market complaints. Use of suitable and validated method for testing and ensuring the quality and the content of the developed dosage products in the pharmaceuticals industry is the integral part of the compliance. It is essential to create novel analytical techniques, particularly for those products that are not included in pharmacopoeia. During the process of drug discovery, release to market, and development, which results in a marketing approval, the creation of sound analytical method(s) is of the utmost importance. It is now important to validate the new analytical technique after development.

Analytical analysis of bulk drug materials, intermediates, drug products, drug formulations, contaminants and degradation products, the pharmaceuticals and their metabolites, is crucial in the field of pharmaceutical research. Analytical assay techniques have been a part of compendia monographs from the inception of formal pharmaceutical analysis, with the goal of defining the quality of bulk drug products by establishing limits for the quantity of their active. There are different techniques of analytical methods. Titrimetric, spectrometry, chromatography, and capillary electrophoresis are some of the test methods used in contemporary monographs.

The process of method development demonstrates that an analytical method is appropriate for application. Information on numerous phases and parameters, such as accuracy, precision, linearity, limit of detection, limit of quantification, specificity, range, and robustness, are provided through the validation of analytical methods (2). Validation should be carried out in accordance with regulatory standards like the ICH standards. To measure the drug ingredients, various instrumental approaches are available. Among them, the HPLC method is chosen because it is quite exact.

The analytical method itself must be properly built, maintained, calibrated, and verified before beginning the work of methods validation. The test method, supporting documentation, and acceptance criteria must be specified for each of the validation characteristics in this document. Specific values must be based on references from the ICH, US-FDA, USP, and relevant literature.

1.2. DRUG MOTIES & DOSAGE FORM:

Ilaprazole (ILA), a newly approved PPI in Asian countries, is reported to be highly effective and safe for the treatment of duodenal ulcer (3). Itopride hydrochloride is highly water soluble novel prokinetic drug that is used in the Gastro esophageal reflux disease (GERD), Non ulcer dyspepsia, chronic gastritis and other gastrointestinal motility disorders. A Simple, rapid, accurate, precise HPLC method was developed and validated for simultaneous estimation of Itopride Hydrochloride and Ilaprazole in a Dosage form.

1.3. ANALYTICAL METHOD DEVELOPMENT & VALIDATION:

The process of confirming the analytical testing strategy utilized for a particular test which is reasonable for its expected use is referred to the method validation. The method used to conduct the analysis is referred to as the analytical technique. The term "analytical method validation" refers to the process of verifying that the analytical testing approach used for a certain test is appropriate for its intended usage. The procedures required to carry out each analytical test should be thoroughly explained. This may involve—but is not limited to— the preparation of the sample, the reference standard, and the reagents; usage of the equipment; creation of the calibration curve; application of the calculation formulae; etc. (1)

In the pharmaceutical industry, analytical method development gives important information on the potency of a drug, the drug's bioavailability, the drug's stability and also its effect.
Analytical methods should be validated unless the method employed is included in the relevant pharmacopoeia or other recognized standard reference.(2)

Analytical methods should be validated unless the method employed is included in the relevant pharmacopoeia or other recognized standard reference. (2)

Validation is a concept developed in the United States in 1978. The concept of validation has been broaden over the years to achieve many activities like from analytical methods used to control quality of drug substances and drug products up to computerized systems for clinical trials, process control or labelling. Validation is best seen as a necessary and prime part of cGMP. (3)

Analytical method validation is defined as the process of establishing documented evidence which provides a high degree of assurance that a specific process such as analytical test method, will consistently produce a product supported by assay results meeting its predetermined specification and quality attributes (i.e. accuracy, precision etc.)(4) The process of doing multiple evaluations is known as method validation, and it is used to determine whether an analysis methodology demonstrates the expected explanation in an appropriate manner and is set up to provide accurate, legal measurements.

Analytic method development and validation are continuous and interconnected activities conducted throughout the drug development process. Analytical methods are required to characterize drug substance and drug product composition during all phases of pharmaceutical development. Early phase methods must support changes in synthetic routes and dosage form and elucidate the structures and levels of impurities. In later phases, goals change to the development of rapid and robust methods for release and stability evaluation that can be transferred to quality units. Analytic methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Analytical method validation is the process of demonstrating that the analytical procedures are suitable for their intended use. According to FDA guideline, analytic method validation is a matter of establishing documented evidence that provides a high degree of assurance that the specified method will consistently provide accurate test results that evaluate a product against its defined specification and quality attributes. The validation process requires quality method development. Whereas validation can be a time-consuming process, methods should not enter the validation phase unless they are fully developed. The relationship of validation and method development can be

Observed as:

- When methods are properly developed, they can be readily validated.
- Validation does not make a method better or more efficient.

A validated method does not necessarily imply that it meets all criteria of a properly developed method. Validation acceptance criteria should be based on method development experience.

Method Validation is required for the following reasons:

1. A new method is been developed.

2. Revision of established method.

3. When established methods are used in different laboratories and different analysts etc.

4. Comparison of methods.

5. When quality control indicates method changes.

Advantages of analytical method validation:

The biggest advantage of method validation is that it builds aϖ degree of confidence, not only for the developer but also to the user.

Although the validation exercise may appear costly and timeϖ consuming, it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end.

Minor changes in the conditions such as reagent supplier or grade, analytical setup are unavoidable due to obvious reasons but the method validation absorbs the shock of such conditions and pays for more than invested on the process.

Guidelines from the following sources provide a framework for performing validation.

- United States pharmacopoeia (USP)
- International conference on harmonization (ICH)ϖ Food and drug administration (FDA)
- Validation according to ICH Guidelines

Typical validation parameters are: (5)

A. Accuracy

B. Precision (Repeatability, Intermediate precision and Reproducibility)

C. Linearity

D. Range

E. Specificity

F. Robustness

G. System suitability testing

H. Limit of detection (LOD) and Limit of quantitation (LOQ)

A. Accuracy:

Definition: It expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. This is sometimes termed trueness.

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. The accuracy of the method was determined by recovery studies. The ICH document on validation methodology recommends accuracy to be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range. Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value.

B. Precision :

Definition: It expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions.

Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability: It expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision. Repeatability must be tested from at least six replications measured at 100 percent of the test target concentration or from at least nine replications covering the complete specified range.

Intermediate precision: It expresses variations within laboratories, such as different days, different analysts, different equipment, and so forth. The objective of intermediate precision validation is to verify that in the same laboratory the method will provide the same results once the development phase is over.

Reproducibility: It expresses the precision between laboratories. The objective of reproducibility is to verify that the method will provide the same results in different laboratories. The reproducibility of an analytical method is determined by analyzing aliquots from homogeneous lots in different laboratories with different analysts.

C. Linearity:

Definition: Linearity of an analytical procedure is its ability (within a given range) to obtain test results that are directly proportional to the concentration of analyte in the sample.

It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/ separate weighing of synthetic mixtures of the drug product components, using the proposed procedure.

D. Range:

Definition: Range of an analytical procedure is the interval from the upper to the lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. For the assay of a drug substance or a finished (drug) product: normally from 80 to 120 percent of the test concentration should be tested/checked for range.

E. Specificity:

Definition: It is the ability to assess unequivocally the analyte in the presence of components, which may be expected to be present. Typically, these might include impurities, degradants, matrix, etc.

F. Robustness:

Definition: It is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

G. System suitability testing:

Definition: The tests, based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated. System suitability testing is an integral part of procedures.

H. Limit of detection and Limit of quantitation:

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value.

The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample, which can be quantitatively determined with a suitable level of precision and accuracy.

Several approaches for determining are possible, depending on whether the procedure is a non-instrumental or instrumental.

- Based on visual evaluation
- Based on signal-to-noise
- Based on the standard deviation of the response and the slope.

The LOD and LOQ were estimated from the set of 5 calibration curves used to determine method linearity. Limit of detection and Limit of quantitation can be calculated by the following equation.

LOD = 3.3 (σ/S), LOQ = 10 (σ/S)

Where,

σ= Standard deviation of y-intercepts of regression lines

S =Slope of the calibration curve

HPLC is one of the most widely used analytical procedures out of all the different analytical techniques. It has a number of benefits over traditional chromatographic methods. HPLC makes precise and quick identification and determination of a variety of natural and synthetic substances possible because of its ease of use and effectiveness. It has a wide range of applications in terms of quantitative and qualitative estimation in many various disciplines, including pharmaceutical, environmental, forensic, food and flavor, and therapeutic. HPLC's only drawbacks are its high cost and length of time.

Normal-Phase Chromatography (NPC) (6)

NPC is the traditional separation mode based on adsorption/desorption of the analyst onto a polar stationary phase (typically silica or alumina). In this technique, non-polar compounds travel faster and are eluted first because of the lower affinity between the non-polar compounds and the stationary phase. Polar compounds are retained for a longer time because of their higher affinity towards the stationary phase. Normal phase mode of separation is, therefore, not generally used for pharmaceutical applications because most of the drug molecules are polar in

nature and hence take longer time to elute.

Reversed-Phase Chromatography (RPC)

Reversed phase mode is the most popular mode for analytical and preparative separations of compounds of interest in chemical, biological, pharmaceutical, food and biomedical sciences. In this mode, the stationary phase is nonpolar hydrophobic packing with octyl or octadecyl functional group bonded to silica gel and the mobile phase is a polar solvent. An aqueous mobile phase allows the use of secondary solute chemical equilibrium (such as ionization control, ion suppression, ion pairing and complexation) to control retention and selectivity. The polar compound gets eluted first in this mode and nonpolar compounds are retained for longer time. As most of the drugs and pharmaceuticals are polar in nature, they are not retained for longer times and hence elute faster. The different columns used are octadecylsilane (ODS) or C8, C4 etc., (in the order of increasing polarity of the stationary phase).

Columns are the heart of HPLC. Liquid chromatographic columns are usually constructed from smooth bore stainless steel tubing. Sometimes made from heavy walled glass tubings and polymer tubings such as PEEK. Guard columns are introduced before analytical columns to increase the life of analytical columns, by removing not only particulate matter and contaminants from solvents but also sample components that bind irreversibly to the stationary phase. Analytical columns ranges from 5 - 25 cm long; inside diameter is often 3 - 5 mm; the most common particle size of packing is 3 - 5 µm. (7)

Two basic types of column packing used in LC are pellicular and porous particles. In pellicular packing, spherical, nonporous, glass or polymer beads are used. A thin layer of silica, alumina, polystyrene – divinylbenzene synthetic resin, or an ion – exchange resin was deposited on the surface of these beads. In the typical porous particle packing of LC is composed of silica, alumina, polystyrene – divinylbenzene synthetic resin, or an ion – exchange resin. Columns for the bonded phase chromatography is prepared by surface functionalization of silica. The surface of fully hydrolysed silica is made up of chemically reactive silanol groups. The most useful bonded phase coatings are siloxanes formed by the reaction of the hydrolyzed surface with organochlorosilanes.

Before beginning validation investigations, the methodology and goal of the analytical techniques should be properly stated and understood. This understanding is obtained from scientifically-based method development and optimization studies. Validation data must be generated under a protocol approved by the sponsor following current good manufacturing practices with the description of methodology of each validation characteristic and

predetermined and justified acceptance criteria, using qualified instrumentation. Protocols for both drug substance and product analytes or mixture of analytes in respective matrices should be developed and executed.

A suitable standard technique should be established for producing reliable analytical results from the competent laboratory. It can only happen if the analytical procedure is validated. Validation of analytical methods is a crucial prerequisite for carrying out the chemical evaluation. Method validation is a process of carrying out several tests to ensure that an analytical test system is appropriate for its intended uses and is able to produce documentation supporting its appropriateness. The validation test should include products excipients that could affect test results in order to accurately evaluate method parameters. Validation of analytical methods is therefore product-specific.

The common method for the development and validation of the analytical method is completed by the following process.

- Planning the appropriate method that must be developed.
- The information related to the work should be collected.
- Qualitative and quantitative analytical methods that can be performed in the lab should be developed.
- The procedure for testing the sample should be created.

A fundamental requirement to play out with the chemical assessment is validation that deals with the analytical procedure. Method validation is a process that involves carrying out several evaluations to see if a method of analysis demonstrates the proper expected explanation and is equipped to provide legitimate, legal measurements. According to the rules and recommendations, the approach should offer useful information that guarantees the product's quality. Such results are determined using many tests on the material. A thoroughly tested procedure should meet each requirement. Testing of the excipients and a focus on standard testing conditions should be part of the validation of the analytical method. These circumstances demonstrate that the analytical method's validation is product-specific.

The Current Good Manufacturing Practices suggest that quality should be built into the product, and testing alone cannot be relied on to ensure product quality. Pharmaceutical products need to maintain high quality in order to provide safe and effective usage. From the analytical point of view, analytical methods used to test these products should have quality attributes built into them. Validation ensures these quality attributes are built into the method. Validation of analytical methods is an essential but time consuming activity for most analytical laboratories. But it results inexpensive, eliminates frustrating repetitions and leads to better time management in the end. The analytical methods need to be validated or revalidated before initial use of the method in routine analysis, when transferred from one laboratory to another, whenever the conditions or method parameters for which the method has been validated change and change is outside the original scope of the method. One of the most acceptable testing method is by chromatographic technique.

Chromatography is defined as a procedure by which solutes are separated by dynamic differential migration process in a system consisting of two or more mobile phases, one of which moves continuously in a given direction and in which the individual substances exhibit different mobilities by reason of differences in absorption, partition, solubility, vapor pressure, molecular size or ionic charge density. When mobile phase used is liquid the type of chromatography is called liquid chromatography. High performance liquid chromatography (HPLC) is a modern form of liquid chromatography that uses small particle columns through which the mobile phase is pumped at high pressure. The

separation of components depends on the extent of interaction between the solute component and the stationary phase. The component that has lowest affinity for the stationary phase will elute first. HPLC is becoming a preferred method of analysis among various analytical methods for pharmaceuticals. HPLC methods provide rapid analysis, greater sensitivity, high resolution, and easy sample recovery, precise and reproducible result Validation of analytical procedure is the legal requirement and is mandatory for non compendial products to perform. ICH guidelines [Q2 (R1)] have set the guidelines for the validation of the analytical method.

The validation of the analytical methods for the product are performed but not limited to identification, assay and dissolution.

1.4. Product for Which the Method to be Validated:

- **Specific Profile Review of Ilaprazole Sodium**

A. Description: Ilaprazole is a substituted benzimidazole prodrug with selective and irreversible proton pump inhibitor activity. A weak base, ilaprazole accumulates in the acidic environment of the secretory canaliculus of the gastric parietal cell where it is converted to an active sulfenamide form that binds to cysteine sulfhydryl groups on the luminal aspect of the proton pump hydrogen-potassium adenosine triphosphatase (H+/K+ ATPase), thereby inhibiting the pump's activity and the parietal cell secretion of H+ ions into the gastric lumen, the final step in gastric acid production.

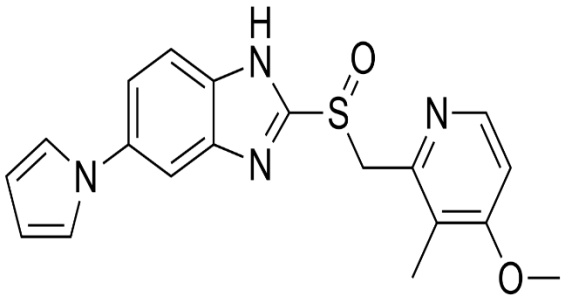
B. Chemicalname:- *2-[(4-methoxy-3-methylpyridin-2-yl)methylsulfinyl]-6-pyrrol-1-yl-1H-benzimidazole*

C. BCS classification:- It belongs to class I (High solubility, High permeability)

D. **Molecular formula:-** C19H18N4O2S

E. **Molecular weight:-** 388.4 g/mol

F. Molecular Structure :-



G. Melting Point :- 149.0 to 153.0 °C

H. Water solubility:- Insoluble

I. Half-life : 4.7-5.3 hr

Specific Profile Review of Itopride Hydrochloride

A. Description:- Itopride hydrochloride is an odourless, white to off white crystalline powder, soluble in water, methanol and sparingly soluble in acetic acid.

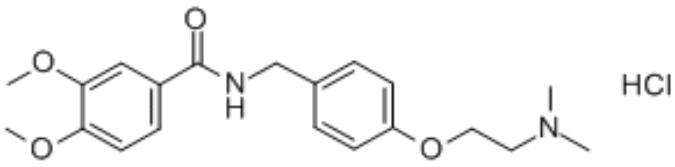
B. Chemicalname:-*N*-**[**[4-(2-Dimethylaminoethoxy)phenyl]methyl**]**-3,4-dimethoxybenzamide.

C. BCS classification:- It belongs to class I (High solubility, High permeability)

D. Molecular formula:- $C_{20}H_{26}N_2O_4$. HCl

E. Molecular weight:- 394.89 g/mol

F. Molecular Structure :-



G. Melting Point :- 194-195°C

H. Water solubility:- 0.0261 mg/mL (20)

I. Pharmacokinetic parameters:-

Absorption

On oral administration, Itopride is rapidly and extensively absorbed and peak serum concentration is achieved within 35 minutes after oral dosing. Thus it has a rapid onset of action.

Metabolism

Itopride is metabolized in the liver by N-oxidation to inactive metabolites by the enzyme flavin-containing monooxygenase (FMO).The half-life of Itopride is about 6 hours.

Excretion

It is excreted mainly by the kidneys as metabolites and unchanged drug.

J. **Pharmacodynamic parameters:-** Itopride has anti-cholinesterase (AchE) activity as well as dopamine D2 receptor antagonistic activity.

Acetylcholine (ACh) released from enteric nerve endings stimulates the contraction of smooth muscle. Enzyme AChE hydrolyses the released ACh, inactivates it and thus inhibits the gastric motility leading to various digestive disorders.

Besides ACh, Dopamine present in the gastrointestinal tract has several inhibitory effects on gastrointestinal motility, including reduction of lower esophageal sphincter and intragastric pressure (suppression of ACh release mediated by the D2 receptors). Itopride, by virtue of its dopamine D2 receptor antagonism, removes the inhibitory effects on Ach release.

It also directly inhibits the enzyme AchE which prevents the degradation of ACh. The net effect is an increase in ACh concentration, which in turn, promotes gastric motility, increases the lower esophageal sphincter pressure, accelerates gastric emptying and improves gastro-duodenal coordination.

This dual mode of action of Itopride is unique and different from the actions of other prokinetic agents (Metoclopramide, Domperidone) available in the market (8).

K. **Therapeutic indications:-**

- Functional dyspepsia
- Non-ulcer dyspepsia (NUD)
- Gastro-esophageal reflux disease (GERD)
- Chronic gastritis
- Diabetic gastroparesis (9).

L. **Dosage and administration:-** The usual daily dosage for adults is 50 mg itopride hydrochloride orally in 3 divided doses before each meal and 150 mg 24 hourly per oral (10).

M. **Drug Interactions:-** Itopride is metabilised by the enzyme flaving containing monooxygenase and not by the cytochrome P450 enzyme system. It is thus devoid of the risk of significant pharmacokinetic drug interaction with cytochrome P450 enzyme inhibitors such as macrolides and azole antifungal agents (9).

N. **Contraindications:-** Itopride should not be used in patients in whom an increase in gastrointestinal motility could be harmful, e.g. gastrointestinal hemorrhage.. Itopride should not be used in patients with known hypersensitivity to it.

O. **Side effects:-** Diarrhea, abdominal pain, increased saliva, headache, irritated feeling, sleep disorder, dizziness and tremor (8).

Methods and Methodology

2.1. Instrumentation

Methods development in HPLC: The following chromatographic conditions were fixed for the simultaneous estimation of Ilaprazole & Itopride. Stationary phase: Phenomenex phenyl hexyl column (250 mm x 4.6mm i.d, 5mm), Mobile phase: Solvent A: water and 0.4%v/v TEA, Solvent. B: Acetonitrile, PH: 4 (adjusted with Orthophosphoric acid). Solvent ratio: 50:50, Detection wavelength: 236nm, Flow rate: 1ml/min, Temperature: Room temperature of 20±2°C.

2.2. 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 Ilaprazole sodium in combination dosage form for routine quality control analysis. No method was found for simultaneous estimation of Itopride hydrochloride and Ilaprazole in combination dosage form.

EXPERIMENTAL SECTION

MATERIALS

TABLE: 1

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

Chemicals

TABLE:2

S. No	Name	Model	Manufacturers/Company
1	**Acetonitrile**	**HPLC**	**Merk**
2	**Methanol**	**HPLC**	**Merk**
3	**Potassium dihydrogen phosphate**	**G.R**	**Merk**
4	**Water**	**-**	**In house production**

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

2.2.A. Preparation of Standard Stock Solution of Ilaprazole

10 mg of standard Ilaprazole drug was weighed accurately and taken in a clean and dry volumetric flask. The drug was dissolved in some amount of Solvent (Acetonitrile: Ethanol; 70:30) and made

upto the mark with the same solvent. This gives 1000 µg/ml solution. From this solution 1ml was taken and transferred to a 10 ml volumetric flask and the volume was made up to the mark by using solvent resulting to a concentration of 100 µg/ml. Next again a 1ml of solution was taken from above dilution in 10ml volumetric flask and made up to 10ml this give concentration of 10 µg/ml.

0.1 N Hydrochloric acid preparation

It was prepared by taking 8.5 ml of 36.5 % of hydrochloric acid and diluted with distilled water to produce 1000 ml in a volumetric flask (26).

6.8 PH Phosphate buffer preparation

It was prepared by dissolving 28.8 gm disodium hydrogen orthophosphate and 11.45 gm potassium dihydrogen orthophosphate (26).

2.2.B. Standard stock solution preparation of Itopride

150 mg of Itopride hydrochloride was dissolved in 100 ml of 0.1 N hydrochloric acid to make 1.5 mg/ml solution and it was further diluted with 0.1 N HCl to produce 1500 µg/ml standard stock solution of Itopride hydrochloride (26).

2.2.C. Preparation of Test Solution

The preparation of synthetic mixture

Table 3 Composition of formulation (Synthetic Mixture)

S. No	Drug/ Excipient Name	Quantity (mg)
1	Itopride Hydrochloride	150
2	Ilaprazole Sodium	10
3	Magnesium Stearate	400
4	Starch	450
Total		**1000**

Above all ingredients were shift and blend to make uniformity of mixing. Take synthetic powder equivalent to 150 mg of Itopride Hydrochloride and 10 mg ilaprazole in 100 ml volumetric flask. Dissolve in 25 ml of Acetonitrile: Ethanol and sonicated for 15 min. Dilute up to 100 ml with

solvent shake vigorously. Filtered through Whatman filter paper No. 42 and further diluted. Finally the solution had concentration of 100 µg/ml and 15000 µg/ml for Itopride and Ilaprazole, respectively. From that pipette out 1ml in 10ml volumetric flask and volume was made up to mark with Methanol to make final concentration of mixture 10 µg/ml and 150 µg/ml for ilaprazole and itopride, respectively.

2.3. Method Development For Assay of Itopride Hydrochloride and Ilaprazole Sodium By Reverse Phase HPLC

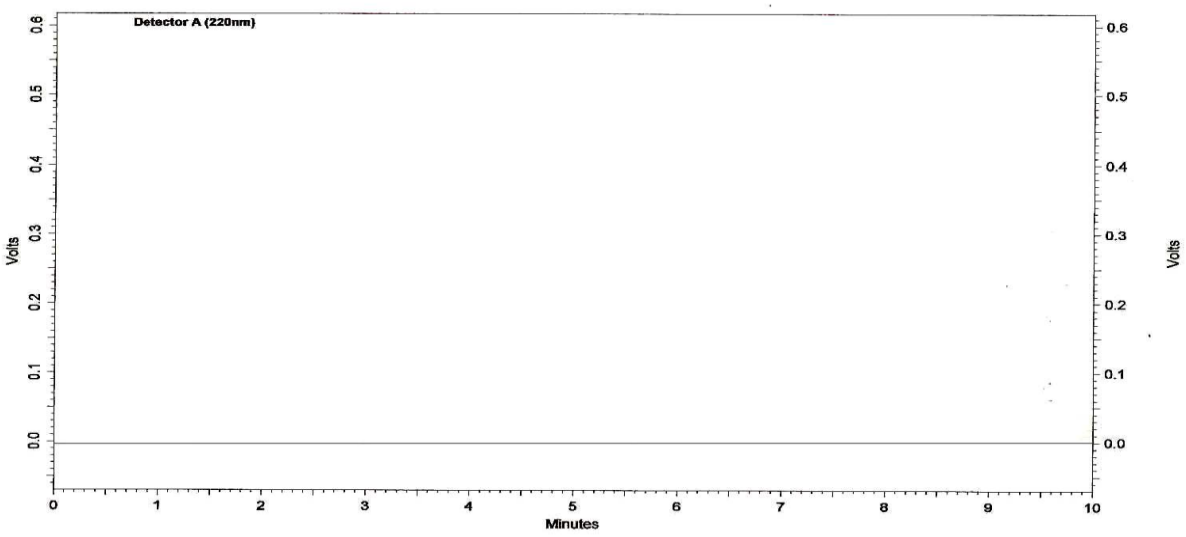
❖ Initialization of the Instrument

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 Ilaprazole 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 Acetonitrile:Ethanol ratio (70:30) filtered and degassed mixture of

the solution *Acetonitrile:Ethanol (70:30)* .

- **Standard Preparation of Itopride Hydrochloride**

Accurately about 150 mg of Itopride Hydrochloride was weighed and transferred to a 10 ml volumetric flask. The volume was then made up to mark with mobile phase to get a standard solution of 150 mg/ml..

- **Standard Solution of Ilaprazole Sodium**

Accurately about 10 mg of IPZ was weighed and transferred to a 10 ml volumetric flask. The volume was then made up to the mark with mobile phase to get a standard solution of ilaprazole at a concentration of 1000µg/mL.

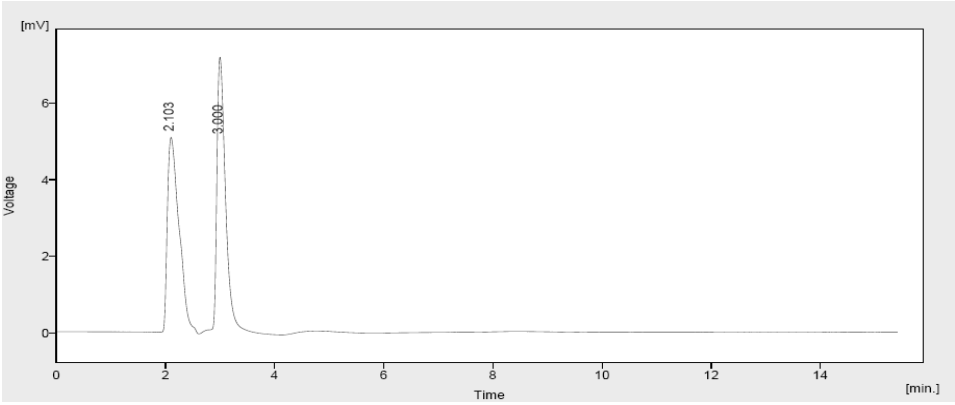
- **Standard Solution of Itopride Hydrochloride and Ilaprazole Sodium Mixture**

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

Table 4

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 Ilaprazole Sodium in the syringe, set the chromatographic condition as per Table 4, saved the chromatographic condition and made the



sequence to run the standard solutionn of Itopride Hydrochloride and Ilaprazole Sodium for15 minutes.

In this mobile phase two peaks were obtained with in 3 mins RT_{ito} 2.1 and RT_{ilz} 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 Ethanol 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 Ilaprazole Sodium**

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

- **Standard solution of Itopride Hydrochloride and Ilaprazole Sodium Mixture**

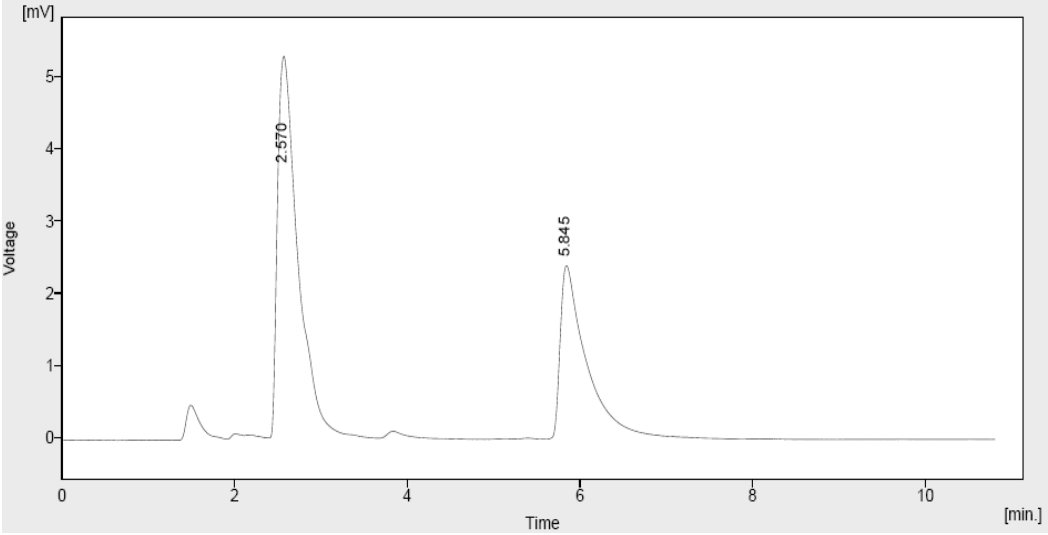
Taken 50ml of Itopride Hydrochloride from the above solution and 25ml of Ilaprazole Sodium and made up to 100 ml with mobile phase.

Table 5

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

Standard solution of Itopride Hydrochloride and Ilaprazole Sodium were filled in the syringe, set

the chromatographic condition as per **Table 5**. Saved the chromatographic conditions and run the standard solution of Itopride Hydrochloride and Ilaprazole Sodium for 10 minutes



In this mobile phase two peaks were obtained with in 6 mins RT_{ito} 2.5 and RT_{ilz} 5.8 though the resolution was good the peaks shape for Itopride and ilaprazole 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 Ilaprazole Sodium as per the above steps.

Table 6

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

Filled the standard solution of Itopride Hydrochloride and Ilaprazole Sodium in the syringe and set the chromatographic conditions as per **Table 6**. Run the standard solutions of Itopride Hydrochloride and Ilaprazole Sodium for 10 mts.

2.4. Validation of Developed Method

Developed method was validated according to ICH guidelines.

Method validation of Itopride Hydrochloride and Ilaprazole Sodium Injection

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

Validation Parameters

A. Precision

- Reproducibility

Five solutions of the test were prepared as per the developed method filled the standard solutions of Itopride Hydrochloride and Ilaprazole 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 Ilaprazole Sodium

- **Standard Preparation of Itopride Hydrochloride**

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

- **Standard Solution of Ilaprazole Sodium**

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

Standard solution of Itopride Hydrochloride and Ilaprazole Sodium Mixture

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

Filled the standard solutions of Itopride Hydrochloride and ilaprazole 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 (Arjun Saraf)

B. 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 & Ilaprazole Sodium for accuracy (100%)

Weighed 44.12mg of Itopride Hydrochloride and 6.83 mg of Ilaprazole 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 & Ilaprazole 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 Ilaprazole 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)

2.5. Validation of Method for Assay of Tablets

1. Linearity and Range

Solutions of the concentration levels of about 100ppm, 80ppm, 60ppm, 40ppm, 20ppm of Itopride Hydrochloride and Ilaprazole Sodium was prepared.

- **Preparation of standard solution of Itopride Hydrochloride and Ilaprazole Sodium for Linearity (100ppm)**

Weighed 44.12g of Itopride Hydrochloride and 6.83 g of Ilaprazole 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 Ilaprazole 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 Ilaprazole 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 Ilaprazole 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 Ilaprazole 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

2. **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 6.83 mg of Ilaprazole Sodium in a volumetric flask and made up the solution to 50 ml with mobile phase.

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

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

3. **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 Ilaprazole Sodium**

Weighed 6.83 mg of Ilaprazole Sodium and dissolved in mobile phase and made up to 100ml with mobile phase

❖ **Standard solution Itopride Hydrochloride and Ilaprazole Sodium Mixture**

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

Run the chromatographic conditions as per the above table

RESULT

3.1. Linearity of Data

S.No	Concentration	Area of Itopride Hydrochloride	Area of Ilaprazole Sodium
1	20 ppm	22572	4285
2	40ppm	41903	8570
3	60ppm	62060	12855
4	80ppm	92959	17141
5	100ppm	11422	21391

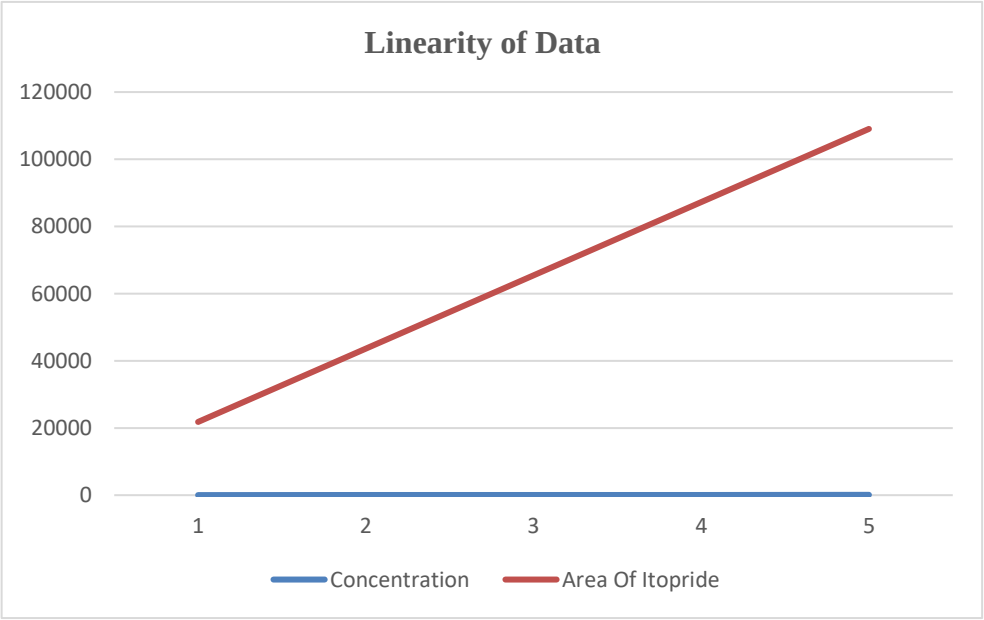
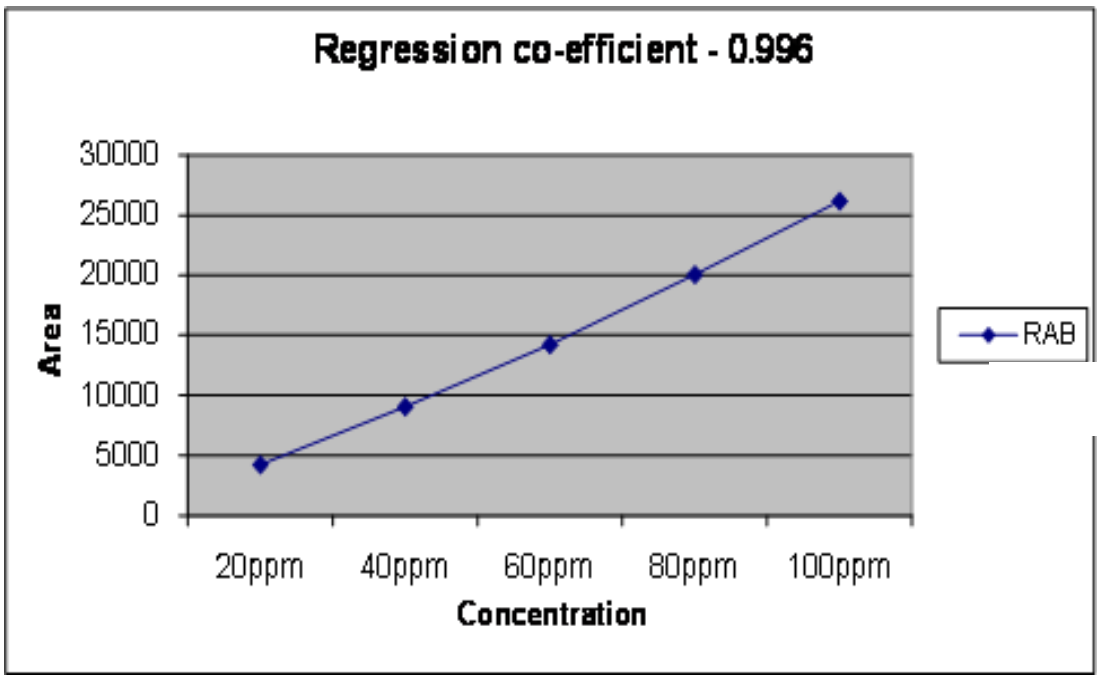


Figure: 4 Calibration curve between the area and concentration of Itopride Hydrochloride.

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 Ilaprazole Sodium.

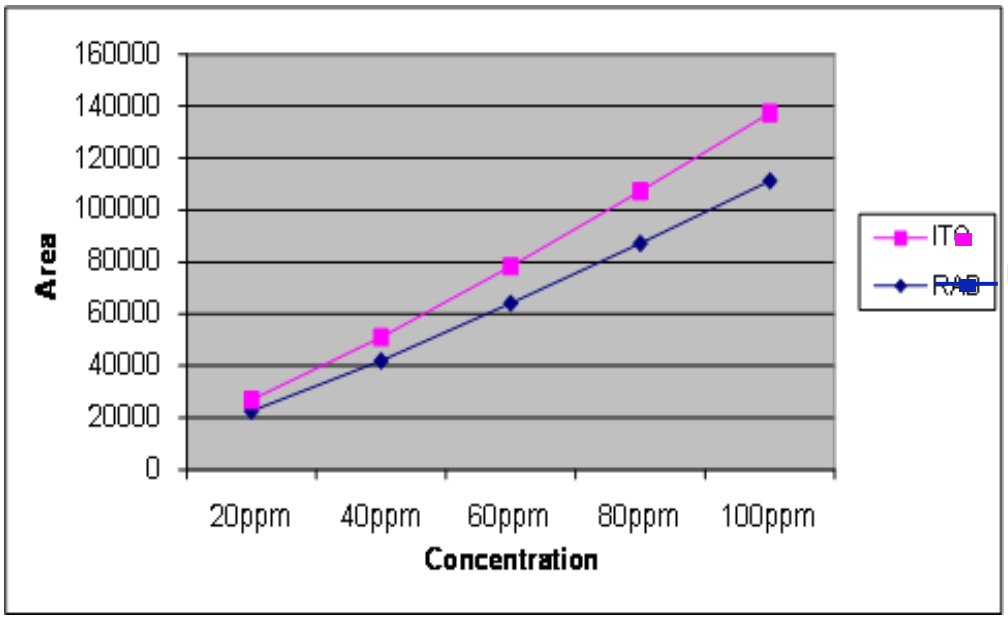
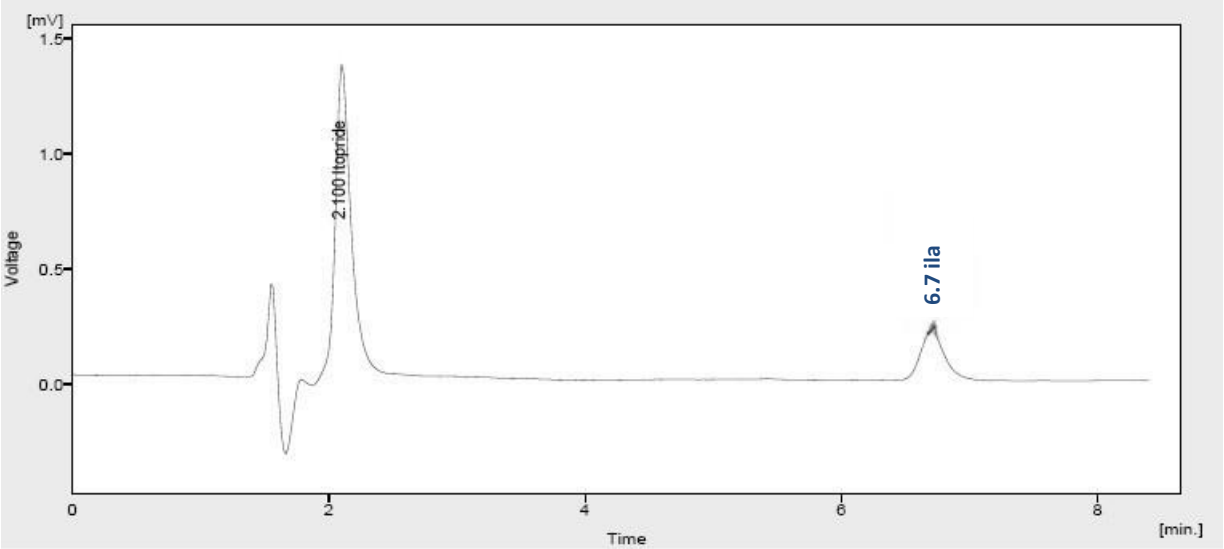


Figure:6 Calibration curve of Itopride hydrochloride and Ilaprazole Sodium

3.2. Specificity

Specificity of itopride hydrochloride and Ilaprazole Sodium

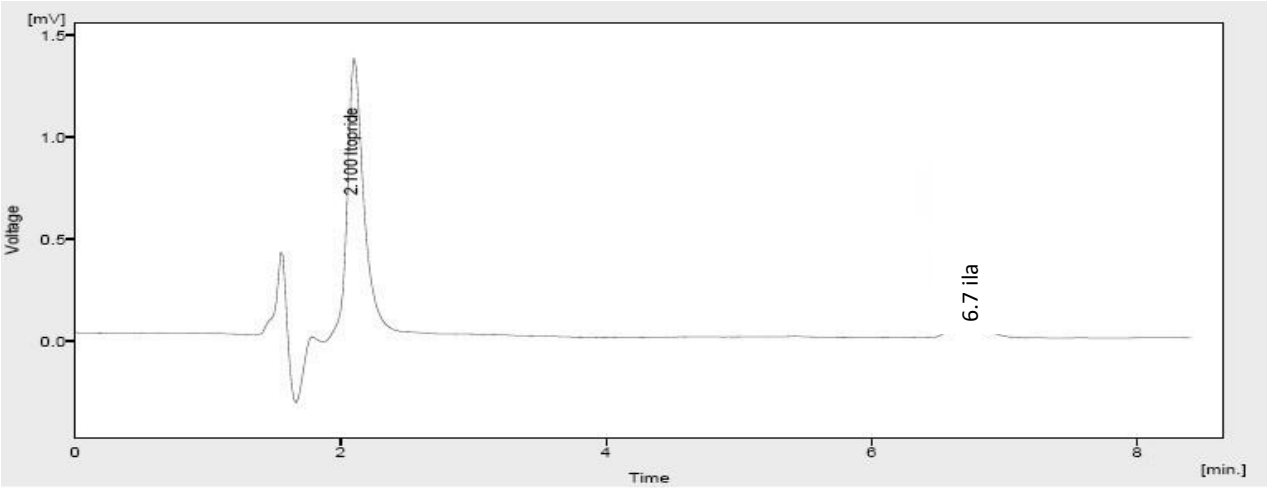


Result table (ESTD sample 80 µg Eth-wat-acet 29-01-09 sst)

S. No	Compound Name	Reten. Time (min)	Area (MV.S)	% Area
1	Itopride	2.140	8.982	81%
2	Ilaprazole	6.700	2.105	19%
Total			11.086	100

3.3. Accuracy

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



S.No.	Compond Name	Reten. Time (Min)	Area (MV.S)	Area %
1	Itopride	2.187	6.206	81.4
2	Ilaprazole	6.663	1.422	18.6
Total			7.628	100%

Result table (uncal sample 60µg eth-wat-ace 29-1-09)

3.3.1 Accuracy

Table :11.2 Accuracy 60% for Ilaprazole sodium

Accuracy 60%				
Drug Name	**S.No**	**Area of Ilaprazole**	**Total Amount**	**% Recovery**
Ilaprazole	**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**

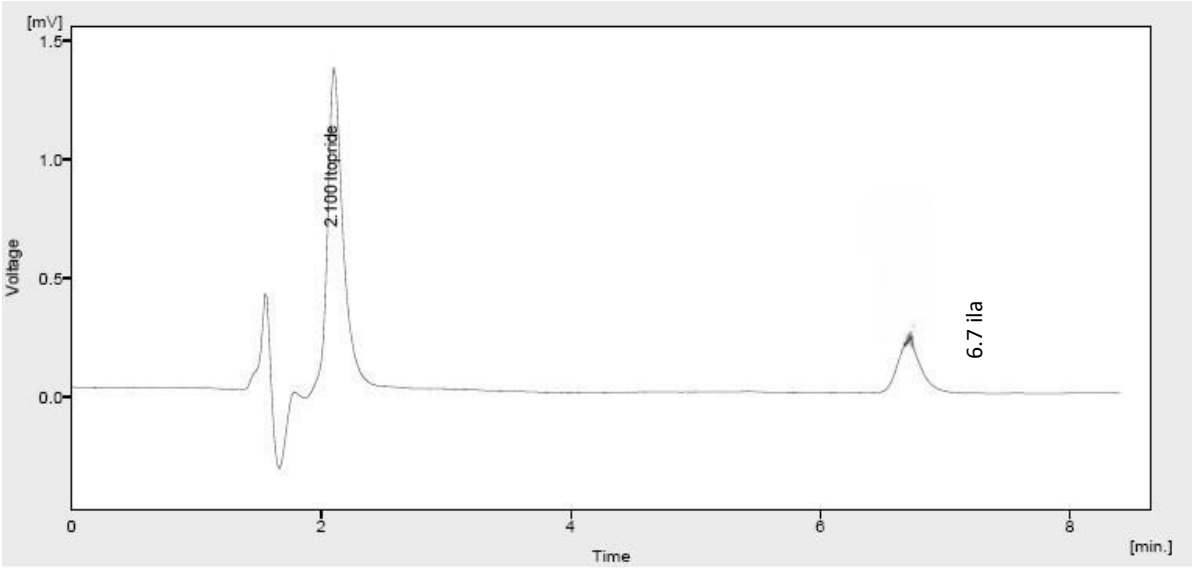
Table :11.3 Accuracy 60% for Itopride hydrochloride

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 Ilaprazole Sodium was 0.808%.The

R.S.D of Itopride Hydrochloride was 0.83 and for Ilaprazole Sodium was 0.803.

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



Result table (uncal sample 60µg eth-wat-ace 29-1-09)

S.No.	Compond Name	Reten. Time (Min)	Area (MV.S)	Area %
1	Itopride	2.187	6.206	81.4
2	Ilaprazole	6.663	1.422	18.6
Total			7.628	100%

Table :11.4 Accuracy for 80% level Ilaprazole sodium

Drug Name	**S.No.**	**Area of Ilaprazole**	**Total Amount**	**% of Recovery**
Ilaprazole	1	4245	36.54	100.52
	2	4260	36.51	100.48
	3	4250	36.43	102.1
Average				**101.033**
S.D				**± 0.755**
RSD				**± 0.75**

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 Ilaprazole sodium was 0.755

R.S.D of % amount of itopride hydrochloride was 0.730 and Ilaprazole sodium was 0.74.

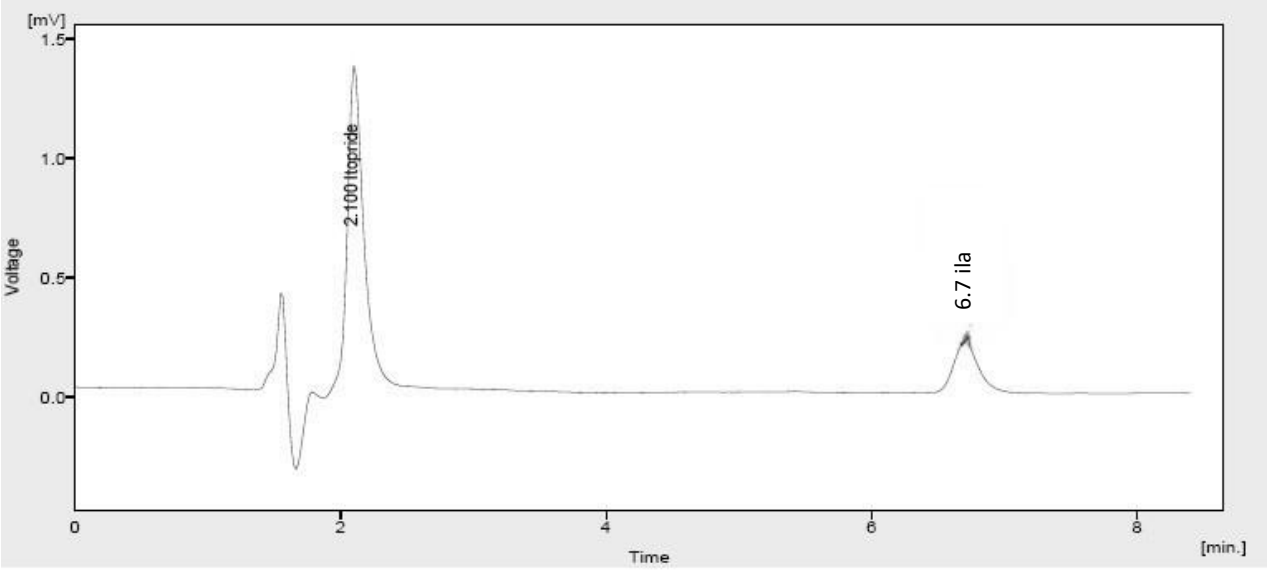


Figure: 9.3 Recovery for itopride hydrochloride and Ilaprazole Sodium at 100% Level

S.No.	Compond Name	Reten. Time (Min)	Area (MV.S)	Area %
1	Itopride	2.187	6.206	81.4
2	Ilaprazole	6.663	1.422	18.6
Total			7.628	100%

Result table (uncal sample 60µg eth-wat-ace 29-1-09)

Table No: 11.6 Accuracy for 100% level Ilaprazole Sodium

Accuracy 100%				
Drug name	**S.No**	**Area of Ilaprazole**	**Total amount**	**% Recovery**
Ilaprazole	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 Ilaprazole sodium was 0.62 .The R.S.D of the % amount of Itopride hydrochloride was 0.30 and % amount of Ilaprazole 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 Ilaprazole 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 Ilaprazole 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. N	**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**	**Ilaprazole Sodium**	
			0.998 (200-1000)ppm	**0.996**	
3	**Precision**	**R.S.D not more than 2%**	**Itopride HCL**	**Ilaprazole Sodium**	
			0.6	**0.5**	
4	**Accuracy**	**R.S.D for % recovery at each accuracy level not more than 2%**	**Accuracy**	**Itopride HCL**	**Ilaprazole Sodium**
			60%	**0.83**	**0.803**
			80%	**0.730**	**0.74**
			100%	**0.367**	**0.18**
		Recovery of Drug	**Avg% Recovery**	**Itopride hydrochloride**	**Ilaprazole 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**	**Ilaprazole Sodium**	
			0.077	**0.506**	

CONCLUSION

Validation is an ongoing and dynamic process that begins prior to an instrument being made available online and continues well beyond method creation and transfer.

An innovative and dependable high-performance liquid chromatography (HPLC) method was developed for the analysis of itopride hydrochloride and Ilaprazole sodium. Various chromatographic settings were used to establish the method. The chromatographic separation was enhanced by using a phenomenex C18 column, with the UV detection wavelength precisely adjusted to 285nm. The mobile phase included a mixture of methanol, water, and acetonitrile at a ratio of 50:40:10. The retention time of itopride hydrochloride and Ilaprazole sodium was determined to be 2.100 and 6.700, respectively.

The validation procedure for itopride hydrochloride and Ilaprazole sodium was developed following the criteria set out by the International Council for Harmonisation (ICH) and the United States Pharmacopeia (USP). The findings obtained were within the specified limitations outlined in the ICH and USP recommendations. Therefore, this approach may be used to concurrently analyze both medications on a consistent way.

An essential prerequisite for conducting chemical assessments is the validation of the analytical process.Technique validation is a rigorous procedure that entails doing many assessments to see whether an analytical technique accurately and reliably provides the intended explanation and is capable of producing valid and legally acceptable results. As per the regulations and suggestions, the method should provide valuable information that ensures the quality of the product. These findings are obtained by extensive testing of the substance. An extensively validated method must fulfill every criterion. In order to validate the analytical technique, it is essential to include the testing of the excipients and adhere to standardized testing conditions. These situations indicate that the validation of the analytical technique is particular to the product.

The Current Good Manufacturing Practices promote the incorporation of quality into the product, stressing that depending just on testing is inadequate to ensure product quality. Pharmaceutical products must adhere to stringent quality standards in order to guarantee their safe and efficient use. From an analytical standpoint, the analytical methodologies used to examine these objects should include quality characteristics. Validation ensures that these quality requirements are included into the method. Validating analytical techniques is an essential but time-consuming activity for most analytical laboratories. Nevertheless, it offers a favorable cost-benefit ratio, minimizes monotonous repetitions, and eventually enhances time allocation. Before the first deployment of the analytical procedures in regular analysis, it is essential to verify or revalidate them. This validation procedure should also be carried out when the techniques are moved from one laboratory to another. Furthermore, validation should be performed anytime there are modifications in the conditions or method parameters that beyond the initial scope of the method. Chromatographic technology is a well-respected method for conducting tests.

Chromatography is a method that separates solutes by taking advantage of variations in their movement caused by differences in absorption, partition, solubility, vapor pressure, molecular size, or ionic charge density. The process involves a system that consists of two or more mobile phases, with one phase experiencing continuous movement in a specified direction. Liquid chromatography is a chromatographic method where the mobile phase consists of a liquid. High performance liquid chromatography (HPLC) is a contemporary technique of liquid chromatography that employs columns filled with diminutive particles. The mobile phase is propelled through the columns at high pressure. The separation of components is contingent upon the degree of interaction between the solute component and the stationary phase. The component exhibiting the least affinity for the stationary phase will be the first one to be liberated. The pharmaceutical industry is increasingly using high-performance liquid chromatography (HPLC) as a precise analytical technique. HPLC procedures provide expedited analysis, enhanced sensitivity, superior resolution, straightforward sample retrieval, and accurate and consistent outcomes. Validation of analytical procedures is a necessary obligation mandated by legislation and is crucial for non-compendial goods. The ICH guidelines, namely Q2 (R1), provide the criteria for verifying analytical processes.

The validation of the analytical techniques for the product encompasses several aspects, such as identification, assay, and dissolution, among others

REFERENCES

1. Agyilirah G.A., Green M., and Ducret R. Evaluation of gastric retention properties of across linked polymer coated tablets versus those of non-disintegrating tablets. *International journal of pharmaceutics***.** 1991;75: p241-247.
2. Hoffman F., Pressman J.H., and Code C.F. Controlled entry of orally administered drugs, physiological considerations. *Drug development and industrial pharmacy*. 1983;9: p1077-1083.
3. Ganji Ashok, V. Uma Masheshwara Rao, K. Mahalakshmi, B. Ajay Kumar , Formulation and evaluation of sustained release Itopride hydrochloride. *International research journal of pharmacy*. 2013;4(10): p70-74.
4. Patel S.S., Ray S, Thakur R.S. Formulation and evaluation of floating drug delivery system containing claithromycin for a helicobacter pylori, *Acta Polonaise Drug Research*. 2006; 63(1): 53-61.
5. Dash A.K., Cudworth G.C. Therapeutic applications of implantable drug delivery systems. *Journal of pharmacological and toxicological methods*. 1998;40(1): p1-12.

6. Bhupendra G.Prajapati, Niklesh Patel, Hitesh K. Patel. Sustained release Itopride hydrochloride matrix tablet. *Asian journal of pharmaceutical research and health care*. 2010;2(1): p75-83.
7. Hokuriko Seiyaku Co. Ltd. Data on file. Revised: April 2001 (2nd version of new form).
8. Ambrish Thosar, Rahul M. Itopride: an updated review of its pharmacological properties and use as a prokinetic. *International Journal of Institutional Pharmacy and Life Sciences*, 2013;3(2): p13-19.
9. Gupta S, Kapoor V, Kapoor B. Itopride, a novel prokinetic agent. *Journal of medical education and research*. 2004;6(2): p106-108.
10. Yong Sung Kim, Tae Hyeon Kim, Chang Soo Choi, Young Woo Kim, Geom Seog Seo, Suck Chei Choi. Effect of Itopride, a new prokinetic, in patients with mild GERD: a pilot study . *World journal of gastroenterology*. 2005;11(27): p4210-4212.
11. Taravat G, Arezoo S, Khosro A, Fatemeh P, Ali N. A drug release study from HPMC matrices using QSPR modeling. *Journal of pharmaceutical sciences*. 2007; 96(12): p3334-3351.
    Devu D , Shalini K.B, Vineela Chadalavada. Development

12. Devu D , Shalini K.B, Vineela Chadalavada. Development and validation of Ilaprazole in bulk and pharmaceutical dosage form by UV spectroscopic method. *International Journal of Advances in Pharmaceutical Analysis. Vol. 2014 ;4(4) : p130-133*
13. J. Jayasree1, S. Sivaneswari, G. Hemalatha, N. Preethi, B. Mounika, S.Vasudeva Murthy. Role of various natural, synthetic and semi-synthetic polymers on drug release kinetics of losartan potassium oral controlled release tablets. *International Journal of Pharmaceutical Investigation*. 2014;4(4): p183-188.
14. Soad A. Yehia, Ahmed H. Elshafeey, Aliaa ElMeshad, Hamoud A. Al Bialey. A pharmaceutical study on different approaches for itopride hydrochloride sustainment: in-vitro and in-vivo evaluation. *Journal of Chemical and Pharmaceutical Research*. 2012;4(2): p1398-1412.
15. Tamam A Hassan, Aly A. Abdel-Rahman, Sayed I. Abdel-Rahman, Jelan A. Abdel-Aleem. Formulation of sustained release itopride hydrochloride matrix tablets using direct compression technique. *Unique journal of pharmaceutical and biological sciences*. 2013;1(3): p72-81

16. Gupta Amit M, Belgamwar Aarti V, Wake Prashant S, Rathi Trivesh P, Mundhada D. R. Design and development of hydrodynamically balanced tablet of Itopride hydrochloride. *Journal of chemical and pharmaceutical research*. 2011;3(6): p856-864 .
17. Ashok G, V. Uma Masheshwara Rao, K. Mahalakshmi, B. Ajay Kumar. Formulation and evaluation of sustained release itopride hydrochloride. *International research journal of pharmacy*. 2013;4(10): p70-74.
18. Bose Anirdeep, Wong Wai T, Singh N. Formulation, development and optimization of sustained release matrix tablet of Itopride hydrochloride by surface response methodology and it's evaluation of release kinetics. *Saudi pharmaceutical journal*. 2012; 21: p201-203.
19. Ratnaparkhi Mukesh P, Pawar Sham A, Somvanshi Fattesingh U, Chaudhari Shilpa P. Formulation and development of floating drug delivery of Itopride HCl. *Journal of Drug Delivery & Therapeutics*. 2013;3(4): p222-228.

    Ahmed M Sayed, Ali Amhed Adel, Hassan A Omiya. Design and in vitro/in vivo evaluation of sustainedrelease floating tablets of Itopride Hydrochloride. *Drug Design, Development and Therapy*. 2016;10: p4061-4071.
20. Prajapati G B, Niklesh Patel, Hitesh K. Patel. Sustained release Itopride Hydrochloride matrix tablets. *Asian journal of pharmaceutical research and health care*. 2010;2(1): p75-83.
21. Pankaj Chhipa, Anil M.Pethe, Satish Upadhyay, Avinash Tekade. Formulation optimization of sustained release pellets of Itopride Hydrochloride using different polymers. *Journal of pharmacy research*. 2009;2(8): p1404-1408.
22. P. Sambha Siva Rao, G. Raveendra Babu, T. Kala Praveen, P. Sri Lakshmi Surekha and M. Chandra Shekhar. Formulation & evaluation of Itopride HCl sustained release pellets. *International journal of pharmaceutical sciences and research*. 2014;5(5): p2074- 2083
23. H. D. Karen, I. S. Anand, B. N. Patel, C. N. Patel. Development and evaluation of floating drug delivery system of Itopride hydrochloride. *American journal of pharmtech research*. 2012;2(2): p602-608.
24. Afaque Raza Mehboob Ansari, Ravikant Yashwantrao Patil, Vijaykumar Jaychand Khandade, Pratik Nilkanth Yewale. Design and evaluation of gastroretentive floating drug delivery system of Itopride hydrochloride. *World journal of pharmacy and pharmaceutical sciences*. 2015;4(7): p823-834.
25. Indian pharmacopeia, 2014; p760.

26. Santos U.Zate, Paresh I. Kothawade, Jitendra W Gajbe, Anantwar S. Pramod, Sahebrao S. Boraste. Spectrophotometric method development and validation of Itopride Hydrochloride in bulk and dosage form. *International Journal of Drug Delivery.* 2010;2:p340-343.
27. Guguloth M, Bomma R, Veerabrahma K. Development of sustained release floating drug delivery system for norfloxacin: in vitro and in vivo evaluation. *Journal of Pharmaceutical Science and Technology*. 2011;65(3): p198-206.
28. Tadros MI. Controlled-release effervescent floating matrix tablets of ciprofloxacin hydrochloride: development, optimization and in vitro-in vivo evaluation in healthy human volunteers. *European journal of pharmaceutics and biopharmaceutics*. 2010;74(2): p332–339.
29. Ganji Ashok, V. Uma Masheshwara Rao, K. Mahalakshmi, B. Ajay Kumar. Formulation and evaluation of sustained release Itopride hydrochloride. *International research journal of pharmacy* 2013;4(10):p70-73.