"DRUG AND EXCIPIENT COMPATIBILITY STUDY OF SELECTED PROTON PUMP INHIBITORS"

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in Partial Fulfillment for the Award of the Degree of

MASTER OF PHARMACY

IN

PHARMACEUTICAL ANALYSIS

BY

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May 2015

CERTIFICATE

This is to certify that the dissertation work entitled "Drug and excipient compatibility study of selected proton pump inhibitors" submitted by Ms. Jinal Shah with Regn. No. (13MPH304) in partial fulfillment for the award of Master of Pharmacy in "Pharmaceutical Analysis" is a bonafide research work carried out by the candidate at the Department of Pharmaceutical analysis, Institute of Pharmacy, Nirma University under our guidance. This work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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DECLARATION

I hereby declare that the dissertation entitled "Drug and excipient compatibility study of selected proton pump inhibitors", is based on the original work carried out by me under the guidance of Dr.Priti J. Mehta, Head of the Department of Pharmaceutical Analysis, Institute of Pharmacy, Nirma University. I also affirm that this work is original and has not been submitted in part or full for any other degree or diploma to this or any other university or institution.

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Date: May, 2015

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LIST OF ABBREVIATIONS

ABBREVIATION	FULL FORM
°C	Degree centigrade
±	Plus or Minus
<	Less than
>	Greater than
λ	Lambda
%	Percentage
µg	Microgram
µL	Microliter
Abs.	Absorbance
API	Active Pharmaceutical Ingredient
AR	Analytical Reagent
ESO	Esomeprazole magnesium
PAN	Pantoprazole sodium
PVP	Polyvinylpyrrolidone
CAS No.	Chemical Abstract Service Number
Cm	Centimeter
Conc.	Concentration
Wt.	Weight
Std	Standatd
IP	Indian pharmacopoeia
Fig.	Figure
UV/VIS	Ulraviolet/Visible
FT-IR	Fourier Transform Infrared spectrometry

g	Gram
H	Hour
HPLC	High Performance Liquid Chromatography
DSC	Differential Scanning Calorimetry
ICH	International Conference on Harmonization
IUPAC	International Union of Pure and Applied Chemistry
SOP	Standard Operating Procedure
L	Litre
Imp	Impurity
RSD	Relative Standard Deviation
SD	Standard Deviation
RH	Relative Humidity
Ref. no	Reference number
w/v	Weight by Volume
v/v	Volume by Volume
min	Minute
pKa	Partition coefficient
R^2	Correlation coefficient

ABSTRACT

The study of drug–excipient's compatibility represents an important phase in the preformulation stage for the development of all dosage forms. Potential physical and chemical interactions between drugs and excipients can affect the chemical nature, the stability and bioavailability of drugs and their therapeutic efficacy and safety.

The class of drugs selected for drug and excipient compatibility study is Proton Pump Inhibitors (PPIs) these agents selectively and irrevocably inhibit the gastric hydrogen/potassium adenosine tri phosphatase (H^+/K^+-exchanging ATPase), part of the 'proton pump' that makes the final step in the acid secretory process. They inhibit both basal and stimulated secretion of gastric acid, exclusively of the nature of parietal cell stimulation.

Esomeprazole magnesium and pantoprazole sodium were selected to check drug and excipient compatibility study of Proton Pump Inhibitors (PPIs). According to literature review PPIs get degraded in very mild oxidative stress condition another conclusion was found that polyvinylpyrrolidone contains organic peroxide as an impurity in it which is more reactive than hydrogen peroxide. Although PVP is widely used as a binder in the tablet formulation of PPIs.

The aim of the present study was to identify the compatibility of selected proton pump inhibitors with different grades of Polyvinylpyrrolidone i.e. PVP K30 and PVP K90.

Accelerated stability study was carried out in accordance with ICH guidelines for drug alone as well as binary mixtures of drug and excipients. Impurities generated were well separated by the chromatographic conditions of esomeprazole magnesium and pantoprazole sodium related substances given in Indian Pharmacopoeia. Compatibility of drug and excipient was checked by two analytical methods High Performace Liquid chromatography (HPLC) and Differential Scanning Calorimetry (DSC). Results obtained were supporting the hypothesis. Impurities generated was beyond the identification threshold specified in ICH Q3A (R1) so it can further be qualified and characterized by suitable analytical techniques.

Key words: Esomeprazole Magnesium, Pantoprazole Sodium, PVP K30, PVP K90, High Performance Liquid Chromatography, Differential Scanning Calorimetry.

CHAPTER 1

INTRODUCTION

1. Introduction

1.1 Introduction to Preformulation study [1, 2, 3]

Preformulation is subdivision of Pharmaceutical science that utilizes biopharmaceutical principles in the determination of physicochemical properties of the drug substance. Preceding to the development of any dosage form of new drug it is essential that definite fundamental physical & chemical properties of drug product are determined.

Definition:

"Investigation of physico-chemical properties of the new drug substances that could affect drug performance and development of an efficacious and stable dosage form".

Preformulation is the first step in the rational development of an active pharmaceutical ingredients (API). It is an investigation of the physico chemical properties of the drug substances, alone and when combined with excipients. Assessment of possible incompatibilities between the drug and different excipients is a core part of preformulation. The formulation of a drug substance often involves it being blended with different excipients to improve manufacturing of dosage form, and to maximize the product's ability to administer the drug dose effectively and accurately. Excipients are known to facilitate administration and moderate release of the active component. They can also stabilize dosage form against degradation from the environment. Most excipients have no direct pharmacological action but they can impart useful properties to the formulation. Excipients can also give rise to inadvertent and/or unintended effects such as increased degradation of the drug. Physical and chemical interactions between drugs and excipients can affect the chemical nature, the stability and bioavailability of drug products, and consequently, their therapeutic efficacy and safety.

1.1.1 Objectives of preformulation study:

- To develop the elegant dosage forms (stable, effective & safe).
- It is important to have an understanding of the physical description of a drug substance before dosage form development.

- It is 1st step in rational development of a dosage form of a drug substances before dosage form development.
- It generates useful information to the formulator to design an optimum drug delivery system.

1.1.2 Goals of preformulation study:

To establish the necessary physicochemical parameters of new drug substances.

To determine kinetic rate profile of dosage form.

To establish physical characteristics. To establish compatibility with common excipients.

1.1.3 Parameters measured in preformulation study:

Bulk characterization:

1. Crystallinity & polymorphism,
2. Hygroscopicity
3. Fine particle characterization,
4. Powder flow properties.

Solubility analysis:

1. Ionization constant –Pka
2. pH solubility profile,
3. Common ion effect-Ksp ,
4. Solubilization
5. Dissolution,
6. Partition co-efficient

Stability analysis:

1. Solution stability,
2. pH rate profile,
3. Solid state stability,
4. Bulk stability,

5. Stability in toxicology formulation
6. Drug and excipient compatibility study

1.1.4 Regulatory perspectives of stability study:

After the initiation of stability testing guidelines by international conference on harmonization (ICH) there has been remarkable progress in implementation of stability studies coss the globe. Now a day, each regulatory authority is giving emphasis that pharmaceutical formulations should be assayed or its purity by use of a SIAM.

Various guidelines are available on designing and as well as conducting stability studies of pharmaceuticals. In the recent years WHO has given a guidelines on the design an conduct a stability testing of pharmaceutical products.

1. Q1A (R2) Stability Testing of New Drug Substances and Products

2. Q1B Photostability Testing of New Drug Substances and Products

3. Q1C Stability Testing for New Dosage Forms

4. Q1D Bracketing and Matrixing Designs for Stability Testing of New Drug Substances and Products

5. Q3A Impurities in New Drug Substances

6. Q3B(R) Impurities in New Drug Products

7. Q3C Impurities: Residual Solvent

8. Q6A International Conference on Harmonization; Guidance on Q6A Specifications: Test Procedures and Acceptance Criteria for New Drug Substances and New Drug Products: Chemical Substances

9. Q7A Good Manufacturing Practice Guidance for Active Pharmaceutical Ingredients

1.2 Introduction to Drug and excipient compatibility study [1, 3, 4]

Study of drug - excipient compatibility is a significant process in development stage of dosage forms. Incompatibility amongst drugs and excipients alter drug stability and bioavailability & thereby affect their safety and efficacy. Dosage form is a pharmaceutical drug delivery system, which is a combination of drug(s) and non-drug components called as excipients. Drug is a chemical substance acquired from either natural, synthetic or semi synthetic source, which is used for the treatment, cure, prevention or mitigation of a disease or disorder in human beings or animals. Excipients are nondrug components which serve specific purposes like shape, stability, solubility, elegance, palatability etc. of a dosage form. These are also called as adjuvants, additives or pharmaceutical aids. Potential physical and chemical interactions between drugs and excipients can affect the chemical nature, stability and bioavailability of drugs and, subsequently, their therapeutic efficacy and safety. Therefore, the evaluation of drug-excipient compatibility is an essential aspect of any preformulation study.

1.2.1 Importance of Drug and Excipient Compatibility Study:

Stability of the dosage form can be maximized. Any physical or chemical interaction between drug and excipient can affect bioavailability and stability of drug.

It helps to avoid the surprise problems. By performing DECS we can identify the possible reaction before formulating final dosage form.

Drug discovery can emerge only new chemical entity. It becomes drug product after formulation and processing with excipients. By using DECS data we can select the appropriate type of the excipient with the chemical entities emerging in drug discovery plans.

DECS data is essential for IND (investigational new drug) submission. Now, USFDA has made it essential to submit DECS data for any new emerging formulation before its approval.

1.2.2 Types of Incompatibility:

A. Physical incompatibility: It involves the alteration in the physical form of the formulation which involves colour changes, liquefaction, phase separation or immiscibility.

B. Chemical incompatibility: It involves unwanted change in formulation which is due to formation of new chemical compound with undesirable activity or any formulation undergoes hydrolysis, oxidation, reduction, precipitation, decarboxylation, and racemization.

C. Therapeutic incompatibility: It is type of in vivo compatibility. It involves change in therapeutic response of the formulation which is undesirable to patient as well as physician.

1.2.3 Analytical techniques used to detect Drug and Excipient Compatibility:

1) Thermal methods of analysis

I. DSC- Differential Scanning Calorimetry

II. DTA- Differential Thermal Analysis

2) Accelerated Stability Study

3) FT-IR Spectroscopy

4) DRS-Diffuse Reflectance Spectroscopy

5) Chromatography

I. SIC-Self Interactive Chromatography

II. TLC-Thin Layer Chromatography

III. HPLC-High Pressure Liquid Chromatography

6) Miscellaneous

I. Radiolabelled Techniques

II. Vapour Pressure Osmometry

III. Flourescence Spectroscopy

1.3 Introduction to methods

1.3.1 Introduction to High Performance Liquid Chromatography (HPLC) [5]

HPLC is a chromatographic technique that is useful for separating ions and molecules that are dissolved in a solvent.

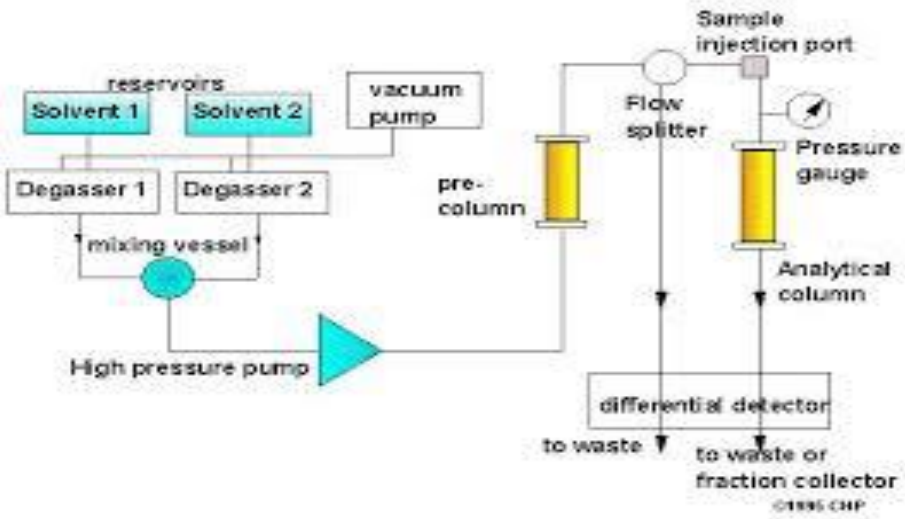


Fig 1.1: Schematic diagram of High Performance Liquid Chromatography (HPLC)

a) Mechanism:

Retention by interaction of nonpolar hydrocarbon chain of stationary phases with nonpolar parts of the sample molecules. HPLC instrument consists of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column.

b) Mobile phase:

Commonly used mobile phase in HPLC

- Methanol (CH_3OH)
- Acetonitrile (ACN)
- Water (H_2O)
- Buffer
- Tetrahydrofuran (THF)

It is very essential to degas the solvents which are used to get stable baseline, enhance the sensitivity of the detector and for stable pump operation.

c) Types of the Pumps:

1) Syringe pumps

2) Reciprocating pump

- Single piston reciprocating pump
- Dual piston reciprocating pump
- Reciprocating diaphragm pump

3) Pneumatic pump

- Direct pressure pump
- Amplifier pump

d) HPLC Columns: A stable and high performance column is essential requisite for rugged, reproducible method. The various stationary phases used in HPLC columns are mentioned in table 1.2

Table: 1.1 Stationary phases used in HPLC column

Group	Type	Particle diameter (µm)
Amino	Normal	5, 10
Nitrile Normal	Normal	5, 10
Amine and Nitrile	Normal	40
Octyl C_8	Reverse phase	3, 4, 5, 10
Octadecyl C_{18}	Reverse phase	4, 5, 10, 40

e) Sample Injection System: Injection systems, includes manual injector, standard auto sampler, high-performance auto sampler, high-performance auto sampler SL plus, micro

well-plate auto sampler, preparative auto sampler and dual loop auto sampler as well as the thermostat. The figure 1.2 shows load and inject position of the the injection system.

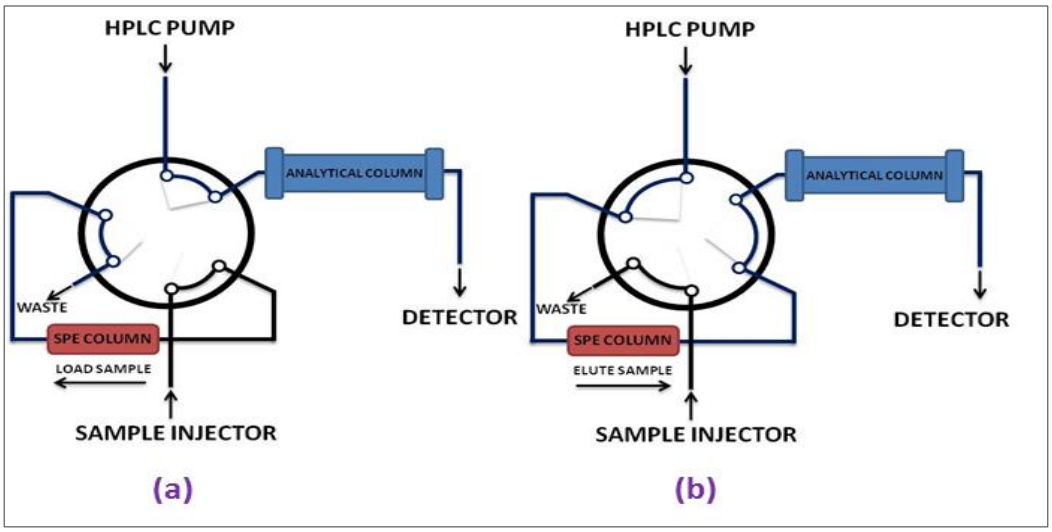


Figure: 1.2 Sample Injection Systems in HPLC

f) Detectors: It is considered as the eye of LC because it measures the separated components. Two types of detectors:

1) Bulk property detectors: They are based on some bulk properties of eluent, such as RI and are not suitable for gradient elution. They are usually less sensitive than solute property detectors.

2) Solute property detectors: Performed by measuring some types of physical or chemical property that is specific to solute only and so can be used with gradient elution.

1.3.2 Introduction to Differential Scanning Calorimetry [5]

DSC is a thermal method whereby the energy necessary to establish a zero temperature difference between a substance and a reference material is recorded as a function of temperature or time when both (substance and reference material) are heated or cooled at a predetermined rate. The DSC curve is recorded with the abscissa indicating the transition temperature. The area of peak measures the total energy transfer to or from the sample.

Principle

Sample and an inert reference heated separately , with the power supply to the sample heater variable so that the temperature difference can be maintained at zero even when endothermic or exothermic changes occur. The difference in power supplied to the two heaters is monitored as the analytical signal.

Instrumentation

This instrument works on the temperature control of two similar specimen holders in the specimen holder's assembly. In its left-half, there is a circuit for differential temperature control while in its right-half there is a circuit for average temperature control.

In the average temperature control circuit, an electrical signal, which is proportional to the dialled temperature of the sample and reference holders, is generated through the programmer.

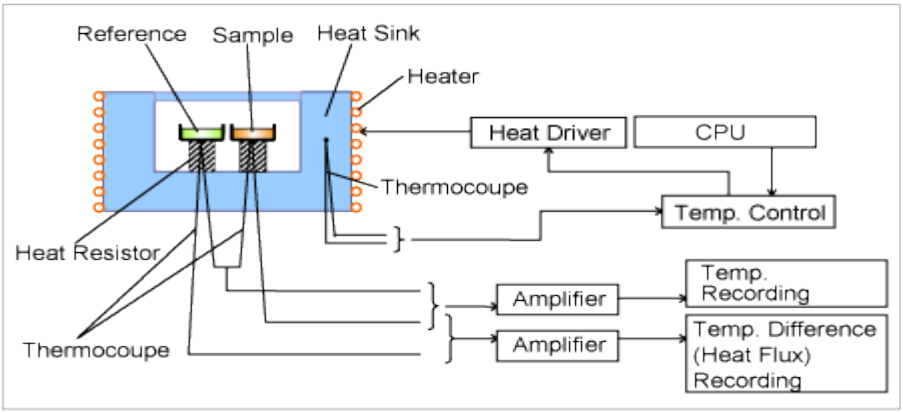


Fig 1.3 Schematic diagram of Differential Scanning Calorimetry (DSC)

In the differential temperature control circuit signals representing the temperature of the sample and reference are compared. If no reaction is taking place in the sample, the differential power input to the sample and reference heater is almost zero. However if the reaction is taking place (H is not zero) a differential power is fed to the heaters. A signal proportional to this differential power along with the sign is transmitted to the recorder pen. The integral of the peak so obtained gives the integral energy change of the sample.

Sample

By DSC, one may analyse liquids and solids in the form of powder, crystals, granules or foil.

Reference material

An inert material like alumina is generally used. An empty pan with lid is also used.

Environment

Generally, DSC measurements are carried out in gas environment. The optimum rate recommended for flowing gas is 20-30 ml/min.

Applications

DSC can be used for all applications of conventional DTA. The advantages lies in the small size of the sample to be used. This technique has found many applications in industry as well.

DSC essentially studies the same thermal phenomena as DTA, albeit using a different principle. Thus DTA and DSC provide very much the same information and their applications are similar. Reference back to the section on the application of DTA will suffice to indicate the scope of DSC. Some differences in the quality of the information obtained sometimes exist however, leading to a preference for one technique over the order for particular purpose.

1.4 Introduction to Proton Pump Inhibitors (PPIs) and Excipient

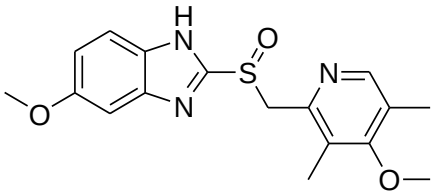
1.4.1 Introduction to Proton Pump Inhibitors (PPIs) [6]

Proton pump inhibitors have inclined the management of acid-peptic conditions dramatically over the last 10 years. Three of these mediators are now widely available esomeprazole (available since 1989), lansoprazole (1995), and pantoprazole (1997). Rabeprazole is now also fetching in some countries.

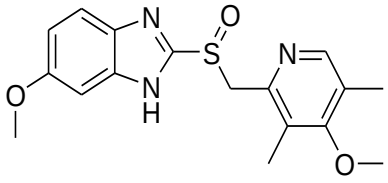
These agents selectively and irrevocably inhibit the gastric hydrogen/potassium adenosine triphosphatase (H^+/K^+-exchanging ATPase), part of the 'proton pump' that makes the final step in the acid secretory process. They inhibit both basal and stimulated secretion of gastric acid, exclusively of the nature of parietal cell stimulation. Clinical uses contain the treatment of peptic ulcer disease, gastro-oesophageal reflux disease, Barrett's oesophagus, Zollinger–Ellison Syndrome, and the eradication of *Helicobacter pylori* as part of mixture regimens.

Structure and mechanism of action of proton pump inhibitors:

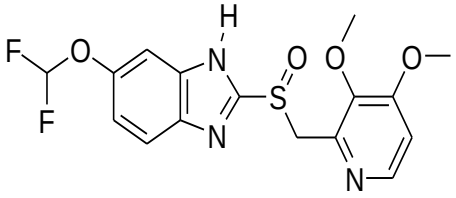
Proton pump inhibitors are all substituted benzimidazole derivatives. They function as pro-drugs, accumulating within the parietal cell canaliculus where acid-catalysed change of the pro-drug to a tetracyclic planar sulphenamide occurs. The sulphenamide then binds covalently to cysteine groups on the proton pump to cause inhibition of gastric acid secretion. Acid production by the proton pump can generally only be reinstated through endogenous synthesis of the H^+K^+-exchanging ATPase, which has a half-life of production of approximately 50 h.



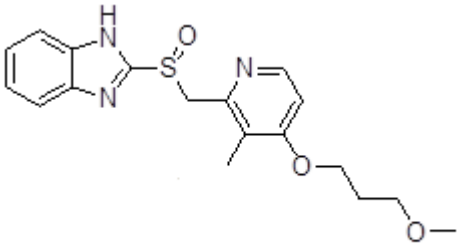
Esomeprazole



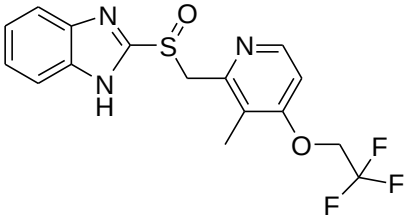
Omeprazole



Pantoprazole



Rabeprazole



Lansoprazole

Fig1.4 Structures of Proton Pump Inhibitors (PPIs)

Proton pump inhibitors are weak bases and accumulate within the acidic parietal cell canaliculus is dependent on the pH gradient and pKa of each agent. The pH of the parietal cell canaliculus is 0.8, whereas that of other acidic compartments such as lysosomes is 4.5–5. The important site of protonation for accumulation of these drugs is the pyridine. All four of the proton pump inhibitors have a pyridine pKa of less than 4.5, which should favour selectivity of the drugs for the parietal cell. The pKa of the pantoprazole pyridine N (3.96) is slightly lower than that of omeprazole (4.13) or lansoprazole (4.01), although this difference has not been shown to be of direct clinical significance. The pKa of the benzimidazole rings are all much lower. The drugs all have similar high levels of activation at a very low pH, whereas in the near neutral pH range of 4–6, pantoprazole is more chemically stable and less activated, and rabeprazole is less stable than the other two drugs. The conversion rate from the pro-drug to the active sulphenamide is slower for pantoprazole.

Acid inhibition is not necessarily maximal after the first dose. Acid catalysed activation of the drug is necessary, so only activated parietal cells will be inhibited, whereas resting parietal cells (approximately 25% of the cell mass) will escape initial inhibition. Both pantoprazole and omeprazole display an increase in acid inhibitory effect over several days of repeated administration, whereas acid inhibition with lansoprazole is maximal after the first dose.

The mechanism of action is similar for all of the proton pump inhibitors, and they all bind to one common distinct site on the alpha subunit of the proton pump. Pantoprazole may also bind to the adjacent cysteine 822, and omeprazole to cysteine 892. Lansoprazole and rabeprazole both bind to additional sites at cysteine 892 and cysteine 321. Pantoprazole has greater selectivity for the cysteine 813/822 sites, but the clinical significance of these differences is unclear.

The drugs are all acid-labile, so when administered orally they must be formulated in an enteric coating to protect them from rapid degradation in the stomach. They are rapidly absorbed in the duodenum.

Uses of Proton Pump Inhibitors (PPIs)

Gastric ulcer
Duodenal ulcers
Helicobacter pylori Eradication
Non ulcer Dyspepsia

1.4.2 Introduction to Excipient [7]

Among synthetic excipients polyvinylpyrrolidone, marketed under the brand name Kollidon®, is one of the most important substances in the pharmaceutical and cosmeticindustries. Starting from the soluble Kollidon® grades which were synthesized by W.Repe in 1939, a number of products followed, including insoluble grades copolymerisates and sustained release preparations for numerous applications. The insoluble grades Kollidon®CL are prepared using a physical cross linking process as popcorn polymers of vinylpyrrolidone. Kollidon®VA is a water soluble copolymerisates vinyl pyrrolidone and vinyl acetates and mainly used as a binderin purposes, a mixture of polyvinyl acetatesand povidone in a ratio of 8:2 is available under the name Kollidon®SR.

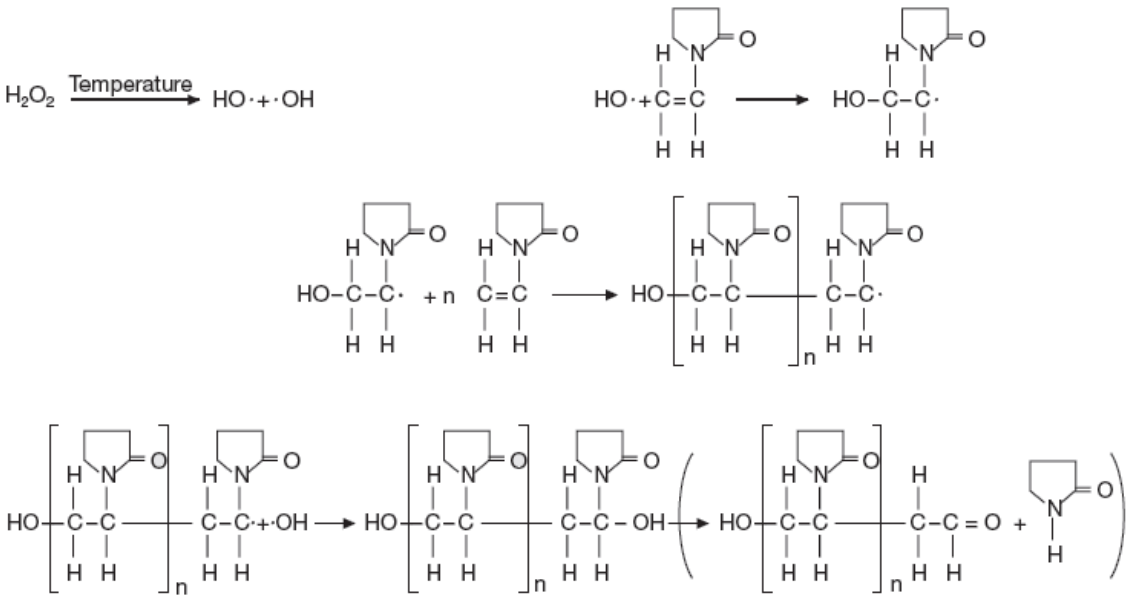


Fig 1.5 the reaction mechanism for the radical polymerization of N-vinylpyrrolidone in water

The mechanism for terminating the polymerization reaction makes it possible to produce soluble polyvinylpyrrolidone of almost any molecular weight.

Apart from the method of production in water shown in figure 1.4 it is also possible to conduct the polymerization in an organic solvent e.g. 2-propanol this technology is used today in the production of low molecular weight polyvinylpyrrolidone for injectable.

The low and medium molecular weight grades of soluble polyvinylpyrrolidone are spray dried to produce the pharmaceutical grade Kollidon® powders, while the high molecular weight grade is roller dried.

Today soluble polyvinylpyrrolidone is one of the most versatile and widely used pharmaceutical auxiliaries.

It is also used in the production of one of the most important topical disinfectant, PVP-Iodine.

Table 1.2 Specifications of the soluble Kollidon® grades

	Kollidon® 12 PF	Kollidon® 17 PF	Kollidon® 25 PF	Kollidon® 30 PF	Kollidon® 90 PF
Clarity and colour (10%) in water	Clear and light	Clear and light	Clear and light	Clear and light	Clear and light
K- value	10.2-13.8	15.3-18	22.5-27	27-32.4	81-96.3
Nitrogen content (%)	11.5-12.8	12.0-12.8	12.0-12.8	12.0-12.8	12.0-12.8
Water (K. Fischer %)	≤ 5.0	≤ 5.0	≤ 5.0	≤ 5.0	≤ 5.0
pH (5% in water)	3.0-5.0	3.0-5.0	3.0-5.0	3.0-5.0	4.0-7.0
Vinylpyrrolidine (ppm)	≤ 5.0	≤ 5.0	≤ 5.0	≤ 10.0	≤ 10.0

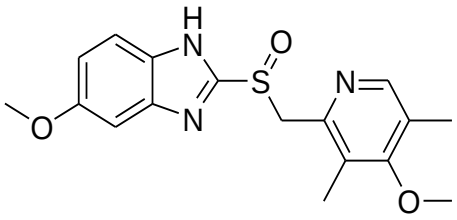
Sulphated ash (%)	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0
Aldehyde (%)	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05	≤ 0.05
Heavy metals (ppm)	≤ 10	≤ 10	≤ 10	≤ 10	≤ 10
Hydrazine (ppm)	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1
Peroxides (ppm)	≤ 400	≤ 400	≤ 400	≤ 400	≤ 400
2- Pyrrolidine (%)	≤1.0	≤1.0	≤ 3.0	≤3.0	≤1.0
Formic acid (%)	-	-	≤0.5	≤0.5	≤0.5
2- Propanol (%)	≤0.5	≤0.5	-	-	-
Microbial status	Passes test	Passes test	Passes test	Passes test	Passes test
Bacterial Endotoxin 6% Solution	≤6 I.U/ ml	≤6 I.U/ ml	-	-	-

CHAPTER 2

DRUG PROFILE

2.1 Drug profile [8, 9]

2.1.1. Esomeprazole Magnesium



- **Description:** Esomeprazole magnesium is trihydrate is the S- inantiomer of Omeprazole and is used for the treatment of PUD, Dyspepsia, GERD.
- **Category:** Proton Pump Inhibitor(PPI)
- **IUPAC Name:** bis (5-methoxy-2-[(S)-[(4-methoxy-3, 5-dimethyl-2pyridinyl) methyl] sulfinyl]-1H-benzimidazole-1-yl)
- **Molecular formula:** $(C_{17}H_{18}N_3O_3S)_2 Mg. 3H_2O$
- **Molecular mass:** 767.16 g/mol
- **Appearance:** White to yellowish powder
- **log (P):** 1.66
- **Chemical class:** Sulfinylbenzimidazole
- **Solubility:** It is soluble in ethanol, methanol, N, N-dimethyl formamide, Acetonitrile slightly soluble in water, acetone and isopropanol.
- **Melting point:** 184-189 ^oC

- **Protein binding:** 97%

- **Ionisation constant pKa:** 4

- **Mechanism of Action:** Esomeprazole is a proton pump inhibitor that suppresses gastric acid secretion by specific inhibition of the H^+/K^+-ATPase pump in the gastric parietal cell.

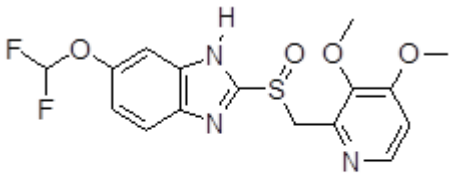
- **Pharmacokinetic profile:** Single 20– to 40-mg oral doses generally give rise to peak plasma esomeprazole concentrations of 0.5-1.0 mg/l within 1–4 hours, but after several days of once-daily administration, these levels may increase by about 50%. The drug is rapidly cleared from the body, largely by urinary excretion.

- **Indication:** for the treatment of peptic ulcer disease dyspepsia, GERD/GORD and zollinger syndrome.

- **Official status:** IP 2014 and USP 2010 monograph

- **Marketed formulation:** Nexium (Esomeprazole Magnessium Trihydrate)

2.1.2 Pantoprazole Sodium



- **Description:** Pantoprazole sodium is a proton pump inhibitor drug used for short-term treatment of erosion and ulceration of the esophagus caused by gastroesophageal reflux disease
- **Category:** Proton Pump Inhibitor(PPI)
- **IUPACName:**6(difluoromethoxy)2{[(3,4dimethoxypyridin2yl)methane]sulfinyl} -1H-1,3-benzodiazole
- **Molecular formula:** $C_{16}H_{15}F_2N_3O_4S$ Na
- **Molecular mass:** 405.35 g/mol
- **Appearance:** White to off-white crystalline powder
- **log (P):** 2.11
- **Chemical class:** Sulfinylbenzimidazole
- **Solubility:** Freely soluble in methanol slightly soluble in water
- **Melting point:** 137.5 -145.5^{o}C
- **Protein binding:** 97%

- **Ionisation constant pKa:** 3.15

- **Mechanism of Action:** Pantoprazole is a proton pump inhibitor (PPI) that suppresses the final step in gastric acid production by forming a covalent bond to two sites of the (H^+,K^+)ATPase enzyme system at the secretory surface of the gastric parietal cell. This effect is dose- related and leads to inhibition of both basal and stimulated gastric acid secretion irrespective of the stimulus

- **Pharmacokinetic profile:** Pantoprazole is extensively metabolized in the liver through the cytochrome P450 (CYP) system. The main metabolic pathway is de methylation, by CYP2C19, with subsequent sulfation; other metabolic pathways include oxidation by CYP3A4. There is no evidence that any of the pantoprazole metabolites have significant pharmacologic activity.

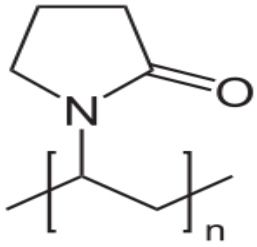
- **Indication:** For the treatment of peptic ulcer disease dyspepsia, GERD/GORD and zollinger syndrome.

- **Official status:** IP 2014 and USP 2010 monograph

- **Marketed formulation:** Protonix (Pantoprazole Sodium)

2.2 Excipient Profile [10]

2.2.1 Polyvinylpyrrolidone:



- **Appearance:** White to light yellow, hygroscopic, amorphous powder
- **Molecular weight:** 2.500 – 2.500.000 $g \cdot mol^{-1}$
- **Chemical formula:** $(C_6H_9NO)_n$
- **Peroxide content:** <400ppm H2O2
- **Description:** PVP binds to polar molecules exceptionally well, owing to its polarity. This has led to its application in coatings for photo-quality ink-jet papers and transparencies, as well as in inks for inkjet printers.
- **Density:** 1.2 g/cm^3
- **Melting point:** 150–180 °C (glass temperature)
- **Solubility:** PVP is soluble in water and other polar solvents. When dry it is a light flaky hygroscopic powder, readily absorbing up to 40% of its weight in atmospheric water. In solution, it has excellent wetting properties and readily forms films. This makes it good as a coating or an additive to coatings
- **Uses:** PVP is a very widely used excipient for the preparation of solid dosage forms. Main application is its function as a binder in wet granulation. It is also useful for the

preparation of effervescent tablets or in direct compression applications. Many other uses, including non-parenteral applications.It is also used in personal care products, such as shampoos and toothpastes in paints, and adhesives that must be moistened, such as old-style postage stamps and envelopes.

CHAPTER 3

LITERATURE REVIEW

3.1 Literature review for Esomeprazole magnesium

Several articles have been reviewed for Esomeprazole magnesium analytical method development and stability indicating assay methods alone as well as in combinations with other drugs for different dosage forms. Various analytical methods and SIAM reported in literature are listed in the following table 3.1

Table3.1 Reported analytical methods and Stability Indicating Assay methods for Esomeprazole magnesium alone and in combination with other drugs

Sr. No	Article	Dosage form	Journal	Ref no.
1	Development and Validation of High Performance Liquid Chromatographic Method for The Determination of Esomeprazole in Tablets	Tablet	Journal of Food and Drug Analysis,Vol. 14, No. 1, 2006, Pages 12-18	11
2	A validated stability indicating ultra-performance liquid chromatographic method for determination of impurities in Esomeprazole magnesium gastro resistant tablets.	Tablet	Journal of Pharmaceutical and Biomedical Analysis 57 (2012) 109– 114	12
3	Spectrophotometric Determination of Esomeprazole Magnesium in Commercial Tablets Using 5-Sulfosalicylic Acid and N -Bromosuccinimide	Tablet	Journal of the Chinese Chemical Society 2008, 55, 557-566	13

4	A Review: Development & Validation of HPLC Method for the Determination of Esomeprazole in Pharmaceuticals	API	Indo global journal of pharmaceutical sciences ISSN 2249- 1023	14
5	Simultaneous estimation of esomeprazole and naproxen in bulk as well as in pharmaceutical formulations by using RP-HPLC.	API	IJPSR (2013), Vol. 4, Issue 8	15
6	Quantification of domperidone,paracetamol, esomeprazole and lansoprazole in pharmaceutical dosage forms by reversed phase high performance liquid chromatography.	Tablet	International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 4, Suppl 3, 2012	16
7	Development and validation of RP-HPLC methods for simultaneous estimation of naproxen and esomeprazole magnesium tri hydrate in combined pharmaceutical formulation.	Tablet	International Journal of Pharmacy and Pharmaceutical Sciences ISSN- 0975-1491 Vol 4, Suppl 3, 2012	17

8	Extensive Study of Aspirin and Its Related Impurities Under Various Stressed Conditions in Low Dose Aspirin and Esomeprazole Magnesium Capsules.	Capsules	Am. J. PharmTech Res. 2012; 2(6) ISSN: 2249-3387	18
9	Stability Indicating RP-HPLC Method for the Estimation of Esomeprazole in Bulk and in its Dosage Forms	API and Tablet	Journal of Advanced Pharmaceutical Research. 2011, 2(4), 170-174.	19
10	Stability indicating assay of Esomeprazole and Naproxen in Tablets by RP-UPLC PDA-Method	Tablet	International Journal of Pharma sciences Vol. 3, No. 2 (2013): 205-210	20
11	Stability Indicating Simultaneous Equation Method for Determination of Domperidone and (S)-Esomeprazole Magnesium in Capsule Dosage Form Using UV-Spectrophotometer	Capsule	British Journal of Pharmaceutical Research 3(3): 435-445, 2013	21
12	Simultaneous estimation of related compounds in esomeprazole and naproxen	Tablet	International Journal of Pharmacy and	22

	tablets by using ion pair reverse phase HPLC		Pharmaceutical Sciences ISSN- 0975-1491 Vol 7, Issue 2, 2015	

3.1.1 Comparison of Stability Indicating Assay Method (SIAM) for Esomeprazole magnesium

Several developed and validated analytical methods and stability indicating assay methods for esomeprazole magnesium combined with other drugs were reviewed. % Degradation of esomeprazole magnesium under various stress conditions were compared, which is summarized in the following table.3.2

Table 3.2 Comparison of Stability Indicating Assay Method for Esomeprazole Magnesium

		Ref[12]	**Ref[19]**	**Ref[20]**	**Ref[21]**
Acid induce degradation	Condition	5 ml 0.1 M HCl 60 °C 1H	0.1N 1 ml HCl 60 °C 30 min	5 ml 1N HCl 60 °C 30 min	5 ml 1N HCl 60 °C 1H
	%degradation	0.9%	13.56%	4%	8%
Alkali induced degradation	Condition	5 ml 0.1M NaOH 60 °C 1H	1ml 0.1N NaOH 60 °C 1H	5 ml 1N NaOH 60 °C 30 min	5 ml 1N NaOH 60 °C 1H
	%degradation	2.7%	5.86%	8%	15%

Peroxide induced degradation	Condition	1% H_2O_2 at 40°C for 1H	3% H_2O_2 at RT for 1H	3% H_2O_2 at RT for 1H	100µL 30% H_2O_2 at RT for 1H
	%degradation	11.7%	10.12%	22%	25%
Dry heat degradation	Condition	60°C for 2h	80°C for 1h	105°C for 2h	105°C for 5h
	%degradation	0.08%	1.2%	0.68%	2%
Photo degradation	Condition	UV light for 5h	75% RH for 3 days	UV 200 watt hours/ sq. meter	Sunlight for 8h
	%degradation	0.2%	0.8%	0.14%	0.5%

3.1.2 Patents available on formulations of Esomeprazole magnesium

Patents available on the formulations comprising Esomeprazole magnesium were reviewed for the list of excipients used in the different dosage forms. Summary of the excipients list in each of the formulations is given in the table 3.3

Table 3.3 Patents available for dosage forms of Esomeprazole magnesium [23]

Sr. No	Title	Inference	Reference
1	Pharmaceutical composition comprising Esomeprazole magnesium dihydrate	Wet granulate pharmaceutical formulationcomprising esomeprazole mag nesium dihydrate with one or more pharmaceutical excipients, wherein the dosage form is essentially free of esomeprazolemagnesium trihydrate. Polyvinylpyrrolidone:1%	WO2013088272 A1
2	Pellet formulations comprising esomeprazole	Stable pharmaceutical formulations comprising esomeprazole and production method of these formulations. Base coating of Esomeprazole magnesium pellets Polyvinylpyrrolidone	WO2013122554 A1
3	Process for the preparation of esomeprazole magnesium in a stable form	There is provided a process for preparing purified esomeprazole magnesium.	US8362259 B2

4	Process for the preparation of esomeprazole magnesium in a stable form	Preparation of purified esomeprazole magnesium comprising the steps of the preparationproviding esomeprazole magne sium. The process is particularly suitable to obtain esomeprazole magnesium di hydrate.	EP2328887 A1
5	Polymorphs of esomeprazole salts	Process for preparation of substantially enantiometrically pure esomeprazole in neutral form or as a pharmaceutically acceptable salt or as its solvates including hydrates. Polyvinylpyrrolidone:0.5%	WO2009047775 A2

3.1.3 Marketed formulations of Esomeprazole magnesium

There are many marketed formulations of Esomeprazole magnesium available in different types of dosage form. Marketed tablet dosage form of esomeprazole magnesium has been reviewed for the list of excipients, which is summarized in the table 3.4

Table 3.4 Marketed formulations of Esomeprazole magnesium [24]

Sr. no	Brand Name	Content	List of excipients
1	NEXIUM	Each gastro-resistant tablet contains 44.5 mg esomeprazole magnesium trihydrate equivalent to 40 mg esomeprazole	Glycerol monostearate 40-55 hypromellose iron oxide (reddish-brown) (E 172)

			magnesium stearate Povidone K90 crospovidone
2	VIMOVO	Each modified-release tablet contains 500 mg naproxen and 20 mg esomeprazole (as	Tablet Core contains Croscarmellose sodium Magnesium stearate Povidone K90 Silica, colloidal anhydrous
3	ESO-TAC	Each gastro-resistant tablet contains 40 mg esomeprazole	Tablet core contains Sodium carbonate, Anhydrous Mannitol (E421) Crospovidone Povidone K90 Calcium stearate
4	ESO-20	Each gastro-resistant tablet contains 20 mg esomeprazole	Tablet Core contains Croscarmellose sodium Magnesium stearate Povidone K90 Silica, colloidal anhydrous

3.2 Literature review for Pantoprazole Sodium

Several articles have been reviewed for Pantoprazole Sodium analytical method development and stability indicating assay methods alone as well as in combinations with other drugs for different dosage forms. Various analytical methods and SIAM reported in literature are listed in the following table 3.5

Table3.5 Reported analytical methods and Stability Indicating Assay methods for Pantoprazole Sodium alone and in combination with other drugs

Sr. No	Article	Dosage form	Journal	Ref no.
1	Effect of Various Salts on the Stability of Lansoprazole, Omeprazole, and Pantoprazole as Determined by High-PerformanceLiquidChromatography.	API	Drug Development and Industrial Pharmacy, 25(9), 1057–1065 (1999)	25
2	A modified high-performance liquid chromatographic method for the analysis of pantoprazole sodium in pharmaceutical dosage forms using lansoprazole as internal standard.	Tablet	Arabian Journal of Chemistry (2011)	26
3	Stability indicating high performance thin-layer chromatographic method for simultaneous estimation of pantoprazole sodium and	Tablet	Journal of Pharmaceutical Analysis 2011;1(4):275–283	27

	itopride hydrochloride in 29combined dosage form.			
4	Development and validation of a reversed-phase hplc method for simultaneous determination of domperidone and pantoprazole in pharmaceutical dosage forms	Tablet	Acta chromatographica, no. 18, 2007	28
5	Stability indicating RP-HPLC method for simultaneous determination of pantoprazole sodium and itopride hydrochloride in bulk and capsule.	Capsule	Orbital the electronic journal of chemistry Vol 2 No. 3 July-September 2010	29
6	Reversed-Phase High Performance Liquid Chromatographic Method forthe Determination of Lansoprazole, Omeprazole and Pantoprazole Sodium Sesquihydrate in Presence of Their Acid-Induced Degradation Products.	API	Chem. Pharm. Bull. 54(6) 814-818 (2006)	30
7	Simultaneous HPLC Estimation of Pantoprazole and Domperidone from Tablets.	Tablet	International Journalof ChemTech 0974-4290	31

			Vol.1, No.2, pp 275-277	
8	Development of UV Spectrophotometric method for estimation of Pantoprazole in pharmaceutical dosage forms	Tablet	J. Chem. Pharm. Res., 2011, 3(2):113-117	32
9	Spectrophotometric determination of omeprazole, lansoprazole and pantoprazole in pharmaceutical formulations.	Tablet	Journal of Pharmaceutical and Biomedical Analysis 30 (2002) 1133-1142	33
10	Stability-Indicating HPLC Methodfor Simultaneous Determination of Pantoprazole and Domperidone from their Combination Drug Product.	Tablet	Chromatographia 2008, 67, January	34
11	Validation of the spectrophotometric determination of omeprazole and pantoprazole sodium via their metal chelates.	API	Journal of Pharmaceutical and Biomedical Analysis 33 (2003) 411_/421	35

12	Stability-indicating HPLC method for the simultaneous determination of pantoprazole, rabeprazole, lansoprazole and domperidone from their combination dosage forms.	Tablet	International Journal of Drug Development & Research October-December 2011 Vol. 3 Issue 4 ISSN 0975-9344	36
13	Development and validation of a stability indicating RP-HPLC method for simultaneous estimation of Pantoprazole sodium sesquihydrate and Levosulpiride in a combined dosage form.	Tablet	Kothapalli, et al., Int J Res Pharm Sci 2014, 4(4) ; 32 – 38	37
14	Improved HPLC Method for Determination of Four PPIs, Omeprazole, Pantoprazole, Lansoprazole and Rabeprazole in Human Plasma.	API	JPharm PharmaceutSci (www.cspsCanada .org) 13(1) 1-10, 2010	38
15	Three-wavelength Spectrophotometric Method for Simultaneous Estimation of Pantoprazole and Domperidone in Pharmaceutical Preparations.	Tablet	International Journal of PharmTech Research ISSN : 0974-4304	39

			Vol.1, No.2, pp 386-389	
16	Design, in vitro evaluation and in vivo studies of novel delayed release tablets of pantoprazole.	Tablet	International Journal of Biomedical and Advance Research IJBAR (2012) 03(11)	40

3.2.1 Comparison of Stability Indicating Assay Method (SIAM) for Pantoprazole Sodium

Several developed and validated analytical methods and stability indicating assay methods for pantoprazole sodium combined with other drugs were reviewed. % Degradation of pantoprazole sodium under various stress conditions were compared, which is summarized in the following table.3.6.

Table 3.6 Comparison of Stability Indicating Assay Method for Pantoprazole Sodium

		Ref [27]	**Ref [34]**	**Ref [36]**	**Ref [37]**
Acid induce degradation	Condition	1 M Hcl 80ºC 6h	0.1 N HCl 6h	1N Hcl 60ºc 30 min	1ml 1M Hcl
	%degradation	25%	15%	22.15%	3%
Alkali induced degradation	Condition	1 M NaOH 80ºC 6h	0.1 N NaOH 6h	1N NaOH 60ºC 30 min	1 ml 1ml NaOH
	%degradation	15%	7%	8.79%	4%

Peroxide induced degradation	Condition	3% H2O2 80ºC 4h	3% H2O2 50ºC 24h	30% H2O2 for 30 min	3% H2O2 for 30 min
	%degradation	27%	25%	24%	15%
Dry heat degradation	Condition	110ºC 2h	60 ºC 24h	105 ºC for 2 h	120ºC for 6h
	%degradation	10%	7%	1.33%	1%
Photo degradation	Condition	UV 255 nm for 24 h	UV 254 nm 24h	UV Light 200 watt h/ square	UV light 255nm 6 h
	%degradation	10%	10%	0.14%	2%

3.2.2 Patents available on formulations of Pantoprazole sodium

Patents available on the formulations comprising Pantoprazole Sodium were reviewed for the list of excipients used in the different dosage forms. Summary of the excipients list in each of the formulations is given in the table 3.7.

Table 3.7 Patents available for dosage forms of Pantoprazole Sodium [41]

Sr. No	Title	Inference	Reference
1	Preparation method for pantoprazole enteric coated tablet	A solid dosage form in tablet or pellet form for oral administration of (S)-Pantoprazole magnesium salt comprising between 5 and 100 mg of the (S)-Pantoprazole magnesium salt, Polyvinyl Pyrrolidone (PVP) as binder and one or more suitable Pharmaceutical excipient.	CA2524979 C
2	An enteric coated mini-pill of pantoprazole sodium	A method for preparing pellets of the core. Formulation and process of the present invention provides greater stability makes the formulation of pantoprazole sodium, sodium bicarbonate crospovidone and more suitable excipient.	CN100553622 C
3	Dosage form containing	A dosage form for oral administration in tablet form consisting of (a) a core consisting of: Pantoprazole magnesium di	US 8703192 B2

	pantoprazole as active ingredient	hydrate, sodium carbonate, mannitol, crospovidone, PVP 90, and calcium stearate.	
4	Compound pant oprazole composite	Pantoprazole with Povidone as a binder sodium bicarbonate and magnesium hydroxide as active ingredients. The composite can be combined with appropriate pharmaceutical excipients and prepared into oral preparations.	CN102114036 Av
5	Dosage form containing S-Pantoprazole as an active ingredient	Dosage form according to claim Pantoprazole sodium in tablet form, where in polyvinyl pyrrolidone and/or hydroxypropyl methyl cellulose is the binder and mannitol is the filler.	WO2004098577 A2

3.2.3 Marketed formulations of Pantoprazole Sodium

There are many marketed formulations of Pantoprazole sodium available in different types of dosage form. Marketed tablet dosage form of Pantoprazole sodium has been reviewed for the list of excipients, which is summarized in the table 3.8

Table 3.8 Marketed formulations of Pantoprazole sodium [42]

Sr. no	Brand Name	Content	List of excipients
1	PROTONIX	Each gastro-resistant tablet contains 20 mg pantoprazole (as sodium sesquihydrate).	Tablet core Calcium stearate Cellulose microcrystalline Crospovidone Hydroxypropylcellulose (type EXF)
2	PANTOLOC	Each gastro-resistant tablet contains 20 mg pantoprazole (as sodium sesquihydrate).	Tablet core Sodium carbonate, anhydrous Mannitol (E421) Crospovidone Povidone K90 Calcium stearate

3	SOMAC	Each gastro-resistant tablet contains 20 mg pantoprazole (as sodium sesquihydrate).	Tablet core Sodium carbonate, anhydrous Mannitol (E421) Crospovidone Povidone K90 Calcium stearate
4	PANPRAZO	Each gastro-resistant tablet contains 40 mg pantoprazole (as sodium sesquihydrate).	Tablet core Sodium carbonate, anhydrous Silica, colloidal anhydrous Crospovidone Hydroxypropylcellulose

3.3 Literature review for Drug and excipient compatibility study of different drugs with povidone

Several articles were reviewed for drug and excipient compatibility study of different drugs (API) with povidone and other excipients. Incompatibilities proven by using analytical method such as HPLC, FT-IR, DSC, XRD, SEM, TEM etc. Summary of the compatibility of drugs with povidone and crospovidone are depicted in table 3.9

Table 3.9 Drug and excipient compatibility study of different drugs with povidone

Sr. no.	Title	Inference	Ref. No.
1	Compatibility study between ketoprofen and pharmaceutical excipients usedin solid dosage forms	Possible interactions between Ketoprofen and each excipients were compared with those of their 1:1 (w/w) physical mixtures. On the basis of thermal results, a possible interaction was found between the Ketoprofen with polyvinylpyrrolidone K30 and magnesium stearate, which could influence the stability of the KT in the binary mixtures. These possible incompatibilities were confirmed by FT-IR and X-ray analysis.	43
2	Application of differential scanning calorimetry and high performance liquid chromatography to determine the effects of	Chemical reactions were present when studying 1:1 mixtures.Niclosamide reacted strongly with Povidone, causing the disappearance of the niclosamide	44

	mixture composition and preparation during the evaluation of niclosamide-excipient compatibility	peak in the DSC thermograms of mixtures.	
3	Drug excipient compatibility studies by physico-chemical techniques The case of indomethacin	Properties of pure compounds with those of binary mixtures drug:excipient which underwent the same treatment were analysed.It was found that interaction occurs between indomethacin and PVP upon simple mechanical mixing. Interaction revealed by the indomethacin melting, Changes appears more clearly in the 20:80 indomethacin: PVP untreated mixture.	45
4	Drug excipient compatibility studies by physico-chemical techniques the case of atenolol	Binaary mixture of atenolol with different excipients were prepared and analysed by different analytical techniques. In the atenolol-PVP mixtures, substantial modifications are seen in the DSC response of atenolol it was mediated, by the hydration water of PVP. The indications of interaction are larger in the PVP-rich mixture.	46
5	Drug-excipient compatibility studies in binary and ternary mixtures by physico-chemical techniques	Compatibility of haloperidol with several excipients (PVP, magnesium stearate and a-lactose) in binary and ternary mixtures was checked. The melting peak of haloperidol is broader	47

		than in the pure drug and its onset temperature is lowered, respectively, to 135.3 °C and to 128.9 °C Furthermore, a 38% decrease of the mean melting enthalpy was recorded.	
6	Influence of Peroxide Impurities in Povidone and Crospovidone on the Stability of Raloxifene Hydrochloride in Tablets: Identification and Control of an Oxidative Degradation Product	Raloxifene hydrochloride underwent an order of magnitude increase in conversion to the N-oxide in the presence of two excipients, povidone and crospovidone, as compared with its conversion in the presence of other excipients. The results from drug–excipient studies and a formulation spiking study support the hypothesis that residual peroxides in povidone and crospovidone promote the formation of the N-oxide impurity of drug.	48

CHAPTER 4

AIM AND OBJECTIVES

Aim of present work

There are patents for different dosage form and marketed formulations claim that Povidone and crospovidone is used as an excipient in different formulation of Esomeprazole magnesium and Pantoprazole sodium. According to literature review of stability indicating assay method of Esomeprazole magnesium and Pantoprazole sodium it reveal that both drugs get degraded in presence of peroxides in very mild conditions because both drugs are prone to oxidation easily. Furthermore, a selected excipient povidone contains peroxides as an organic impurity in it. The purpose of this study is to prove the compatibility of API Esomeprazole magnesium and Pantoprazole sodium with povidone of different grades PVPK30 and PVPK90.

Objective of present work

To determine degradation product in the binary mixture of Esomeprazole magnesium and Pantoprazole sodium with Povidone K30 and Povidone K90.

CHAPTER 5

INSTRUMENTS, REAGENTS & MATERIALS

5.1 Instruments and equipments

All the instruments and equipments used throughout project were calibrated periodically as per in house SOP of department of pharmaceutical analysis, Institute of pharmacy, Nirma University.

Following instruments were used throughout the project:

- **High performance Liquid Chromatography** with model No. JASCO 200 series, manufactured by Jasco Inc, JAPAN with pump (Jasco PU 2080 plus); Mixer (Jasco MX 2080-31); Injector (Rheodyne valve 20µL fixed loop); Detector (Jasco MD-2015 plus); Software (Borwin Jasco 1.5 version) and Column for Esomeprazole magnesium (Agilent Zorbax Eclipse C8 250* 4.6 mm 5µm) column for Pantoprazole sodium (AGILENT Zorbax eclipse XDB C8 4.6*150mm 5µm)
- **UV Visible Spectro photometer**, with model no. UV-2450; Double beam manufactured by Jasco Inc., Japan
- **Fourier transform Infrared Spectrophotometer (FT-IR)**, with model no. JASCO FT/IR-6100 series, manufactured by Jasco Inc., Japan
- **Analytical balance**, with model CITIZEN Scale CX-220, manufactured by CITIZEN Private Ltd., India having capacity of 10 mg to 220 mg.
- **Sonicator**, with model Trans-o- sonic, D impact 936 having capacity of 2 litres
- **pH meter**, Manufacture by Lutron, Taiwan; Model ph-mV-Temperature measurement Probe.
- **Melting Point Apparatus**, T0603160, manufactured by EIE Instruments Pvt. Ltd Ahmedabad India
- **Hot Air Oven**, EIE 108, Manufactured EIE Instruments Pvt. Ltd., Ahmedabad India
- **Water bath**, manufactured by EIE instruments Pvt. Ltd., Ahmedabad India
- **Vaccum Pump**, manufactured by Rockers/shah brothers.

5.2 Reagents and Materials:

- Esomeprazole Magnesium and Pantoprazole Sodium API were supplied as a gift sample by Nirlife Healthcare Ltd. Ahmedabad.

- Excipient Povidone K30 and Povidone K90 were supplied as a gift sample from Nirlife healthcare Ltd. Ahmedabad.

- The HPLC grade Acetonitrile, Methanol, and analytical grade Orthophosphoric Acid, Triethyl amine were purchased from Merck, specialities Pvt, Ltd, Worli, Mumbai.

- Hydrogen Peroxide (6 % w/v) analytical grade, from CDH chemicals Mumbai India.

- Water used was obtained by using Millipore Milli Q Plus water purification system.

- The membrane filter paper 0.45µm used for the mobile phase filtration were supplied by Millipore Ltd, Banglore.

- All glasswares volumetric flask, beaker, measuring cylinder, pipette were of class A borosilicate glass.

CHAPTER 6

EXPERIMENTAL WORK FOR ESOMEPRAZOLE MAGNESIUM

6.1 Identification of drug

The identification of Esomeprazole magnesium was carried out by following techniques.

1. Melting point
2. UV- spectroscopy
3. FT-IR spectroscopy

6.1.1 Identification of drug by melting point

Melting Point of ESO has been determined using capillary melting point apparatus.

Melting Point obtained were compared with that available in literature as shown in table**.**

Table: 6.1 Comparison of reported and observed melting point of ESO

Drug	Reported(°c) [8]	Observed(°c)
ESO	184-189	185

6.1.2 Identification of drug by UV Spectroscopy

UV spectrum of ESO (100 µg/ml) in methanol was recorded using UV-VIS spectrophotometer.By scanning the sample in the range of 200-400 nm against methanol as a blank.

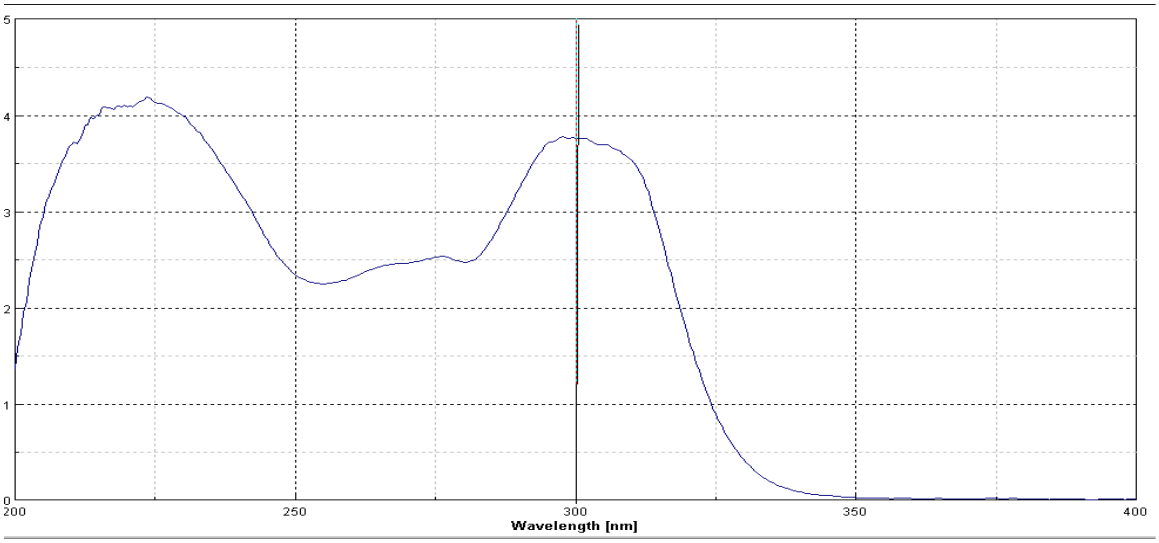


Figure 6.1 UV spectrum of ESO (100 µg/ml) in methanol

Table 6.2 Comparison of reported absorption maxima with observed absorption maxima

Drug	Reported Absorption maxima [49]	Observed absorption maxima
ESO	302 nm	300 nm

6.1.3 Identification of drug by FT-IR

FT-IR spectrum of ESO was recorded in diffused reflectance mode. Theoretical values of wave numbers responsible for functional groups are compared with observed values of wave numbers as summarized in table 6.3

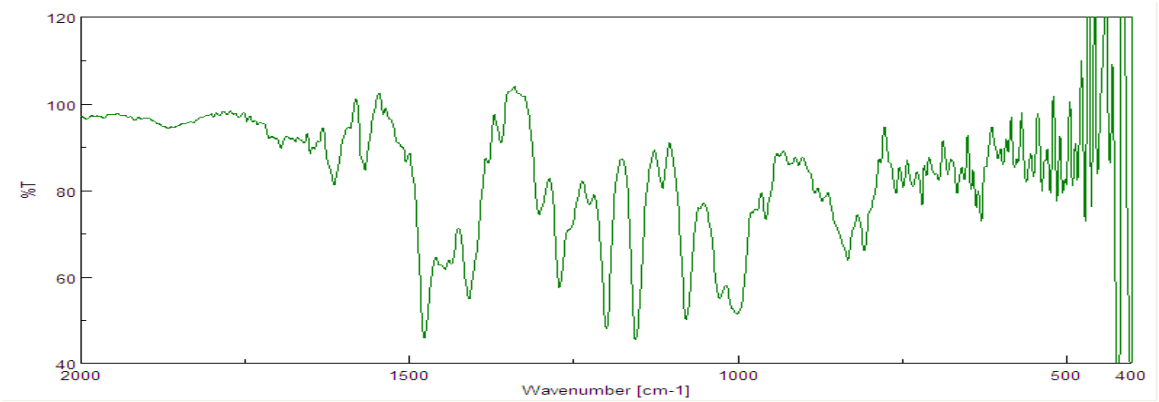


Fig 6.2 Recorded FT-IR spectra of ESO

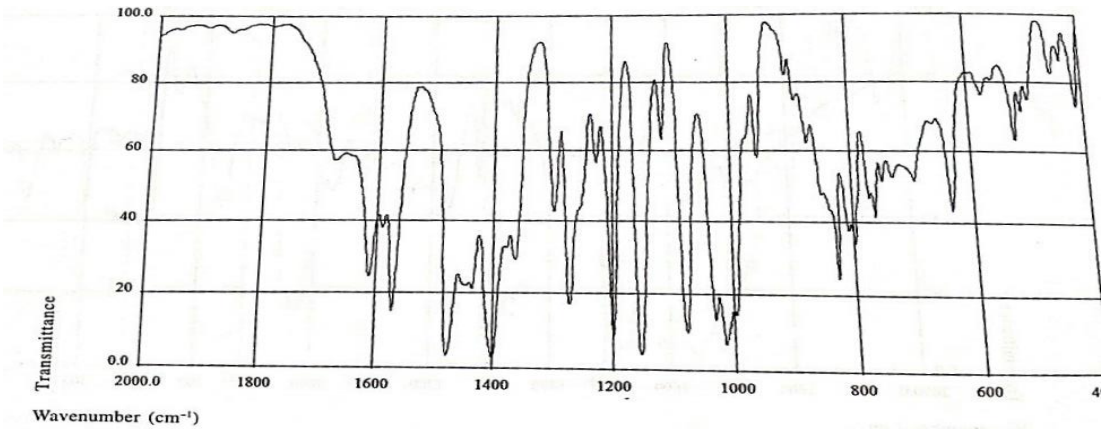


Fig 6.3 Standard FT-IR spectra of ESO (IP-2014) [50]

Table 6.3 Observations for FT-IR spectra of ESO

Theoretical frequency(cm^{-1})	Observed frequency(cm^{-1})
1610	1615
1569	1567
1475	1477
1410	1409
1270	1271
1200	1200
1150	1156

By performing the identification tests and comparing with the standard values it can be concluded that procured sample was of Esomeprazole magnesium.

6.2 Accelerated stability study

Accelerated stability studies were carried out in accordance with ICH guidelines (Q1A (R2) ICH). ESO alone as well as in combination with excipients were exposed under accelerated stability condition such as 40°C 75%RH.

6.2.1 Sample preparation for accelerated stability study

To identify the stability of Esomeprazole magnesium with excipient Povidone, Binary mixture of ESO and excipient (1:1) i.e.5 g API and 5 g excipient ,were prepared with proper mixing and kept in closed amber colored vial and exposed to 40ºC and 75%RH in stability chamber to extrapolate probable chemical incompatibility between drug and excipient. Along with the mixture, ESO, PVP K30 and PVP K90 alone were also kept in the stability chamber. Control samples were kept in refrigerator. Sampling was planned to be done at 7, 15, 30 and 45 days and continued for 6 months. Assay and %impurity has been found for each samples.

Table 6.4 Accelerated stability study for Esomeprazole magnesium with Povidone K30 and Povidone K90

Sample no.	Sample	Condition
1	Esomeprazole (5g)	40 °C & 75%RH
		Control(15°C)
2	Esomeprazole(5g)+ PVP K30(5g) (1:1)	40 °C & 75%RH
		Control(15°C)
3	Esomeprazole(5g)+ PVP K90(5g) (1:1)	40 °C & 75%RH
		Control(15°C)
4	PVP K30(2g)	40 °C & 75%RH
		Control(15°C)
5	PVP K90(2g)	40 °C & 75%RH
		Control(15°C)

6.3 Esomeprazole magnesium and povidone compatibility study

6.3.1 Chromatographic conditions

The chromatographic method followed was official in IP 2014, Government of India Ministry of Health and family Welfare. Column used was a C_8 250 mm × 4.6 mm, 5µm. The separation was achieved on an isocratic method. Mobile phase A contains 10 mM Phosphate buffer (pH 7.0) and the Mobile phase B contains acetonitrile the ratio was 70:30 (v/v); respectively. The flow rate was 1 mL/min and the detection wavelength was 305 nm. The column temperature was maintained at 25°C (ambient) and the detection was monitored at a wavelength 302 nm. The injection volume was 20µL. A mixture of triple distilled water and acetonitrile in the proportion of 70:30 (v/v); respectively used as a solvent or diluent.

6.3.2 Sample preparation:

6.3.2.1 Sample preparation for Esomeprazole magnesium

1) Preparation of stock solutions:

A stock solution of Esomeprazole magnesium 25 mg/mL was prepared by dissolving 25 mg of ESO in 10 ml of methanol.

2) Preparation of working stock solutions:

Sample solutions having concentration 1000 µg/mL were prepared from stock solution.4 ml of the stock solution was diluted up to 10 ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of impurities generated.

3) Preparation of working solutions:

Working solutions having concentration 50 µg/mL were prepared from the sample solution. 0.5ml of the sample solution was diluted up to 10ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of assay of ESO.

6.3.2.2 Sample preparation for Esomeprazole magnesium with Excipient

1) Preparation of stock solutions:

A stock solution of Esomeprazole magnesium 25 mg/mL was prepared by dissolving 50 mg of drug and excipient mixture in 10ml of methanol.

2) Preparation of working stock solutions:

Sample solutions having concentration 1000µg/mL were prepared from stock solution. 4 ml of the stock solution was diluted up to 10 ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of impurities generated.

3) Preparation of working solutions:

Working solutions containing 50 µg/mL were prepared from the sample solution. 0.5ml of the sample solution was diluted up to 10 ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of assay of ESO.

6.4 Results and discussion

6.4.1 Compatibility study of Esomeprazole magnesium with povidone

Esomeprazole magnesium is a Proton Pump Inhibitor which was selected to carry out drug and excipient compatibility study with different grades of povidone. Esomeprazole magnesium was mixed with excipient 1:1 separately as described in table 6.2.1 and exposed to 40°C 75%RH. Sampling was planned to be done at 7, 15, 30, 45 days and continued for 6 months. Exposed samples were analysed for new impurity generated and assay determination of ESO.

6.4.2 HPLC analysis of Esomeprazole magnesium alone

As per section 6.2.1 along with ESO and excipient mixture samples, Esomeprazole magnesium alone was also exposed to accelerated stability conditions. After completion of the time period stability samples were prepared for HPLC analysis. In case of Esomeprazole magnesium alone 25mg of drug was weighed and transferred it to 10 ml volumetric flask to get concentration of 2500µg/ml. Further dilutions were made as described in the section 6.3.2.

Chromatographic conditions

Following chromatographic conditions are described for related substance method of Esomeprazole magnesium monograph official in Indian pharmacopoeia-2014.

Table 6.5 Chromatographic conditions

Column	C_8 250 mm*4.6 i.d. ,5 µm Particle size
Mobile phase	10 mM Phosphate buffer (pH 7.0) and acetonitrile 70:30 (v/v)
Flow rate	1ml/ min
Temperature	Ambient
Detection wavelength	302 nm
Injection volume	20µL

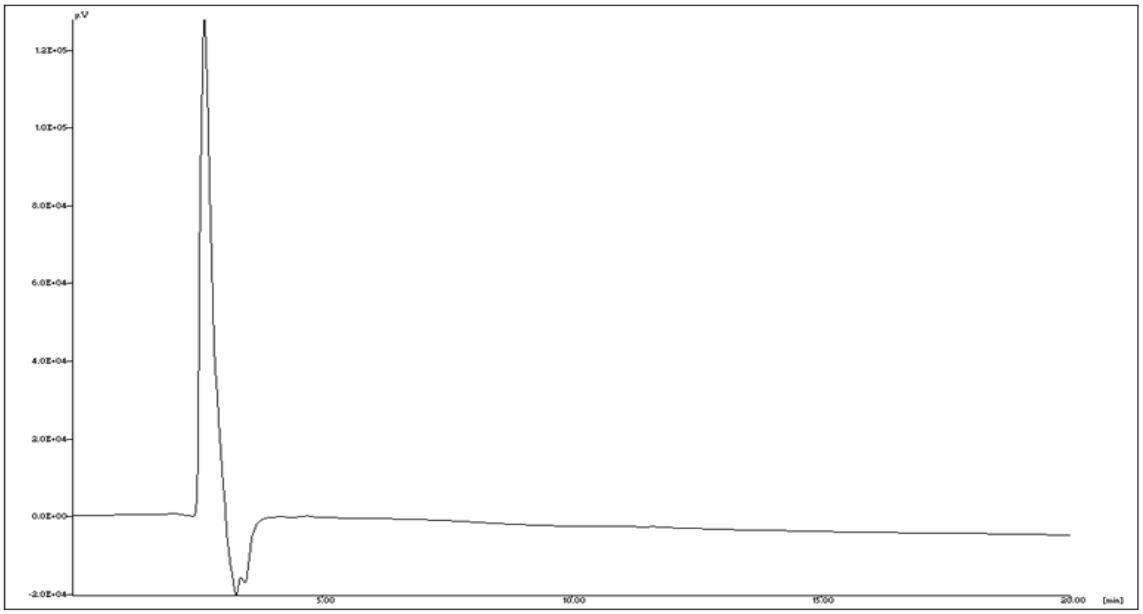


Fig 6.4 HPLC chromatogram of blank (Methanol)

6.4.3 System suitability parameters for Esomeprazole Magnesium

System suitability for Esomeprazole magnesium was checked by injecting sample solutions having concentration of 50 µg/ml for 6 times. System suitability parameters are depicted in table 6.6.

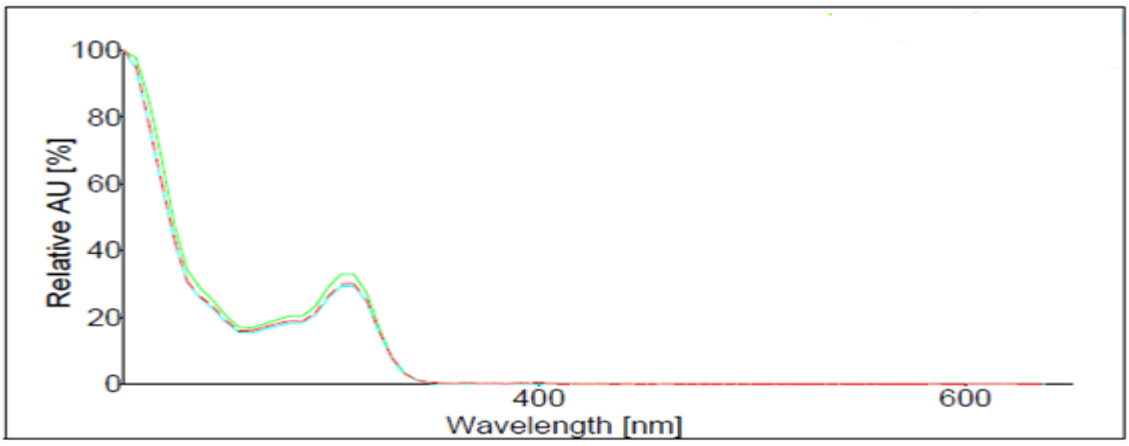


Figure 6.5 Peak purity spectra of ESO (50 µg/ml)

Table: 6.6 HPLC data of peak purity for Esomeprazole magnesium in methanol (50 µg/ml)

Parameters	Inference	Specification criteria
Peak name	ESO (50 µg/ml)	-
%RSD	0.998	≤ 2
Purity tail	997.877	(1….1000)
Purity front	997.326	(1….1000)
Number of plates	5268	Not less than 5000
Tailing factor	1.19	Not more than 2

Table 6.6 shows that parameters for peak purity of esomeprazole magnesium passes the specified limits as per Indian pharmacopoeia-2014, which indicates that chromatographic conditions are suitable for analysis of Esomeprazole magnesium.

6.4.4 Determination of impurities from stability samples of Esomeprazole magnesium alone

For the determination of impurities in the stability samples of Esomeprazole magnesium, samples having concentration of 1000 µg/ml were made prior to inject it into the HPLC system.

Fig 6.6 HPLC chromatogram of ESO (1000 µg/ml)

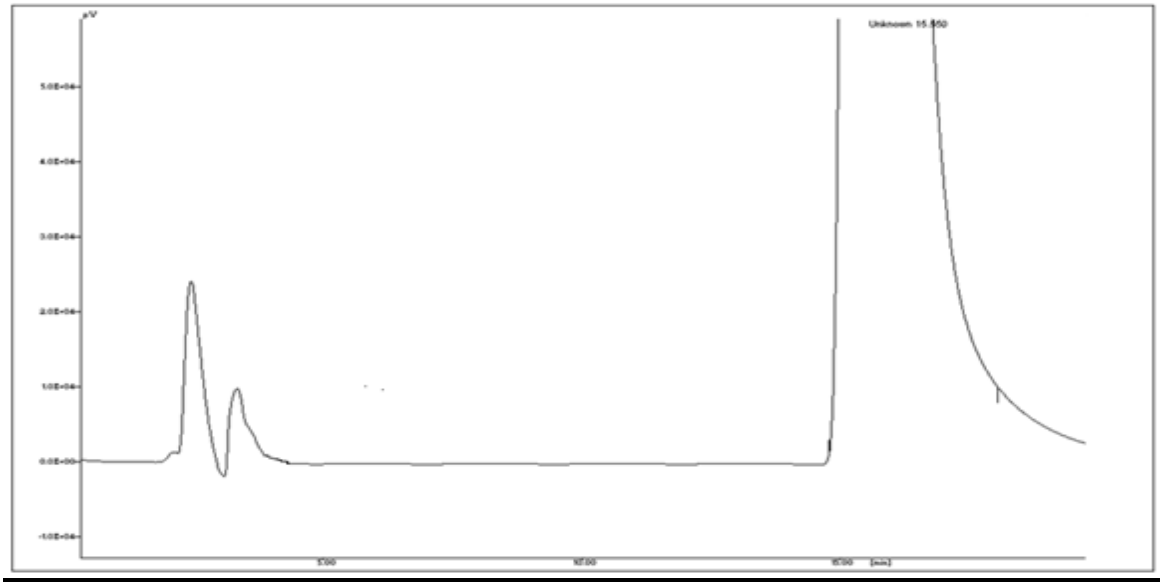


Fig 6.7 HPLC chromatogram of ESO (1000 µg/ml) (zoomed view)

Fig 6.7 shows that no impurities get generated from Esomeprazole magnesium when exposed to accelerated stability conditions 40°C & 75%RH.

6.4.5 Determination of Esomeprazole magnesium from stability samples of Esomeprazole magnesium alone

For the determination of ESO from the stability samples of Esomeprazole magnesium alone, samples having concentration of 50 µg/ml were made prior to inject it into the HPLC system. Stability sample of ESO were correlated with the control samples for determination of % assay of drug.

Calculation:

%Assay = (Area of test/ Area of std)* (weight of std/ Weight of test) *100

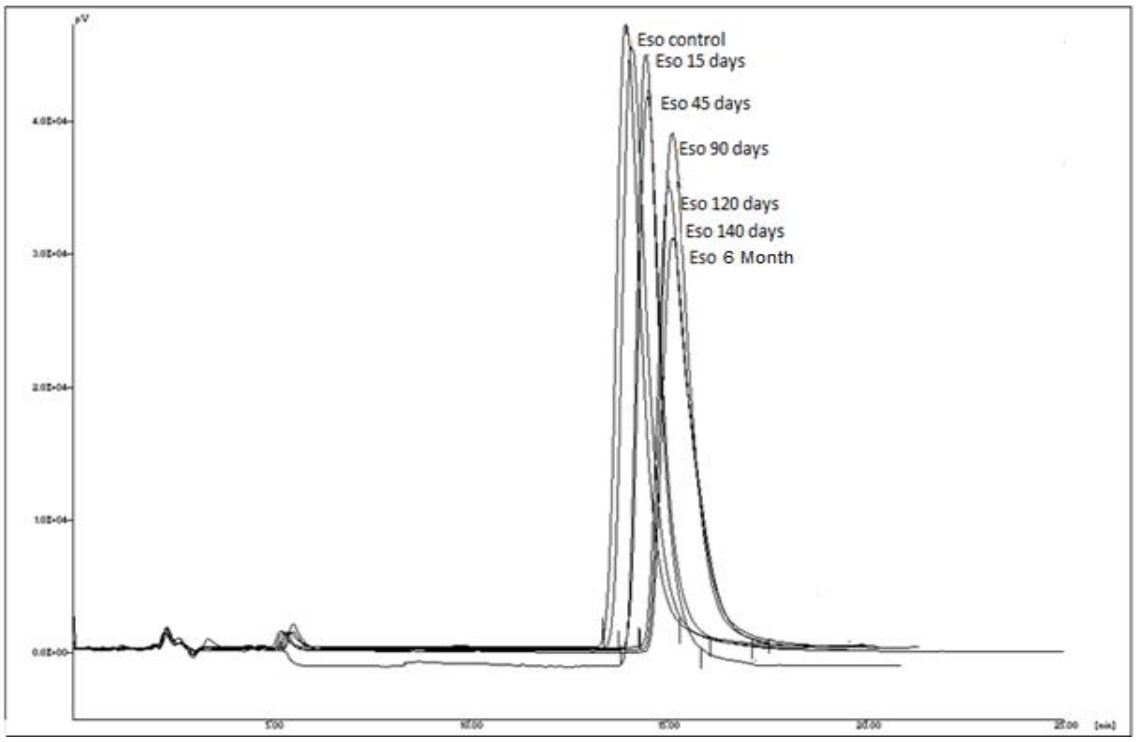


Fig 6.8 HPLC overlay chromatogram of ESO stability samples (50 µg/ml)

Fig 6.8 Shows overlay chromatogram of Esomeprazole control sample along with the accelerated stability study samples.

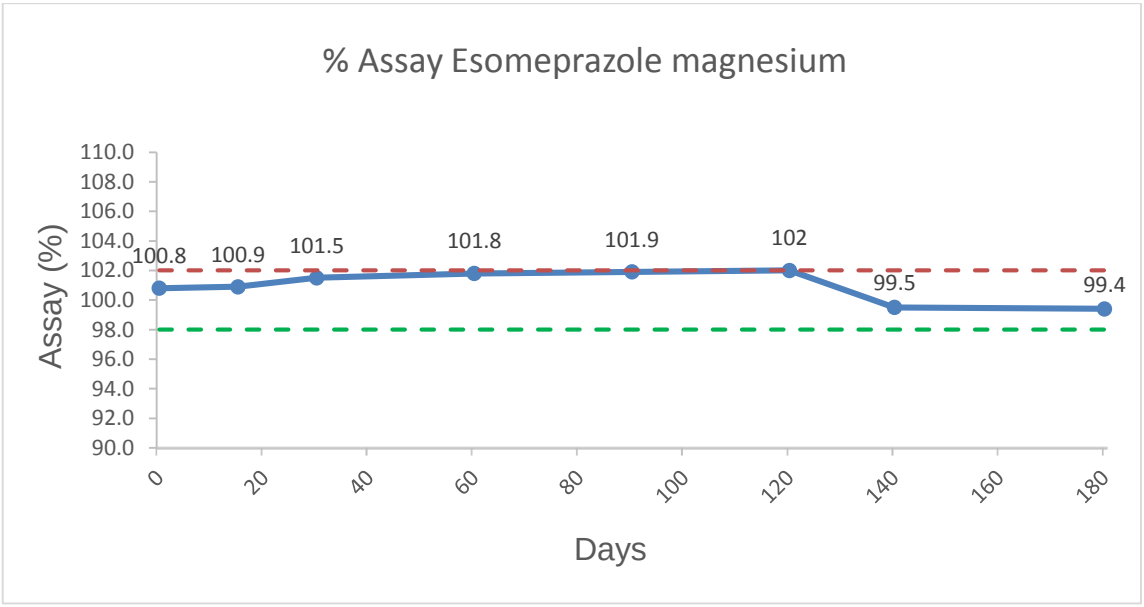


Fig 6.9 %Assay of ESO

Fig 6.9 shows there is no significant decrease in assay of esomeprazole magnesium. Specification criteria for Esomeprazole magnesium as per Indian pharmacopoeia is not less than 98% and not more than 102% of esomeprazole magnesium calculated on anhydrous basis. However, after 6 months of accelerated stability study of Esomeprazole magnesium alone the content of ESO is not significantly decreasing.

6.4.6 Compatibility study of Esomeprazole magnesium with Povidone K30

To identify the stability of Esomeprazole magnesium with Povidone K30, Binary mixture of ESO and PVP K30 (1:1) i.e. 5 g ESO and 5 g PVP K30 were prepared with proper mixing and kept in closed amber colored vial and exposed to 40ºC and 75%RH in stability chamber to extrapolate probable chemical incompatibility between ESO and PVP K30. Along with the mixture, dug and excipient alone were also kept in the stability chamber. Control samples were kept in refrigerator. Assay and %impurity has been found for each samples.

6.4.7 HPLC analysis of Esomeprazole magnesium and Povidone K30

Exposed accelerated stability samples of Esomeprazole magnesium and PVP K30 binary mixtures were used to prepare samples for HPLC analysis. In case of Esomeprazole magnesium and Povidone K30, 50 mg of drug and excipient mixture was weighed and transferred to 10 ml volumetric flask to get concentration of 2500µg/ml. Further dilutions were made with the diluent Acetonitrile: Water to get concentration of 1000 µg/mL for determination of impurities generated and 50 µg/mL for determination of assay of ESO as described in the section 6.3.2. Chromatographic conditions were kept constant as described in table 6.5. The responses of ESO and PVP K30 binary mixture obtained in each accelerated stability samples were compared with the responses of respective initial samples (control) and the % of generated impurities has been found for each samples.

To check the interference of excipient i.e. PVP K30, sample solution of PVP K30 in methanol was injected into the proposed HPLC system.

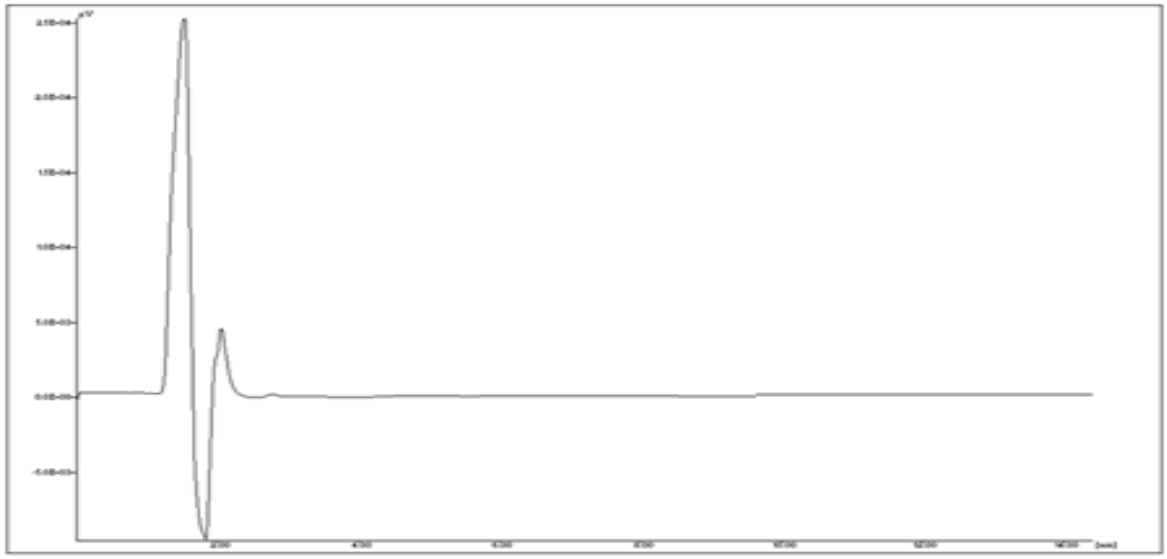


Fig 6.10 HPLC chromatogram of PVP K30 (Placebo)

Fig 6.10 indicates that there is no interferences of excipient PVP K30 in the analysis of esomeprazole magnesium using the proposed method.

6.4.8 Determination of impurities from stability samples of Esomeprazole magnesium and PVP K30

For the determination of impurities from the stability samples of ESO and PVP K30 binary mixtures, samples having concentration of 1000µg/ml were made as described in the section 6.3.2. Responses of impurities appeared in the samples were recorded. % of impurities generated has been calculated for each samples.

Calculation:

%Impurity= (Area of Impurity/Area of std)* (conc. Of std/ Conc. of test)*100

Where,

Conc. of std = 10 µg/mL

Conc. of test = 1000µg/mL

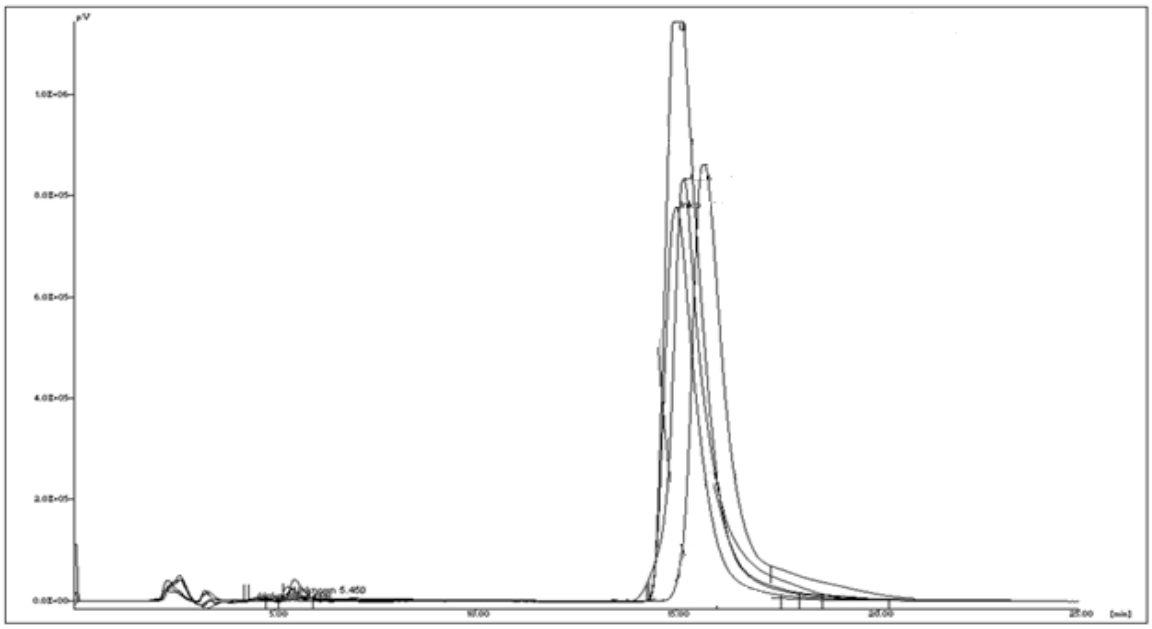


Fig 6.11 HPLC overlay chromatogram of ESO + PVP K30 (1000 µg/ml)

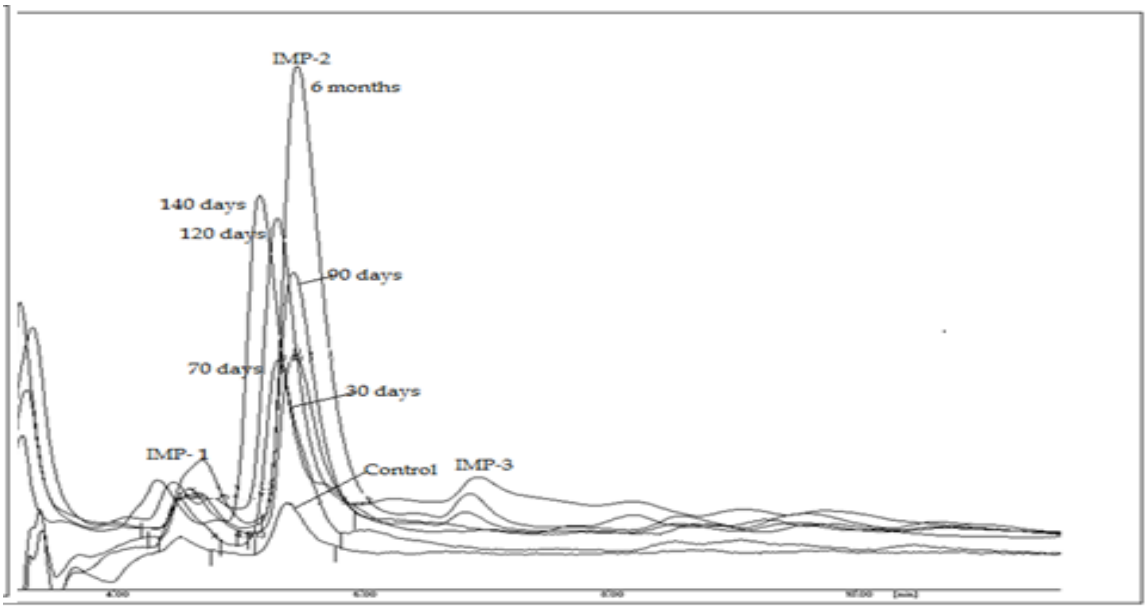


Fig 6.12 HPLC overlay chromatogram of ESO + PVP K30 (1000 µg/ml)

(Zoomed view)

Fig 6.12 shows zoomed view of overlay chromatogram of ESO + PVP K30. Total 3 impurities get generated in the mixture of ESO with PVP K30 samples exposed to accelerated stability conditions. Chromatogram reveals that the area of impurities is gradually

increasing starting fom the controlled sample to the accelerated stability study samples of 6 months.Total % of impurities has been calculated for each of the stability samples.

Table 6.7 Increase in total % Impurity in mixture of ESO + PVP K30

Condition	Days	Area of Imp-1 Rt=4.5	%Imp	Area of Imp-2 Rt=5.3	%Imp	Area of Imp-3 Rt=6.2	% Imp	Area of drug peak at Rt-=15.5	Total IMP	Single max
40 °C 75 RH	0	-	-	-	-	-	-	45892357	-	-
	7	29700	0.072	72265	0.18	-	-	48239201	0.25	0.18
	15	47476	0.116	138697	0.34	-	-	38562555	0.45	0.34
	30	59047	0.144	244821	0.60	-	-	37938498	0.74	0.60
	45	61777	0.150	277819	0.68	-	-	33250415	0.83	0.68
	60	67316	0.164	326017	0.79	-	-	47984121	0.96	0.79
	90	55658	0.136	352348	0.86	-	-	39875163	0.99	0.86
	120	56983	0.139	399655	0.97	4938	0.001	41542579	1.11	0.97
	140	68697	0.167	419177	1.02	5847	0.002	40039698	1.19	1.02
	180	79852	0.187	42987	1.05	6526	0.003	40863982	1.24	1.05
Control	180	14904	0.036	67811	0.17	-	-	49724958	0.2	0.17

Data depicted in table 6.7 shows that area of IMP-1 and IMP-2 is gradually increasing on the exposure of accelerated stability conditions whereas IMP-3 gets generated after the long term exposure of accelerated stability conditions. Total impurities get generated in the binary mixture of ESO and PVP K30 starting from 0.25% to 1.24%. after six months study. Control sample of ESO and PVP K30 binary mixture shows impurity after 180 days though this impurity can not be considered as significant.

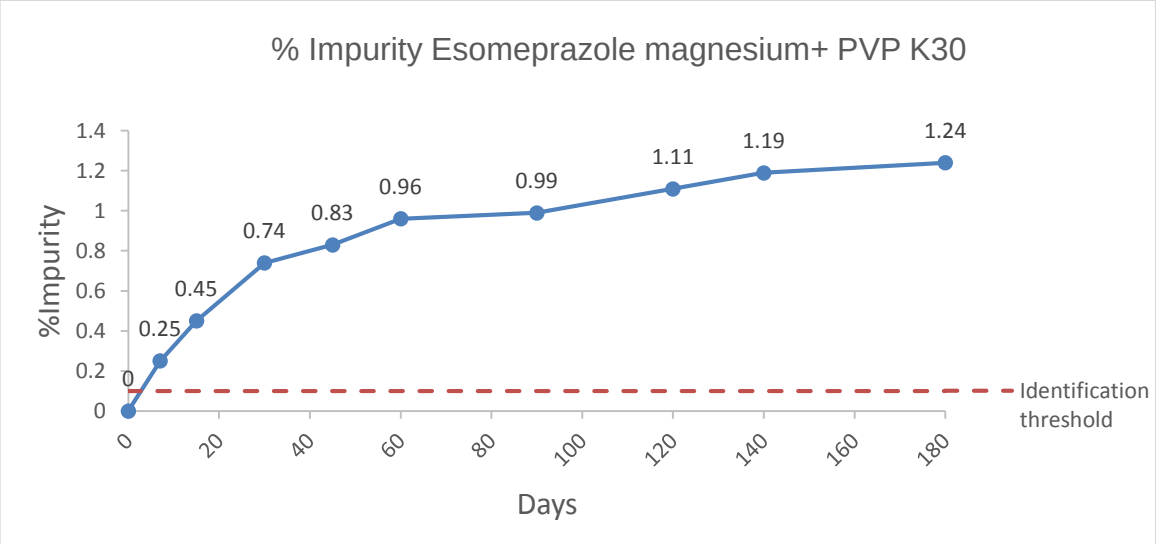


Fig 6.13 Increase in %Impurity for ESO + K30 stability samples

6.4.9 Determination of Esomeprazole magnesium from the stability samples of Esomeprazole magnesium with PVP K30

For the determination of ESO from the stability samples of Esomeprazole magnesium and PVP K30, samples having concentration of 50 µg/ml were made as described in section 6.3.2 prior to inject it into the HPLC system. Stability sample of ESO+ PVP K30 were correlated with the control samples for determination of % assay of drug.

Calculation: %Assay = (Area of test/ Area of std)* (weight of std/ Weight of test) *100

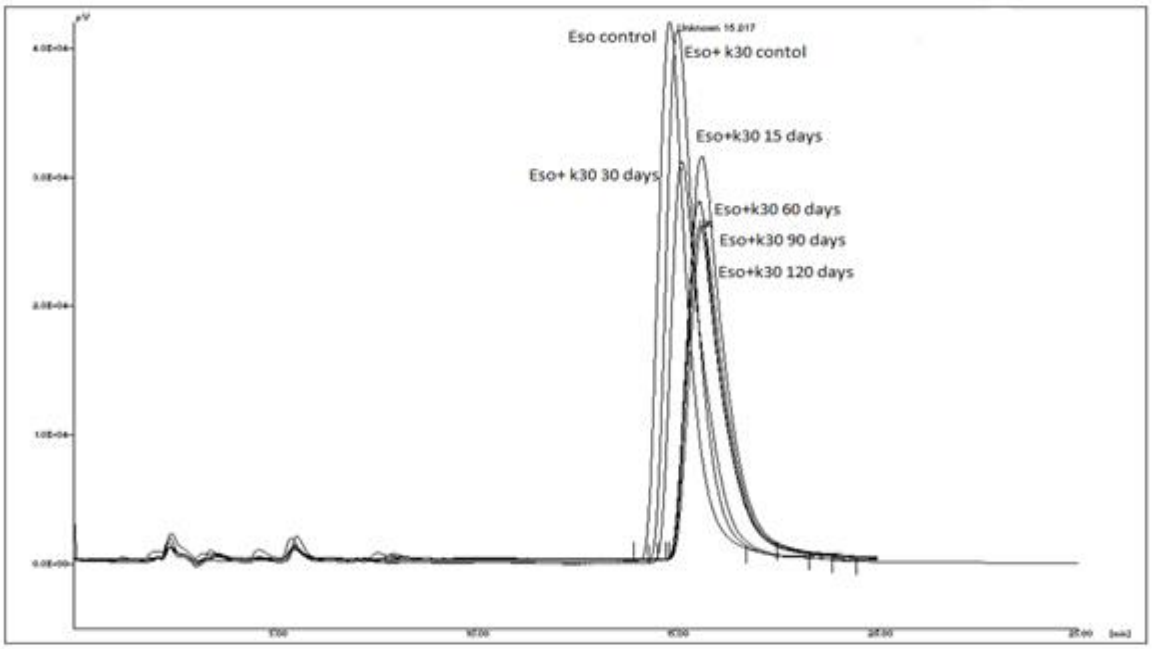


Fig 6.14 HPLC overlay chromatogram of stability samples of ESO + PVP K30 (50 µg/ml)

Fig 6.14 shows Esomeprazole + PVP K30 (50 µg/ml) overlay chromatogram of stability samples along with control sample. The chromatogram reveals that the area of drug peak is gradually decreasing after 6 months of the study.

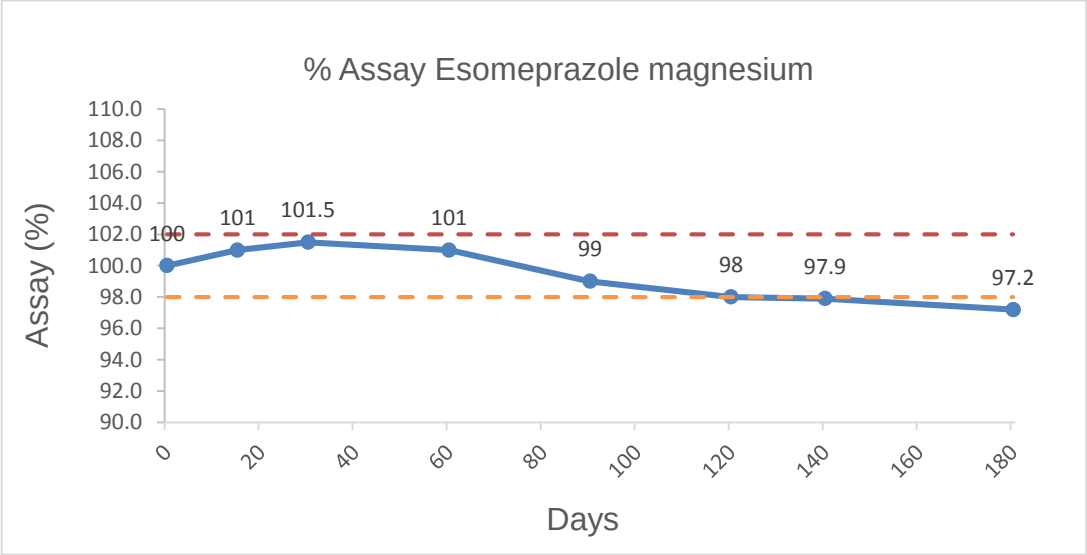


Fig 6.15 % Assay of ESO from ESO+ PVP K30

Fig 6.15 shows % assay of Esomeprazole magnesium in the binary mixture of ESO and PVP K30. Though the assay is not decreasing much and it lies within the limits specified by Indian Pharmacopoeia but the total generated impurities are beyond the identification threshold described in ICHQ3A (R1). Even the single max of Impurity-2 is also beyond the identification threshold so qualification and characterization of the impurities generated by Esomeprazole magnesium and PVP K30 should be carried out.

6.4.10 Compatibility study of Esomeprazole magnesium with Povidone K90

To identify the stability of Esomeprazole magnesium with Povidone K90, Binary mixture of ESO and PVP K90 (1:1) i.e. 5 g ESO and 5 g PVP K90 were prepared with proper mixing and kept in closed amber colored vial and exposed to 40ºC and 75%RH in stability chamber to extrapolate probable chemical incompatibility between ESO and PVP K90. Along with the mixture, dug and excipient alone were also kept in the stability chamber. Control samples were kept in refrigerator. Assay and % impurity has been found for each samples.

6.4.11 HPLC analysis of Esomeprazole magnesium and Povidone K90

Exposed accelerated stability samples of Esomeprazole magnesium and PVP K90 binary mixtures were used to prepare samples for HPLC analysis. In case of Esomeprazole magnesium and Povidone K90, 50 mg of drug and excipient mixture was weighed and transferred to 10 ml volumetric flask to get concentration of 2500µg/ml. Further dilutions were made with the diluent Acetonitrile: Water to get concentration of 1000 µg/mL for determination of impurities generated and 50 µg/mL for determination of assay of ESO as described in the section 6.3.2. Chromatographic conditions were kept constant as described in table 6.5. The responses of ESO and PVP K90 binary mixture obtained in each accelerated stability samples were compared with the responses of respective initial samples (control) and the % of generated impurities has been found for each samples.

To check the interference of excipient i.e. PVP K90, sample solution of PVP K90 in methanol was injected into the proposed HPLC system.

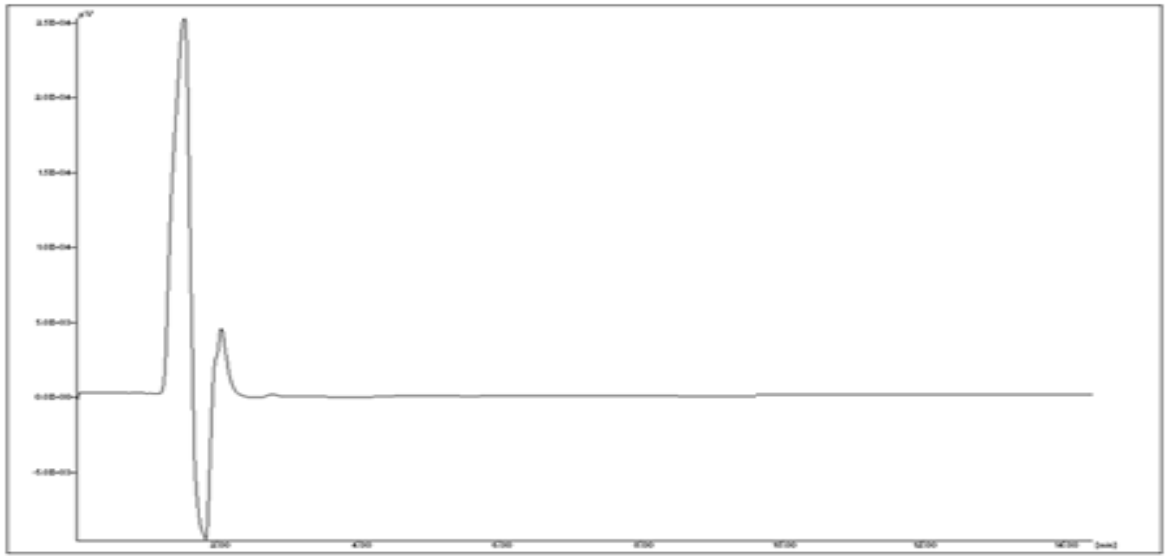


Fig 6.16 HPLC chromatogram of PVP K90 (Placebo)

Fig 6.16 indicates that there is no interferences of excipient PVP K90 in the analysis of esomeprazole magnesium using the proposed method.

6.4.12 Determination of impurities from stability samples of Esomeprazole magnesium and PVP K90

For the determination of impurities from the stability samples of ESO and PVP K90 binary mixtures, samples having concentration of 1000µg/ml were made as described in the section 6.3.2. Responses of impurities appeared in the samples were recorded. % of impurities generated has been calculated for each samples.

Calculation:

%Impurity= (Area of Impurity/Area of std)* (conc. Of std/ Conc. of test)*100

Where,

Conc. of std = 10 µg/mL

Conc. of test = 1000µg/mL

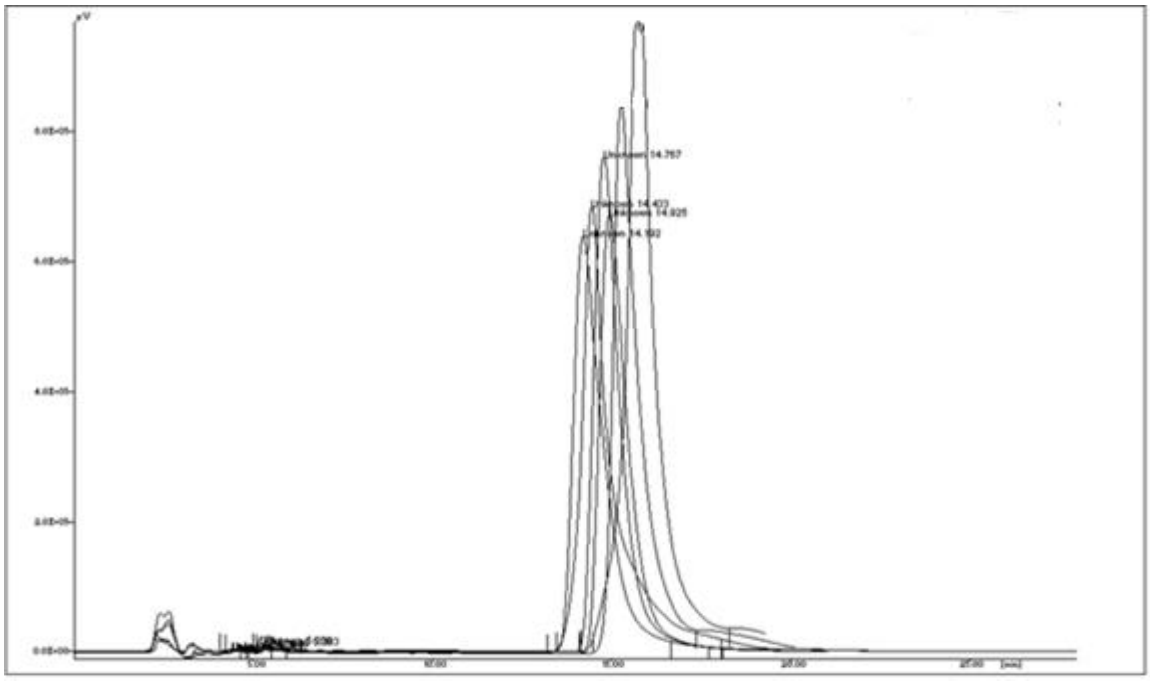


Fig 6.17 HPLC overlay chromatogram of ESO + PVP K90 (1000 µg/ml)

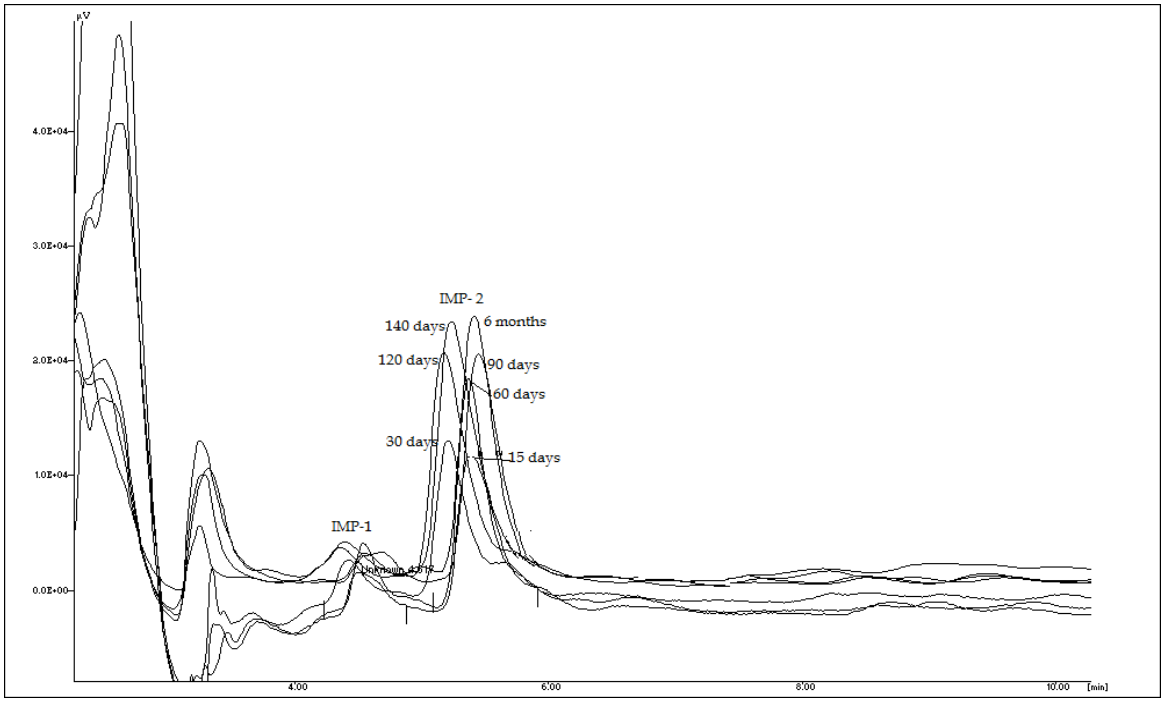


Fig 6.18 HPLC overlay chromatogram of ESO + PVP K90 (1000 µg/ml) (Zoomed view)

Fig 6.18 shows zoomed view of overlay chromatogram of ESO + PVP K90. Total 2 impurities get generated in the mixture of ESO with PVP K90 samples exposed to accelerate stability conditions. Chromatogram reveals that the area of impurities is gradually increasing on exposure of accelerated stability study samples of 6 months.Total % of impurities has been calculated for each of the stability samples.

Table 6.8 Increase in total % Impurity in mixture of ESO + PVP K90

Condition	Days	Area of Imp-1 Rt=4.5	%Imp	Area of Imp-2 Rt=5.3	%Imp	Area of drug peak at Rt-=15.5	Total IMP	Single max
40 °C 75 RH	**0**	-	-	-	-	40521895	-	-
	7	38885	0.095	166815	0.41	39193862	0.50	0.41
	15	49219	0.120	185561	0.45	38262638	0.57	0.45
	30	69426	0.169	247406	0.60	45518640	0.77	0.60
	45	67816	0.165	222740	0.54	41289049	0.79	0.54
	60	86480	0.211	242693	0.59	35175760	0.80	0.59
	90	65174	0.159	257861	0.63	35123879	0.89	0.63
	120	78995	0.192	389951	0.95	35693388	1.04	0.95
	140	74957	0.183	406741	0.99	35447486	1.07	0.99
	180	75860	0.185	412563	1.0	40552368	1.19	1.0
Control	180	35383	0.086	186220	0.15	39295934	0.24	0.45

Data depicted in table 6.8 shows that area of IMP-1 and IMP-2 is gradually increasing on the exposure of accelerated stability conditions. Total impurities get generated in the binary mixture of ESO and PVP K90 starting from 0.50% to 1.19%. after six months study.

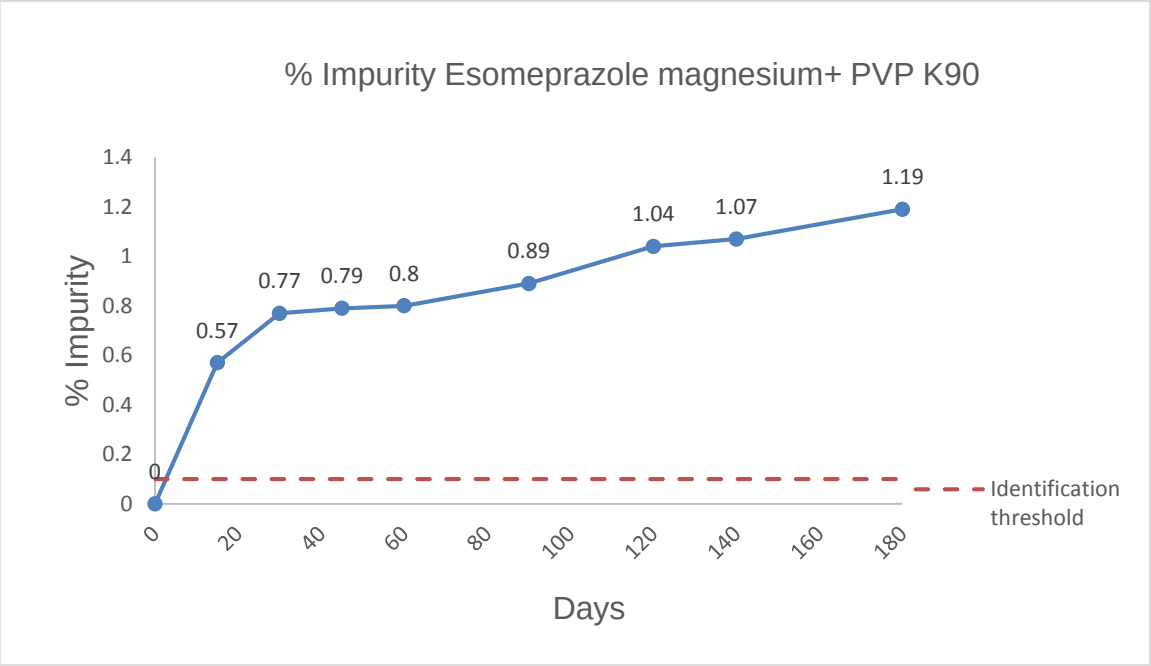


Fig 6.19 Increase in %Impurity for ESO + K90 stability samples

6.4.13 Determination of Esomeprazole magnesium from the stability samples of Esomeprazole magnesium with PVP K90

For the determination of ESO from the stability samples of Esomeprazole magnesium and PVP K90, samples having concentration of 50 µg/ml were made as described in section 6.3.2 prior to inject it into the HPLC system. Stability sample of ESO+ PVP K90 were correlated with the control samples for determination of % assay of drug.

Calculation: %Assay = (Area of test/ Area of std)* (weight of std/ Weight of test) *100

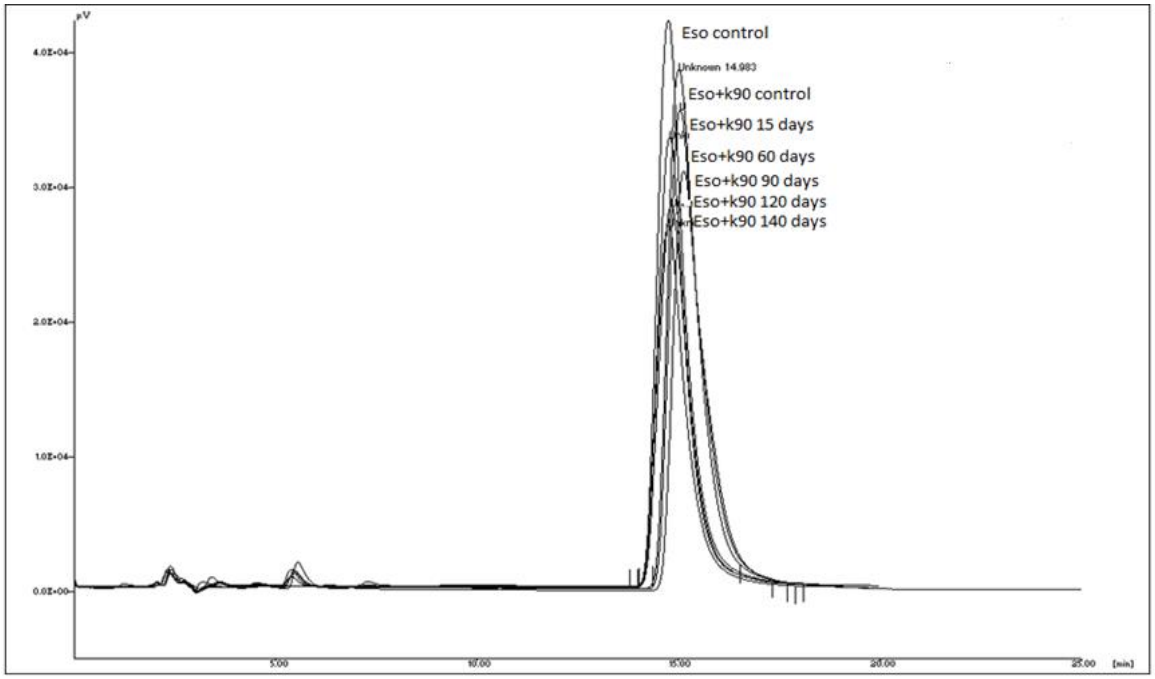


Fig 6.20 HPLC chromatogram of stability samples of ESO + PVP K90 (50 µg/ml)

Fig 6.20 shows Esomeprazole + PVP K90 (50 µg/ml) overlay chromatogram of stability samples along with control sample. The chromatogram reveals that the area of drug peak is gradually decreasing after 6 months of the study.

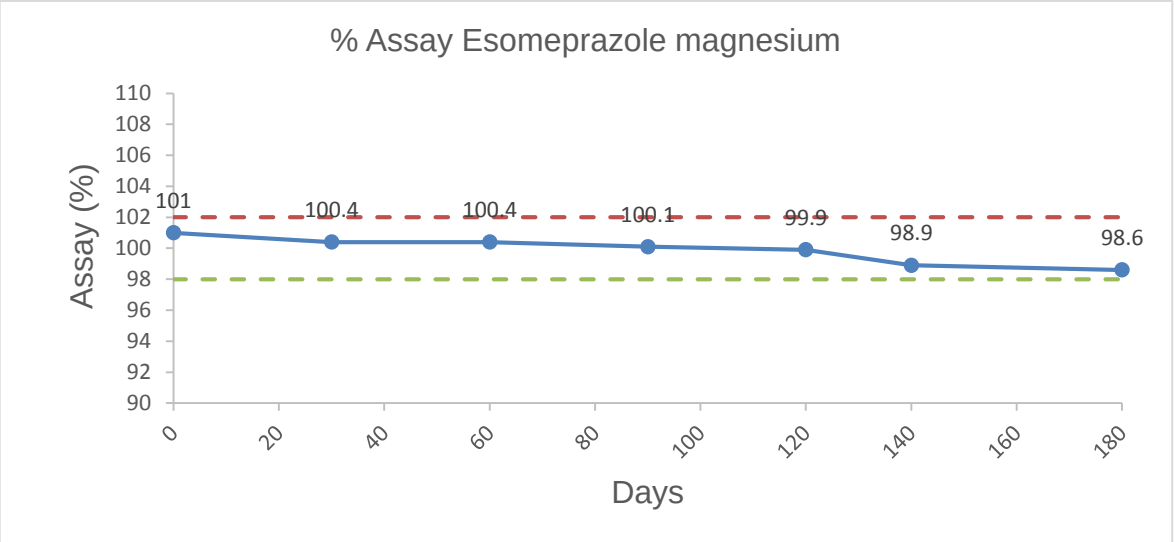


Fig 6.21 % Assay of ESO from ESO + PVP K90

Fig 6.21 shows %assay of Esomeprazole magnesium in the binary mixture of ESO and PVP K90. Though the assay is not decreasing much and it lies within the limits specified by Indian Pharmacopoeia but the total generated impurities are beyond the identification threshold described in ICHQ3A (R1). Even the single max of Impurity-2 is also beyond the identification threshold so qualification and characterization of the impurities generated by Esomeprazole magnesium and PVP K90 should be carried out.

6.4.14 Oxidative degradation of Esomeprazole magnesium [12]

To investigate the impact of peroxides on esomeprazole magnesium, oxidative degradation of ESO was carried out. 25 mg of ESO dissolved in 25 ml of methanolic H_2O_2 (3% hydrogen peroxide). Volumetric flask kept in the water bath at 40°C for 60 min. Further dilutions were made with methanol. Sample concentration was made 1000 µg/ml prior to inject it into the HPLC system.

Table6.9 Comparison of oxidative degradation with reported degradation

% Degradation	Reported	Observed
ESO	11.7%	19%

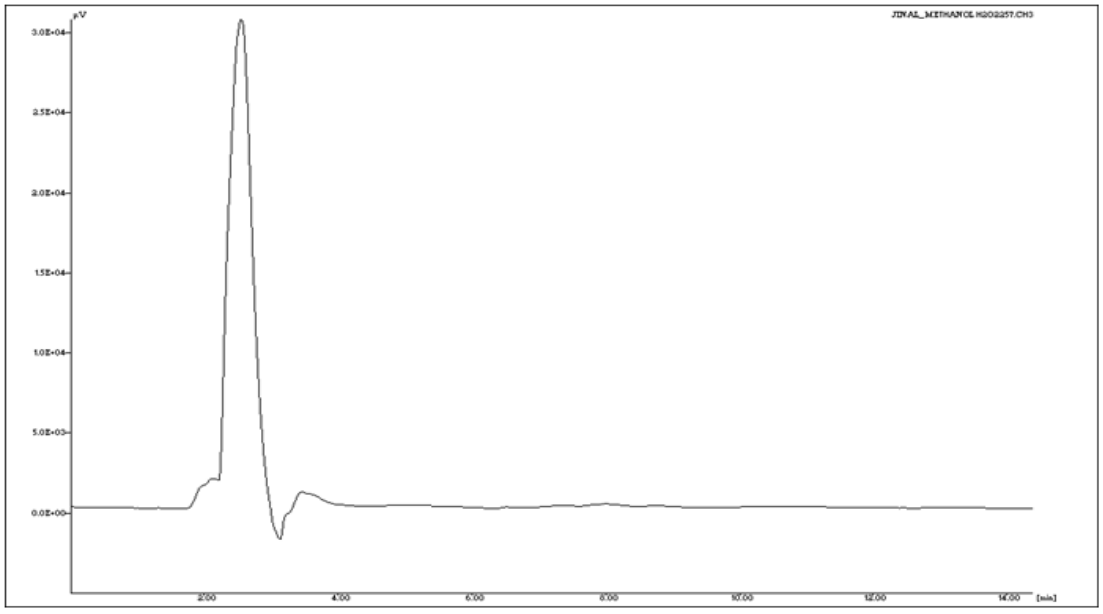


Fig 6.22 HPLC chromatogram of Methanolic H_2O_2 (Blank)

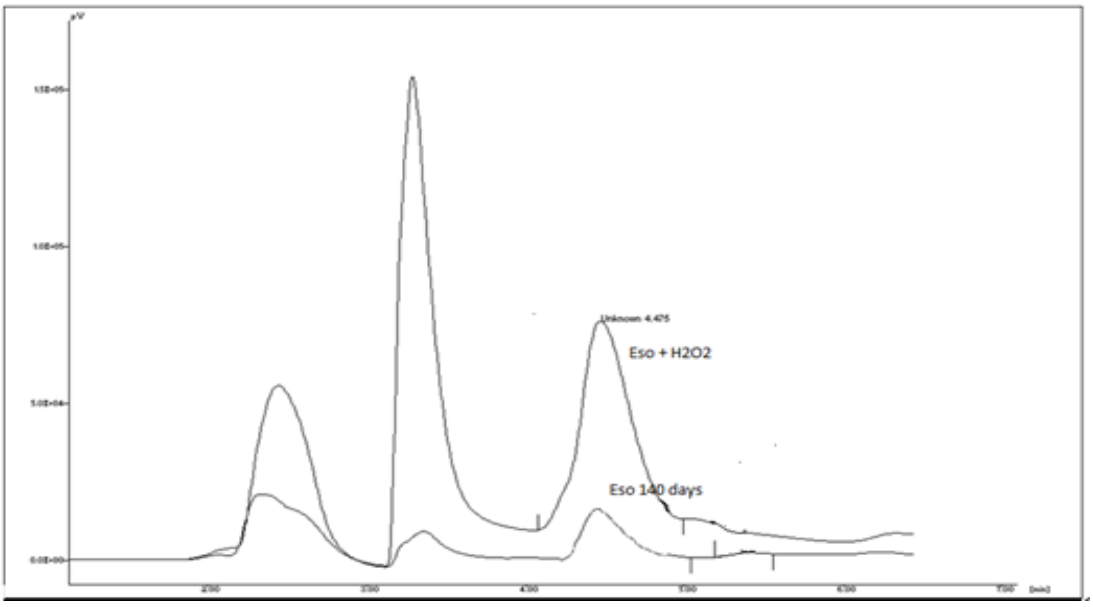
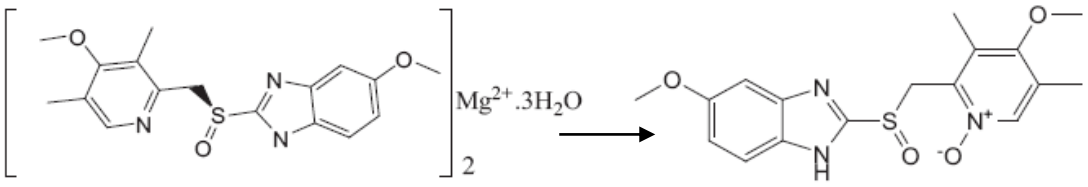


Fig 6.23 comparison of degradation profile of Esomeprazole magnesium with oxidative degradation

Degradation profile of the exposed samples of esomeprazole magnesium with PVP K30 and PVP K90 was compared with the oxidative degradation of pure drug. The retention time of impurities generated in accelerated stability study samples resembles the retention time of degradation product by oxidative degradation of esomeprazole magnesium. So it can be said that the degradation of esomeprazole magnesium with excipients was mediated by organic peroxides present as an impuritiy in Excipient PVP K30 and PVP K90.

Probable chemical reactions occurring between Esomeprazole and Hydrogen peroxide



Esomeprazole Magnesium Trihydrate

+ R.OH

6.5 Differential Scanning Calorimetry (DSC) Analysis

Differential scanning calorimetry (DSC) is used to investigate the drug-excipient interactions. It is a useful tool to carry out preformulation studies and to know possible interaction between drug and excipients. The results obtained by HPLC were compared with the thermal analysis results. But the changes in DSC thermogram cannot always be sufficient data to prove that some interaction occurs between drug and excipient.

DSC data can be analysed by appearance, shift or disappearance of endothermic or exothermic peaks and/or variations in the corresponding enthalpy values in thermal curves of drug–excipient mixtures.

To analyse the compatibility of Esomeprazole with PVP K30 and PVP K90 by DSC, exposed binary mixtures of Esomeprazole magnesium and Povidone were run under thermal analysis. Thermogram obtained of exposed samples were compared with the thermogram of controlled samples. Following data shows the thermogram of drug and excipient alone as well as exposed samples of binary mixtures.

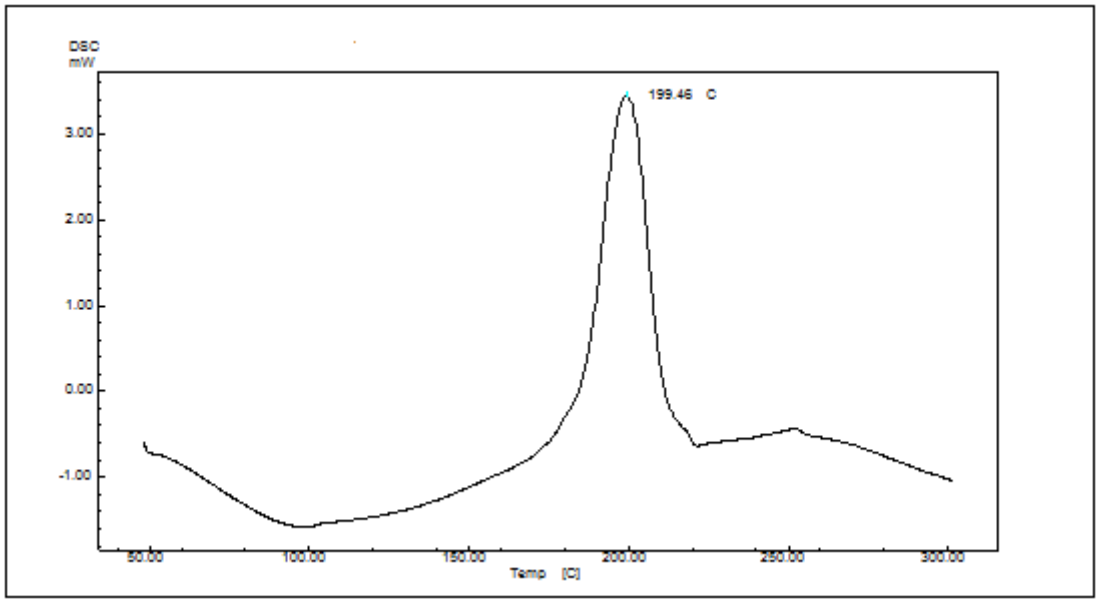


Fig 6.24 Thermogram of Esomeprazole magnesium control sample

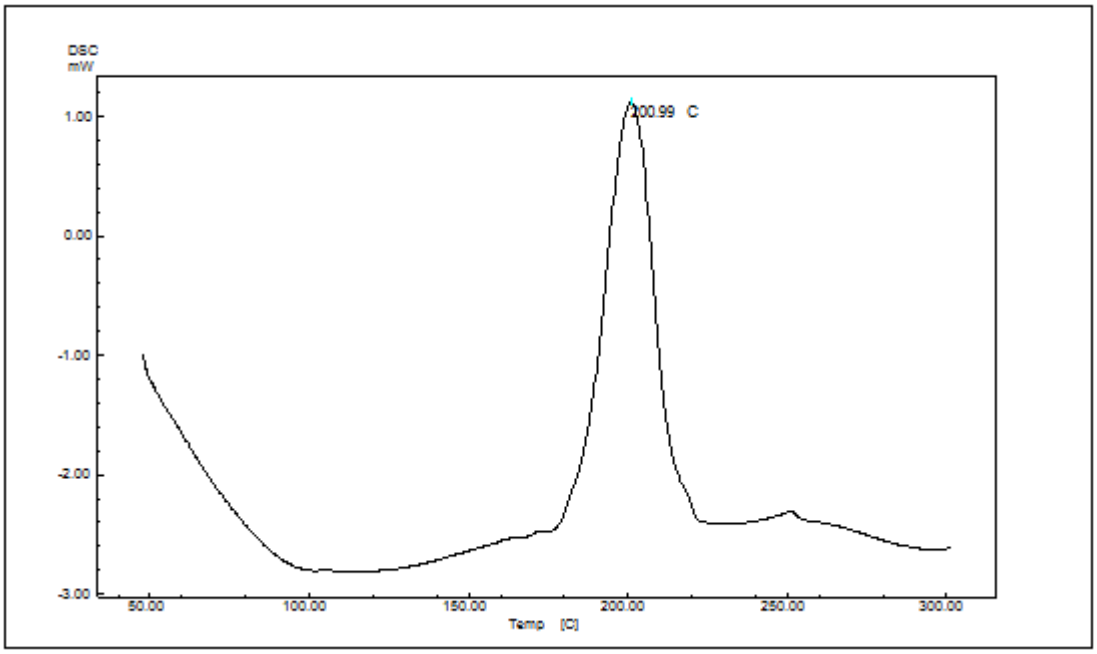


Fig 6.25 Thermogram of Esomeprazole magnesium exposed sample

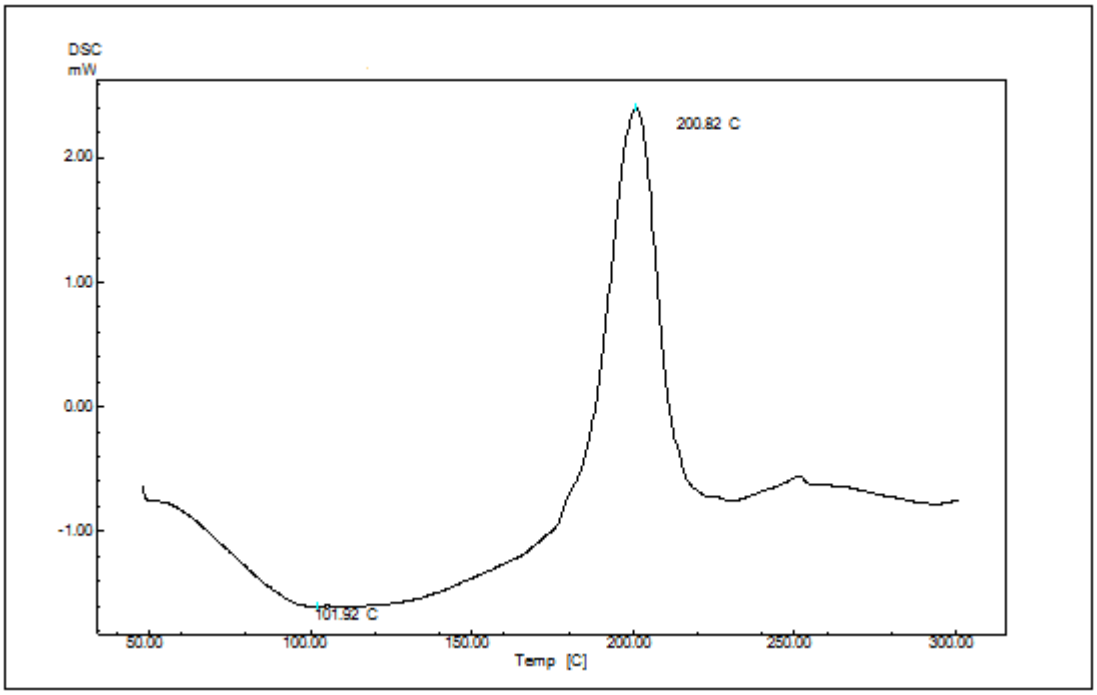


Fig 6.26 Thermogram of Esomeprazole magnesium and PVP K30 exposed sample

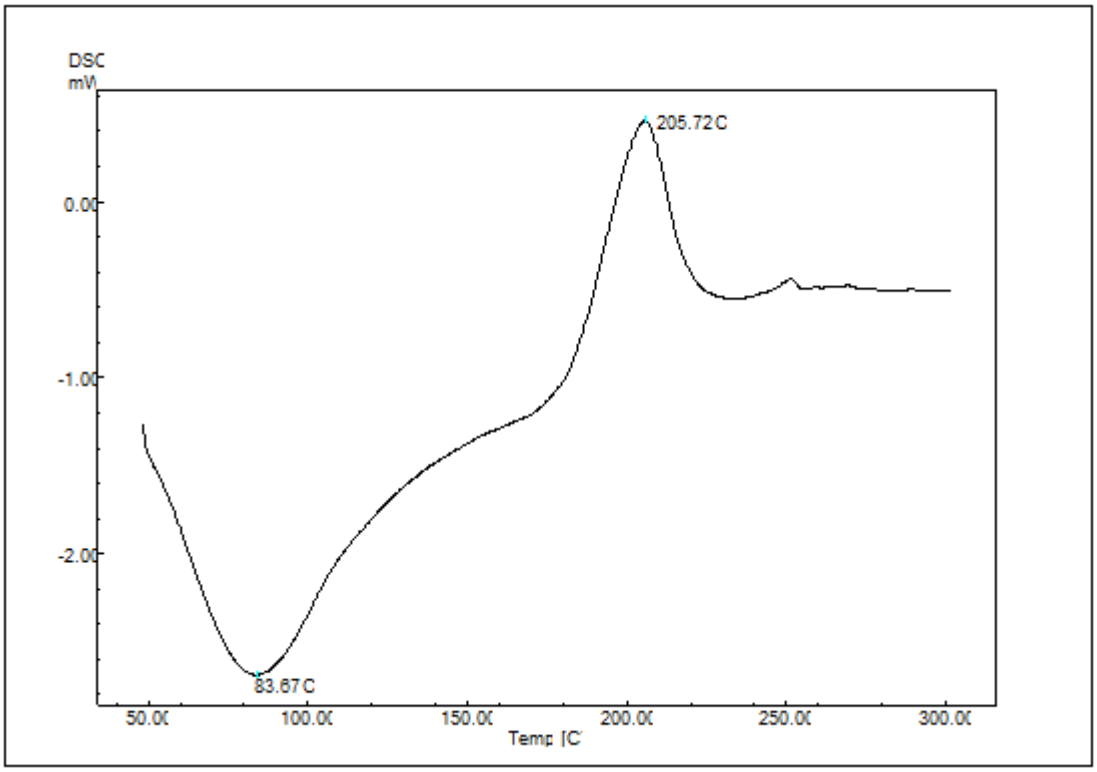


Fig 6.27 Thermogram of Esomeprazole magnesium and PVP K90 exposed sample

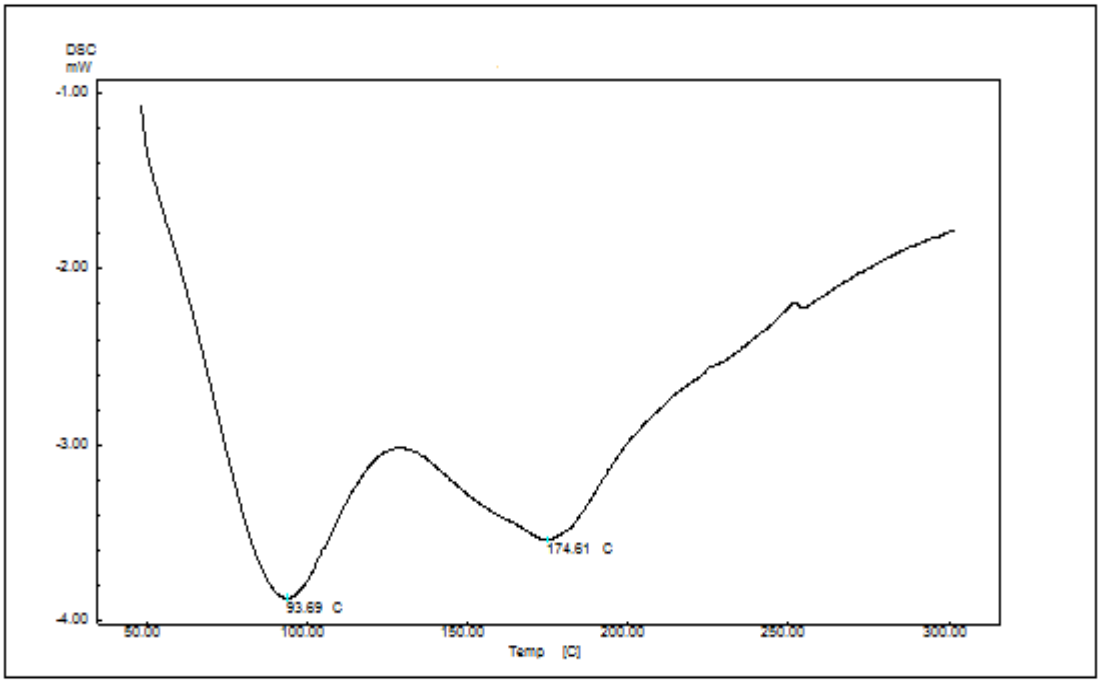


Fig 6.28 Thermogram of PVP K30

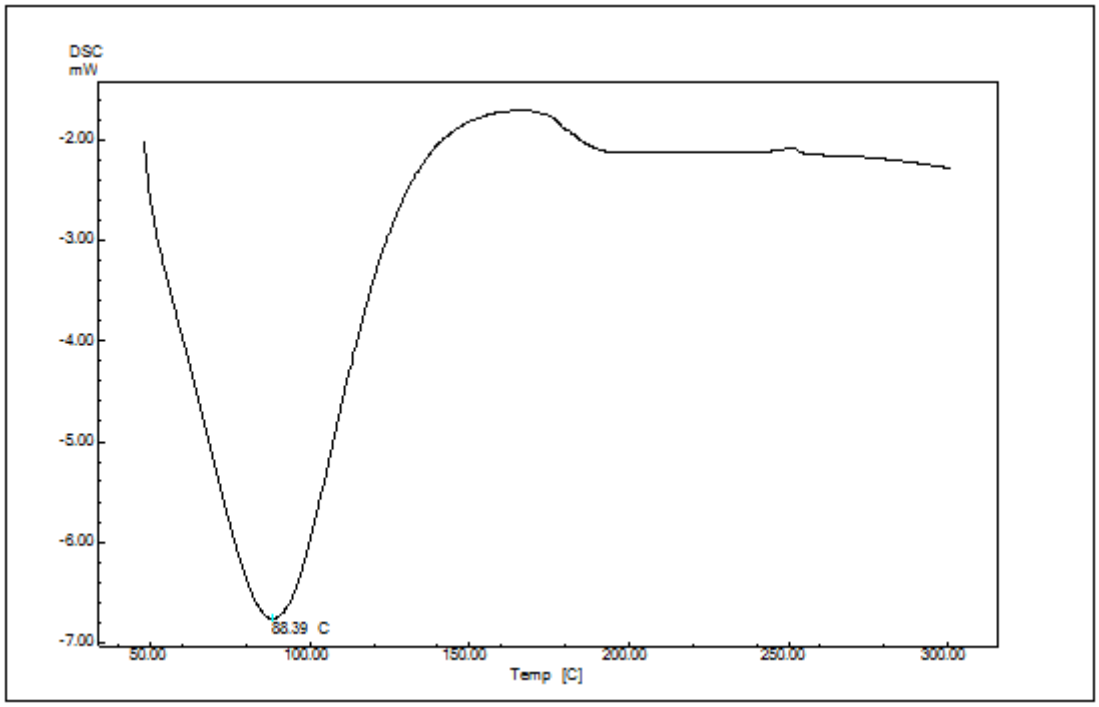


Fig 6.29 Thermogram of PVP K90

Above thermogram shows very little changes compared to the control samples of Esomeprazole magnesium.

Table 6.10 Summary of DSC data

Condition	**Esomeprazole**	**Esomeprazole+ PVP K30**		**Esomeprazole+ PVP K90**		**PVP K30**		**PVP K90**
Fresh	199.46	92.91	203.92	88.70	204.62	93.69	174.61	88.39
Stressed	200.99	101.92	200.82	83.67	205.72			

Data depicted in table 6.10 shows that there is no physical interaction between Esomeprazole magnesium and PVP K30 as well as PVP K90. So it can be concluded that chemical interaction is there between drug and organic peroxides present in excipient as an impurity, which is degrading Esomeprazole magnesium which supports the data obtained in HPLC analysis.

CHAPTER 7

EXPERIMENTAL WORK FOR PANTOPRAZOLE SODIUM

7.1 Identification of drug

The identification of Pantoprazole sodium was carried out by following techniques.

1. Melting point
2. UV- spectroscopy
3. FT-IR spectroscopy

7.1.1 Identification of drug by melting point

Melting Point of PAN has been determined using capillary melting point apparatus.

Melting Point obtained were compared with that available in literature as shown in table**.**

Table: 7.1 Comparison of reported and observed melting point of PAN

Drug	Reported(°c)[9]	Observed(°c)
PAN	137.5-145.5	142

7.1.2 Identification of drug by absorption maxima

UVspectrum of PAN (100 µg/ml) in methanol was recorded using UV-VIS spectrophotometer.

By scanning the sample in the range of 200-400 nm against methanol as a blank.

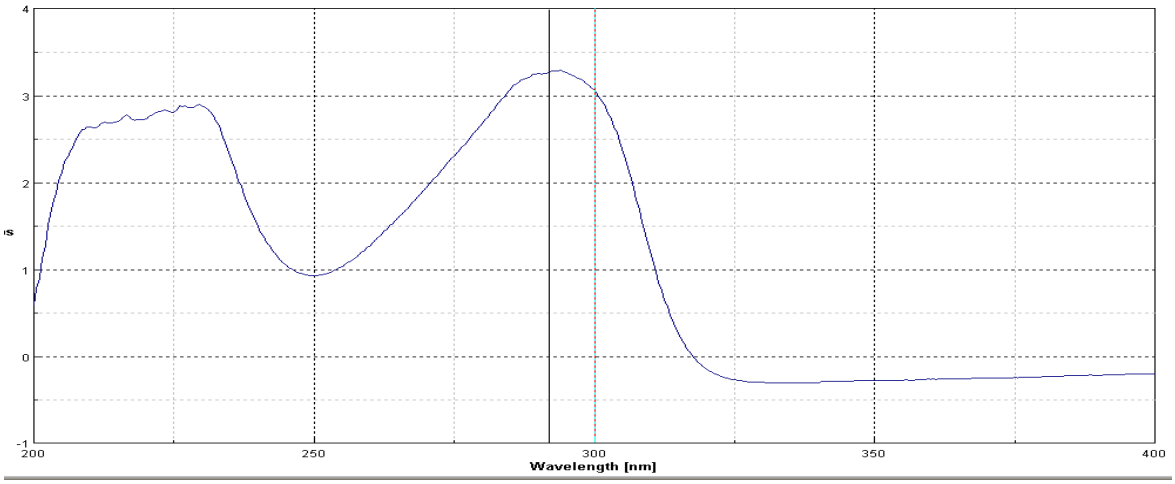


Figure 7.1 UV spectrum of PAN (100 µg/ml) in methanol

Table 7.2 Comparison of reported absorption maxima with observed absorption maxima

Drug	Reported Absorption maxima[51]	Observed absorption maxima
PAN	290 nm	291nm

7.1.3 Identification of drug by FT-IR

FT-IR spectrum of PAN was recorded in diffused reflectance mode. Theoretical values of wave numbers responsible for functional groups are compared with observed values of wave numbers as summarized in table 7.3

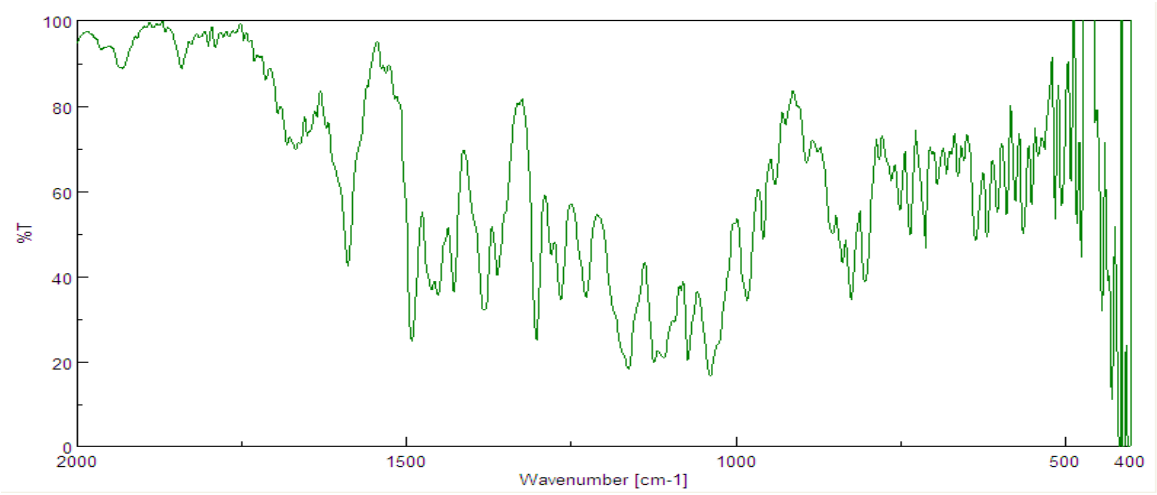


Fig 7.2 Recorded FT-IR spectra of PAN

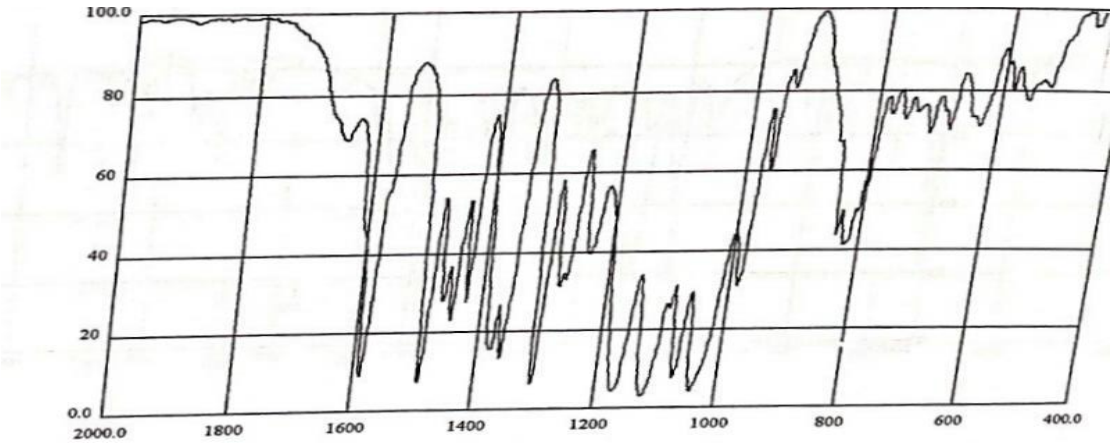


Fig 7.3 Standard FT-IR spectra of PAN (IP-2014) [50]

Table 7.3 Observations for FT-IR spectra of PAN

Theoretical frequency(cm^{-1})	Observed frequency(cm^{-1})
1590	1589
1489	1491
1165	1162
1125	1124
1070	1073
1040	1038

By performing the identification tests and comparing with the standard values it can be concluded that procured sample was of Pantoprazole sodium.

7.2 Accelerated stability study

Accelerated stability studies were carried out in accordance with ICH guidelines (Q1A (R2) ICH). PAN alone as well as in combination with excipients were stressed under accelerated stability condition such as 40°C & 75%RH.

7.2.1 Sample preparation for accelerated stability study

To identify the stability of Pantoprazole Sodium with excipient Povidone, Binary mixture of PAN and excipient (1:1) i.e. 5 g API and 5gm excipient, were prepared with proper mixing and kept in closed amber colored vial and exposed to 40ºC and 75%RH in stability chamber to extrapolate probable chemical incompatibility between drug and excipient. Along with that mixture, PAN, PVP K30 and PVP K90 alone were also kept in the stability chamber. Control samples were kept in refrigerator. Sampling was planned to be done at 7, 15, 30 and 45 days and continued for 6 months. Assay and %impurity has been found for each samples.

Table 7.4 Accelerated stability study for Pantoprazole sodium with Povidone K30 and Povidone K90

Sample no.	Sample	Condition
1	Pantoprazole (5g)	40°C&75%RH
		Control(15°C)
2	Pantoprazole(5g) + PVP K30(5g) (1:1)	40°C&75%RH
		Control(15°C)
3	Pantoprazole(5g) + PVP K90(5g) (1:1)	40°C&75%RH
		Control(15°C)
4	PVP K30 (2g)	40°C&75%RH
		Control(15°C)
5	PVP K90 (2g)	40°C&75%RH
		Control(15°C)

7.3 Pantoprazole sodium and povidone compatibility study

7.3.1 Chromatographic conditions

The chromatographic method followed was reported []. Column used was C_8 150 mm × 4.6 mm, 5µm. The separation was achieved on an isocratic method. Mobile phase A contains 10 mM Phosphate buffer (pH 7.0) and the Mobile phase B contains acetonitrile and the ratio was 70:30 (v/v); respectively. The flow rate was 1 mL/min and the detection wavelength was 290 nm. The column temperature was maintained at 25 (ambient) and the detection was monitored at a wavelength 290 nm. The injection volume was 20µL. A mixture of triple distilled water and acetonitrile in the proportion of 70:30 (v/v); respectively used as a solvent or diluent.

7.3.2 Sample preparation

7.3.2.1 Sample preparation for Pantoprazole sodium

1) Preparation of stock solutions:

A stock solution of Pantoprazole sodium 25 mg/mL was prepared by dissolving 25 mg of PAN in 10 ml of methanol.

2) Preparation of working stock solutions:

Sample solutions having concentration 1000µg/mL were prepared from stock solution. 4 ml of the stock solution was diluted up to 10 ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of impurities generated.

3) Preparation of working solutions:

Working solutions having concentration 50 µg/mL were prepared from the sample solution. 0.5ml of the sample solution was diluted up to 10 ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of assay of PAN.

7.3.2.2 Sample preparation for Pantoprazole sodium with Excipient

1) Preparation of stock solutions:

A stock solution of Pantoprazole sodium 25 mg/mL was prepared by dissolving 50 mg of drug and excipient mixture in 10 ml of methanol.

2) Preparation of working stock solutions:

Sample solutions having concentration 1000µg/mL were prepared from stock solution.4 ml of the stock solution was diluted up to 10 ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of impurities generated.

3) Preparation of working solutions:

Working solutions having concentration 50 µg/mL were prepared from the sample solution. 0.5ml of the sample solution was diluted up to 10 ml with solvent mixture containing Triple Distilled Water and ACN (70:30) for the determination of assay of PAN.

7.4 Results and discussion

7.4.1 Compatibility study of Pantoprazole sodium with povidone

Pantoprazole sodium is a Proton Pump Inhibitor which was selected to carry out drug and excipient compatibility study with different grades of povidone. Pantoprazole sodium was mixed with excipient 1:1 separately as described in table 7.2.1 and exposed to 40°C 75%RH. Sampling was planned to be done at 7, 15, 30, 45 days and continued for 6 months. Exposed samples were analysed for new impurity generated and assay determination of PAN.

7.4.2 HPLC analysis of Pantoprazole sodium alone

As per section 7.2.1 along with PAN and excipient mixture samples, Pantoprazole sodium alone was also exposed to accelerated stability conditions. After completion of the time period stability samples were prepared for HPLC analysis. In case of Pantoprazole sodium alone 25mg of drug was weighed and transferred it to 10 ml volumetric flask to get concentration of 2500µg/ml. Further dilutions were made as described in the section 7.3.2.

Chromatographic conditions

Anthony Ekpe et al [25] reported following chromatographic conditions for the stability indicating assay method of pantoprazole sodium.

Table 7.5 Chromatographic conditions

Column	C_8 150 mm*4.6 i.d. ,5 µm Particle size
Mobile phase	10 mM Phosphate buffer (pH 7.0) and acetonitrile 70:30 (v/v)
Flow rate	1ml/ min
Temperature	Ambient
Detection wavelength	290nm
Injection volume	20µL

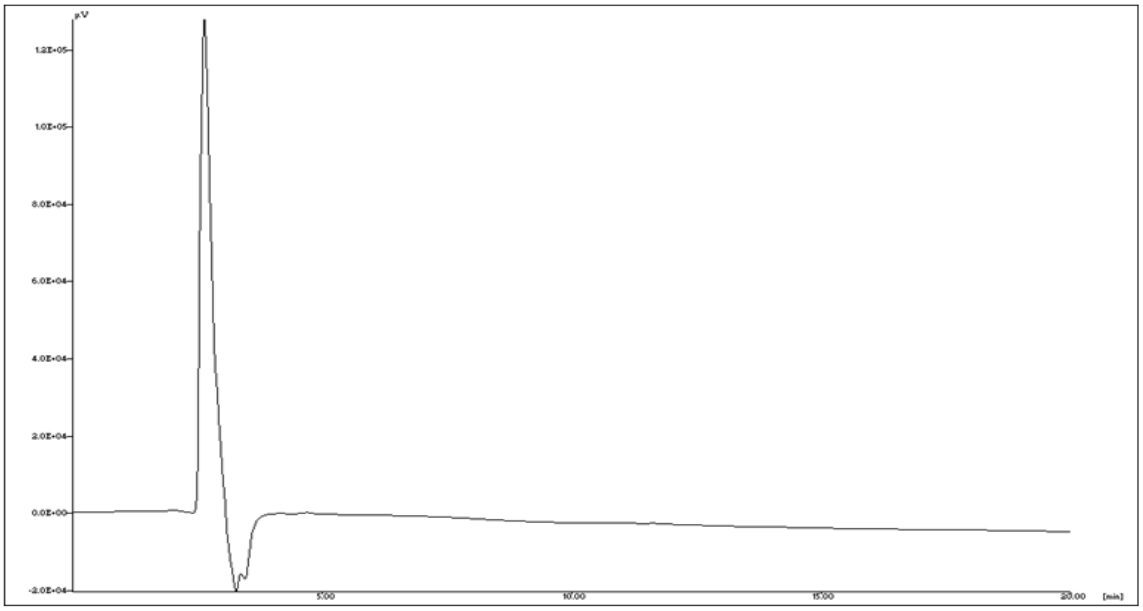


Fig 7.4 HPLC chromatogram of blank (Methanol)

7.4.3 System suitability parameters for Pantoprazole Magnesium

System suitability for Pantoprazole sodium was checked by injecting sample solutions having concentration of 50 µg/ml for 6 times. System suitability parameters are depicted in table 7.6.

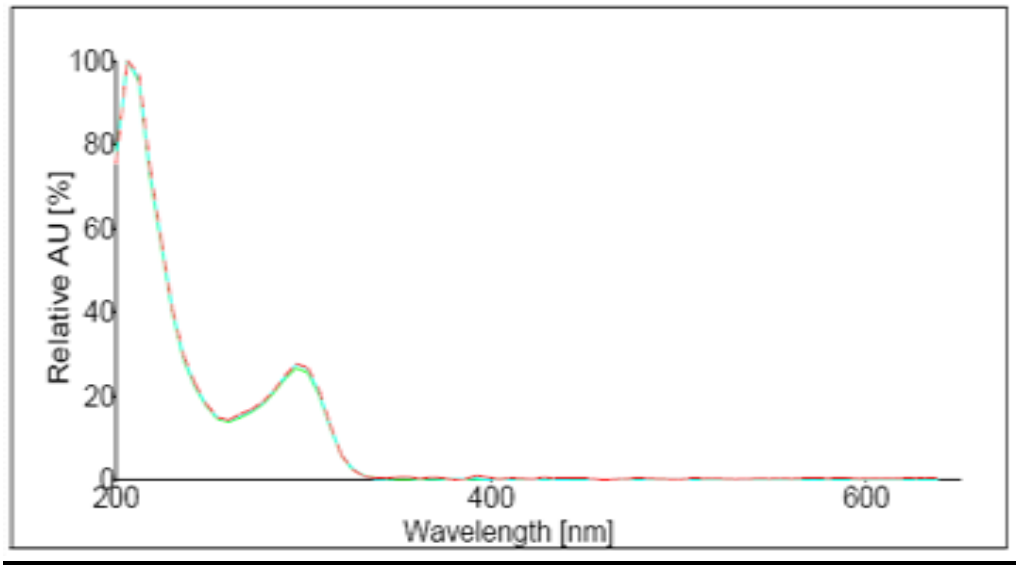


Figure 7.5 Peak purity spectra of PAN (50 µg/ml)

Table: 7.6 HPLC data of peak purity for Pantoprazole sodium in methanol (50 µg/ml)

Parameters	Inference	Specification criteria
Peak name	PAN (50 µg/ml)	-
%RSD	0.989	≤ 1
Purity tail	997.525	(1….1000)
Purity front	997.822	(1….1000)
Number of plates	5385	Not less than 5000
Tailing factor	1.10	Not more than 2

Table 7.6 shows that parameters for peak purity of Pantoprazole sodium passes the specified limits as per Indian pharmacopoeia-2014, which indicates that chromatographic conditions are suitable for analysis of Pantoprazole sodium.

7.4.4 Determination of impurities from stability samples of Pantoprazole sodium alone

For the determination of impurities in the stability samples of Pantoprazole sodium, samples having concentration of 1000 µg/ml were made prior to inject it into the HPLC system.

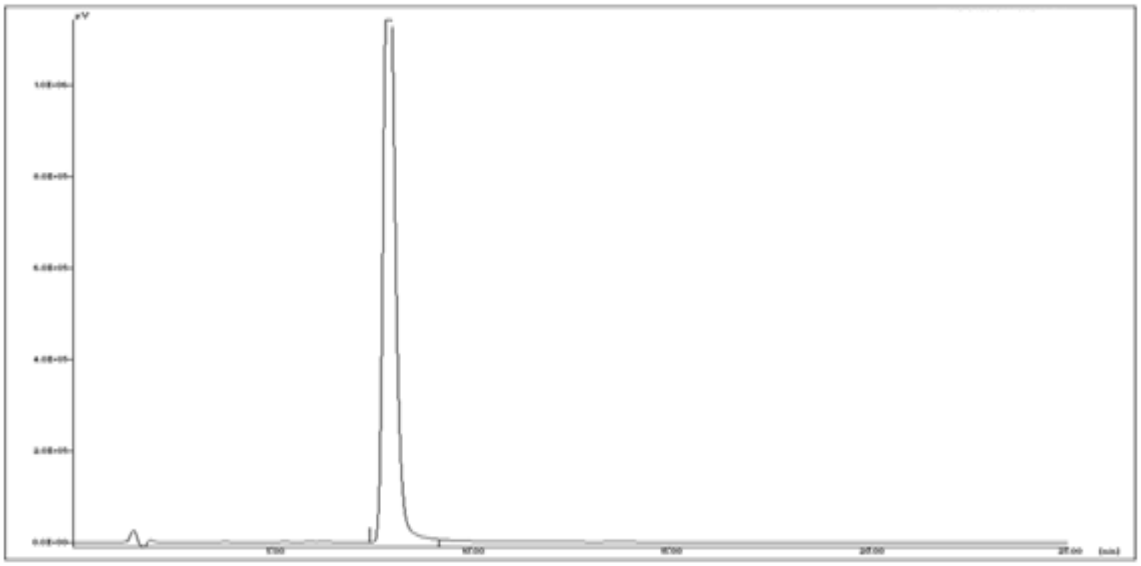


Fig 7.6 HPLC chromatogram of PAN (1000 µg/ml)

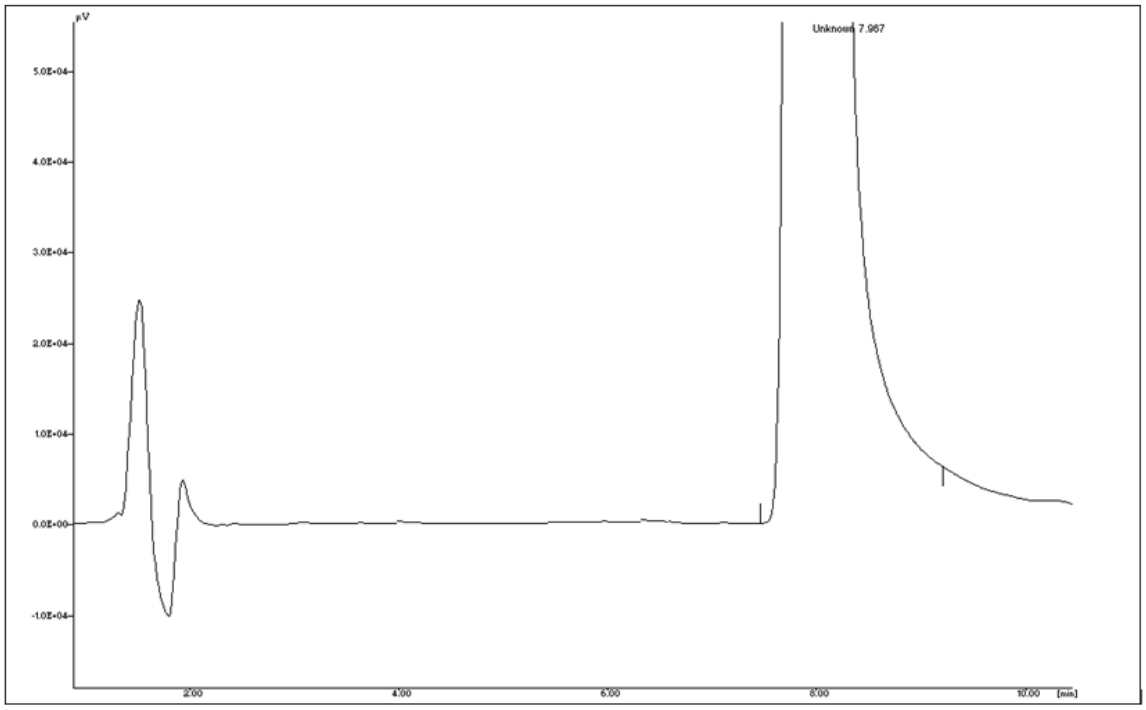


Fig 7.7 HPLC chromatogram of PAN (1000 µg/ml) (Zoomed view)

Fig 7.7 shows that no impurities get generated on exposure of Pantoprazole sodium when exposed to accelerated stability conditions 40°C 75%RH.

7.4.5 Determination of Pantoprazole sodium from stability samples of Pantoprazole sodium alone

For the determination of PAN from the stability samples of Pantoprazole sodium alone, samples having concentration of 50 µg/ml were made prior to inject it into the HPLC system. Stability sample of PAN were correlated with the control samples for determination of % assay of drug.

Calculation:

%Assay = (Area of test/ Area of std)* (weight of std/ Weight of test) *100

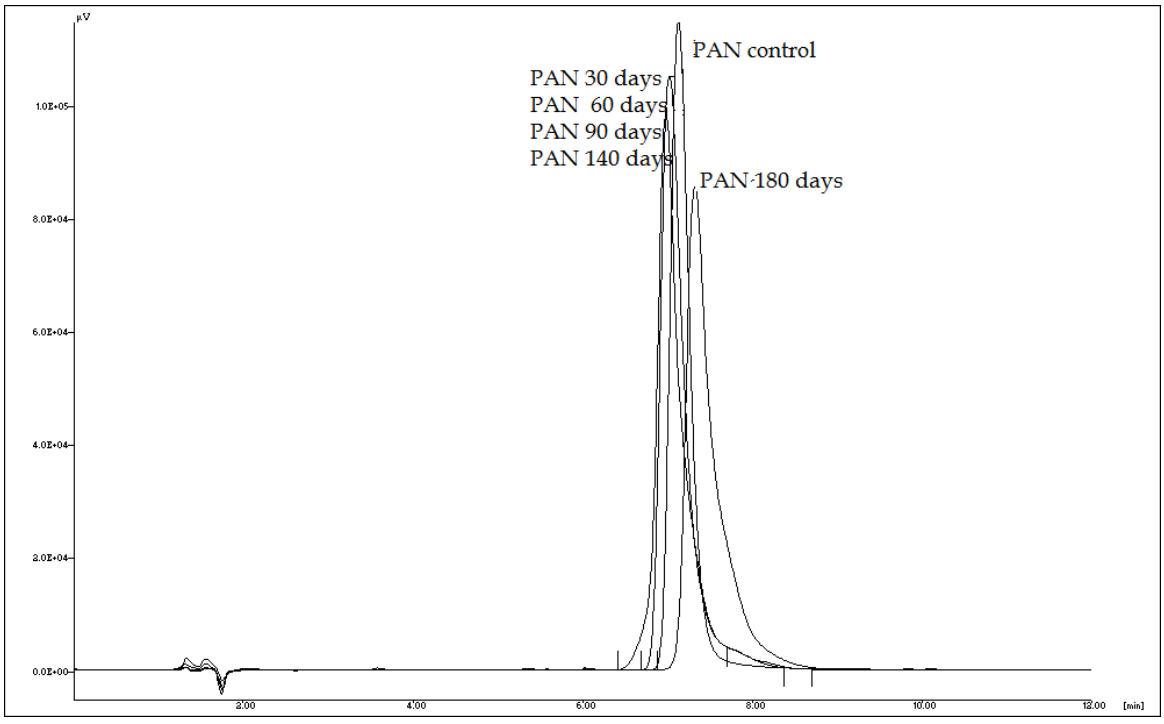


Fig 7.8 HPLC overlay chromatogram of PAN stability samples (50 µg/ml)

Fig 7.8 Shows overlay chromatogram of Pantoprazole control sample along with the accelerated stability study samples.

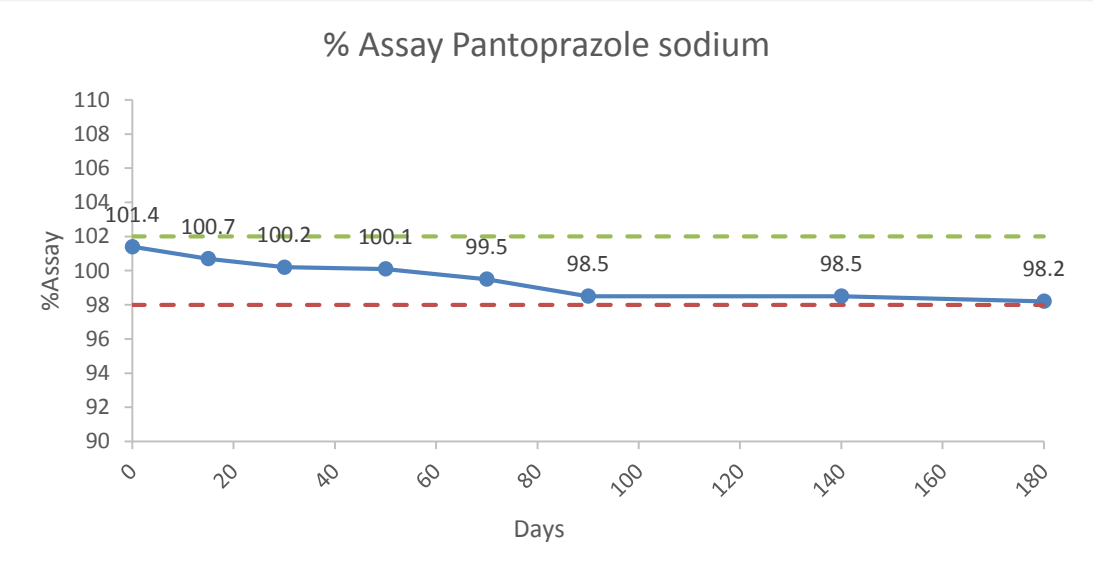


Fig 7.9 %Assay for PAN

Fig 7.9 shows there is no significant decrease in assay of Pantoprazole magnesium. Specification criteria for Pantoprazole sodium as per Indian pharmacopoeia is not less than 98% and not more than 102% of Pantoprazole sodium calculated on anhydrous basis. However, after 6 months of accelerated stability study of Pantoprazole sodium alone the content of PAN is not significantly decreasing.

7.4.6 Compatibility study of Pantoprazole sodium with Povidone K30

To identify the stability of Pantoprazole sodium with Povidone K30, Binary mixture of PAN and PVP K30 (1:1) i.e. 5 g PAN and 5 g PVP K30 were prepared with proper mixing and kept in closed amber colored vial and exposed to 40ºC and 75%RH in stability chamber to extrapolate probable chemical incompatibility between PAN and PVP K30. Along with the mixture, dug and excipient alone were also kept in the stability chamber. Control samples were kept in refrigerator. Assay and %impurity has been found for each samples.

7.4.7 HPLC analysis of Pantoprazole sodium and Povidone K30

Exposed accelerated stability samples of Pantoprazole sodium and PVP K30 binary mixtures were used to prepare samples for HPLC analysis. In case of Pantoprazole sodium and Povidone K30, 50 mg of drug and excipient mixture was weighed and transferred to 10 ml volumetric flask to get concentration of 2500µg/ml. Further dilutions were made with the diluent Acetonitrile: Water to get concentration of 1000 µg/mL for determination of impurities generated and 50 µg/mL for determination of assay of PAN as described in the section 7.3.2. Chromatographic conditions were kept constant as described in table 7.5. The responses of PAN and PVP K30 binary mixture obtained in each accelerated stability samples were compared with the responses of respective initial samples (control) and the % of generated impurities has been found for each samples.

To check the interference of excipient i.e. PVP K30, sample solution of PVP K30 in methanol was injected into the proposed HPLC system.

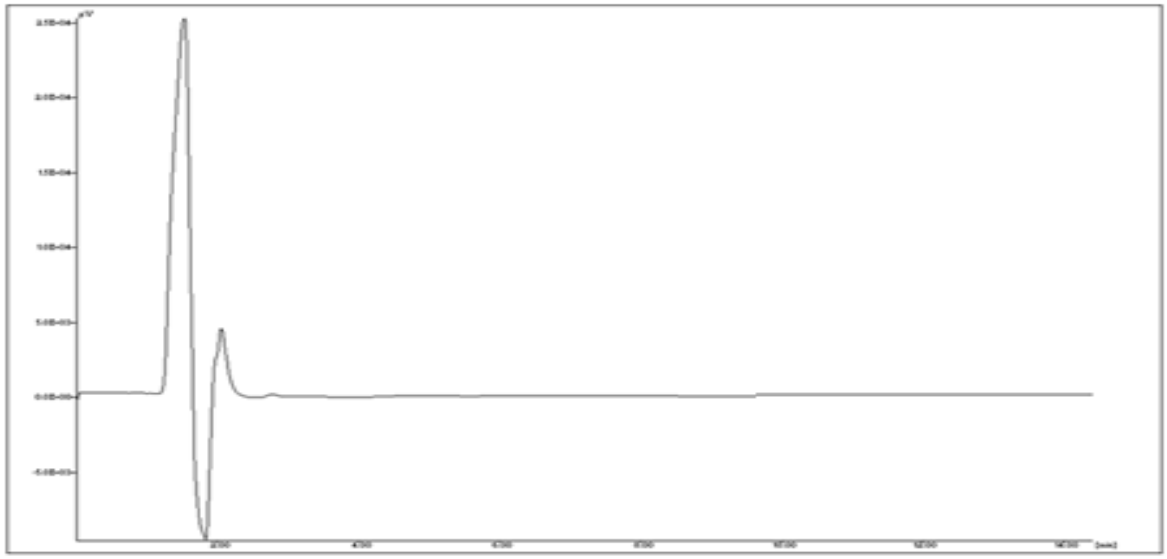


Fig 7.10 HPLC chromatogram of PVP K30 (Placebo)

Fig 7.10 indicates that there is no interferences of excipient PVP K30 in the analysis of Pantoprazole sodium using the proposed method.

7.4.8 Determination of impurities from stability samples of Pantoprazole sodium and PVP K30

For the determination of impurities from the stability samples of PAN and PVP K30 binary mixtures, samples having concentration of 1000µg/ml were made as described in the section 7.3.2. Responses of impurities appeared in the samples were recorded. % of impurities generated has been calculated for each samples.

Calculation:

%Impurity= (Area of Impurity/Area of std)* (conc. Of std/ Conc. of test)*100

Where,

Conc. of std = 10 µg/mL

Conc. of test = 1000µg/mL

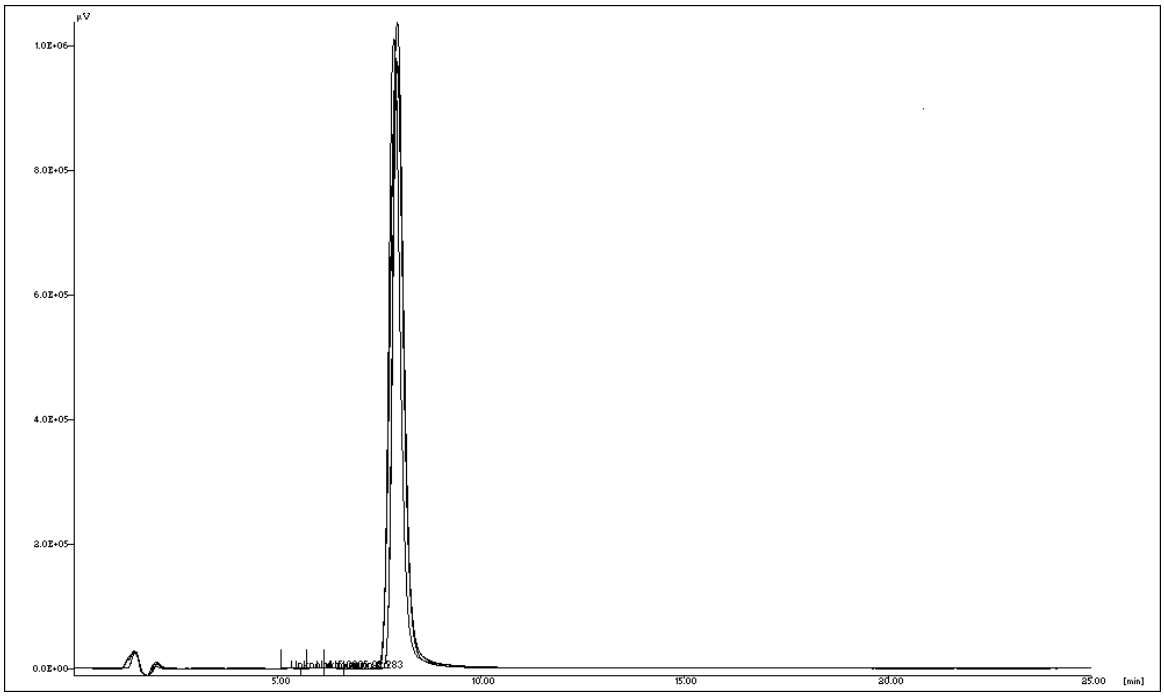


Fig 7.11 HPLC overlay chromatogram of PAN + PVP K30 (1000 µg/ml)

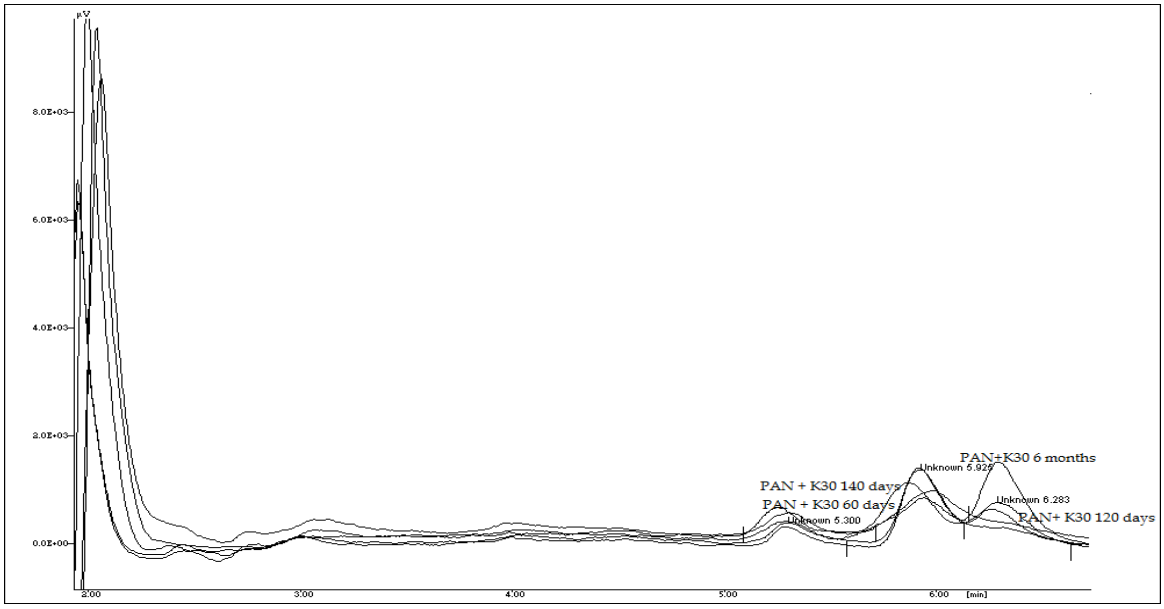


Fig 7.12 HPLC overlay chromatogram of PAN + PVP K30 (1000 µg/ml) (Zoomed view)

Fig 7.12 shows zoomed view of overlay chromatogram of PAN + PVP K30. Total 3 impurities get generated in the mixture of PAN with PVP K30 samples exposed to accelerated stability conditions. Chromatogram reveals that the area of impurities is gradually increasing starting fom the controlled sample to the accelerated stability study samples of 6 months.Total % of impurities has been calculated for each of the stability samples.

Table 7.7 Increase in total % Impurity in mixture of PAN + PVP K30-

Condition	**Days**	**Area of Imp-1 Rt=5.3**	**%Imp**	**Area of Imp-2 Rt=5.9**	**%Imp**	**Area of Imp-3 Rt=6.2**	**%Imp**	**Area of drug peak at Rt-=7.3**	**Total IMP**	**Single max**
40 °C 75 RH	**0**	-	-	-	-	-	-	31101871	-	-
	15	10112	0.03	23481	0.056	10260	0.04	19114584	0.126	0.056
	30	10523	0.05	21460	0.052	10869	0.058	28916203	0.16	0.052
	60	10652	0.056	23529	0.062	11853	0.056	29985516	0.174	0.0624
	90	10541	0.062	23009	0.065	12563	0.052	38034432	0.179	0.0652
	140	10654	0.065	36388	0.075	13452	0.089	26438556	0.229	0.0754
	180	10785	0.069	39847	0.085	18654	0.091	34862849	0.245	0.0852
Control	180	-	-	-	-	-	-	34562318	-	-

Data depicted in table 7.7 shows that area of IMP-1, IMP-2 and IMP-3 is gradually increasing on the exposure of accelerated stability conditions Total impurities get generated in the binary mixture of PAN and PVP K30 starting from 0.126% to 0.245%. after six months study.

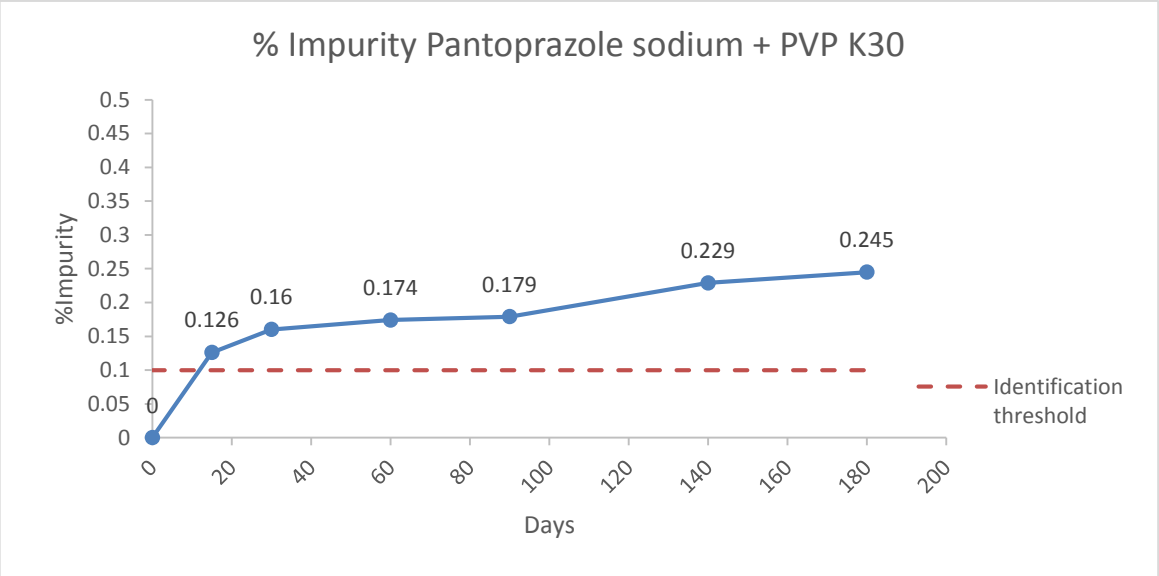


Fig 7.13 Increase in %Impurity for PAN + K30 stability samples

7.4.9 Determination of Pantoprazole sodium from the stability samples of Pantoprazole sodium with PVP K30

For the determination of PAN from the stability samples of Pantoprazole sodium and PVP K30, samples having concentration of 50 µg/ml were made as described in section 7.3.2 prior to inject it into the HPLC system. Stability sample of PAN+ PVP K30 were correlated with the control samples for determination of % assay of drug.

Calculation: %Assay = (Area of test/ Area of std)* (weight of std/ Weight of test) *100

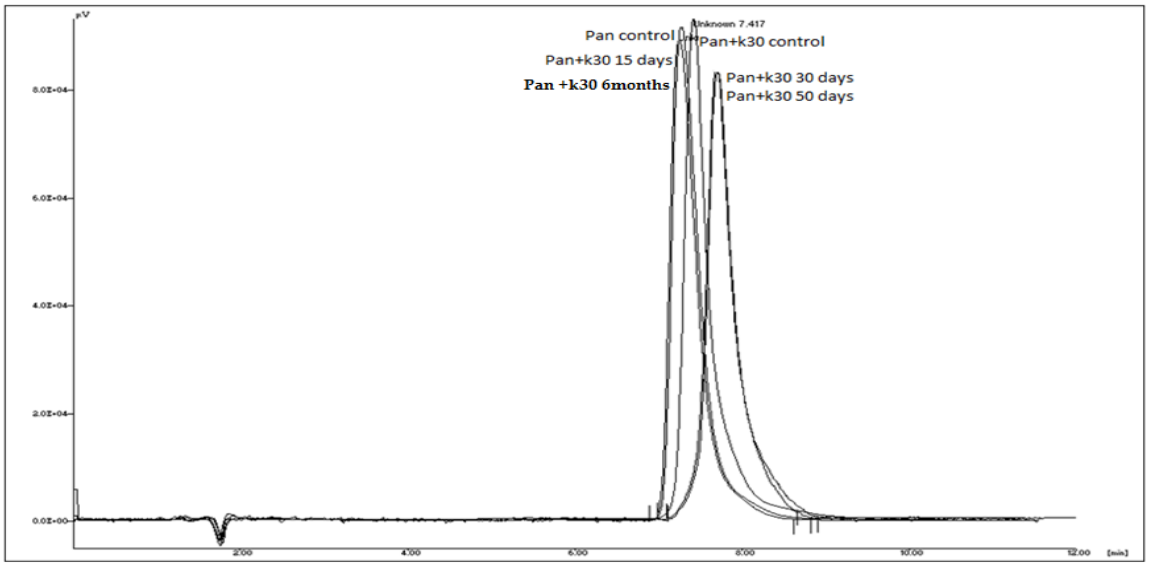


Fig 7.14 HPLC overlay chromatogram of stability samples of PAN + PVP K30 (50 µg/ml)

Fig 7.14 shows Pantoprazole + PVP K30 (50 µg/ml) overlay chromatogram of stability samples along with control sample. The chromatogram reveals that the area of drug peak is gradually decreasing after 6 months of the study.

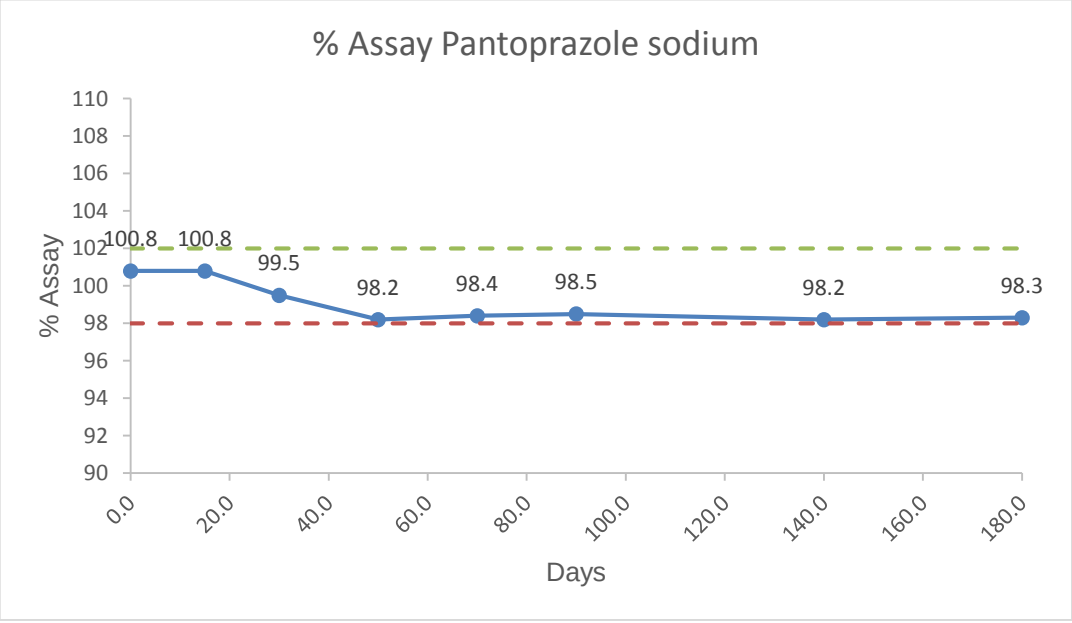


Fig 7.15 % Assay of PAN from PAN+ PVP K30

Fig 7.15 shows %assay of Pantoprazole sodium in the binary mixture of PAN and PVP K30. Though the assay is not decreasing much and it lies within the limits specified by Indian Pharmacopoeia but the total generated impurities are beyond the identification threshold described in ICHQ3A (R1) so qualification and characterization of the impurities generated by Pantoprazole sodium and PVP K30 should be carried out.

7.4.10 Compatibility study of Pantoprazole sodium with Povidone K90

To identify the stability of Pantoprazole sodium with Povidone K90, Binary mixture of PAN and PVP K90 (1:1) i.e. 5 g PAN and 5 g PVP K90 were prepared with proper mixing and kept in closed amber colored vial and exposed to 40ºC and 75%RH in stability chamber to extrapolate probable chemical incompatibility between PAN and PVP K90. Along with the mixture, dug and excipient alone were also kept in the stability chamber. Control samples were kept in refrigerator. Assay and % impurity has been found for each samples.

7.4.11 HPLC analysis of Pantoprazole sodium and Povidone K90

Exposed accelerated stability samples of Pantoprazole sodium and PVP K90 binary mixtures were used to prepare samples for HPLC analysis. In case of Pantoprazole sodium and Povidone K90, 50 mg of drug and excipient mixture was weighed and transferred to 10 ml volumetric flask to get concentration of 2500µg/ml. Further dilutions were made with the diluent Acetonitrile: Water to get concentration of 1000 µg/mL for determination of impurities generated and 50 µg/mL for determination of assay of PAN as described in the section 7.3.2. Chromatographic conditions were kept constant as described in table 7.5. The responses of PAN and PVP K90 binary mixture obtained in each accelerated stability samples were compared with the responses of respective initial samples (control) and the % of generated impurities has been found for each samples.

To check the interference of excipient i.e. PVP K90, sample solution of PVP K90 in methanol was injected into the proposed HPLC system.

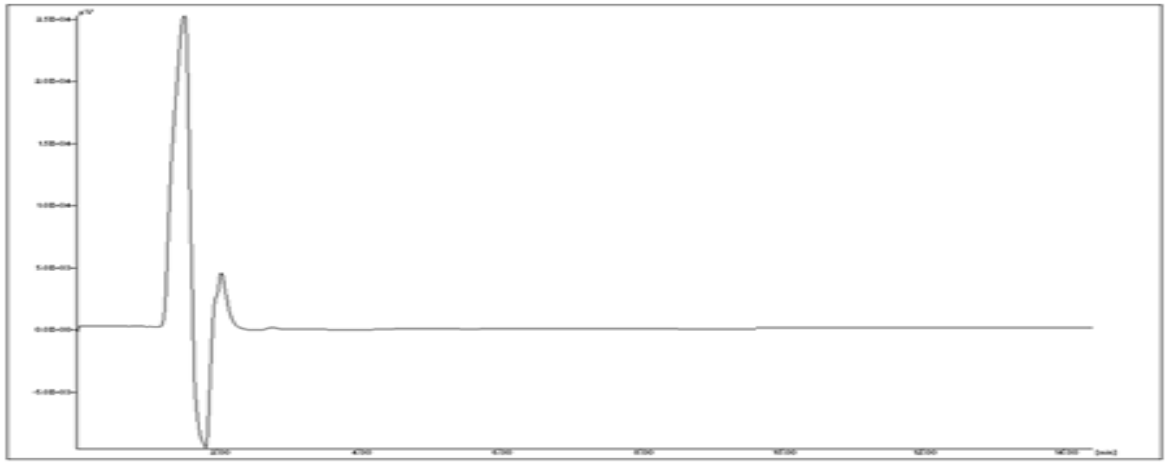


Fig 7.16 HPLC chromatogram of PVP K90 (Placebo)

Fig 7.16 indicates that there is no interferences of excipient PVP K90 in the analysis of Pantoprazole sodium using the proposed method.

7.4.12 Determination of impurities from stability samples of Pantoprazole sodium and PVP K90

For the determination of impurities from the stability samples of PAN and PVP K90 binary mixtures, samples having concentration of 1000µg/ml were made as described in the section 7.3.2. Responses of impurities appeared in the samples were recorded. % of impurities generated has been calculated for each samples.

Calculation:

%Impurity= (Area of Impurity/Area of std)* (conc. Of std/ Conc. of test)*100

Where,

Conc. of std = 10 µg/mL

Conc. of test = 1000µg/mL

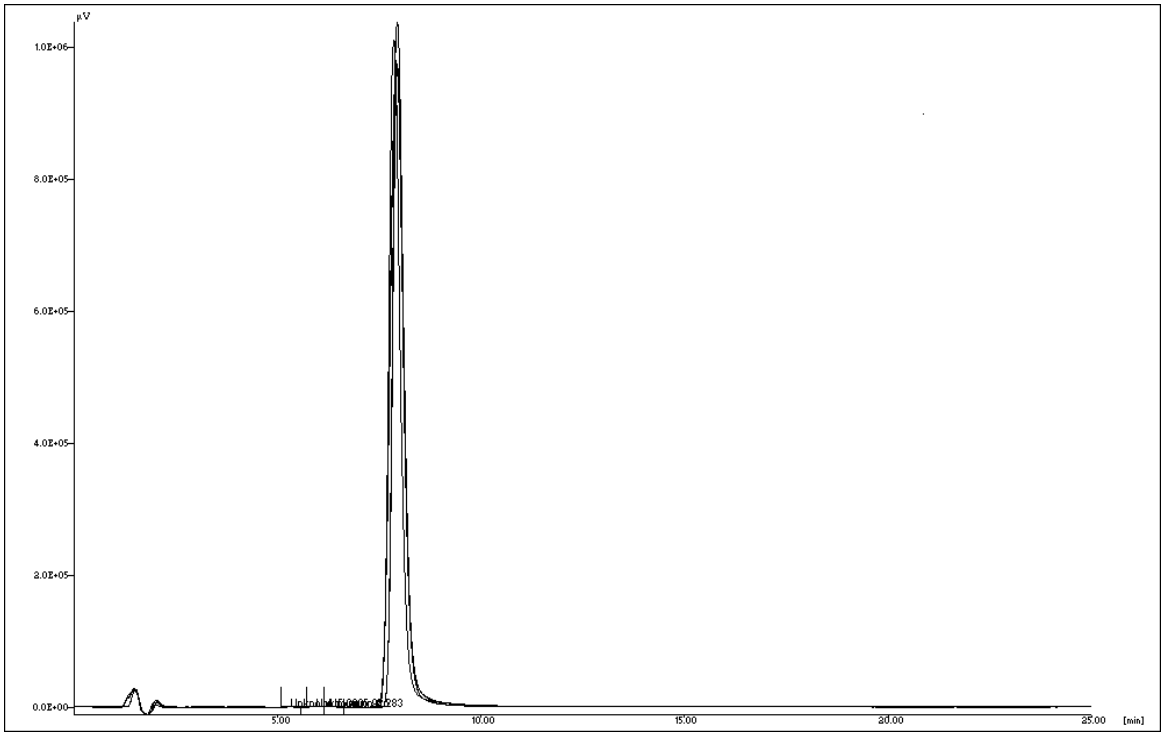


Fig 7.17 HPLC overlay chromatogram of PAN + PVP K90 (1000 µg/ml)

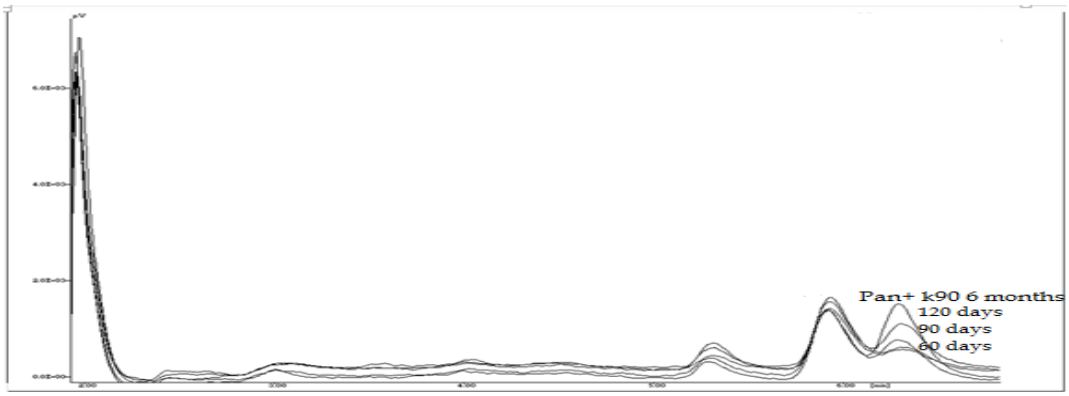


Fig 7.18 HPLC overlay chromatogram of PAN + PVP K90 (1000 µg/ml) (Zoomed view)

Fig 7.18 shows zoomed view of overlay chromatogram of PAN + PVP K90. Total 3 impurities get generated in the mixture of PAN with PVP K90 samples exposed to accelerate stability conditions. Chromatogram reveals that the area of impurities is gradually

increasing on exposure of accelerated stability study samples of 6 months.Total % of impurities has been calculated for each of the stability samples.

Table 7.8 Increase in total % Impurity in mixture of PAN + PVP K90

Condition	Days	Area of Imp-1 Rt=5.3	%Imp	Area of Imp-2 Rt=5.9	%Imp	Area of Imp-3 Rt=6.2	%Imp	Area of drug peak at Rt-=7.3	Total IMP	Single max
40 °C 75 RH	**0**	-	-	-	-	-	-	32145369	-	-
	15	9685	0.02	22145	0.048	10265	0.03	31245258	0.098	0.048
	30	9945	0.025	20847	0.042	10523	0.027	31258469	0.094	0.042
	60	10254	0.03	24129	0.052	11453	0.031	34156278	0.113	0.052
	90	10421	0.032	24745	0.056	11258	0.032	25478615	0.12	0.056
	140	10457	0.033	25741	0.059	12452	0.035	32187567	0.127	0.059
	180	10562	0.04	29812	0.17	12658	0.038	36541897	0.252	0.17
Control	180	-	-	-	-	-	-	32159545	-	-

Data depicted in table 7.8 shows that area of IMP-1,IMP-2 and IMP-3 is gradually increasing on the exposure of accelerated stability conditions. Total impurities get generated in the binary mixture of PAN and PVP K90 starting from 0.098% to 0.252%. after six months study.

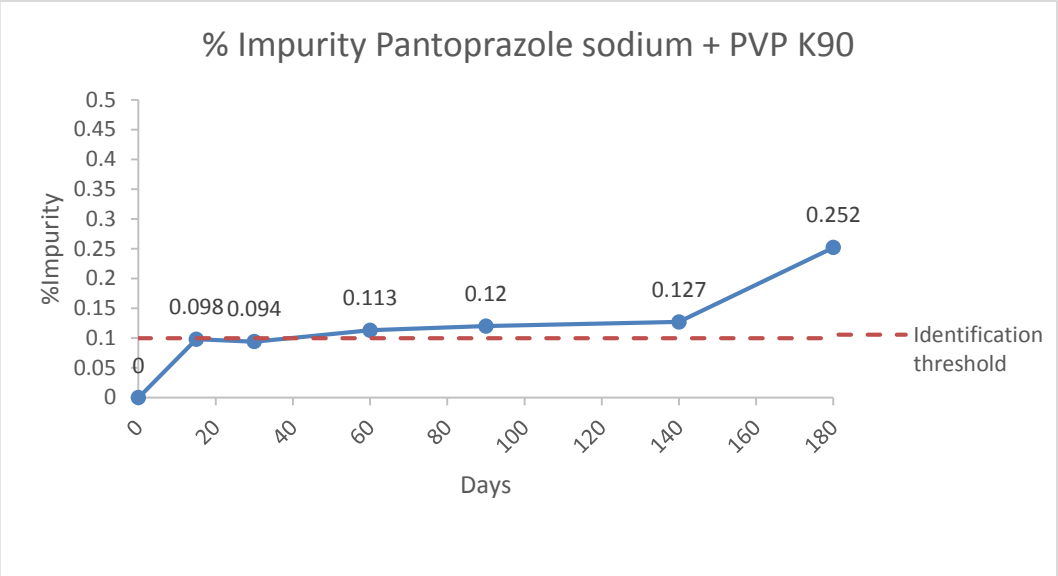


Fig 7.19 Increase in %Impurity for PAN + K90 stability samples

7.4.13 Determination of Pantoprazole sodium from the stability samples of Pantoprazole sodium with PVP K90

For the determination of PAN from the stability samples of Pantoprazole sodium and PVP K90, samples having concentration of 50 µg/ml were made as described in section 7.3.2 prior to inject it into the HPLC system. Stability sample of PAN+ PVP K90 were correlated with the control samples for determination of % assay of drug.

Calculation: %Assay = (Area of test/ Area of std)* (weight of std/ Weight of test) *100

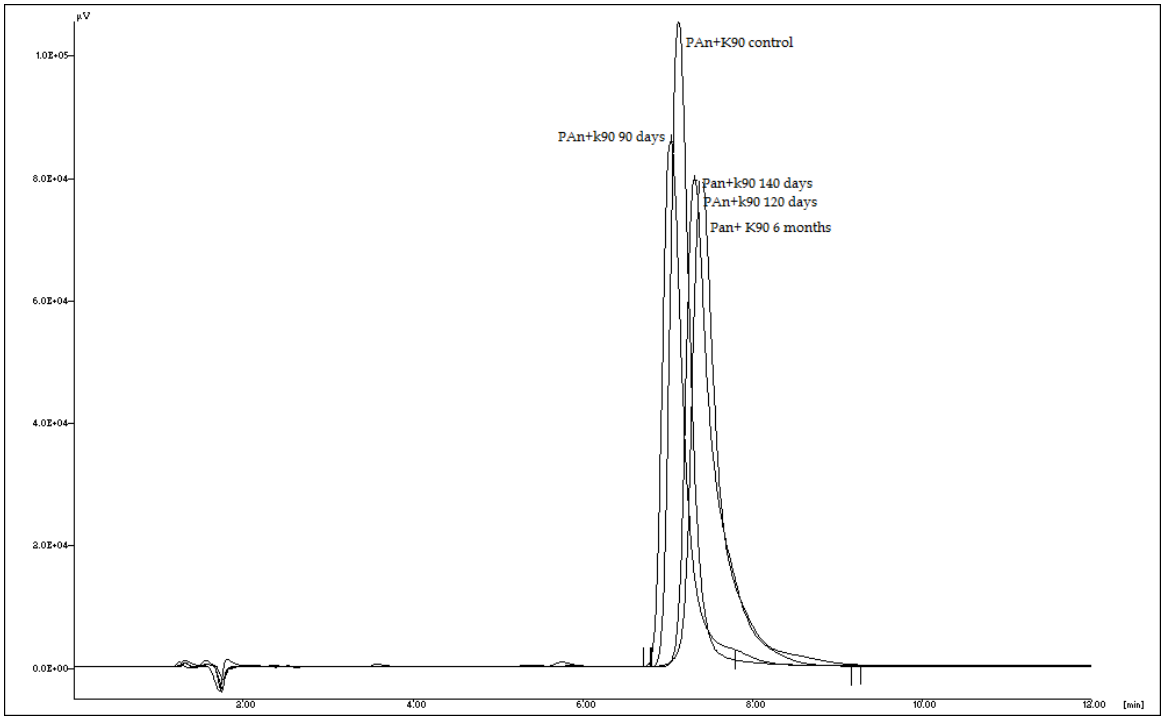


Fig 7.20 HPLC overlay chromatogram of stability samples of PAN + PVP K90 (50 µg/ml)

Fig 7.20 shows Pantoprazole + PVP K90 (50 µg/ml) overlay chromatogram of stability samples along with control sample. The chromatogram reveals that the area of drug peak is gradually decreasing after 6 months of the study.

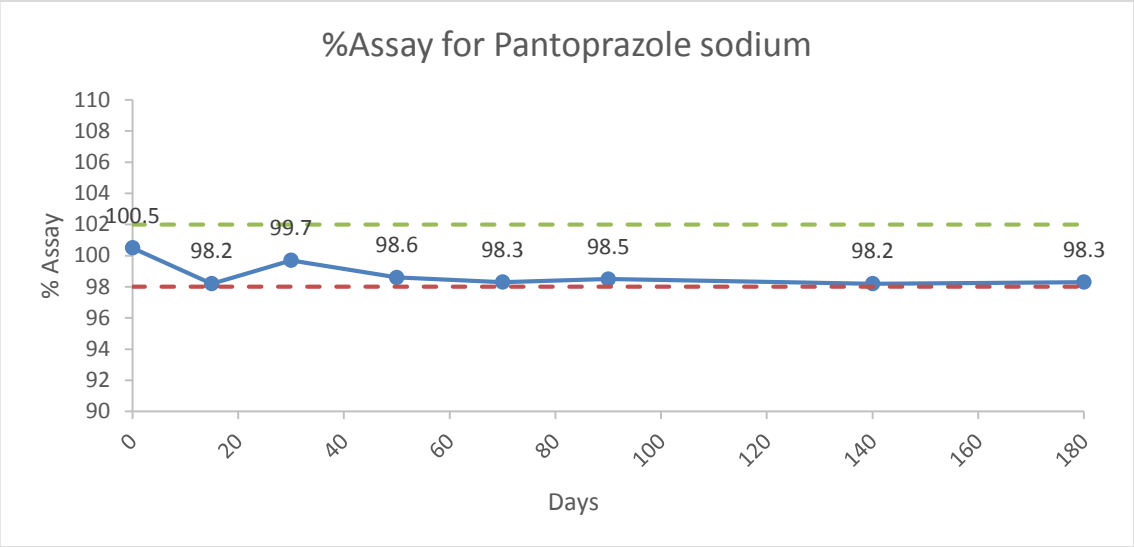


Fig 7.21 % Assay of PAN from PAN + PVP K90

Fig 7.21 shows %assay of Pantoprazole sodium in the binary mixture of ESO and PVP K90. Though the assay is not decreasing much and it lies within the limits specified by Indian Pharmacopoeia but the total generated impurities are beyond the identification threshold described in ICHQ3A (R1) so qualification and characterization of the impurities generated by Pantoprazole sodium and PVP K90 should be carried out.

7.4.14 Oxidative degradation of Pantoprazole sodium [37]

To investigate the impact of peroxides on Pantoprazole sodium, oxidative degradation of PAN was carried out. 25 mg of PAN dissolved in 25 ml of methanolic H_2SO_4 (3% hydrogen peroxide). Volumetric flask kept in the water bath at 40°C for 30 min. further dilutions were made with methanol. Sample concentration was made 1000 µg/ml prior to inject it into the HPLC system.

Table7.9 comparison of oxidative degradation with reported degradation

% Degradation	Reported	Observed
PAN	15%	20%

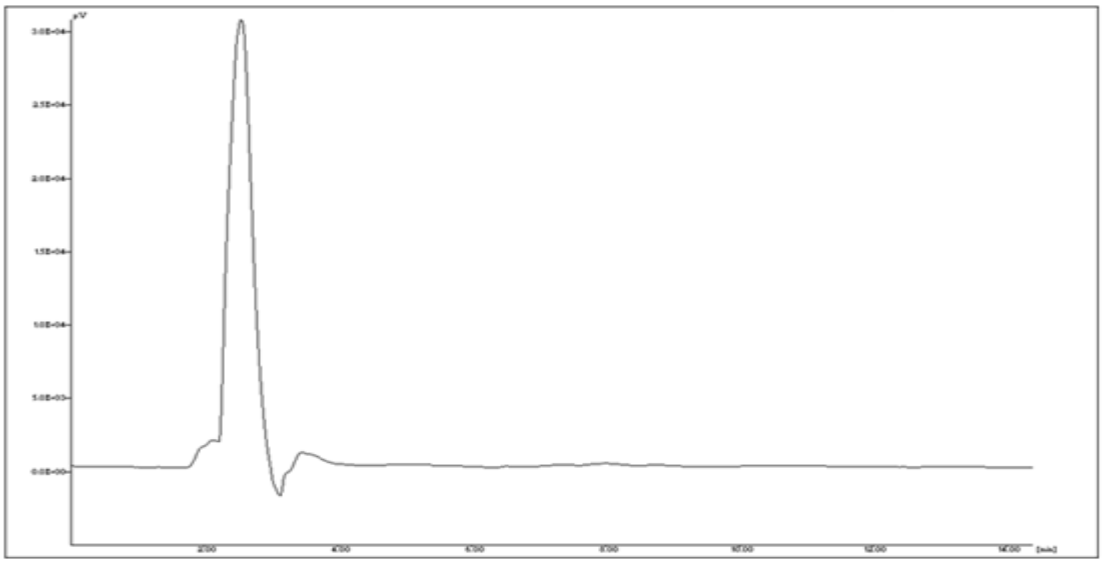


Fig 7.22 HPLC chromatogram of Methanolic H_2O_2 (Blank)

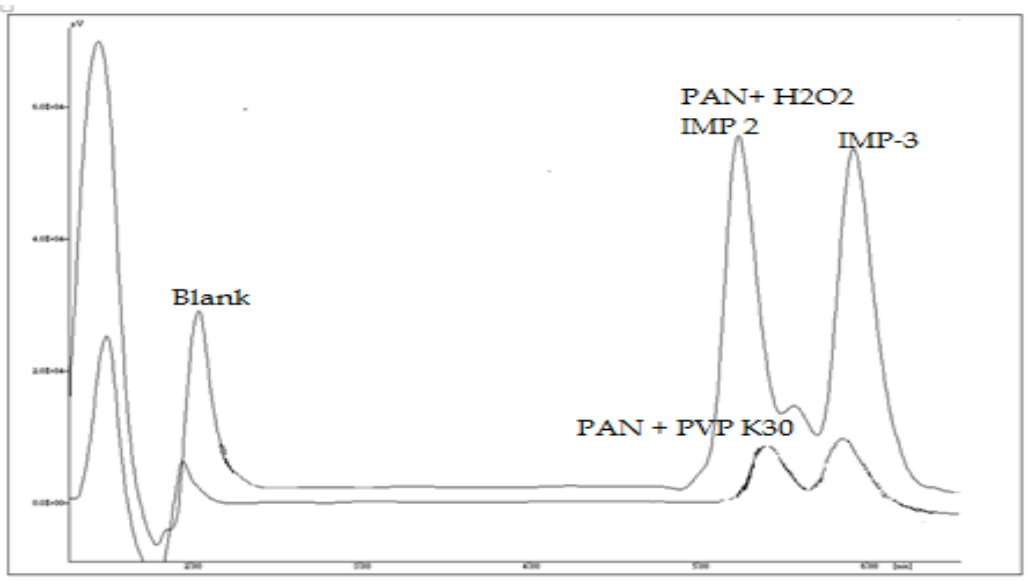
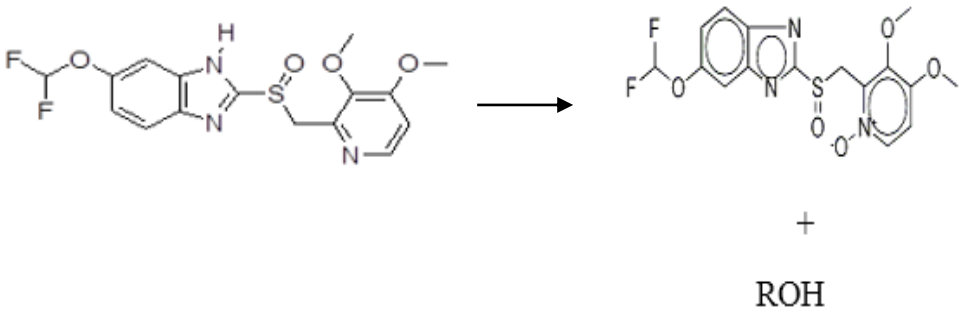


Fig 7.23 comparison of degradation profile of Pantoprazole sodium with oxidative degradation

Degradation profile of the exposed samples of Pantoprazole sodium with PVP K30 and PVP K90 was compared with the oxidative degradation of pure drug. The retention time of impurities generated in accelerated stability study samples resembles the retention time of

degradation product by oxidative degradation of Pantoprazole sodium. So it can be said that the degradation of Pantoprazole sodium with excipients was mediated by organic peroxides present as an impurity in Excipient PVP K30 and PVP K90.

Probable chemical reactions occurring between Pantoprazole sodium and Hydrogen Peroxide



7.5 Differential Scanning Calorimetry (DSC) Analysis

Differential scanning calorimetry (DSC) is used to investigate the drug-excipient interactions. It is a useful tool to carry out preformulation studies and to know possible interaction between drug and excipients. The results obtained by HPLC were compared with the thermal analysis results. But the changes in DSC thermogram cannot always be sufficient data to prove that some interaction occurs between drug and excipient.

DSC data can be analysed by appearance, shift or disappearance of endothermic or exothermic peaks and/or variations in the corresponding enthalpy values in thermal curves of drug–excipient mixtures.

To analyse the compatibility of Pantoprazole with PVP K30 and PVP K90 by DSC, exposed binary mixtures of Pantoprazole sodium and Povidone were run under thermal analysis. Thermogram obtained of exposed samples were compared with the thermogram of controlled samples. Following data shows the thermogram of drug and excipient alone as well as exposed samples of binary mixtures.

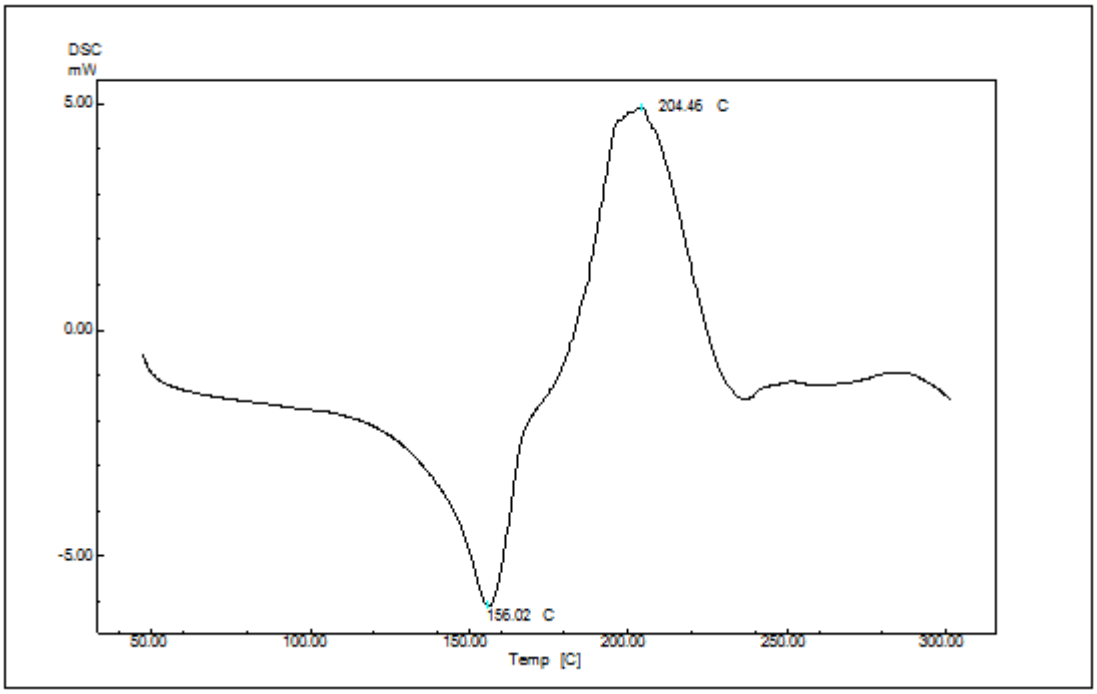


Fig 7.24 Thermogram of Pantoprazole control sample

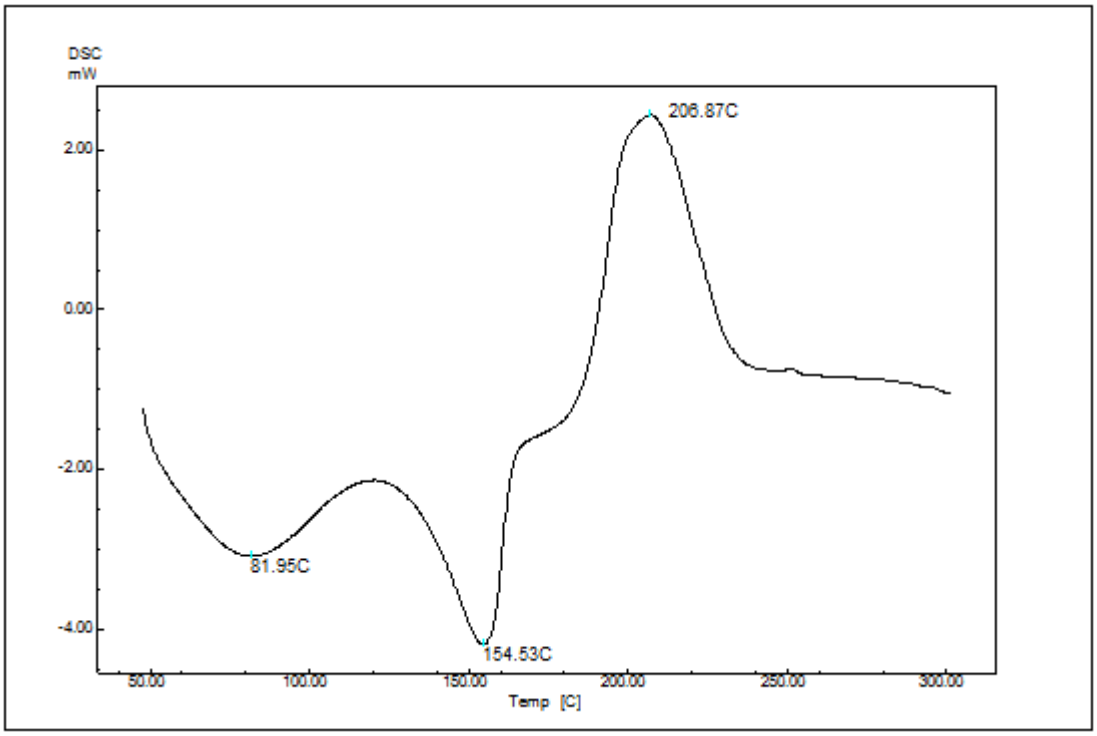


Fig 7.25 Thermogram of Pantoprazole exposed sample

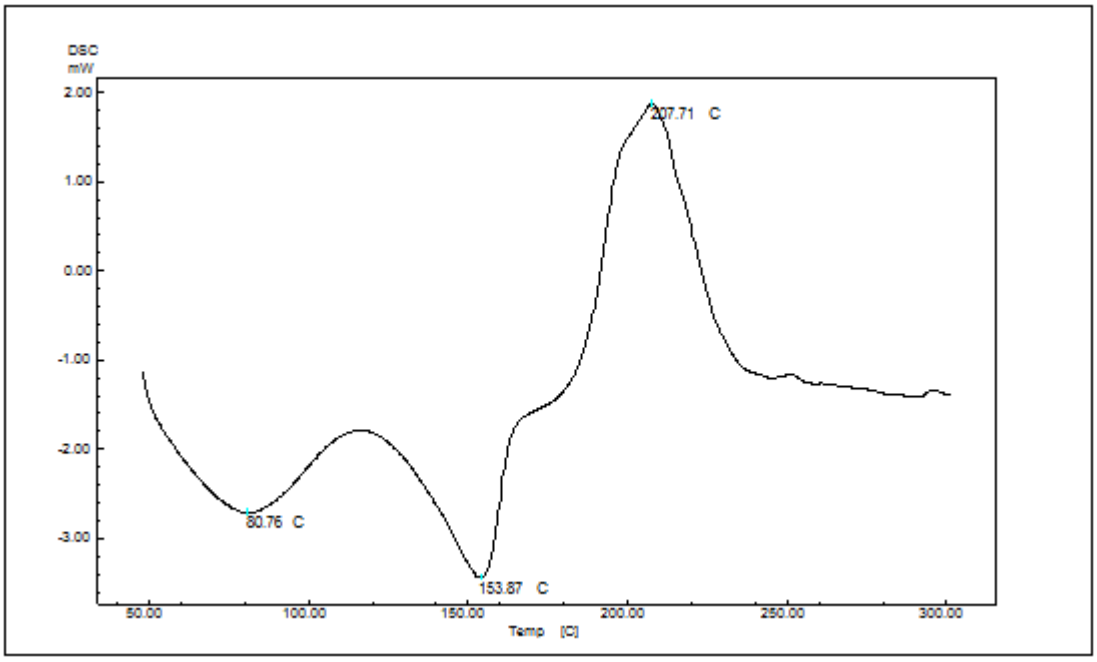


Fig 7.26 Thermogram of Pantoprazole sodium and PVP K30 exposed sample

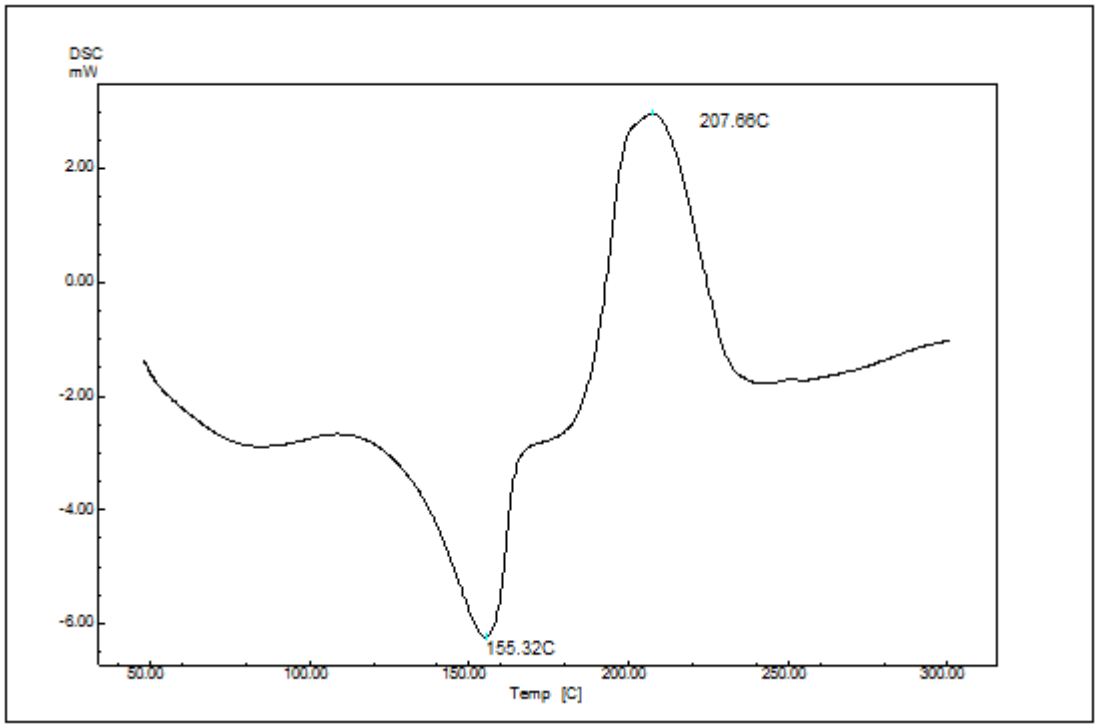


Fig 7.27 Thermogram of Pantoprazole sodium and PVP K90 exposed sample

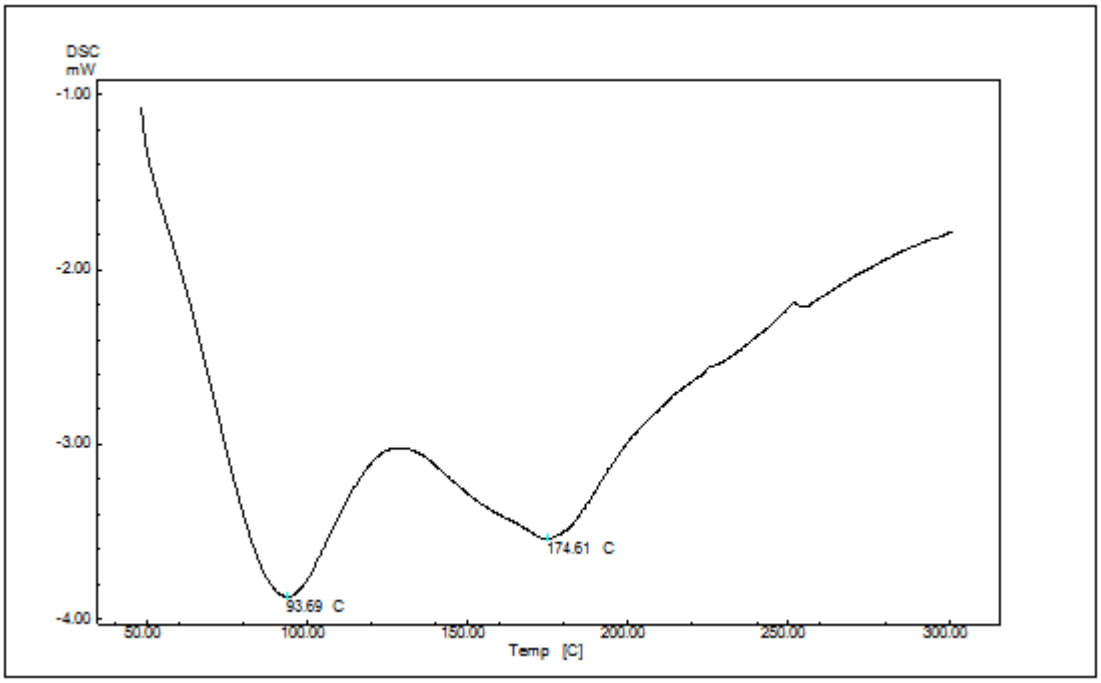


Fig 7.28 Thermogram of PVP K30

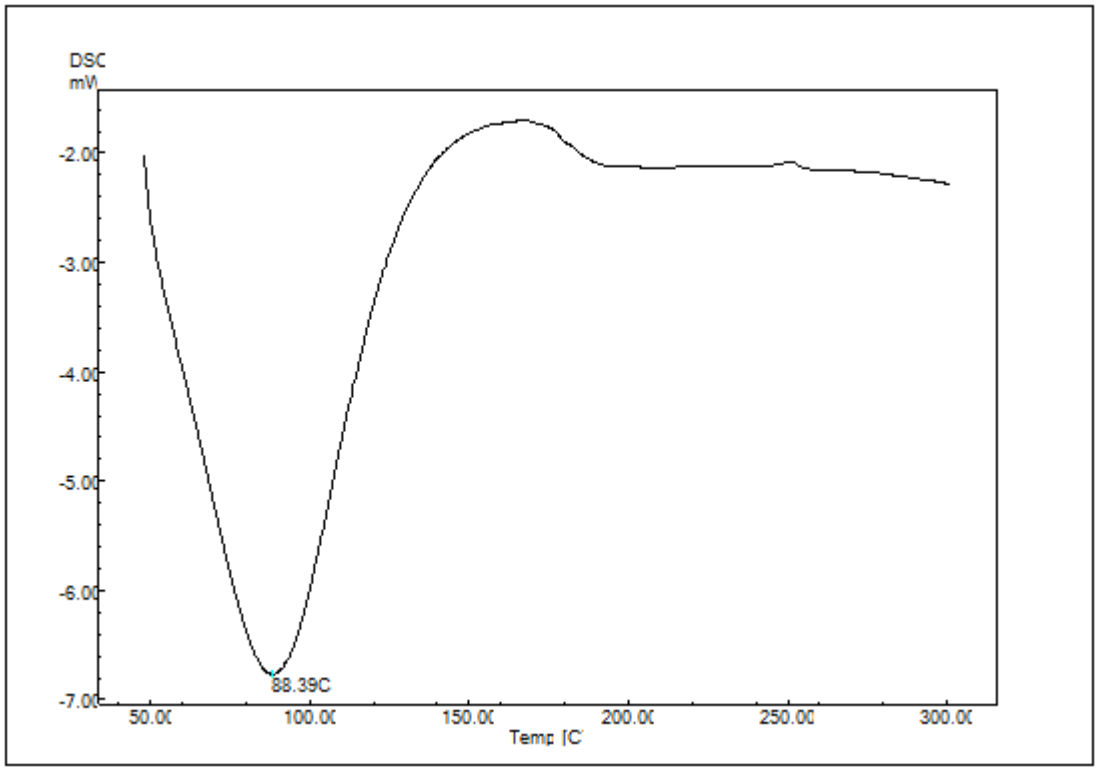


Fig 7.29 Thermogram of PVP K90

Above thermogram shows very little changes compared to the control samples of Pantoprazole magnesium.

Table 7.10 Summary of DSC data

Condition	**Pantoprazole**		**Pantoprazole+ PVP K30**			**Pantoprazole+ PVP K90**			**PVP K30**		**PVP K90**
Fresh	156.02	204.46	84.45	153.19	208.17	81.95	154.53	206.87	93.69	174.61	88.39
Stressed	155.32	207.66	80.76	153.87	207.71	79.21	153.92	206.53			

Data depicted in table 7.10 shows that there is no physical interaction between Pantoprazole sodium and PVP K30 as well as PVP K90. So it can be concluded that chemical interaction is there between drug and organic peroxides present in excipient as an impurity, which is degrading Pantoprazole sodium which supports the data obtained in HPLC analysis.

CHAPTER 8

CONCLUSION

8. Conclusion

The study of drug–excipient's compatibility represents an important phase in the preformulation stage for the development of all dosage forms. Potential physical and chemical interactions between drugs and excipients can affect the chemical nature, the stability and bioavailability of drugs and, consequently, their therapeutic efficacy and safety.

The class of drugs selected for drug and excipient compatibility study is Proton Pump Inhibitors (PPIs) these agents selectively and irrevocably inhibit the gastric hydrogen/potassium adenosine tri phosphatase (H^+/K^+-exchanging ATPase), part of the 'proton pump' that makes the final step in the acid secretory process. They inhibit both basal and stimulated secretion of gastric acid, exclusively of the nature of parietal cell stimulation.

Esomeprazole magnesium and pantoprazole sodium were selected to check drug and excipient compatibility study of Proton Pump Inhibitors (PPIs). According to literature review PPIs get degraded in very mild oxidative stress condition another conclusion was found that polyvinylpyrrolidone contains organic peroxide as an impurity in it which is more reactive than hydrogen peroxide. Although PVP is widely used as a binder in the tablet formulation of PPIs.

So the aim of the present study was to identify the compatibility of selected proton pump inhibitors with different grades of Polyvinylpyrrolidone i.e. PVP K30 and PVP K90.
Accelerated stability study was carried out in accordance with ICH guidelines for drug alone as well as binary mixtures of drug and excipients.
Impurities generated were well separated by the chromatographic conditions of esomeprazole magnesium and pantoprazole sodium related substances given in Indian Pharmacopoeia.

For Esomeprazole Magnesium, in presence of PVP K30 and PVP K90 total 3 impurities get generated when ESO was exposed to accelerated stability condition.

Total % Impurity was found to be 1.24% and 1.19% for Esomeprazole magnesium with PVP K30 and with PVP K90 respectively.

For Pantoprazole Sodium, in presence of PVP K30 and PVP K90 total 3 impurities get generated when PAN was exposed to accelerated stability condition.

Total % Impurity was found to be 0.245% and 0.252% for Pantoprazole sodium with PVP K30 and with PVP K90 respectively.

As per the ICH Q3A (R1) guideline impurity of new drug product which has maximum daily dose ≤2 g/day Reporting threshold is 0.05% Identification threshold is 0.10% and Qualification threshold is 0.15%.

The total impurity generated in Esomeprazole magnesium with PVP K30 and PVP K90 is 1.24% and 1.19% respectively and total impurity generated in Pantoprazole sodium with PVP K30 and PVP K90 is 0.245 % and 0.252% respectively which is beyond the limits specified by ICH guidelines.

The impurity profile of Esomeprazole magnesium and Pantoprazole sodium was compared with oxidative degradation profile in presence of Hydrogen peroxide and it was found similar which indicates that degradation of Proton Pump Inhibitors (PPIs) is due to the organic peroxide content present in excipient povidone as an impurity in it.

Furthermore a compatibility was checked by Differential Scanning Calorimetry (DSC) it also supports the hypothesis.

CHAPTER 9

FUTURE SCOPE

9. Future scope

- Further qualification and characterization of generated impurities using hyphenated analytical techniques.
- To check incompatibility of other Proton Pump Inhibitors (PPIs) with povidone.
- Formulation and development of Esomeprazole magnesium and Pantoprazole sodium dosage form by selecting the good quality of excipient povidone which is free from hydrogen peroxide as a chain terminator in polymerization reaction.

CHAPTER 10

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