Generic Formulation Development of Misoprostol 200 Tablet and Its Evaluation

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A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Masters of Pharmacy

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled "Generic Formulation Development of Misoprostol 200 Tablet and Its Evaluation" is an authentic and genuine thesis project carried out by me under the guidance of Md. Anisur Rahman, Assistant Professor, Department of Pharmacy, East West University, Dhaka.

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ENDORSEMENT BY THE CHAIRPERSON

This is to certify that the dissertation entitled "Generic Formulation Development of Misoprostol 200 Tablet and Its Evaluation" is a genuine research work carried out by Sumaiya Ahmed Bhasha, under the supervision of Md. Anisur Rahman (Assistant Professor, Department of Pharmacy, East West University, Dhaka). I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in thus connection are duly acknowledged.

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CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled "Generic Formulation Development of Misoprostol 200 Tablet and Its Evaluation", submitted to the Department of Pharmacy, East West University, Dhaka, in partial fulfillment of the requirements for the Degree of Masters of Pharmacy, was carried out by Sumaiya Ahmed Bhasha, ID # 2015-1-79-013 under my supervision and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree/Diploma.

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Sumaiya Ahmed Bhasha

This Research Paper is Dedicated

To

My Beloved Parents

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ABSTRACT

This thesis paper was accomplished in a way to establish generic formulations with Misoprostol in the dosage form of 200 microgram tablet. Direct compression method is used for the tablet manufacturing same as innovator's. This dissertation has been carried out with a view to getting some information, and to know the changes in physical and chemical attributes of finished dosage form (tablet) using different generic formulations. Since the onset of action of this product needs to be within 30 minutes, dissolution, an in-vitro technique to assess bioavailability, is emphasized over other attributes in each formulation. The results obtained from the analysis have been compared with that of the innovator's product and proceeded according to further requirement. The generic formulas presented here are developed using the same excipients as innovator's but there are changes in terms of amount of disintegration and commercial grade of diluents. On this note, besides justifying the generic formulations with physical and chemical analysis, the changes in excipient quantity and grade are justified by evaluating API-excipient compatibility in terms of potency degradation and impurity generation. In this thesis work, the values determined by assay and dissolution tests of the products, potency and impurity analysis of API-excipient mix are represented graphically along with tabular presentation. All of the studies presented in this thesis have been performed following the direction for controlled room condition and storage condition in order to proceed for further study. This developed generic formulation for the thesis project has shown better dissolution and lesser generation of impurities with a slight changes in excipients in comparison to innovators formula. The formula may be beneficial for manufacturing the product containing Misoprostol 200 microgram.

Keywords: Generic formulation, compatibility study, cervical ripening, binary mixture, dissolution, impurities, 8-epi Misoprostol, A-type Misoprostol, B-type Misoprostol.

Chapter One

INTRODUCTION

1.1 INTRODUCTION

Misoprostol is a synthetic analog of natural prostaglandin E1, that exerts many indications and it is also used for some off-label medical purposes. Misoprostol is marketed as finished pharmaceutical dosage form, tablets, and it is administered to patients in oral, sublingual, vaginal or through rectal route. World Health Organization (2009) published a Model List of Essential Medicines including Misoprostol due to its wide-ranging applications in reproductive health. As per, cited by The American Society of Health-System Pharmacists this drug is available as generic forms. Various generic formulations have different impact on the finished dosage form as well as on the patient due its inherent characteristic.

The objective of this dissertation is to establish generic formulations of Misoprostol and justify the formula with analyzing the finished product based on different physical and chemical attributes. The experiment may turn out of great importance as the choice of excipients will be vindicated by analyzing its effect on Misoprostol (API) degradation and impurity generation. Innovator's product (Cytotec 200 Tablet) will be used as reference and for comparison with the developed generic formulations. Based on the nature of Misoprostol, generic formulation will be chosen to manufacture only by direct compression. The trials for formulation development will be proceeded step by step. Any changes of the excipients, or physical parameters will be rationalized with evaluation and further tests so that it does not have impact on finished product ultimately. Objective of the experiment is to determine compatibility of Misoprostol with different excipients in terms of impurity generation followed by potency degradation, by using compatibility method. Based on this study, we can have an overview on impact of excipients and storage condition on degradation of the drug product. This trial results can also justify the use of excipient in the finished product. As per World Health Organization guidelines for product development, the compatibility of the drug product with other excipients should be address to justify the formula, if there is any additional excipient in the finished dosage form other than claimed by innovator. Since the generic formulation will have the same excipients as claimed by innovator, the evaluation of few points can be omitted, eg. differential scanning calorimetry, photolysis etc., from the compatibility study.

1.2 INNOVATOR'S PRODUCT (CYTOTEC 200 TABLET)

The innovator's drug product (Cytotec 200 Tablet) is manufactured by G. D. Searle LLC, under marketing authorization of Pfizer Inc.

The product contains Misoprostol USP as active pharmaceutical ingredient (stabilized with Hydroxypropylmethylcellulose). Inactive ingredients are Microcrystalline Cellulose, Sodium Starch Glycolate, and Hydrogenated Castor Oil. (FDA Product Monograph)

Manufacturer of Cytotec 200 Tablet specifies about the raw materials used in the product in US Patent, filed on July 1980, which are:

Table 1.1: Formulation of Cytotec 200 Tablet

Ingredients	Amount per dose (in mg)
Misoprostol solid dispersion (in Hydroxypropyl Methylcellulose)	20.47
Microcrystalline Cellulose 103 (from FMC Corp)	175.53
Sodium Starch Glycolate	3.00
Hydrogenated Castor Oil	1.00

(G. D. Searle & Co., Skokie, 1980)

The drug production will be formulated using Misoprostol and some excipients.

1.3 MISOPROSTOL

Misoprostol is used as active ingredient in the generic formulations. Misoprostol is developed and discovered in 1973 by G.D. Searle. Prostaglandin E_1 has long been recognized as an effective inhibitor of gastric acid secretion when administered intravenously. However, three major problems have prevented the use of natural Prostaglandin E_1 as a therapeutic treatment for peptic ulcer disease. Each of these problems, lack of oral activity, side-effects, and short duration of action, has been overcome by the chemical development of misoprostol from Prostaglandin E_1 (Collins P.W., 1990). Misoprostol produces a dose-related inhibition of gastric acid as well as pepsin secretion, and enhances mucosal resistance to injury. It is an effective anti-ulcer agent and

also has oxytocic properties. Misoprostol is only found in individuals that have used or taken this drug (DrugBank, Misoprotol)

1.3.1 Characteristic of Misoprostol

Misoprostol is clear, colorless or yellowish, oily liquid, which is hygroscopic in nature. This is practically insoluble in water, soluble in ethanol (96%), and sparingly soluble in acetonitrile. Molecular formula of Misoprostol is $C_{22}H_{38}O_5$ with relative molecular mass of 382.5 $gmol^{-1}$ (BP Monograph, 2016).

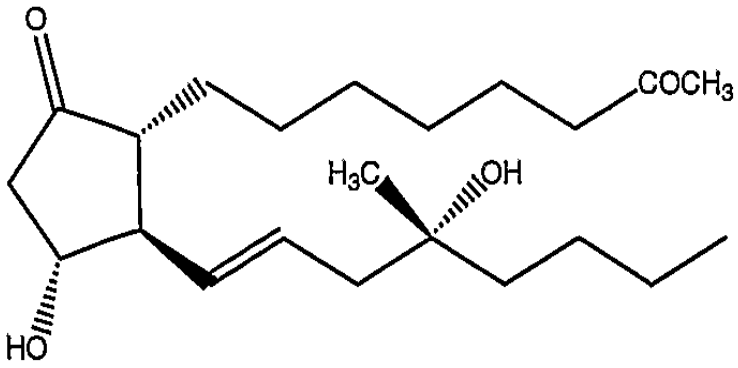


Fig 1.1: Structure of Misoprostol (BP Monograph, 2016)

1.3.2 Synthesis of Misoprostol

The manufacturing process of Misoprstol active substance involves mainly three stages:

Stage-I: Protection of the hydroxyl functional group of Norprostol

Protected Norprostol (Triethylsilyl Norprostol) is generated by reaction of Norprostol with triethylsilychloride in the presence of triethylamine and imidazole.

Stage-II: Formation of protected Misoprostol

Protected Misoprostol is formed by utilizing a series of organometallic reactions on trimethylsilyloctynol to produce a non-isolated intermediate, which preferentially undergoes 1,4 addition to Triethylsilyl Norprostol to form the protected Misoprostol.

Stage-III: Deprotection reaction to generate Misoprostol

Silyl groups are removed by acid hydrolysis of protected Misoprostol. The crude Misoprostol is then further purified to generate the pure Misoprostol active substance. (DMF, Misoprostol, Piramal Healthcare Inc)

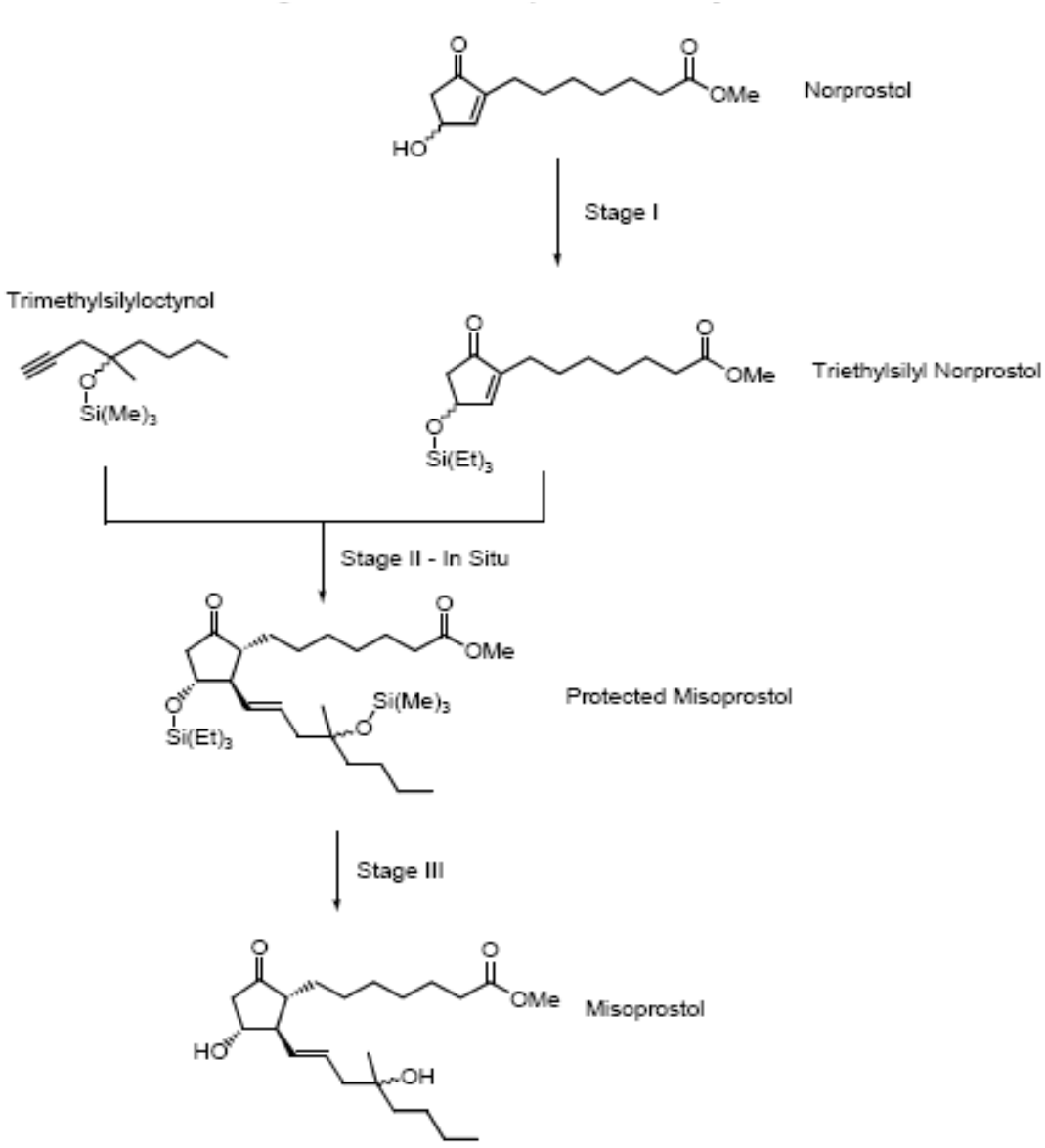
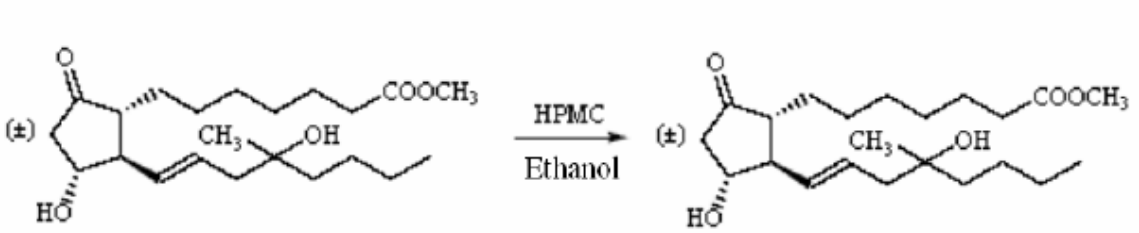


Fig 1.2: Manufacturing Process Steps of Misoprostol (DMF, Misoprostol, Piramal Healthcare Inc)

1.3.3 Stabilization of Misoprostol

Due to its inherent stability nature and handling difficulties of the active substance, it is commercially supplied in its viable stabilized form as stabilized active substance, named as MISOPROSTOL DISPERSION. The stability of Misoprostol is significantly improved by diluting it with Hydroxy Propyl Methylcellulose (HPMC) or Hypromellose with the ratio of 1:100 parts. This stabilization process does not alter the structure and/or chemical properties of the active substance. (DMF, Misoprostol, Piramal Healthcare Inc)



Misoprostol Active Substance (100%) Misoprostol Dispersion (1%)

Fig 1.3: Root of Synthesis for stabilization of Misoprostol active substance

The manufacturing process of stabilized active substance involves only one stage:
A mixture of hypromellose (HPMC), Misoprostol active substance and ethanol are blended in a suitable blender/drier. The solvent is evaporated by vacuum drying. A sample is taken for in-process testing for loss on drying and the resulting dispersion is milled and blended.

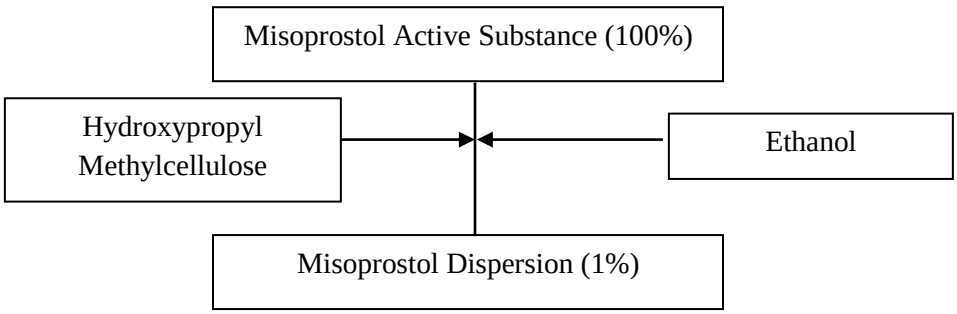


Fig 1.4: Raw material flow chart for stabilization of Misoprostol active substance

1.3.4 Impurities of Misoprostol

During the synthesis of Misoprostol, a final chromatographic purification step is performed. This purification step removes non-prostaglandin-related materials which are used during synthesis of

Misoprostol and removes or reduces the level of other prostaglandin-related materials. (DMF, Misoprostol, Piramal Healthcare Inc)

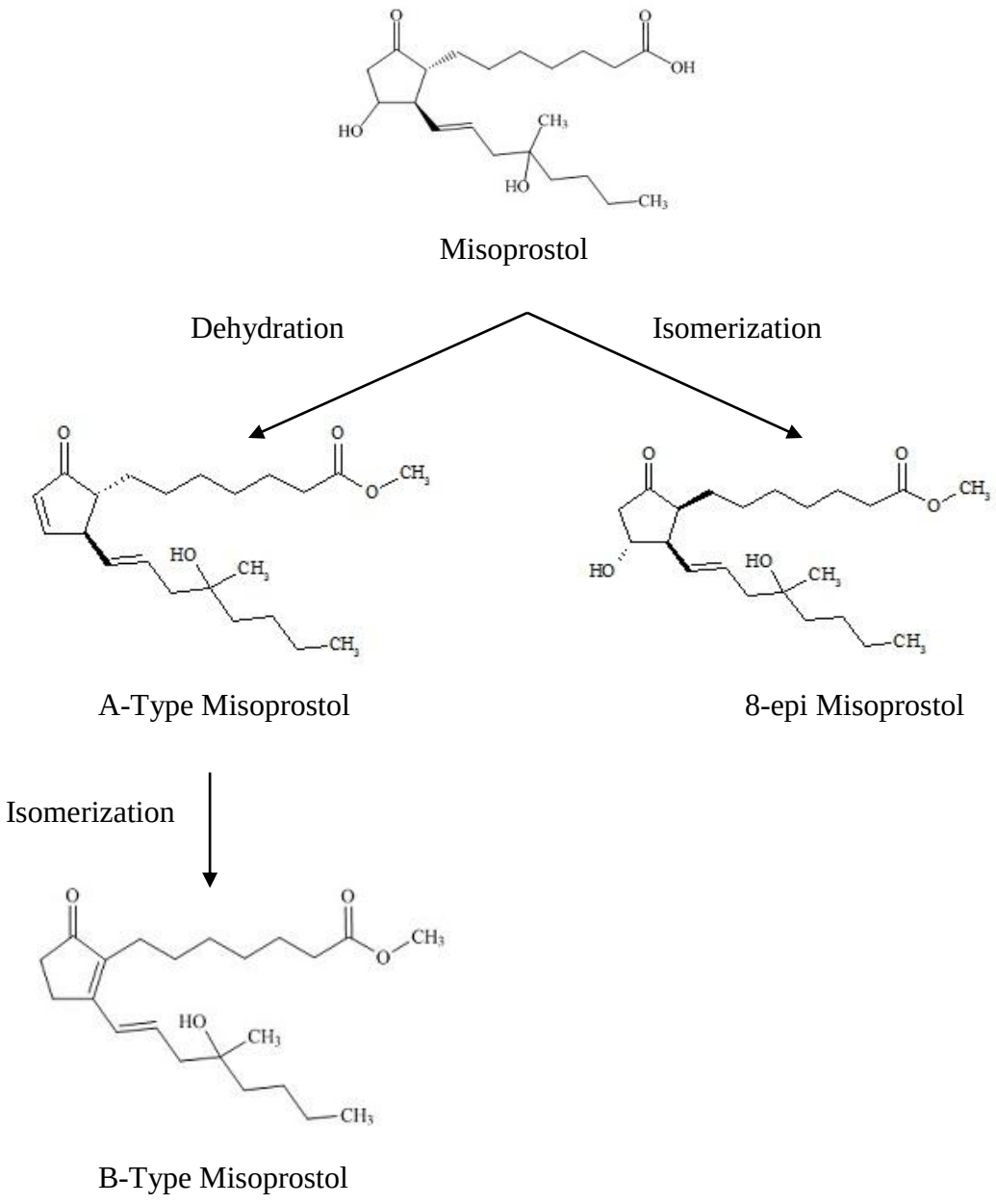


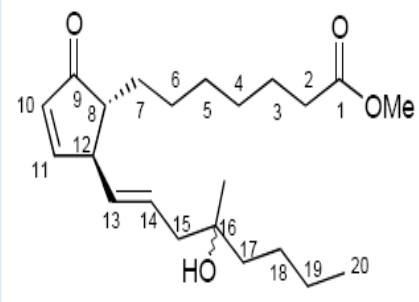
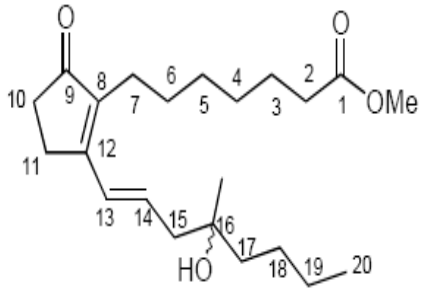
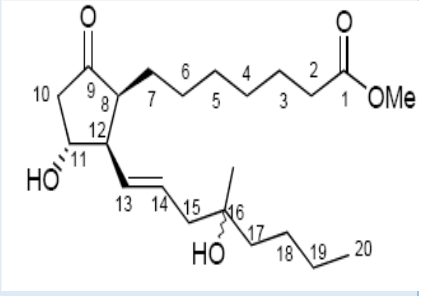
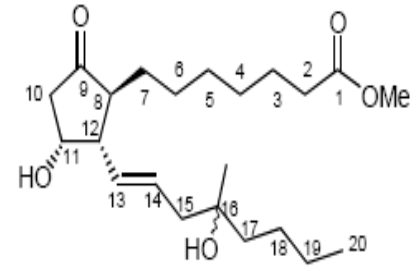
Fig 1.5: Impurity generation from Misoprostol (DMF, Misoprostol, Piramal Healthcare Inc)

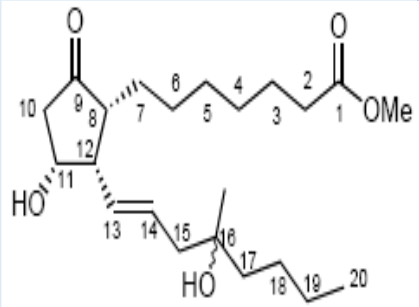
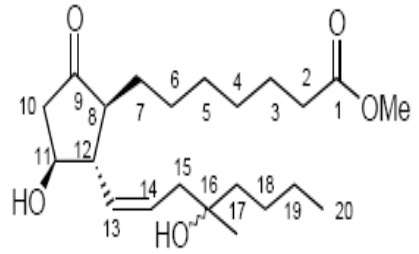
The inactive type A misoprostol is obtained by dehydration, and 8-epi misoprostol by isomerization, which are both catalysed by water; type B Misoprostol is the result of isomerisation of inactive type A.

Potential impurities are known to be associated with the manufacturing process including

genotoxic impurities and inorganic residual metals. Some of the degraded and process generated impurities of Misoprostol are described below:

Table 1.2: Different Degradation & Process Generated Impurities of Misoprotol

Type of Impurities	Overview	Structure
A-Type Misoprostol	It is a process impurity formed during Stage III production of Misoprostol and is also a degradation impurity. The level of this impurity is restricted in the Misoprostol active substance oil to be NMT 0.10%.	
B-Type Misoprostol	B-Form Misoprostol is a degradation impurity and is the result of isomerisation of A-Form Misoprostol to the more stable tetra substituted olefin. The level of this impurity is restricted in the Misoprostol oil is NMT 1.0%.	
8-epi Misoprostol	It is a process impurity formed during Stage II production of Misoprostol and is also a degradation impurity. The level of this impurity is restricted in the Misoprostol active substance to be NMT 0.30%.	
11-epi Misoprostol	11-epi Misoprostol can be isolated from the Misoprostol process or manufactured by the coupling of Norprostol with Vinyl Tin Adduct followed by deprotection of the C16 hydroxyl group.	

Type of Impurities	Overview	Structure
12-epi Misoprostol	It is a process impurity formed during Stage II. The level of this impurity is restricted in the Misoprostol Active substance to be NMT 1.0%. It is the kinetic product of the work up after the addition of cuprate cis to the triethylsilyl protected alcohol of protected Norprostol.	
13, 14 *cis*-Misoprostol	It is a process impurity formed during Stage-II. The level of this impurity is restricted in the Misoprostol active substance to be NMT 0.10% as Individual other impurity.	

(DMF, Misoprostol, Piramal Healthcare Inc)

In the unfavorable storage conditions and over the time of shelf life, misoprostol turns into 3 main inactive degradation products: type A and type B and 8-epimer misoprostol (Collins P.W., et. al, 1985). 12-epimer of Misoprostol is present in the API dispersion at a controlled quantity as process impurity.

1.3.5 Medicinal Uses of Misoprostol

Misoprostol is marketed as an oral preparation used to prevent and treat gastroduodenal damage induced by nonsteroidal anti-inflammatory drugs (NSAIDs). However, misoprostol is used off-label for a variety of indications in the practice of obstetrics and gynecology, including medication abortion, medical management of miscarriage, induction of labor, cervical ripening before surgical procedures, and the treatment of postpartum hemorrhage (Allen, O'Brien, 2009). Misoprostol's effects are dose dependent and include cervical softening and dilation, uterine contractions, nausea, vomiting, diarrhea, fever, and chills. (Goldberg, Greenberg, Darney, 2001). Some of the medicinal uses of the drug are as follows:

1.3.5.1 Ulcer Prevention

Misoprostol is used for the prevention of NSAID-induced gastric ulcers. It acts upon gastric parietal cells, inhibiting the secretion of gastric acid by G-protein coupled receptor-mediated inhibition of adenylate cyclase, which leads to decreased intracellular cyclic AMP levels and decreased proton pump activity at the apical surface of the parietal cell. Misoprostol has a dose depended effect. At higher dose Misoprostol is effective to reduce gastric acid secretion, but at lower dose misoprostol only stimulate increased secretion of the protective mucus that lines the gastrointestinal tract and increase mucosal blood flow, thereby increasing mucosal integrity.

1.3.5.2 Medical Abortion

Misoprostol is used either alone or in combination with another medication, like mifepristone or methotrexate, for medical abortions as an alternative to surgical abortion. Medical abortion is preferable to users because it feels more "natural," as the drugs induce a miscarriage. If the woman has an intrauterine device in place, it must be removed before treatment. Medication abortion necessarily involves heavy bleeding and cramping as the pregnancy is expelled. Other transient side effects from misoprostol include nausea, vomiting, diarrhea, fever, and chills (Allen, O'Brien, 2009).

1.3.5.3 Management of Miscarriage

Misoprostol is an option for the medical management of early pregnancy failure, including anembryonic pregnancies and embryonic demise, and incomplete abortion for women at 12 weeks or less of gestation. Misoprostol is sometimes used to treat early fetal death in the absence of spontaneous miscarriage, but further research is needed to establish a safe, effective protocol. Contraindications include pelvic infection or sepsis, hemodynamic instability or shock, allergy to misoprostol, known bleeding disorder, concurrent anticoagulant therapy, and confirmed or suspected ectopic or molar pregnancy (Allen, O'Brien, 2009).

1.3.5.4 Cervical Ripening and Labor Induction

Misoprostol is effective in labor induction through cervical ripening (effacement) at 1^{st}, 2^{nd} or 3^{rd} trimester. This mechanism is shown to be effective for induction of labor with a viable fetus as well as for fetal death or termination of pregnancy (Allen, O'Brien, 2009).

1.3.5.5 Postpartum Bleeding

Misoprostol is also used to prevent and treat post-partum bleeding. Orally administered misoprostol was marginally less effective than oxytocin. The use of rectally administered misoprostol is optimal in cases of bleeding; it was shown to be associated with lower rates of side effects compared to other routes. A randomised control trial of misoprostol use found a 38% reduction in maternal deaths due to *postpartum* haemorrhage in resource-poor communities. Misoprostol is recommended due to its cost, effectiveness, stability, and low rate of side effects. Oxytocin must also be given by injection, while misprostol can be given orally or rectally for this use, making it much more useful in areas where nurses and physicians are less available. (Villar, et. al, 2002).

1.4 MICROCRYSTALLINE CELLULOSE (MCC)

Microcrystalline Cellulose (MCC) is a very popular binder and diluent in tablet and capsule formulations due to its excellent compressibility, stability and safety. It is a purified, partially depolymerized cellulose produced from α-cellulose treated with mineral acids by controlled hydrolysis and subsequent micronization and size fractionation. Following hydrolysis, the hydrocellulose is purified by filtration and the aqueous slurry is spray dried to form dry, porous particles of a broad size distribution. It is chemically identical to native cellulose, but differs in particle size and crystallinity due to the physical and chemical treatments.

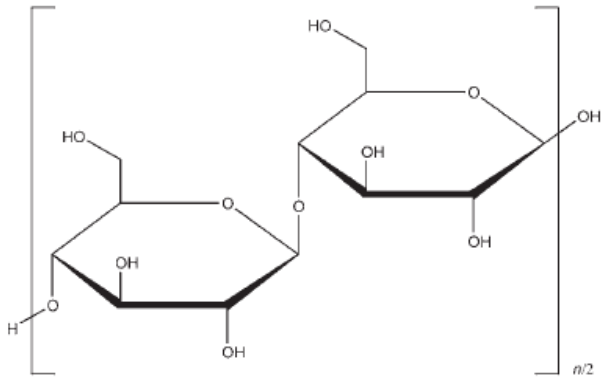


Fig 1.6: Structure of Microcrystalline Cellulose (Rowe, Sheskey, Quinn, 2009)

MCC is widely used in oral pharmaceutical and food products and can be considered as very safe. After oral consumption, it is neither absorbed nor digested. It is available as a white,

odorless, tasteless powder, composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different properties and applications. Microcrystalline cellulose is a stable though hygroscopic material (Rowe, Sheskey, Quinn, 2009).

Commercially some sources are available that manufacture DMF grade of Microcrystalline Cellulose. From those, FMC Biopolymer is picked as the source and they have wide ranges of commercials grade of this diluent under the name of Avicel® based on physical characteristic, eg. particle size distribution and moisture content. For this research, two grades of MCC to be used. Avicel® was introduced by FMC in 1964 in selected particle sizes and moisture contents as an ingredient for direct compression tableting. Avicel® has overcome the problems which were earlier faced by using Lactose. They are:

- ✓ A brown color that developed when used in tablets containing basic amine drugs, caused by an impurity in the lactose which chemically reacted with amines.
- ✓ Lumping of the lactose in bulk drums on storage. (FMC Biopolymer)

1.4.1 Microcrystalline Cellulose 103 (Avicel® 103)

This is used for direct compression tableting, wet granulation and spheronization. It can also be used in capsule filling processes, especially those employing tamping or other means of consolidation as part of the process. It has nominal particle size of 50 micron with bulk density of 0.26 – 0.31 g/cc. Moisture content of this grade is not more than 3% so this is used in case of products where moisture sensitive active pharmaceutical ingredients are present. (FMC Health & Nutrition)

1.4.2 Microcrystalline Cellulose 113 (Avicel® 113)

This is also used for direct compression tableting, wet granulation and spheronization and also in capsule filling processes as like as MCC 103. It has nominal particle size of 50 micron with bulk density of 0.27 – 0.34 g/cc. Moisture content of this grade is not more than 2%. (FMC Health & Nutrition)

1.5 SODIUM STARCH GLYCOLATE (SSG)

Sodium Starch Glycolate is widely used in oral pharmaceuticals as a superdisintegrant in capsule and tablet formulations. It is commonly used in tablets prepared by either direct-compression or wet-granulation processes. The usual concentration employed in a formulation is between 2% and 8%, with the optimum concentration about 4%. Disintegration occurs by rapid uptake of water followed by rapid and enormous swelling.

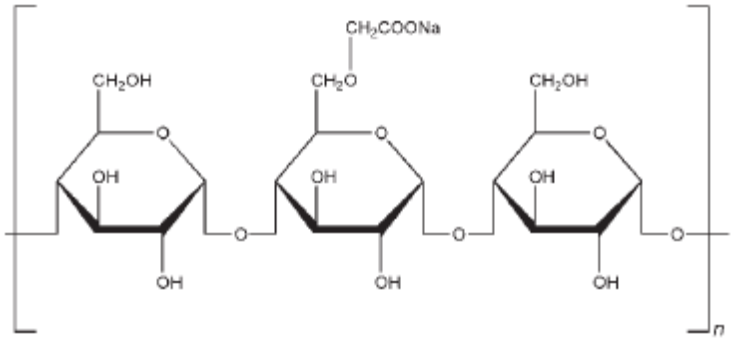


Fig 1.8: Structure of Sodium Starch Glycolate (Handbook of Pharmaceutical Excipients)

Although the effectiveness of many disintegrants is affected by the presence of hydrophobic excipients such as lubricants, the disintegrant efficiency of Sodium Starch Glycolate is unimpaired. Increasing the tablet compression pressure also appears to have no effect on disintegration time. (Gebre, et. al, 1996)

The USP32–NF27 describes two types of sodium starch glycolate, Type A and Type B, and states that sodium starch glycolate is the sodium salt of a carboxymethyl ether of starch or of a crosslinked carboxymethyl ether of starch. The Ph. Eur 6.0 describes three types of material: Type A and Type B are described as the sodium salt of a crosslinked partly O-carboxymethylated potato starch. Type C is described as the sodium salt of a partly O-carboxymethylated starch, crosslinked by physical dehydration. Types A, B, and C are differentiated by their pH, sodium, and sodium chloride content. The Ph. Eur and USP–NF monographs have been harmonized for Type A and Type B variants. Sodium starch glycolate is a white or almost white free-flowing very hygroscopic powder. The Ph. Eur 6.0 states that when examined under a microscope it is seen to consist of: granules, irregularly shaped, ovoid or pear-

shaped, 30–100 mm in size, or rounded, 10–35 mm in size; compound granules consisting of 2–4 components occur occasionally. The granules show considerable swelling in contact with water.

1.5.1 Method of Manufacture

Sodium starch glycolate is a substituted derivative of potato starch. Typically, commercial products are also crosslinked using either sodium trimetaphosphate (Types A and B) or dehydration (Type C). (Bolhuis, et. al, 1986) Starch is carboxymethylated by reacting it with sodium chloroacetate in an alkaline, nonaqueous medium, typically denatured ethanol or methanol, followed by neutralization with citric acid, acetic acid, or some other acid.

1.6 HYDROGENATED CASTOR OIL (HCO)

Hydrogenated castor oil is a hard wax with a high melting point used in oral and topical pharmaceutical formulations. In oral formulations, hydrogenated castor oil is used to prepare sustained-release tablet and capsule preparations;(2,3) the hydrogenated castor oil may be used as a coat or to form a solid matrix. Hydrogenated castor oil is additionally used to lubricate the die walls of tablet presses and is similarly used as a lubricant in food processing. Hydrogenated castor oil is also used in cosmetics.

Hydrogenated castor oil occurs as a fine, almost white or pale yellow powder or flakes. The Ph. Eur 6.0 describes hydrogenated castor oil as the oil obtained by hydrogenation of virgin castor oil. It consists mainly of the triglyceride of 12-hydroxystearic acid. (Handbook of Pharmaceutical Excipients, 6th edition)

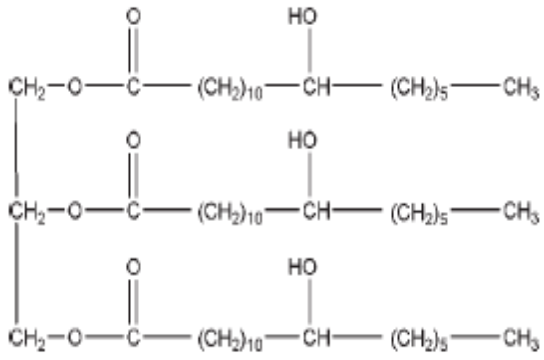


Fig 1.8: Structure of Hydrogenated Castor Oil (Handbook of Pharmaceutical Excipients)

Chapter Two

Literature Review

Misoprostol 200 Tablet is worldwide used as prostaglandin E1 analog, for medical abortion, alone or with other drugs, as well as for other gynecological purposes. Several researches have been conducted to establish its various indications, route of administration along with required dose, formulation development of this drug, and treatment of raw material itself to prevent it from degradation and for making it convenient for handling during manufacturing of drugs with this API.

Due to physical properties of misoprostol, for formulation of drug it is recommended to use a stabilized form of dispersion in appropriate diluent for making is convenient for using in formulation. Based on researches, the stability of misoprostol oil is significantly improved in a hydroxypropyl methylcellulose (HPMC) dispersion (1:100). In order to assess the effect of water on misoprostol stability, the rate of misoprostol degradation was investigated in the misoprostol/HPMC dispersion at 55°C, along with the water sorption isotherm, under seven different relative humidity (RH) conditions ranging from 0 to 81%. The results indicated that the first-order rate constants of misoprostol degradation increased in a concave-up fashion as the water content of the dispersion increased. Below 30% relative humidity (±2% water), the first-order rate constants of misoprostol degradation were found to be minimum. The results of the stability study were interpreted in terms of the changing structure of HPMC as it related to the mobility of water and misoprostol within the HPMC dispersion. (Kararli, T.T. & Catalano, T., 1990)

Another research was performed by the same duo regarding API:HPMC dispersion. In order to understand the enhanced stability of misoprostol oil in HPMC, during 1990 Kararli with his fellow research member Catalano investigated physical state of misoprostol oil in HPMC films using differential scanning calorimetry (DSC), dynamic mechanical analysis (DMA), and transmission IR (TIR). Further, to determine the effect of polymer structure and the mobility of both water and misoprostol on misoprostol stability, the rate of misoprostol degradation was investigated in the misoprostol/HPMC dispersion (1:100) at 55°C. The water sorption isotherm of the dispersion at 55°C was determined, at seven different relative humidities, ranging from zero to 81%. The DSC and DMA measurements indicated that misoprostol oil, up to 29% in dry weight, is molecularly dispersed in the glassy HPMC. The TIR studies showed no evidence of complications between misoprostol and HPMC. Stability studies of the misoprostol with HPMC

dispersion (in 1:100 ratio) directed that the first-order rate constants for misoprostol degradation became higher in a concave-up fashion as the water content of the dispersion increased. Below two percent water content, the rate of misoprostol degradation was found to be minimal. Overall, it is suggested that misoprostol is stabilized in the dispersion by being molecularly dispersed in HPMC. Further, the glassy state of HPMC should reduce the mobility of misoprostol and water, leading to a minimal rate of degradation for misoprostol at low moisture levels. (Kararli, Catalano, 1990)

This commentary reviews the documented obstetric/gynecological benefits of misoprostol as well as the difficulties inherent to deciphering the available data. The authors note that regimens used in clinical trials are difficult to compare and often cumbersome for women. They also cite a lack of data on pharmacokinetics as well as observed differences in the success rates of various regimens. These issues prompt the authors to ask, "How good is good enough?" They call for more thorough assessments of misoprostol's benefits (e.g., success rates, easy access, and increased privacy) and risks (including treatment failure, side effects, and the possibility of incomplete abortions or ongoing pregnancies). They suggest that acceptability may increase by improving misoprostol's benefits, reducing its risks, or both. The authors conclude that simplified misoprostol regimens, including self-administration, should be evaluated. They also recommend that researchers identify reasons for the differences in reported success rates and develop a coherent research strategy for the future. (Blanchard, et. al, 2000)

Later, in the same year another research was published which is suggesting use another drug along with misoprostol to serve the purpose of abortion. This document provides a comprehensive review of the use of misoprostol for obstetric and gynecological purposes over the last 15 years. The author reviews misoprostol's effectiveness as an agent for cervical priming before a surgical abortion, and as a cervical primer before hysteroscopy and endometrial biopsy. The article discusses misoprostol's use as an effective agent alone or as an adjunct to mifepristone or methotrexate for medical first- and second trimester pregnancy termination. It also describes misoprostol's potential effectiveness for treatment of incomplete or inevitable abortion, prevention and treatment of postpartum hemorrhage, induction of fetal death in all trimesters, and cervical ripening and labor induction after viability. The author notes that use of misoprostol is associated with tachysystole/ hypertension and uterine rupture, especially for

patients with a previous uterine scar. Because misoprostol is stable, inexpensive, and easily stored, the author describes it as an inexpensive life saving alternative to other prostaglandins and oxytocics in low-resource settings. On the other hand, he notes, its medically unsupervised and unregulated use as an abortifacient has created obstacles to its acceptance worldwide. The author concludes that, as the only inexpensive oral prostaglandin alternative, misoprostol has found widespread use in the clinical practice of obstetrics and gynecology in the developed and developing world. (Broekhuizen, 2000)

Misoprostol is also used during 3rd stage of labor successfully and the efficacy was systematically reviewed and compared with placebo or other uterotonics in preventing maternal morbidity associated with the third stage of labor. Some abstracted data were identified, retrieved, evaluated and assessed the quality of all published studies (from January 1996 to May 2002) which assessed misoprostol's efficacy in minimizing uterine blood loss during the third stage of labor. Seventeen studies included 28 170 subjects; of these, approximately one-half received misoprostol with the remainder receiving either a placebo or another uterotonic agent. An estimate of the odds ratio (OR) and risk difference for dichotomous outcomes was calculated using a random- and fixed-effects model. Continuous outcomes were pooled using a variance-weighted average of within-study difference in means. In assessing studies comparing misoprostol with placebo, those who received oral misoprostol had a decreased risk of needing additional uterotonics. Compared with placebo, use of misoprostol was associated with an increased risk for shivering and pyrexia. In contrast, in studies comparing misoprostol with oxytocin, oxytocin was associated with significantly lower rates of postpartum hemorrhage, maternal shivering and pyrexia. In studies comparing misoprostol with Syntometrine, misoprostol was associated with higher rates of the need for additional uterotonic agent as well as shivering. Misoprostol was inferior to oxytocin and other uterotonics with regard to any of the third stage of labor outcomes assessed. However, when compared to placebo, misoprostol had a decreased risk of needing additional uterotonics. Thus, in less-developed countries where administration of parenteral uterotonic drugs may be problematic, misoprostol represents a reasonable agent for the management of the third stage of labor. Additional randomized clinical trials examining objective outcome measures (i.e. need for blood transfusion or 10% hemoglobin change) may further define benefits and risks of misoprostol use during the third stage of labor. (Joy, Ramos, Kaunitz, 2003)

In the year 2003, a research was conducted by Khan and El-Rafaey to distinguish the pharmacokinetics and adverse-effect profile of rectally administered misoprostol. To evaluate absorption of rectally administered misoprostol, 20 women were randomized to receive misoprostol 600 μg by either oral or rectal administration after delivery. Their blood samples were collected at certain time intervals and analyzed for serum concentrations of misoprostol free acid. The research concluded as misoprostol tablets are absorbed rectally even though they are formulated for oral use. As an adverse effect, rate of shivering is 73% in rectally administerd drug in compared to orally taken misoprostol. Misoprostol administered rectally is associated with lower peak levels and a reduction in adverse effects compared with the oral route. Increasing rectal doses may achieve higher efficacy without reducing the acceptability of the treatment. (Khan, El-Refaey, 2003)

In 2004 Creinin with his fellow researchers conducted a trial to assess if there was any potential relationship between endometrial thickness and final treatment outcome in women successfully treated with misoprostol for a first trimester anembryonic gestation, embryonic demise or fetal demise. Eighty women were selected and treated with up to two doses of Misoprostol 800 μg vaginally for early pregnancy failure. Transvaginal ultrasonography was performed at 2 (range 1–4), 7 (range 5–9) and 14 (range 12–17) days after treatment. The median endometrial thickness at each of the follow-up visits for women who had expelled the gestational sac was 14 mm, 10 mm, and 7 mm, respectively. The endometrial thickness at the first follow-up visit exceeded 15 mm in 20 subjects (36%) and 30 mm in four subjects (7%). Only three women had a suction aspiration for bleeding after documented expulsion. The endometrial thickness for these women was 11, 13, and 14 mm at the first follow-up visit. There is no obvious relationship between increasing endometrial thickness and the need for surgical intervention in women treated with misoprostol for early pregnancy failure. (Creinin, et. al, 2004)

A study was conducted to compare vaginal versus oral administration of misoprostol for labor induction. The study was conducted on two groups of women with 20 persons each using the drug misoprostol. One group was administered vaginal misoprostol at 100 μg dose whereas the patients from other group (group II) were provided the same dose (100 μg) via the oral route.

The doses were repeated for both the groups at every 3 hours interval. If no response was identified under continuous cardiotocographic (CTG) tracings, the dose was doubled. At the end the study, it was detected that the vaginal route of administration induced a higher accomplishment rate in a shorter time interval using a lower dose but was associated with more abnormal fetal heart rate patterns and instances of uterine hyperstimulation. Based on this study, it was recommended to use vaginal approach with cardiotocographic monitoring continuously. (Toppozada, et al., 1997)

In 1998, researcher Singh and his team conducted a trial to identify the optimum dose of vaginally administered misoprostol for cervical priming before vacuum aspiration abortion. One hundred twenty women were selected randomly to receive 200, 400, 600, or 800 µg of misoprostol given at vaginal route. Vacuum aspiration was performed 3–4 hours after the insertion of misoprostol tablets. The degree of cervical dilation before operation was measured with a Hegar dilator. Preoperative and intraoperative blood loss and associated side effects also were assessed. 96.7% women in the 400-µg group and all in the 600-µg and 800-µg groups achieved cervical dilation of at least 8 mm. The success rate for the 200-µg group was only 23.3%. The research could conclude on the fact that there was no significant difference among the 400, 600, and 800 µg groups in terms of achieving cervical dilation at least 8 mm. However, higher dose of misoprostol was associated with significantly more side effects than 600 and 400 µg (preoperative and intraoperative blood loss, abdominal pain, fever). It is quite visible through the research that vaginal application of 400 µg of misoprostol is the optimal dose for vacuum aspiration preabortion cervical dilation in first-trimester nulliparas. (Singh, Fong, Prasad, Dong, 1998)

In the year 1999, a study was conducted with a purpose to compare the abortifacient effect of vaginally administered moistened misoprostol tablets with that of the combination regimen of mifepristone and oral misoprostol. One group of women at ≤56 days' gestation received 800 µg misoprostol intravaginally in the form of sodium chloride solution–moistened tablets. Another group of women had received 600 mg mifepristone followed by 400 µg misoprostol orally. Subjects were monitored for abortion success, adverse side effects, and bleeding characteristics. Abortion failure was defined as persistence of an intrauterine sac or the need to perform a surgical evacuation of the uterus for hemorrhage, for incomplete abortion, or at the subject's

request. Abortion occurred in 88% women in 1st group receiving sodium chloride moistened tablet and 94% women in other group taking combination of drug misoprostol and mifepristone and a surgical procedure was not required. Abortion rates were not influenced by gestational age in either group. Prostaglandin-related side effects of fever and chills, vomiting, diarrhea, and uterine pain were all significantly higher in group 1. Excessive uterine bleeding was uncommon in both groups, and no subjects received blood transfusions. The research concluded with the statement that abortion rate with vaginally an administered moistened misoprostol tablet is similar to that with the combination of mifepristone and oral misoprostol. However, vaginally administration of misoprostol is associated with significantly more prostaglandin-related side effects since it contained higher dose than orally taken misoprostol in combined drug. (Jain, et. al, 1999)

Another study was conducted to compare the pharmacokinetic profiles of orally, rectally, and vaginally administered misoprostol tablets in some pregnant women who are between 7 and 14 completed weeks of gestation. Women were randomly assigned to be given 400 µg misoprostol orally, rectally, or vaginally 3 hours before surgical termination of pregnancy. Blood samples were obtained different time points till 240 minutes and later analyzed for plasma concentrations of misoprostol free acid. Vaginal misoprostol was present in the circulation longer than oral misoprostol and had a greater area under curve at 240 minutes. Rectal misoprostol had a similar pattern but a much lower area under curve at 240 minutes. Oral misoprostol had a significantly greater peak plasma concentration and a shorter duration to maximum concentration than either rectal or vaginal misoprostol. Finally the study was concluded to that oral misoprostol tablet is also absorbed by the rectal and vaginal routes. Misoprostol administered in early pregnancy has route-dependent pharmacokinetics and is absorbed best when administered vaginally. (Khan, et. al, 2004)

Another research was done to identify better route of administration of Misoprostol, whether it is orally or vaginally. The aim of the study is to compare the efficacy and safety of 100 µg oral and 50 µg vaginal misoprostol for labor induction. Ninety-nine patients with indications for labor induction randomly received 100 µg oral misoprostol every 4 h or 50 µg vaginal misoprostol every 4 h, using maximum six doses. Few physical changes, like rates of tachysystole, hypertonus and hyperstimulation syndrome, oxytocin use, number of doses used, failed induction

rate and neonatal outcomes were monitored and compared for the two groups. There were also no significant differences for intrapartum complications and neonatal outcomes between the oral and vaginal misoprostol groups. The findings specify that, in a closely supervised hospital setting with adequate monitoring, 100 µg oral misoprostol has the potential to induce labor as safely and effectively as its 50 µg vaginal analogue. As oral use of the drug is easier for the patient and the doctor, oral misoprostol will probably be more preferable than the vaginal route for labor induction. (Uludag et. al, 2005)

Postpartum hemorrhage accounts for 17% to 40% of maternal mortality in some parts of the world (El-Refaey et al., 1997). Most of the articles confirm the effectiveness of oral and rectal misoprostol for the prevention and management of postpartum hemorrhage.

In the study by O'Brien et al. (1998), 14 women were selected with postpartum hemorrhage unresponsive to oxytocin and ergometrine or, when ergometrine was contraindicated, oxytocin alone. Women received 1000 µg of Misoprostol in rectal route. In all the 14 women, hemorrhage was controlled and sustained uterine contractions were produced within 3 minutes of administration. No women required any further uterotonic treatment and all the women made a full recovery. Other intrapartum complications included preeclampsia, diabetes mellitus, abruption, retained placenta, asthma, and malpresentation. The authors suggest that, because the absorption of misoprostol is mucous membrane dependent, absorption from the rectal mucosa is just as effective as from vaginal or oral administration. Additionally, because oral medication cannot be administered to women under general anesthesia and vaginal administration during heavy bleeding is unlikely to be effective, there is considerable potential for misoprostol to reduce maternal mortality from postpartum hemorrhage, particularly in developing countries.

The data obtained by El-Refaey et al., in 1997, also demonstrates the effectiveness of 600 µg of misoprostol for management of postpartum hemorrhage. The authors note misoprostol's advantages over the medicine 0.5 mg Ergometrine, with 5 units of oxytocin, which is routinely used in the developed world. Unlike misoprostol, this medicine is contraindicated in women with hypertension in pregnancy, frequently causes nausea and vomiting, and must be administered by intramuscular injection. The authors found misoprostol to be a safe and effective alternative.

Since Misoprostol drug product is very prone to be degraded at uncontrolled storage condition, several researches were performed regarding its stability and efficacy. One of these important studies was conducted with innovator's product Cytotec 200 Tablet by researcher Berard and his team. This study had a purpose to compare the physical characteristics (weight, friability), water content, misoprostol content and decomposition product content (type A misoprostol, type B misoprostol and 8-epi misoprostol) of misoprostol tablets Cytotec (Pfizer) exposed to air for periods of 1 hour to 720 hours (30 days), to those of identical non exposed tablets. 420 tablets of Cytotec (Pfizer) were removed from their aluminium blister and stored at 25°C/60% relative humidity. Water content and misoprostol degradation products were evaluated in tablets exposed from 1 to 720 hours (30 days). Comparison was made with control tablets from the same batch stored in non-damaged blisters. By 48 hours, exposed tablets demonstrated increased weight (+4.5%), friability (+13.00%), and water content (+80%) compared to controls. Exposed tablets also exhibited a decrease in Misoprostol quantity (−5.1% after 48 hours) and an increase in the inactive degradation products (+25% for type B, +50% for type A and +11% for 8-epi misoprostol after 48 hours) compared to controls. Exposure of Cytotec tablets to regular European levels of air and humidity results in significant time-dependent changes in physical and biological composition that could impact adversely upon clinical efficacy. (Berard, et. al, 2014)

Chapter Three

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Materials Collection

For the research purpose, active ingredient, excipient, instruments, equipment were required as necessary. These were collected from different suppliers.

3.1.2 Raw Materials

Some raw materials, including active pharmaceutical ingredient (API) were required for preparation of generic formulation and further development trials. The list of raw materials those were used during this research is given below with their individual source (supplier name):

Table 3.1: Source of Raw Materials Used

Material Name	Specification	Supplier's Name
Misoprostol Dispersion	USP	Piramal Healthcare UK Ltd.
Microcrystalline Cellulose 103	BP/Ph.Eur	FMC Biopolymer
Microcrystalline Cellulose 113	BP/Ph.Eur	FMC Biopolymer
Sodium Starch Glycolate (Type-A)	BP/Ph.Eur	DMV Fonterra Excipients B.V.
Hydrogenated Castor Oil	BP/Ph.Eur	BASF Personal Care & Nutrition

3.1.3 Equipments and Instruments

Few equipments and instruments were used during formulation and analysis of the trial batches. These are listed as following:

Table 3.2: List of Instruments Used Throughout Research Work

Serial No.	Equipments	Source (Supplier Name)	Origin
1.	Electronic Balance	Mettler Toledo	Germany
2.	Laminar Air Flow	Esco Lab	USA
3.	Drum Blender	Chamunda Pharma	India
4.	Compression Machine (Clit Tab Press)	Chamunda Pharma	India
5.	Friability Tester	Electrolab	India
6.	Hardness Tester	Electrolab	India
7.	Disintegration Tester	Electrolab	India
8.	High Performance Liquid Chromatography	Agilent Laboratories	Germany
9.	Dissolution Apparatus	Agilent Laboratories	Germany

3.1.4 Images of Instruments

Some images of important instruments those were used in different times during this research work.

Fig 3.1: Electronic Balance (Mettler Toledo)

Fig 3.2: Laminar Air Flow (Esco Lab)

Fig 3.3: Drum Blender

Fig 3.4: Tablet Press (Lab Scale) (Chamunda Pharma)

Fig 3.5: Friability & Hardness Tester (Electrolab India)

Fig 3.6: Disintegration Tester (Electrolab India)

Fig 3.7: Dissolution Tester (Agilent Laboratories)

Fig 3.8: High Performance Liquid Chromatography (Agilent Laboratories)

3.1.5 Apparatus

Some apparatus are listed in the following table those were used through the research work.

Table 3.3: List of Apparatus Used Throughout Research Work

Serial No.	Apparatus
1.	Spatula, Spoon
2.	Polybags
3.	30 mesh SS screen
4.	15 mL Glass Vial (Type-1)
5.	20 mm Rubber Stopper
6.	20 mm Flip Off Seal

3.2 METHODS

Initially trial was done to establish generic formula of Misoprostol 200 µg Tablet which are similar to innovator's product in terms of assay results and dissolution profile. The formulation sets of active and excipients were made up of relying patent of innovator's that have mentioned about the API-excipients and in what amount they are used in there tablet. The formula was slightly modified in both amount and grade of excipients for trial purpose. In generic formulations, the quantity of Misoprostol Dispersion was considered to be 20.20 mg per tablet based on declaration by supplier, Piramal Healthcare. As per the declaration, the dispersion is of 1:100 ratio of API with Hydroxypropyl Methylcellulose. The generic formulation sets were blended and compressed individually at a controlled room condition, temperature not more that 25°C, and relative humidity not more than 45%, to prevent the product from moisture.

The study for the generic formulation development was done following gradual steps so that purpose and outcome of the research do not overlap the trial phases. The study was done under three phases following roughly a design as follows:

Table 3.4: Study Design

	Phase One Trial	Phase Two Trial	Phase Three Trial
Study	Finished dosage forms were manufactured with different formulations.	Finished dosage forms were manufactured with different formulations.	Compatibility study was done between API and excipients used.
Purpose	- To establish the increased amount of disintegrant, Sodium Starch Glycolate. - To identify appropriate hardness range for the generic formula.	- To establish the use of different grade of diluent. - To justify different hardness range (lower than innovator's) by chemical analysis.	- To evaluate impact of excipients, and storage condition on impurity generation in Misoprostol. - To evaluate potency of Misoprostol at different storage condition.
Parameters evaluated	Physical Parameters, Assay, Dissolution	Physical Parameters, Assay, Dissolution	Assay, Impurity analysis

3.2.1 Phase-One Trial

In this set of trial, disintegrating agent was used in higher amount. As per patent, Sodium Starch Glycolate can be used up to 12% in case of Misoprostol Tablet. (Sekar Selvaraj, et. al, 2015). Based on previous trials, amount of disintegrating agent was finalized Sodium Starch Glycolate was used in larger amount (4.5% w/w) after gradually increasing from the innovator's (1.5% w/w) with a view to obtaining better dissolution profile. Since moisture presence inside compressed tablet initiates impurity generation faster, a different grade of diluent (Microcrystalline Cellulose 113) was used in a set of trial and physical and chemical attributes were evaluated. Besides, formulation with the diluent grade used by innovators was also evaluated based on the same physical and chemical attributes.

3.2.1.1 Formulation With Microcrystalline Cellulose 103

Formula was prepared with using this grade of diluent and tablets were compressed following two different ranges of hardness, eg. 120 N to 140 N, and 160 N to 180 N. Different ranges of hardness were chosen to observe the impact of dissolution. Physical parameters and potency were checked in the compressed tablets.

Table 3.5: Generic Formula with Microcrystalline Cellulose 103

Ingredients	Amount per dose (in mg)	
	Formula1.1 (a) 120 N to 140 N	Formula1.1 (b) 160 N to 180 N
Misoprostol Dispersion	20.20	20.20
Microcrystalline Cellulose 103	179.80	179.80
Sodium Starch Glycolate	9.00	9.00
Hydrogenated Castor Oil	1.00	1.00

3.2.1.2 Formulation With Microcrystalline Cellulose 113

Another set of formula was prepared using this grade of diluent (with lower moisture content) and tablets were compressed following two different ranges of hardness, eg. 120 N to 140 N, and

160 N to 180 N. Different ranges of hardness were chosen to observe the impact of dissolution. Physical parameters and potency were evaluated checked in the compressed tablets.

Table 3.6: Generic Formula with Microcrystalline Cellulose 113

Ingredients	Amount per dose (in mg)	
	Formula1.2 (a) 120 N to 140 N	Formula1.2 (b) 160 N to 180 N
Misoprostol Dispersion	20.20	20.20
Microcrystalline Cellulose 113	179.80	179.80
Sodium Starch Glycolate	9.00	9.00
Hydrogenated Castor Oil	1.00	1.00

3.2.1.3 Procedure

- Misoprostol Dispersion, small amount of Microcrystalline Cellulose (MCC 103 or MCC 113), Sodium Starch Glycolate were blended in a drum blender (lab scale) for 10 minutes. The mix was passed through #30 mesh SS screen and placed into the same drum blender.
- Rest amount of Microcrystalline Cellulose (MCC 103 or MCC 113) was added to the drum blender containing the mix of previous step and again blended for 5 minutes.
- Hydrogenated Castor Oil was added to the drum blender and mixed for 2 minutes.
- The blended mix was compressed into tablets using hexagonal punch, having bisect line on both sides (similar to innovator's product) at two different hardness ranges.

3.2.2 Phase-Two Trial

After phase-one trial, hardness range 120 N to 140 N was chosen to proceed for further development work. Other two formulas were prepared using both grades of Microcrystalline Cellulose. Potency and dissolution profile of finished product were compared with innovator's

product along with evaluation of physical characteristic of the finished product with generic formulas.

Table 3.7: Generic Formulas Used in Phase-Two Trial

Ingredients	Amount per dose (in mg)	
	Formula 2.1	Formula 2.2
Misoprostol Dispersion	20.20	20.20
Microcrystalline Cellulose 103	179.80	-
Microcrystalline Cellulose 113	-	179.80
Sodium Starch Glycolate	9.00	9.00
Hydrogenated Castor Oil	1.00	1.00

Both the formulas were blended and compressed using the same procedure described in section 3.2.1.3. The compressed tablets were used for chemical evaluation and compared with innovator's product, Cytotec 200 Tablet.

3.2.3 Phase-Three Trial

In this phase of trial compatibility study between API and excipients was done to evaluate effect of excipients in case of impurity generation followed by API degradation. Several combination set was prepared to assess the impurity generation over the time and to analyze potency of active ingredients. The combination sets used for the trial purpose were kept in certain storage conditions for predefine period of time. Samples from each storage conditions were tested at certain intervals (days).

3.2.3.1 Procedure of API : Excipients Sets Preparation (Each Vial)

- Required amount of API and excipients (as applicable) was dispensed in electronic balance accurately under Laminar Air Flow using spatula.

Room condition was strictly maintained (Temp.: not more than 25° C; Relative Humidity: not more than 45% RH) and the total process was done under closed condition to avoid moisture entraption.

- At first, defined amount of excipients was place in a 15 ml vials individually and kept at tightly closed condition till active ingredient is added.
- Exact amount of API, Misoprostol Dispersion, was added in each vial. Close the vials with rubber stopper and mix the materials with gentle shake.
  In case of control, there was only API, without any trace of excipients.
- In this way, required amount of vials was prepared for the trial. Seal the vials with Flip Off Seal and place in different storage conditions with proper labeling.

3.2.3.2 Misoprostol Dispersion (Active Ingredient)

Active Pharmaceutical Ingredient (API), Misoprostol Dispersion was kept as a control along with other API:Excipient set to evaluate impact of excipients over potency degradation and impurity generation in API itself. The API was also kept under different storage condition for defined period of time.

Table 3.8: Storage Condition and Time Points of Misoprostol Dispersion

API Name	Amount/ Test	Excipients Name	API & Excipient Ratio/ vial	Storage Condition	Time Points
Misoprostol Dispersion	2 g	-	Not Applicable	25° C Temp./ 60% RH	7, 14 and 21 days
				30° C Temp./ 75% RH	7, 14 and 21 days
				40° C Temp./ 75% RH	7, 14 and 21 days
				60° C Temp.	7, 14 and 21 days

3.2.3.3 Trial Sets of Combination 01 (API : Microcrystalline Cellulose 103 = 1 : 1)

Microcrystalline Cellulose 103 is used as diluents of Misoprostol Tablet by innovators. A binary combination set was prepared for evaluation of impact of this diluent on potency degradation and impurity generation in Misoprostol Dispersion in finished dosage form. The API : MCC 103 sets were also kept under different storage condition for defined period of time.

Table 3.9: Storage Condition and Time Points of Misoprostol Dispersion: Microcrystalline Cellulose 103

API Name	Amount/ Test	Excipients Name	API & Excipient Ratio/ vial	Storage Condition	Time Points
Misoprostol Dispersion	2 g	Microcrystalline Cellulose 103	1 : 1	25° C Temp./ 60% RH	7, 14 and 21 days
				30° C Temp./ 75% RH	7, 14 and 21 days
				40° C Temp./ 75% RH	7, 14 and 21 days
				60° C Temp.	7, 14 and 21 days

3.2.3.4 Trial Sets of Combination 02 (API : Microcrystalline Cellulose 113 = 1 : 1)

Microcrystalline Cellulose 113 is used as diluents in generic formulation of Misoprostol Tablet since water content is lower in this diluents grade. A binary combination set was prepared for evaluation of impact of this diluent on potency degradation and impurity generation in Misoprostol Dispersion in finished dosage form. The API : MCC 113 sets were also kept under different storage condition for defined period of time.

Table 3.10: Storage Condition and Time Points of Misoprostol Dispersion: Microcrystalline Cellulose 113

API Name	Amount/ Test	Excipients Name	API & Excipient Ratio/ vial	Storage Condition	Time Points
Misoprostol Dispersion	2 g	Microcrystalline Cellulose 113	1 : 1	25° C Temp./ 60% RH	7, 14 and 21 days
				30° C Temp./ 75% RH	7, 14 and 21 days
				40° C Temp./ 75% RH	7, 14 and 21 days
				60° C Temp.	7, 14 and 21 days

3.2.3.5 Trial Sets of Combination 03 (API : Sodium Starch Glycolate)

Sodium Starch Glycolate (SSG) is used as disintegrating agent in both innovator's and generic formulation of Misoprostol Tablet. Since there is difference in quantity of this ingredient in both innovator's and generic formulations, three individual sets were prepared with API and Sodium Starch Glycolate combination to conduct the trial. In first set, API and SSG were used in binary ratio (1 : 1). Another two set were rationalized with refer to the amount used by innovator's and generic formula, which are 1.5% and 4.5% of Sodium Starch Glycolate per tablet. The use of this extra amount of Sodium Starch Glycolate was justified in the previous sets of trial.

All the three sets were evaluated for defined period of time, from the vials kept in different storage conditions, to check the impact of this disintegrant on potency degradation and impurity generation in Misoprostol Dispersion in finished dosage form.

Table 3.11: Storage Condition and Time Points of Misoprostol Dispersion: Sodium Starch Glycolate

API Name	Amount/ Test	Excipients Name	API & Excipient Ratio/ vial	Storage Condition	Time Points
Misoprostol Dispersion	2 g	Sodium Starch Glycolate	1 : 1	25° C Temp./ 60% RH	7, 14 and 21 days
				30° C Temp./ 75% RH	7, 14 and 21 days
				40° C Temp./ 75% RH	7, 14 and 21 days
				60° C Temp.	7, 14 and 21 days
Misoprostol Dispersion	1.15 g	Sodium Starch Glycolate	1 : 0.15	25° C Temp./ 60% RH	7, 14 and 21 days
				30° C Temp./ 75% RH	7, 14 and 21 days
				40° C Temp./ 75% RH	7, 14 and 21 days
				60° C Temp.	7, 14 and 21 days

API Name	Amount/ Test	Excipients Name	API & Excipient Ratio/ vial	Storage Condition	Time Points
Misoprostol Dispersion	1.45 g	Sodium Starch Glycolate	1 : 0.45	25° C Temp./ 60% RH	7, 14 and 21 days
				30° C Temp./ 75% RH	7, 14 and 21 days
				40° C Temp./ 75% RH	7, 14 and 21 days
				60° C Temp.	7, 14 and 21 days

3.2.3.6 Trial Sets of Combination 04 (API : Hydrogenated Castor Oil)

Hydrogenated Castor Oil is used as lubricating agent in both innovator's and generic formulation of Misoprostol Tablet. The amount of this ingredient has not been changed in any generic formualation of previous trials. A binary combination set was prepared for evaluation of impact of this lubricating agent on potency degradation and impurity generation in Misoprostol Dispersion in finished dosage form. The combination sets were also kept under different storage condition for defined period of time.

Table 3.12: Storage Condition and Time Points of Misoprostol Dispersion: Hydrogenated Castor Oil

API Name	Amount/ Test	Excipients Name	API & Excipient Ratio/ vial	Storage Condition	Time Points
Misoprostol Dispersion	2 g	Hydrogenated Castor Oil	1 : 1	25° C Temp./ 60% RH	7, 14 and 21 days
				30° C Temp./ 75% RH	7, 14 and 21 days
				40° C Temp./ 75% RH	7, 14 and 21 days
				60° C Temp.	7, 14 and 21 days

Chapter Four

RESULTS

4.1 RESULTS

Evaluation of compressed tablets with each generic formula was carried out along with the reference innovator's product. Both physical and chemical parameters were tested and compared with the reference to justify the use of different grade and amount of excipients. The blends used for compatibility study, were evaluated against a set of control (Misoprostol Dispersion).

4.1.1 Results of Phase-One Trial

In Phase-One set of trial, two different formulations were prepared, with tablet compressed in two different hardness ranges respectively. The finished dosage forms of generic formulas were evaluated and Cytotec 200 Tablet was used for comparison intra batches.

4.1.1.1 Physical Parameters Evaluation

Appearance of the compressed tablets of the different formulation as well as other physical characteristics of the tablets was checked. Brand product evaluation was also done and compared with that of generic formulations. The evaluated parameters as well as its results are captured in following table:

Table 4.1: Values of Physical Parameters of Formulations of Phase-One Trial With Innovator's Product (Cytotec 200 Tablet)

Physical Parameters	Innovator's Product	Formula 1.1 (a)	Formula 1.1 (b)	Formula 1.2 (a)	Formula 1.2 (b)
Description	White tablet, having bisectline on both sides	White tablet, having bisectline on both sides	White tablet, having bisectline on both sides	White tablet, having bisectline on both sides	White tablet, having bisectline on both sides
Tablet Weight (Average of 10 tablets)	200.1 mg	200.2 mg	200.1 mg	200.0 mg	199.9 mg
Disintegration Time	8 sec	8 sec	9 sec	8 sec	7 sec
	8 sec	10 sec	10 sec	11 sec	9 sec
	10 sec	10 sec	12 sec	11 sec	11 sec
	11 sec	13 sec	12 sec	14 sec	14 sec
	15 sec	17 sec	16 sec	18 sec	15 sec
	21 sec	18 sec	17 sec	20 sec	18 sec

Physical Parameters	Innovator's Product	Formula 1.1 (a)	Formula 1.1 (b)	Formula 1.2 (a)	Formula 1.2 (b)
Hardness	162 N	165 N	131 N	168 N	132 N
	169 N	163 N	137 N	172 N	130 N
	175 N	174 N	128 N	167 N	126 N
	165 N	168 N	126 N	164 N	134 N
	168 N	172 N	127 N	175 N	128 N
	174 N	177 N	140 N	169 N	138 N
Friability	0.0%	0.0%	0.0%	0.0%	0.0%

4.1.1.2 Chemical Parameters Evaluation

Besides evaluation of physical parameters of each generic formulation, the potency and dissolution profile of generic formulations were also checked and compared with innovator's product (Cytotec 200 Tablet). Assay test was conducted in High Performance Liquid Chromatography (HPLC).

4.1.1.2.1 Assay Result Calculation

Assay of compressed tablet of each formula was evaluated and compared with that of innovator's product. The results were put in the following table and presented graphically.

Table 4.2: Assay Results of Innovator's Product and Formulations of Phase-One Trial

Parameter	Innovators Prodcuct	Formula 1.1 (a)	Formula 1.1 (b)	Formula 1.2 (a)	Formula 1.2 (b)
Assay	99.54%	99.42%	100.12%	99.97%	100.45%

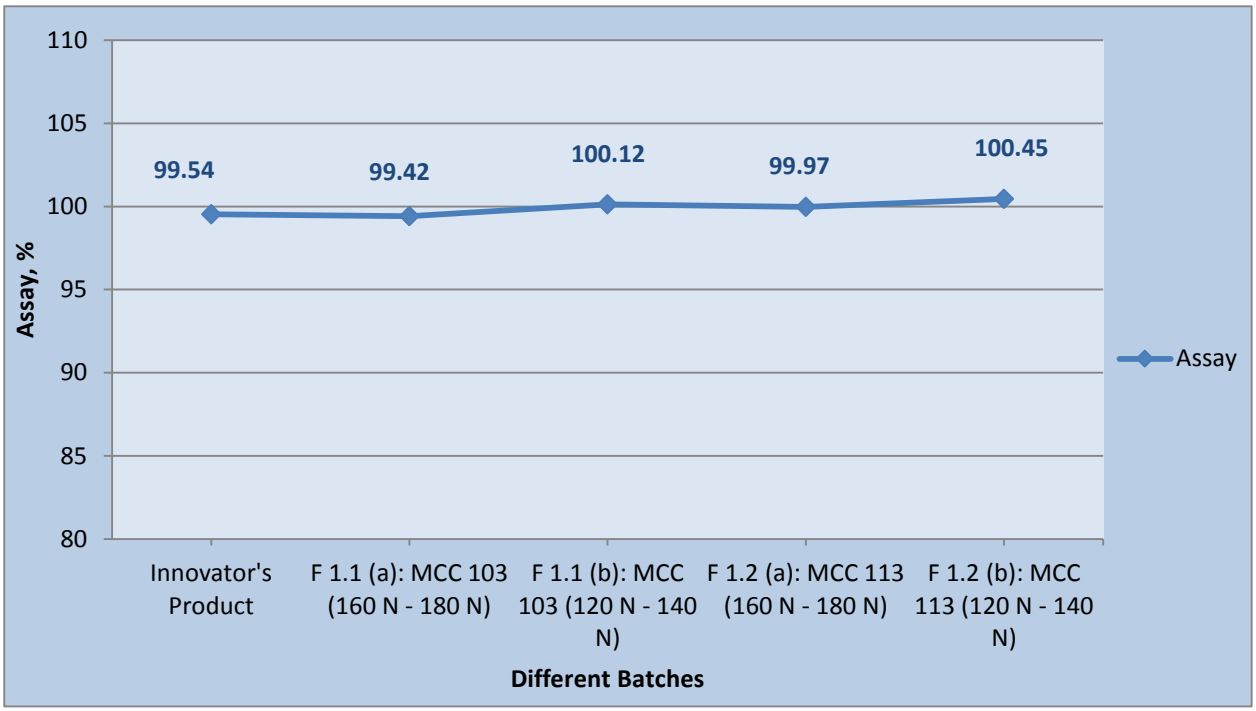


Fig 4.1: A plot showing assay results (% average) of Innovators product and formulations of Phase-One trial

4.1.1.2.2 Dissolution Profile Evaluation

Dissolution of compressed tablets, of each generic formulations of Phase-One trial as well as innovator's product, was checked in Water media, with Apparatus II at 50 rpm paddle speed (USP Monograph). Sampling for dissolution was done at 10 minutes, 15 minutes and 20 minutes interval of the dissolution apparatus run. Total twelve tablets were checked from each batch for dissolution, and average value was calculated. The results were presented in tabular manner and average value of every formula of each time point was presented graphically.

Table 4.3: Dissolution Profile of Innovator's Product and Formulations of Phase-One Trial (After 10 minutes)

Tablets	Innovator's Product	Formula 1.1 (a)	Formula 1.1 (b)	Formula 1.2 (a)	Formula 1.2 (b)
Tablet-01	81%	85%	90%	88%	91%
Tablet-02	80%	82%	94%	85%	90%
Tablet-03	84%	88%	85%	84%	89%
Tablet-04	91%	85%	82%	85%	91%
Tablet-05	90%	85%	86%	85%	93%
Tablet-06	84%	81%	88%	85%	91%
Tablet-07	84%	82%	81%	80%	92%
Tablet-08	88%	89%	86%	84%	92%
Tablet-09	89%	86%	82%	85%	88%
Tablet-10	82%	85%	89%	86%	87%
Tablet-11	81%	84%	86%	86%	94%
Tablet-12	84%	85%	85%	85%	86%
Average	**84.83%**	**84.75%**	**86.17%**	**84.17%**	**90.33%**

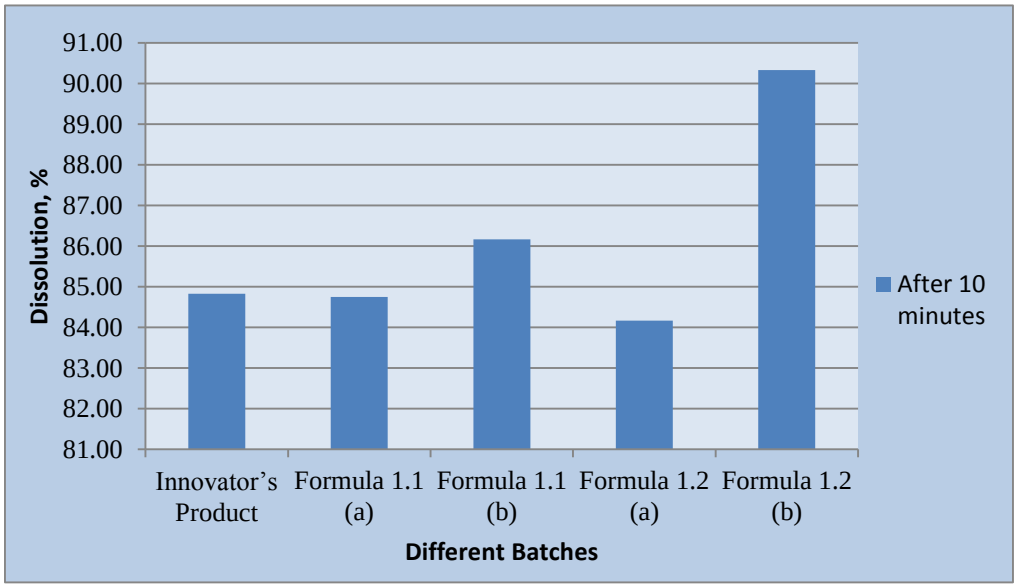


Fig 4.2: A plot showing dissolution results (% average value) of Innovators product and formulations of Phase-One trial after 10 minutes

Table 4.4: Dissolution Profile of Innovator's Product and Formulations of Phase-One Trial (After 15 minutes)

Tablets	Innovator's Product	Formula 1.1 (a)	Formula 1.1 (b)	Formula 1.2 (a)	Formula 1.2 (b)
Tablet-01	94%	92%	90%	93%	96%
Tablet-02	93%	89%	88%	94%	98%
Tablet-03	96%	89%	86%	94%	93%
Tablet-04	94%	92%	83%	89%	92%
Tablet-05	92%	90%	88%	95%	98%
Tablet-06	95%	87%	91%	90%	93%
Tablet-07	97%	91%	94%	90%	93%
Tablet-08	96%	88%	84%	90%	95%
Tablet-09	94%	92%	100%	89%	92%
Tablet-10	98%	91%	99%	92%	95%
Tablet-11	97%	92%	97%	89%	94%
Tablet-12	94%	91%	102%	92%	98%
Average	**95.00%**	**90.33%**	**91.83%**	**91.42%**	**94.75%**

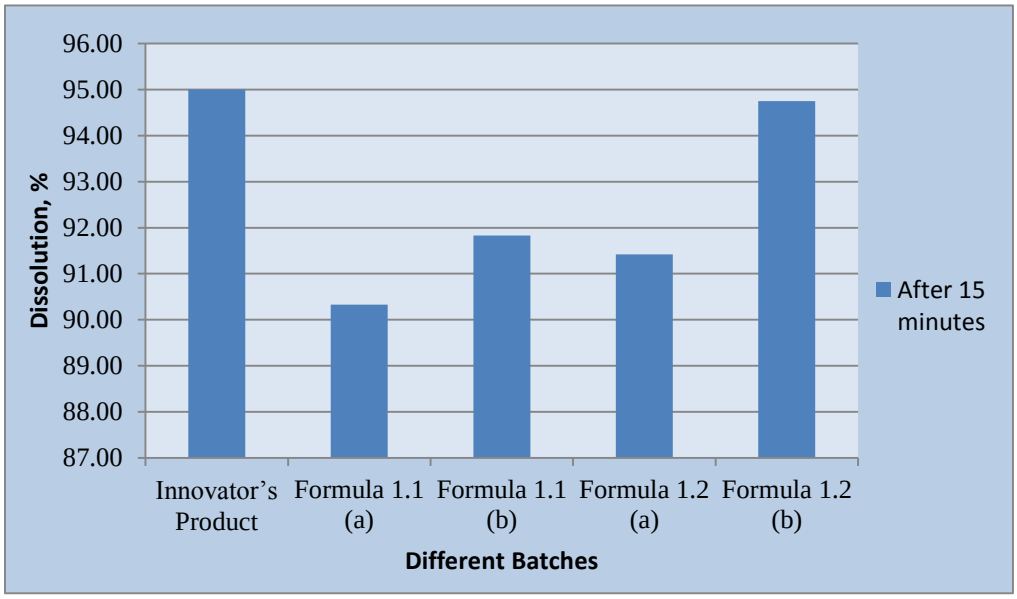


Fig 4.3: A plot showing dissolution results (% average value) of Innovators product and formulations of Phase-One trial after 15 minutes

Table 4.5: Dissolution Profile of Innovator's Product and Formulations of Phase-One Trial (After 20 minutes)

Tablets	Innovator's Product	Formula 1.1 (a)	Formula 1.1 (b)	Formula 1.2 (a)	Formula 1.2 (b)
Tablet-01	97%	90%	96%	92%	96%
Tablet-02	93%	89%	95%	93%	96%
Tablet-03	86%	92%	96%	92%	99%
Tablet-04	95%	94%	84%	89%	95%
Tablet-05	97%	94%	98%	92%	95%
Tablet-06	100%	93%	88%	93%	94%
Tablet-07	98%	92%	96%	91%	96%
Tablet-08	99%	94%	93%	92%	95%
Tablet-09	94%	89%	89%	92%	100%
Tablet-10	98%	92%	91%	91%	101%
Tablet-11	92%	91%	101%	96%	96%
Tablet-12	99%	96%	103%	94%	99%
Average	**95.67%**	**92.17%**	**94.17%**	**92.25%**	**96.83%**

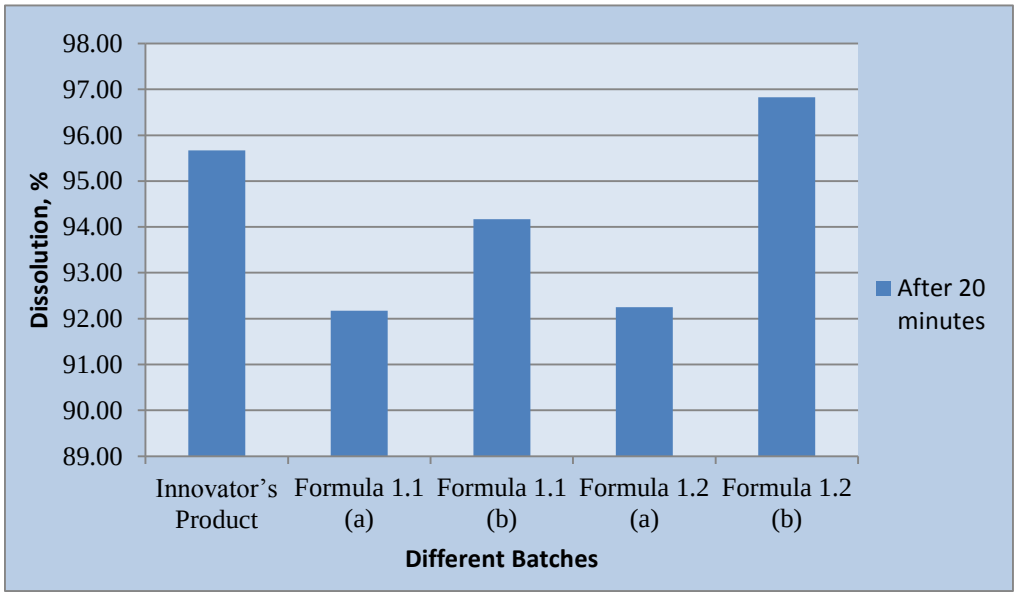


Fig 4.4: A plot showing dissolution results (% average value) of Innovators product and formulations of Phase-One trial after 20 minutes

4.1.2 Results of Phase-Two Trial

Similarly, in Phase-Two set of trial, two different formulations were prepared using different grades of Microcrystalline Cellulose. The range of hardness was kept in lower range (120 N to 140 N) after evaluating dissolution profile of phase-one trial formulations. Amount of excipients and API was kept unchanged from previous trial. Physical and chemical evaluation of the generic formulas of phase-two trial was done.

4.1.2.1 Physical Parameters Evaluation

Appearance of the compressed tablets of the different formulation as well as other physical characteristics of the tablets was checked. The finished dosage forms of generic formulas were again evaluated physically, and data of innovator's product was considered from previous evaluation mentioned in Table 4.1. The evaluated parameters as well as its results are captured in following table:

Table 4.6: Values of Physical Parameters of Formulations of Phase-Two Trial With Innovator's Product (Cytotec 200 Tablet)

Physical Parameters	Innovator's Product	Formula 2.1 (Avicel 103)	Formula 2.2 (Avicel 113)
Description	White tablet, having bisectline on both sides	White tablet, having bisectline on both sides	White tablet, having bisectline on both sides
Tablet Weight (Average of 10 tablets)	200.1 mg	200.0 mg	199.9 mg
Disintegration Time	8 sec	7 sec	9 sec
	8 sec	10 sec	12 sec
	10 sec	11 sec	14 sec
	11 sec	14 sec	15 sec
	15 sec	18 sec	18 sec
	21 sec	20 sec	18 sec

Physical Parameters	Innovator's Product	Formula 2.1 (Avicel 103)	Formula 2.2 (Avicel 113)
Hardness	162 N	129 N	133 N
	169 N	132 N	130 N
	175 N	130 N	128 N
	165 N	134 N	132 N
	168 N	128 N	134 N
	174 N	135 N	138 N
Friability	0.0%	0.0%	0.0%

4.1.2.2 Chemical Parameters Evaluation

Besides evaluation of physical parameters of each generic formulation, the potency and dissolution profile of generic formulations were also checked and compared with innovator's product (Cytotec 200 Tablet). Assay test was conducted in High Performance Liquid Chromatography (HPLC).

4.1.2.2.1 Assay Result Calculation

Assay of compressed tablet of each formula was evaluated and compared with that of innovator's product. The results were put in the following table and presented graphically.

Table 4.7: Assay Results of Innovator's Product and Formulations of Phase-Two Trial

Parameter	Innovators Prodcuct	Formula 2.1 (Avicel 103)	Formula 2.2 (Avicel 113)
Assay	99.54%	100.08%	100.21%

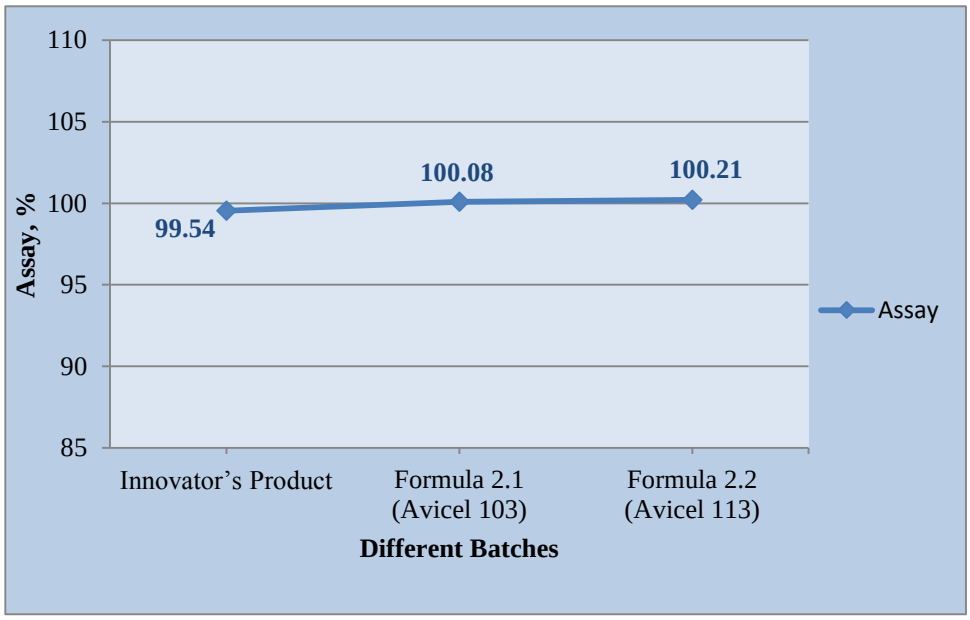


Figure 4.5: A plot showing assay results (% average) of Innovators product and formulations of Phase-Two trial

4.1.2.2.2 Dissolution Profile Evaluation

Dissolution of compressed tablets, of each generic formulations of Phase-One trial as well as innovator's product, was checked in Water media, with Apparatus II at 50 rpm paddle speed (USP Monograph). Sampling for dissolution was done at 10 minutes, 15 minutes, 20 minutes and 30 minutes interval of the dissolution apparatus run. Total twelve tablets were checked from each batch for dissolution, and average value was calculated. The results were presented in tabular manner and average value of every formula of each time point was presented graphically.

Table 4.8: Dissolution Profile of Innovator's Product and Formulations of Phase-Two Trial (After 10 minutes)

Tablets	Innovator's Product	Formula 2.1 (Avicel 103)	Formula 2.2 (Avicel 113)
Tablet-01	87%	94%	97%
Tablet-02	96%	89%	97%
Tablet-03	91%	88%	94%
Tablet-04	95%	94%	94%
Tablet-05	93%	90%	90%
Tablet-06	85%	86%	90%
Tablet-07	94%	90%	97%
Tablet-08	84%	88%	98%
Tablet-09	97%	88%	88%
Tablet-10	93%	86%	81%
Tablet-11	91%	88%	93%
Tablet-12	93%	88%	98%
Average	**91.48%**	**88.99%**	**93.12%**

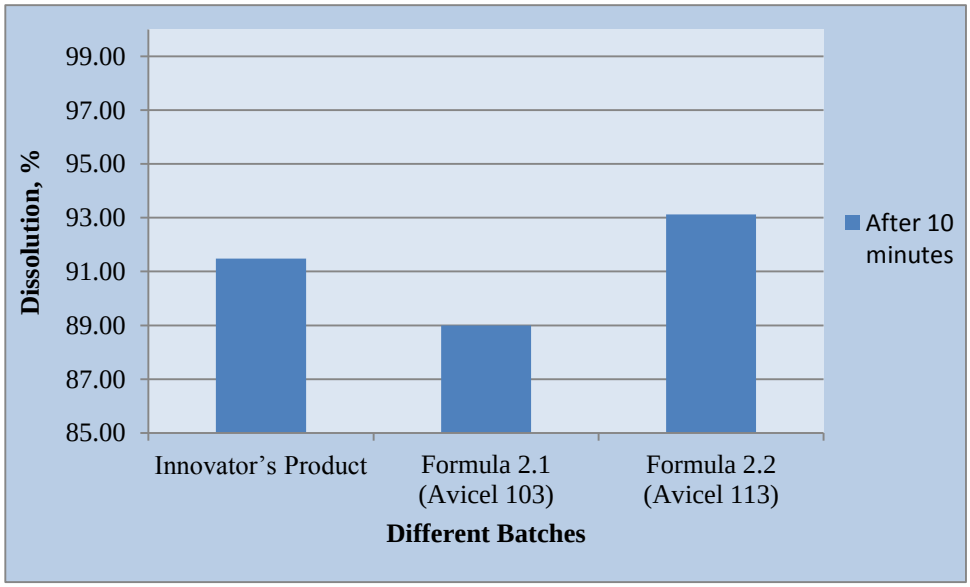


Fig 4.6: A plot showing dissolution results (% average value) of Innovators product and formulations of Phase-Two trial after 10 minutes

Table 4.9: Dissolution Profile of Innovator's Product and Formulations of Phase-Two Trial (After 15 minutes)

Tablets	Innovator's Product	Formula 2.1 (Avicel 103)	Formula 2.2 (Avicel 113)
Tablet-01	89%	87%	102%
Tablet-02	94%	93%	100%
Tablet-03	94%	96%	99%
Tablet-04	98%	98%	96%
Tablet-05	96%	97%	102%
Tablet-06	92%	97%	98%
Tablet-07	94%	100%	101%
Tablet-08	97%	89%	101%
Tablet-09	95%	93%	95%
Tablet-10	93%	94%	98%
Tablet-11	95%	96%	100%
Tablet-12	92%	99%	90%
Average	**93.96%**	**94.85%**	**98.49%**

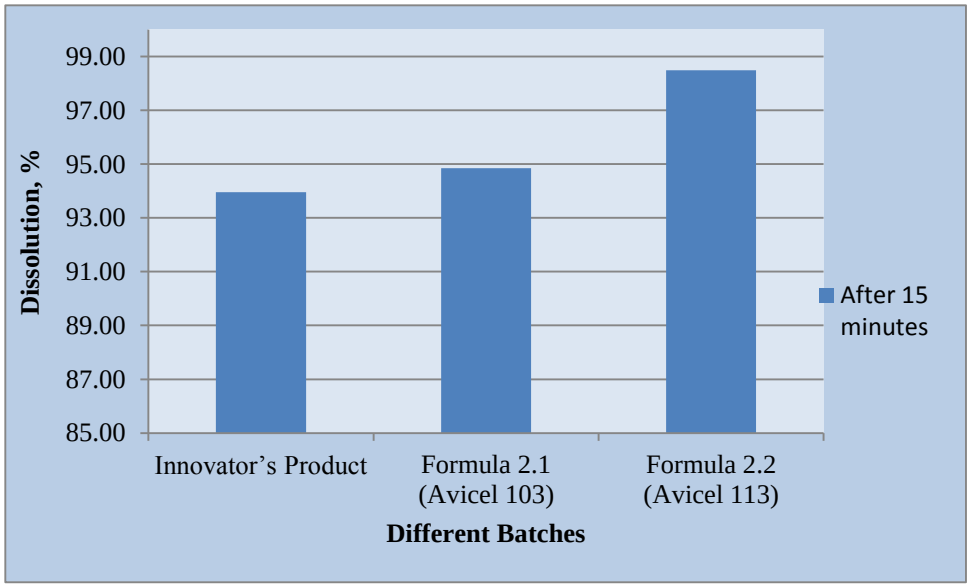


Fig 4.7: A plot showing dissolution results (% average value) of Innovators product and formulations of Phase-Two trial after 15 minutes

Table 4.10: Dissolution Profile of Innovator's Product and Formulations of Phase-Two Trial (After 20 minutes)

Tablets	Innovator's Product	Formula 2.1 (Avicel 103)	Formula 2.2 (Avicel 113)
Tablet-01	97%	97%	102%
Tablet-02	93%	96%	103%
Tablet-03	86%	96%	103v
Tablet-04	95%	98%	88%
Tablet-05	97%	100%	104%
Tablet-06	100%	95%	101%
Tablet-07	98%	102%	95%
Tablet-08	99%	97%	94%
Tablet-09	94%	97%	102%
Tablet-10	98%	87%	101%
Tablet-11	92%	86%	97%
Tablet-12	99%	89%	96%
Average	**95.78%**	**94.95%**	**98.77%**

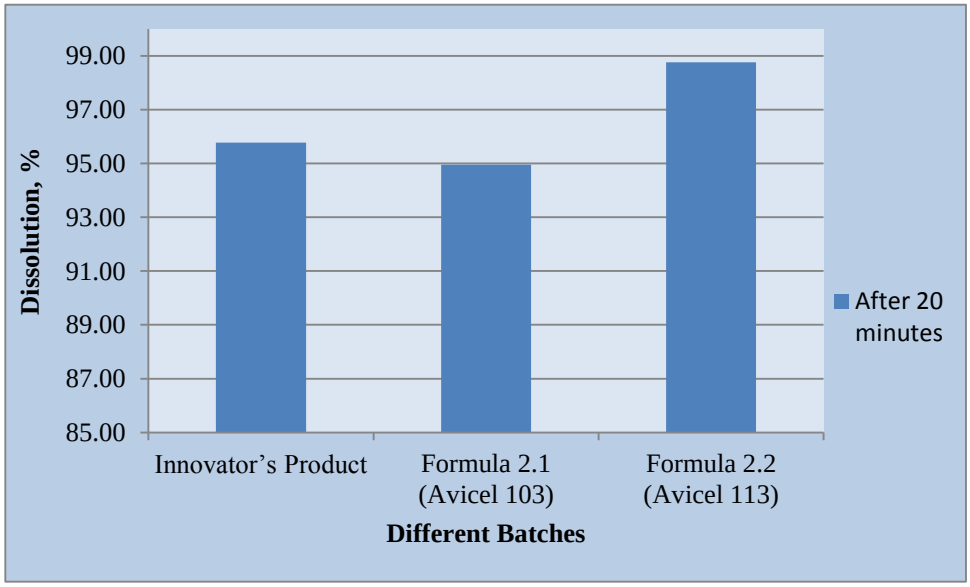


Fig 4.8: A plot showing dissolution results (% average value) of Innovators product and formulations of Phase-Two trial after 20 minutes

Table 4.11: Dissolution Profile of Innovator's Product and Formulations of Phase-Two Trial (After 30 minutes)

Tablets	Innovators Product	Formula 2.1 (Avicel 103)	Formula 2.2 (Avicel 113)
Tablet-01	97%	101%	97%
Tablet-02	94%	96%	96%
Tablet-03	99%	101%	97%
Tablet-04	94%	94%	100%
Tablet-05	91%	99%	100%
Tablet-06	100%	97%	97%
Tablet-07	94%	100%	101%
Tablet-08	100%	100%	100%
Tablet-09	100%	97%	101%
Tablet-10	96%	95%	104%
Tablet-11	100%	94%	99%
Tablet-12	101%	104%	101%
Average	**97.14%**	**98.18%**	**99.40%**

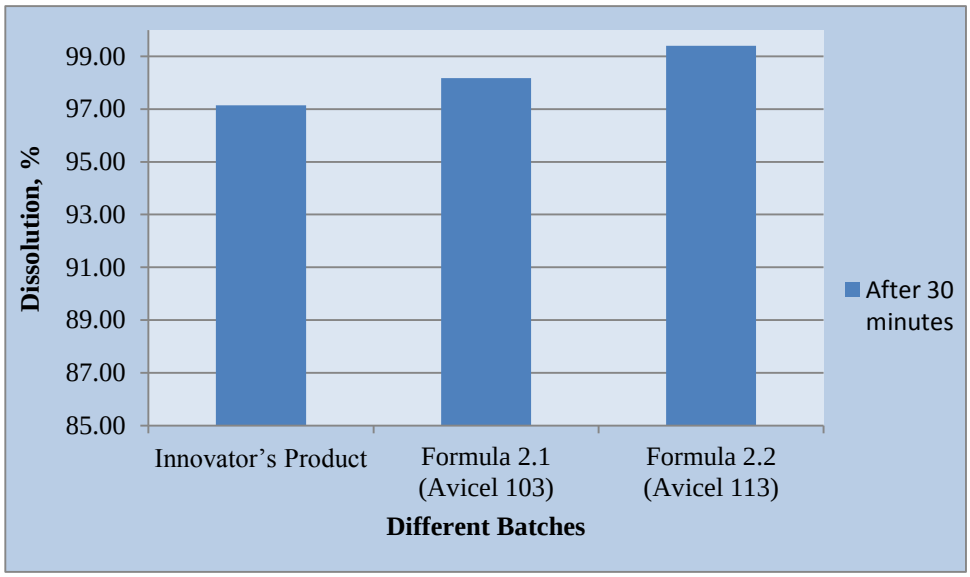


Fig 4.9: A plot showing dissolution results (% average value) of Innovators product and formulations of Phase-Two trial after 30 minutes

4.1.3 Results of Phase-Three Trial

Assay and Related Substances (impurities) of Misoprostol Dispersion (API) and API with different excipients mix were analyzed. 8-wpi Misoprostol, A-Type Misoprostol, B-Type Misoprostol and 12-epi Misoprostol were considered as related substances. Since, 12-epi Misoprostol is a process impurity, it is kept out from the summed amount of other impurities (Total Impurity). The results are presented in tabular format and as graphical presentations.

4.1.3.1 Chemical Results Misoprostol Dispersion

Table 4.12: Assay & Related Substances of Misoprostol Dispersion at Different Storage Conditions

Materials Present	Storage Condition	Time Interval (Days)	Results						Remarks
			Assay	Related Substances					
				8-epi Misoprostol	B-Type impurity	A-Type impurity	12-epi Misoprostol	Total Impurity*	
Misoprostol Dispersion	N/A	Initial	102.6%	0.02%	ND	ND	0.52%	0.02%	OK
	25° C/ 60% RH	07	102.1%	0.07%	ND	0.13%	0.43%	0.20%	OK
		14	100.7%	0.07%	ND	0.14%	0.46%	0.21%	OK
		21	99.9%	0.08%	ND	0.15%	0.50%	0.23%	OK
	30° C/ 75% RH	07	101.9%	0.07%	ND	0.17%	0.45%	0.24%	OK
		14	101.0%	0.08%	ND	0.19%	0.51%	0.27%	OK
		21	99.7%	0.10%	ND	0.22%	0.58%	0.32%	OK
	40° C/ 75% RH	07	101.1%	0.07%	ND	0.23%	0.45%	0.40%	OK
		14	100.4%	0.45%	ND	1.37%	0.50%	1.82%	OK
		21	99.7%	0.77%	ND	2.68%	0.56%	3.45%	OK
	60° C Temp.	07	101.1%	0.17%	ND	0.71%	0.42%	0.88%	OK
		14	99.8%	0.46%	ND	1.49%	0.46%	1.95%	OK
		21	99.1%	0.84%	ND	2.80%	0.54%	3.64%	OK

* Excluding 12-epi Misoprostol.

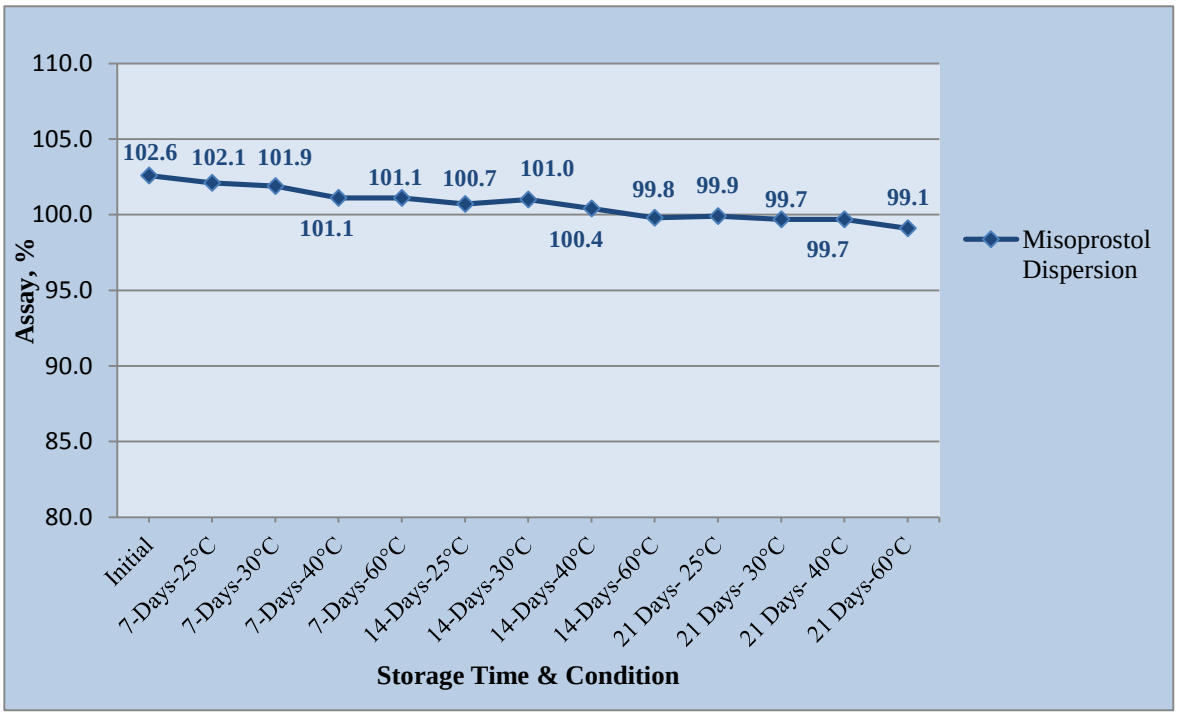


Fig 4.10: Assay Results of Misoprostol Dispersion at diffetrent storage conditions

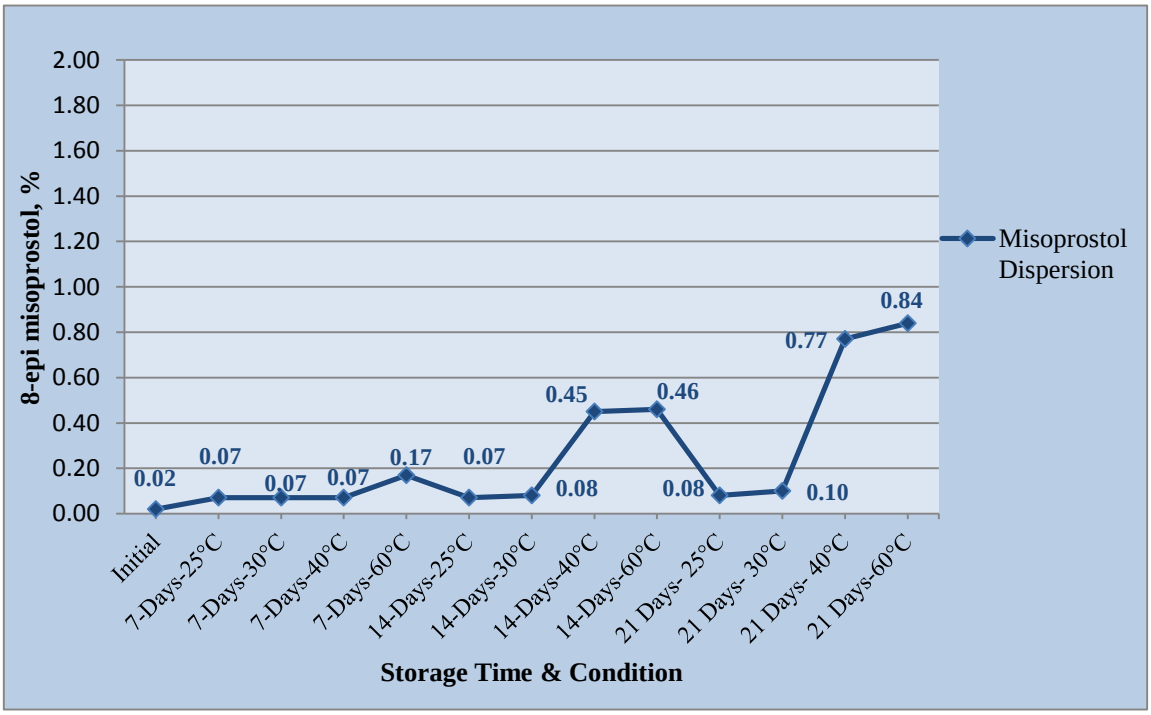


Fig 4.11: 8-epi Misoprostol Results of Misoprostol Dispersion at diffetrent storage conditions

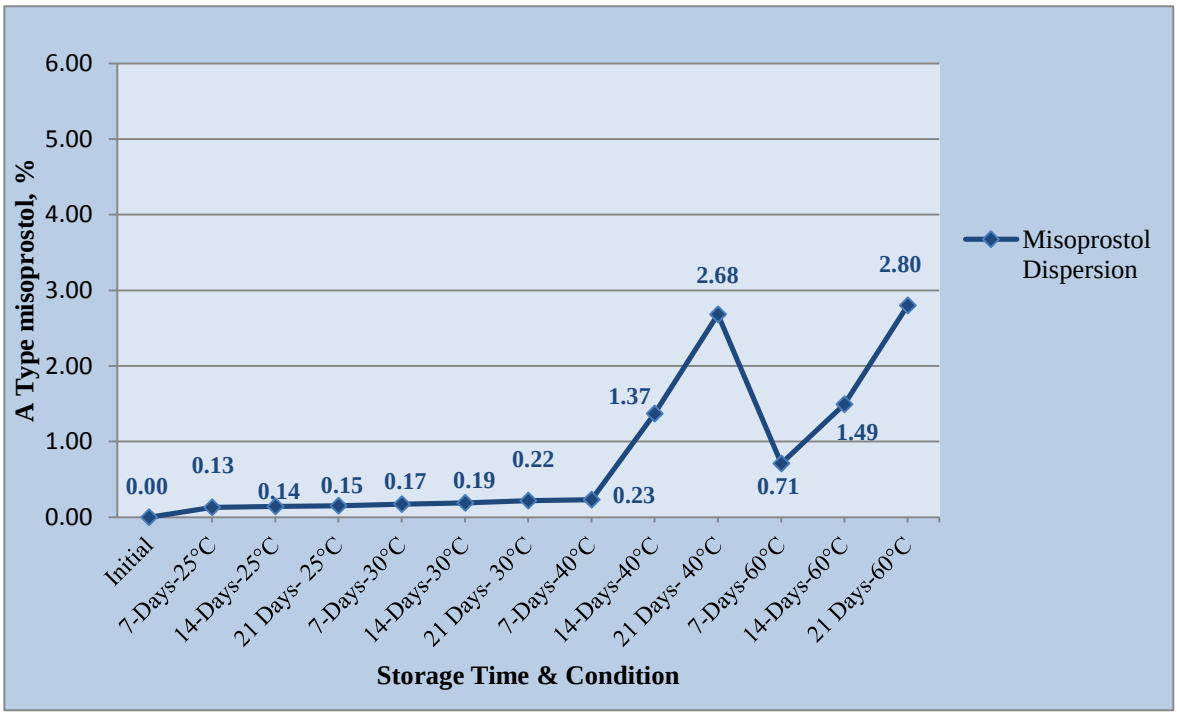


Fig 4.12: A-Type Misoprostol Results of Misoprostol Dispersion at diffetrent storage conditions

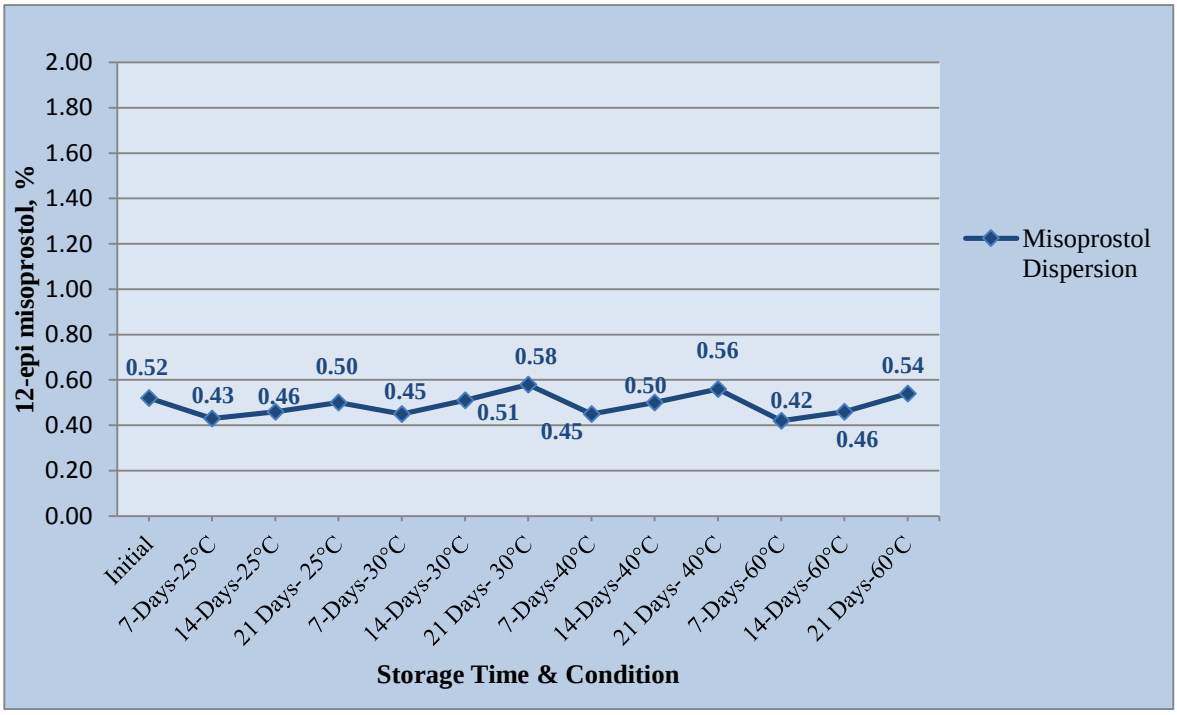


Fig 4.13: 12-epi Misoprostol Results of Misoprostol Dispersion at diffetrent storage conditions

4.1.3.2 Chemical Results of Combination 01 (API : Microcrystalline Cellulose 103 = 1 : 1)

Table 4.13: Assay & Related Substances of Combination 01 at Different Storage Conditions

Materials Present	Storage Condition	Time Interval (Days)	Results						Remarks
			Assay	Related Substances					
				8-epi Misoprostol	B-Type impurity	A-Type impurity	12-epi Misoprostol	Total Impurity*	
Misoprostol Dispersion : Microcrys-talline Cellulose 103 (1 : 1)	N/A	Initial	100.1%	0.02%	ND	ND	0.45%	0.02%	OK
	25° C/ 60% RH	07	100.0%	0.07%	ND	0.13%	0.48%	0.20%	OK
		14	99.7%	0.07%	ND	0.13%	0.50%	0.20%	OK
		21	99.3%	0.08%	ND	0.13%	0.53%	0.21%	OK
	30° C/ 75% RH	07	99.8%	0.08%	ND	0.14%	0.46%	0.22%	OK
		14	99.1%	0.08%	ND	0.16%	0.49%	0.24%	OK
		21	98.6%	0.09%	ND	0.19%	0.52%	0.28%	OK
	40° C/ 75% RH	07	99.7%	0.12%	ND	0.22%	0.45%	0.34%	OK
		14	96.8%	0.51%	ND	2.04%	0.51%	2.55%	OK
		21	94.7%	1.03%	1.13%	3.92%	0.56%	6.08%	OK
	60° C Temp.	07	98.7%	0.16%	0.18%	0.76%	0.44%	1.10%	OK
		14	96.0%	0.64%	1.06%	2.91%	0.55%	4.62%	OK
		21	93.8%	1.29%	1.46%	4.48%	0.61%	7.23%	OK

* Excluding 12-epi Misoprostol.

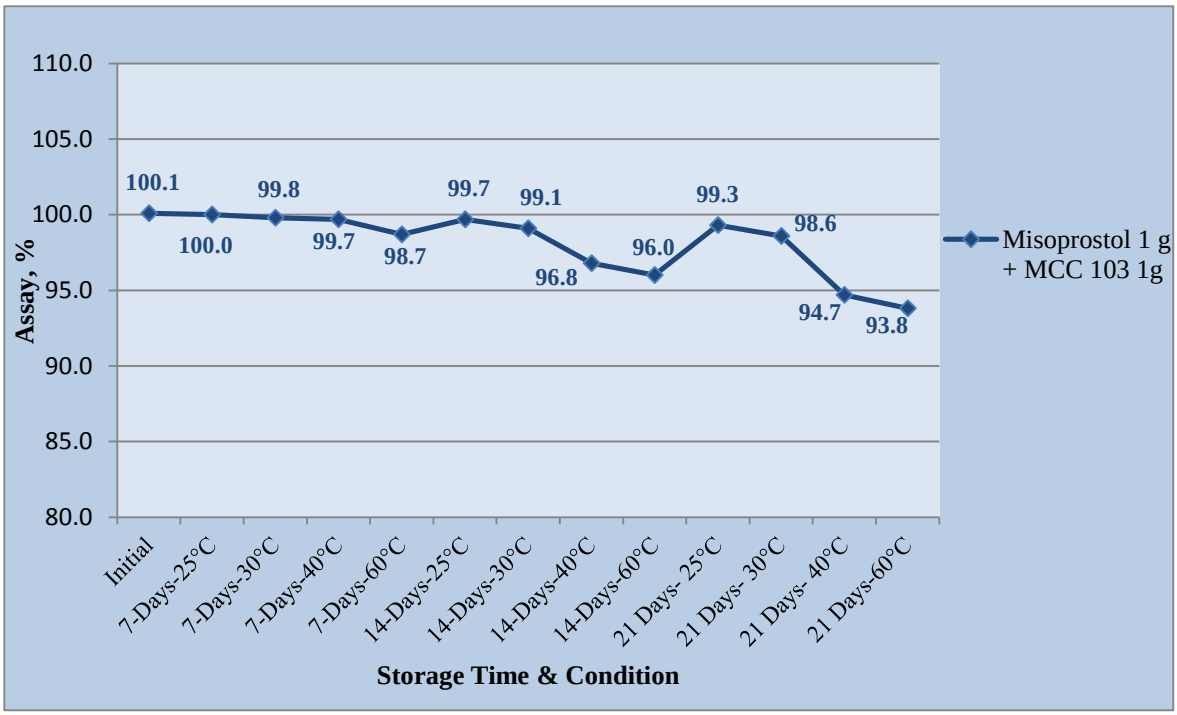


Fig 4.14: Assay Results of Combination 01 at diffetrent storage conditions

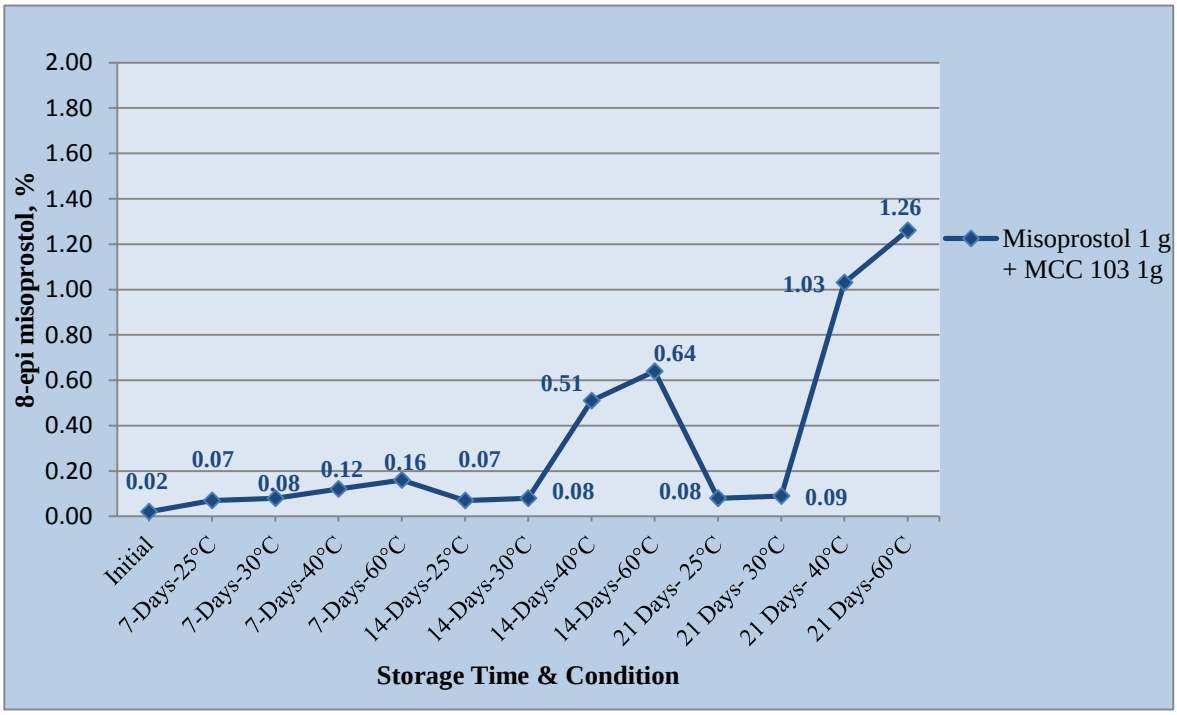


Fig 4.15: 8-epi Misoprostol Results of Combination 01 at diffetrent storage conditions

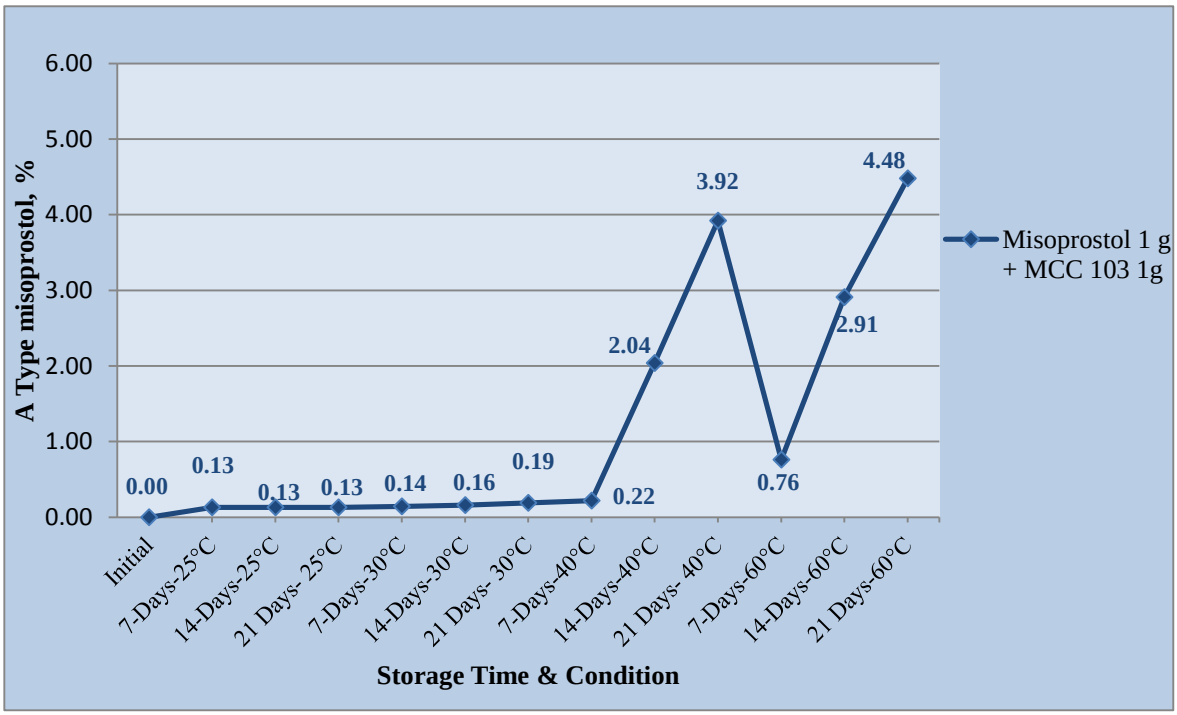


Fig 4.16: A-Type Misoprostol Results of Combination 01 at diffetrent storage conditions

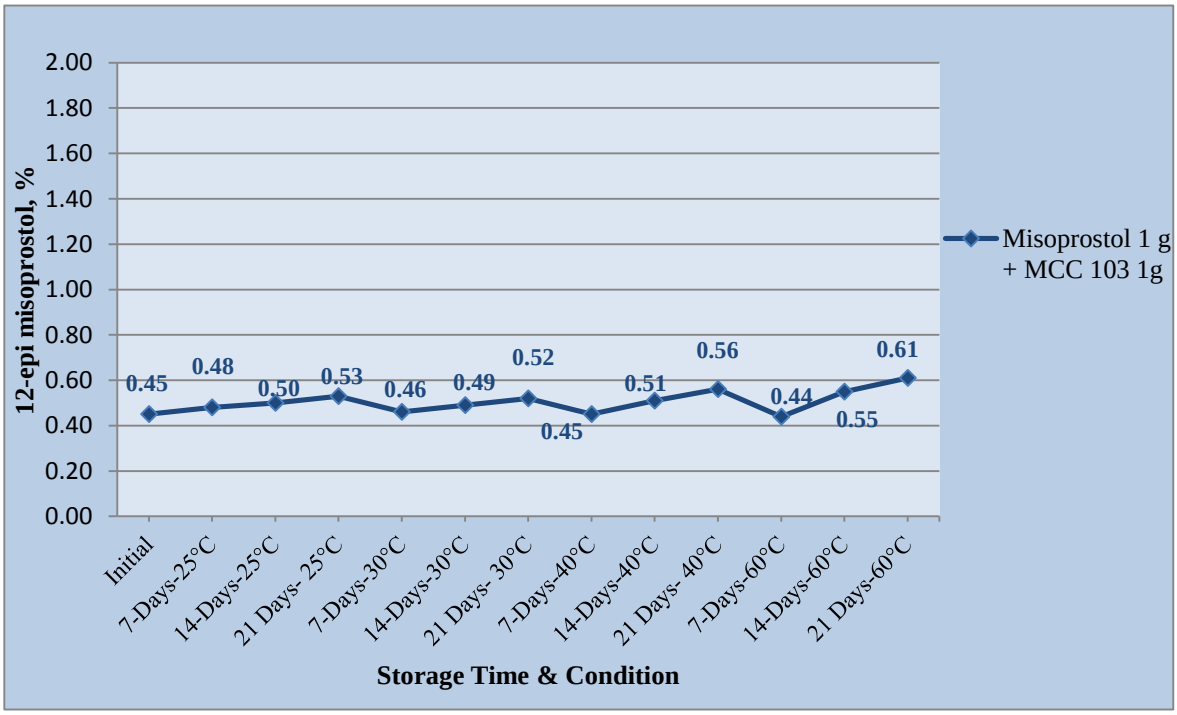


Fig 4.17: 12-epi Misoprostol Results of Combination 01 at diffetrent storage conditions

4.1.3.3 Chemical Results of Combination 02 (API : Microcrystalline Cellulose 113 = 1 : 1)

Table 4.14: Assay & Related Substances of Combination 02 at Different Storage Conditions

Materials Present	Storage Condition	Time Interval (Days)	Results						Remarks
			Assay	Related Substances					
				8-epi Misoprostol	B-Type impurity	A-Type impurity	12-epi Misoprostol	Total Impurity*	
Misoprostol Dispersion : Microcrys-talline Cellulose 113 (1 : 1)	N/A	Initial	100.3%	0.01%	ND	ND	0.42%	0.01%	OK
	25° C/ 60% RH	07	100.1%	0.05%	ND	0.11%	0.45%	0.16%	OK
		14	99.8%	0.06%	ND	0.12%	0.47%	0.18%	OK
		21	99.9%	0.08%	ND	0.13%	0.49%	0.21%	OK
	30° C/ 75% RH	07	99.7%	0.08%	ND	0.12%	0.45%	0.20%	OK
		14	99.1%	0.08%	ND	0.15%	0.50%	0.23%	OK
		21	98.7%	0.09%	ND	0.17%	0.53%	0.26%	OK
	40° C/ 75% RH	07	99.8%	0.09%	ND	0.20%	0.46%	0.29%	OK
		14	97.2%	0.39%	ND	1.29%	0.51%	1.68%	OK
		21	94.9%	0.71%	0.57%	2.19%	0.55%	3.47%	OK
	60° C Temp.	07	99.0%	0.13%	0.13%	0.49%	0.46%	0.75%	OK
		14	96.4%	0.43%	0.53%	1.94%	0.55%	2.90%	OK
		21	93.9%	0.77%	0.60%	2.45%	0.60%	3.82%	OK

* Excluding 12-epi Misoprostol.

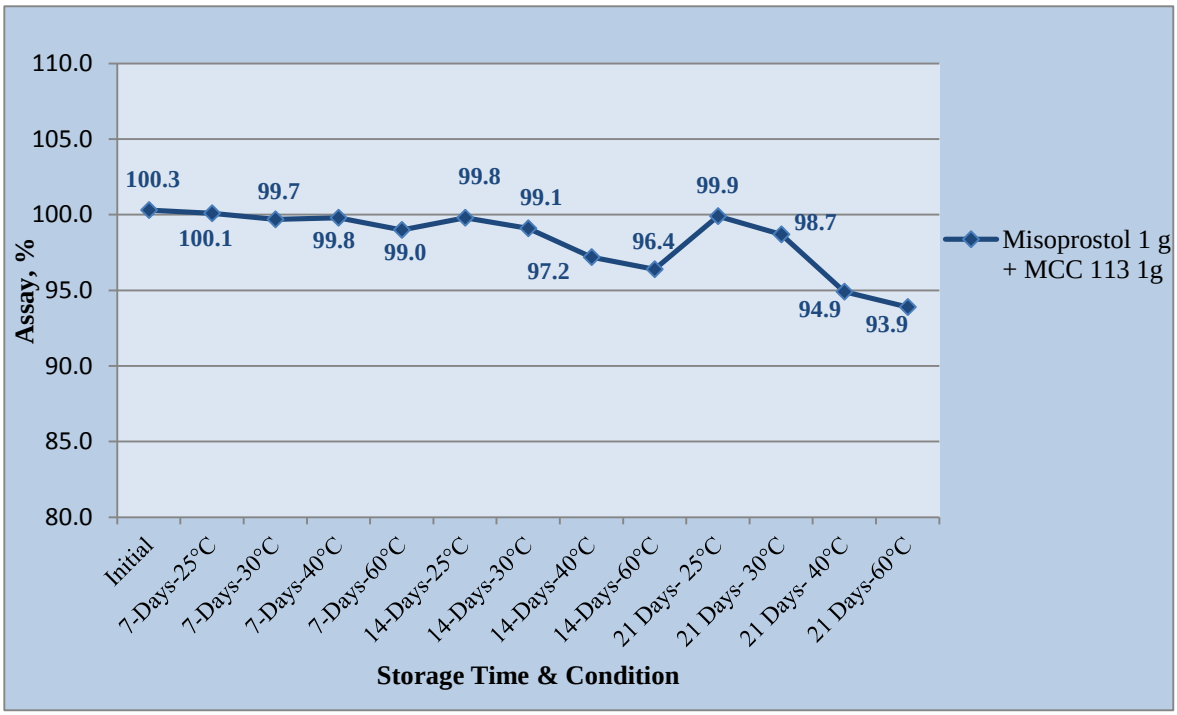


Fig 4.18: Assay Results of Combination 02 at diffetrent storage conditions

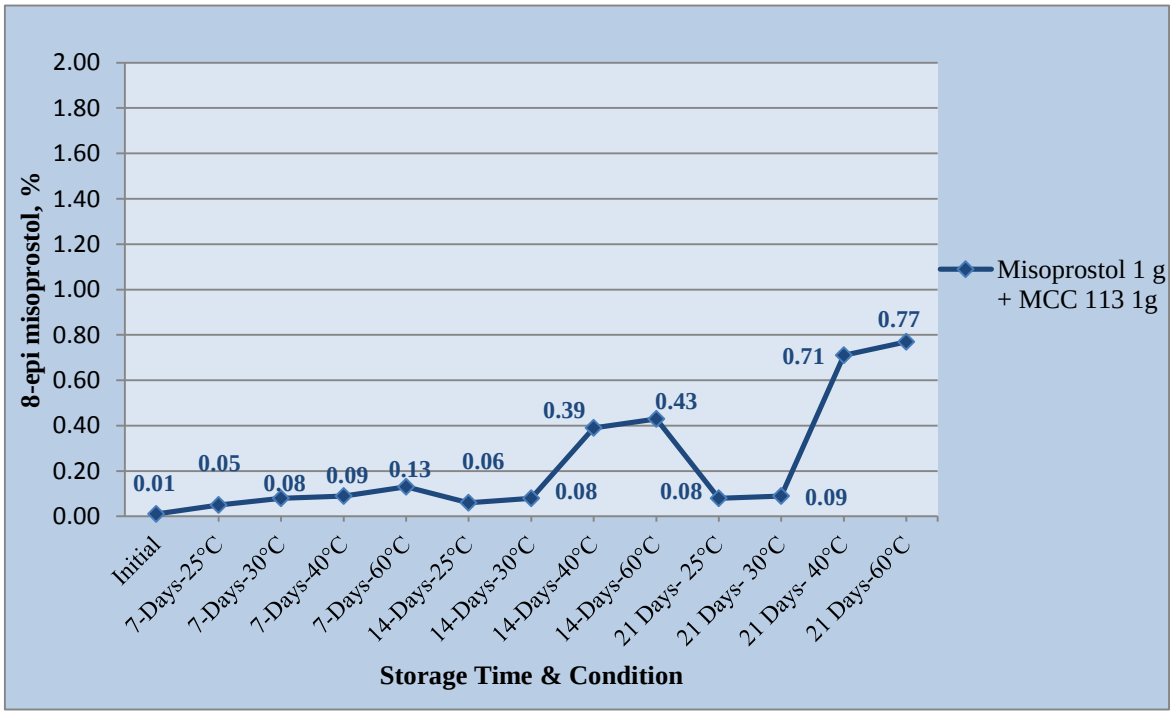


Fig 4.19: 8-epi Misoprostol Results of Combination 02 at diffetrent storage conditions

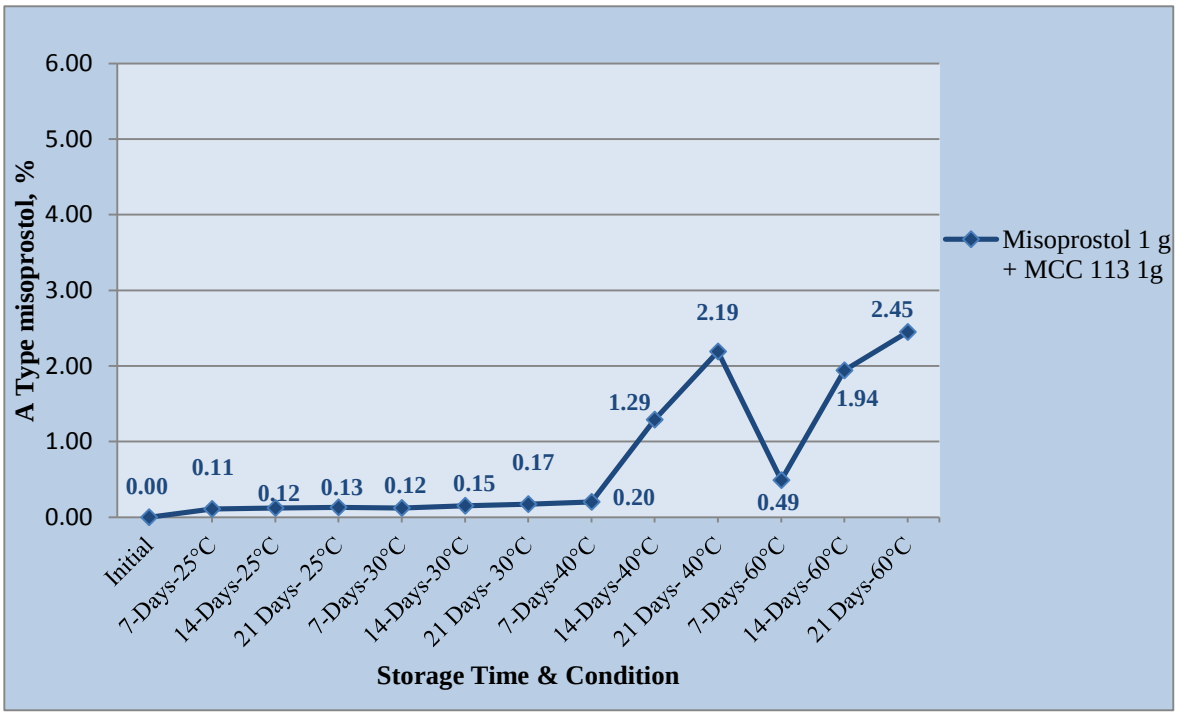


Fig 4.20: A-Type Misoprostol Results of Combination 02 at diffetrent storage conditions

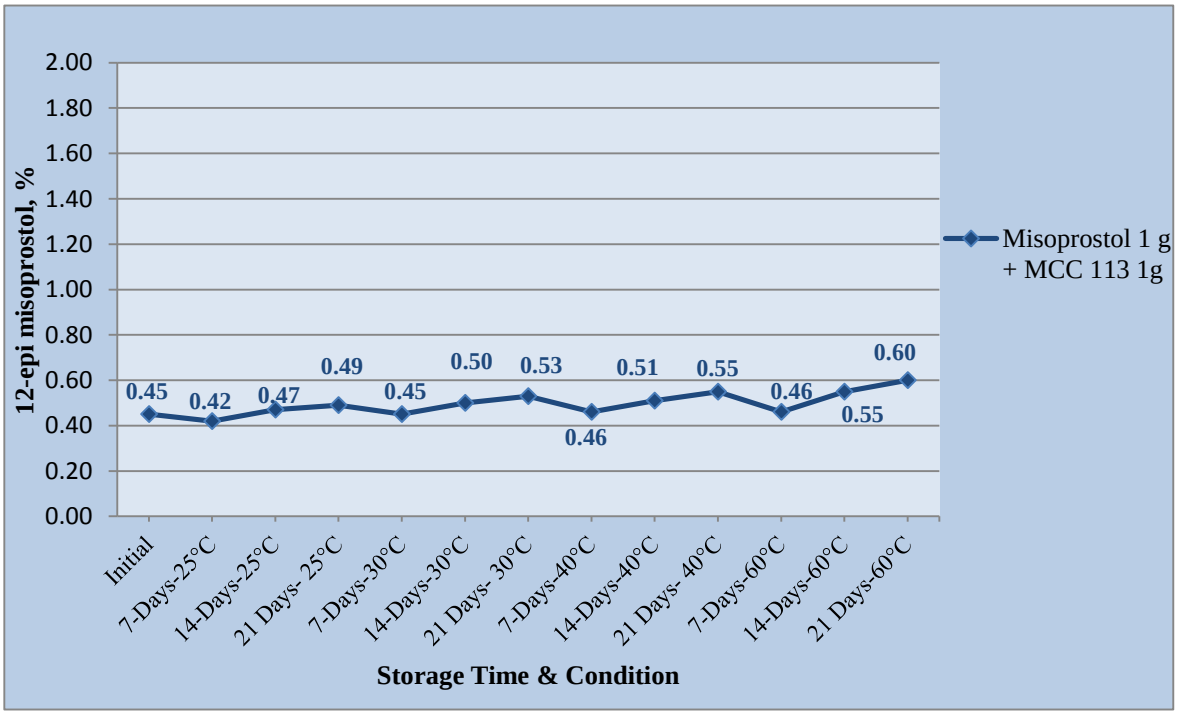


Fig 4.21: 12-epi Misoprostol Results of Combination 02 at diffetrent storage conditions

4.1.3.4 Chemical Results of Combination 03 (a) (API : Sodium Starch Glycolate = 1 : 1)

Table 4.15: Assay & Related Substances of Combination 03 (a) at Different Storage Conditions

Materials Present	Storage Condition	Time Interval (Days)	Results						Remarks
			Assay	Related Substances					
				8-epi Misoprostol	B-Type impurity	A-Type impurity	12-epi Misoprostol	Total Impurity*	
Misoprostol Dispersion : Sodium Starch Glycolate (1 : 1)	N/A	Initial	100.8%	0.02%	ND	ND	0.32%	0.02%	OK
	25° C/ 60% RH	07	100.5%	0.05%	ND	0.10%	0.34%	0.15%	OK
		14	99.9%	0.07%	ND	0.12%	0.39%	0.19%	OK
		21	99.5%	0.09%	0.10%	0.16%	0.44%	0.35%	OK
	30° C/ 75% RH	07	100.4%	0.05%	ND	0.12%	0.36%	0.17%	OK
		14	98.6%	0.07%	ND	0.17%	0.40%	0.24%	OK
		21	97.4%	0.10%	0.11%	0.21%	0.43%	0.42%	OK
	40° C/ 75% RH	07	98.1%	0.07%	ND	0.20%	0.37%	0.27%	OK
		14	97.0%	0.41%	ND	2.47%	0.48%	2.88%	OK
		21	95.7%	0.95%	0.64%	4.21%	0.58%	5.80%	OK
	60° C Temp.	07	100.2%	0.20%	0.13%	1.30%	0.40%	1.63%	OK
		14	97.7%	0.48%	0.57%	3.63%	0.42%	4.68%	OK
		21	94.9%	1.17%	0.68%	4.83%	0.54%	6.68%	OK

* Excluding 12-epi Misoprostol.

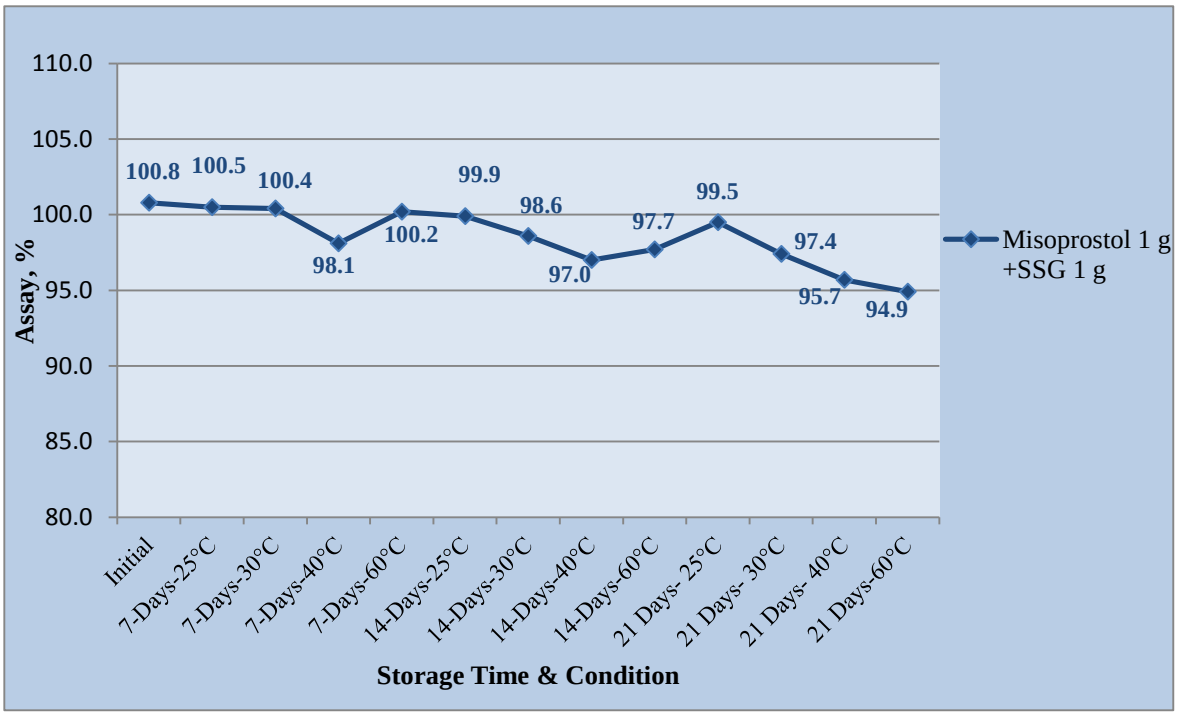


Fig 4.22: Assay Results of Combination 03 (a) at diffetrent storage conditions

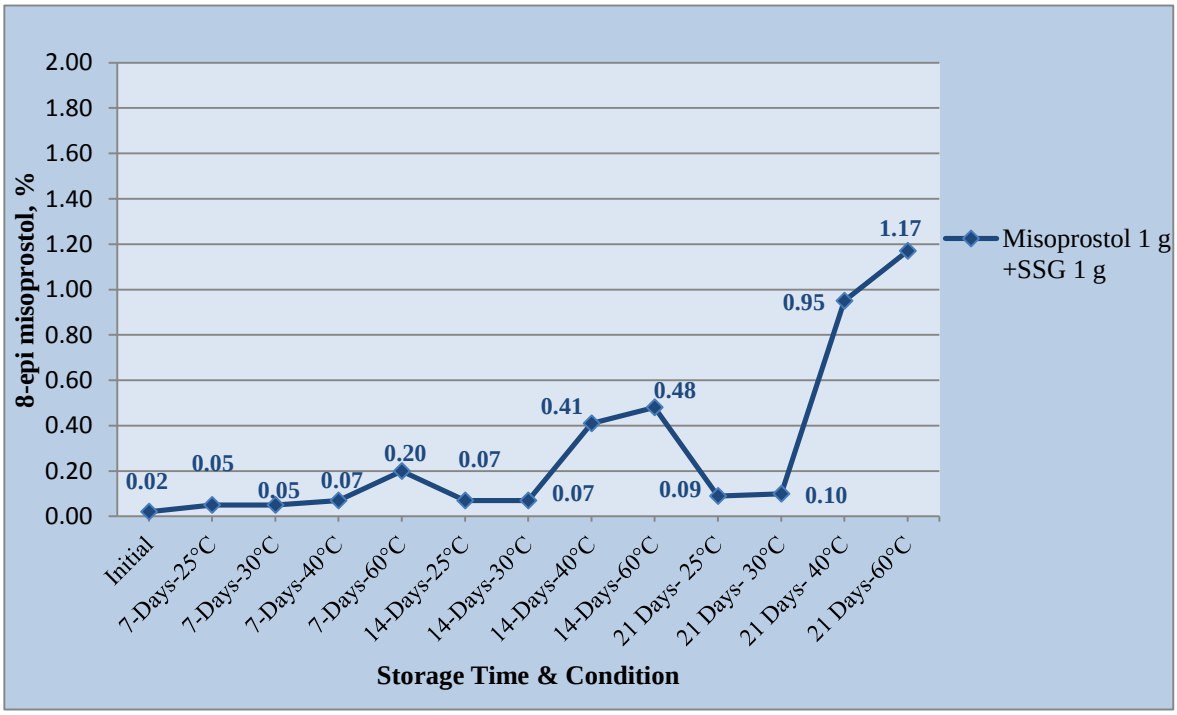


Fig 4.23: 8-epi Misoprostol Results of Combination 03 (a) at diffetrent storage conditions

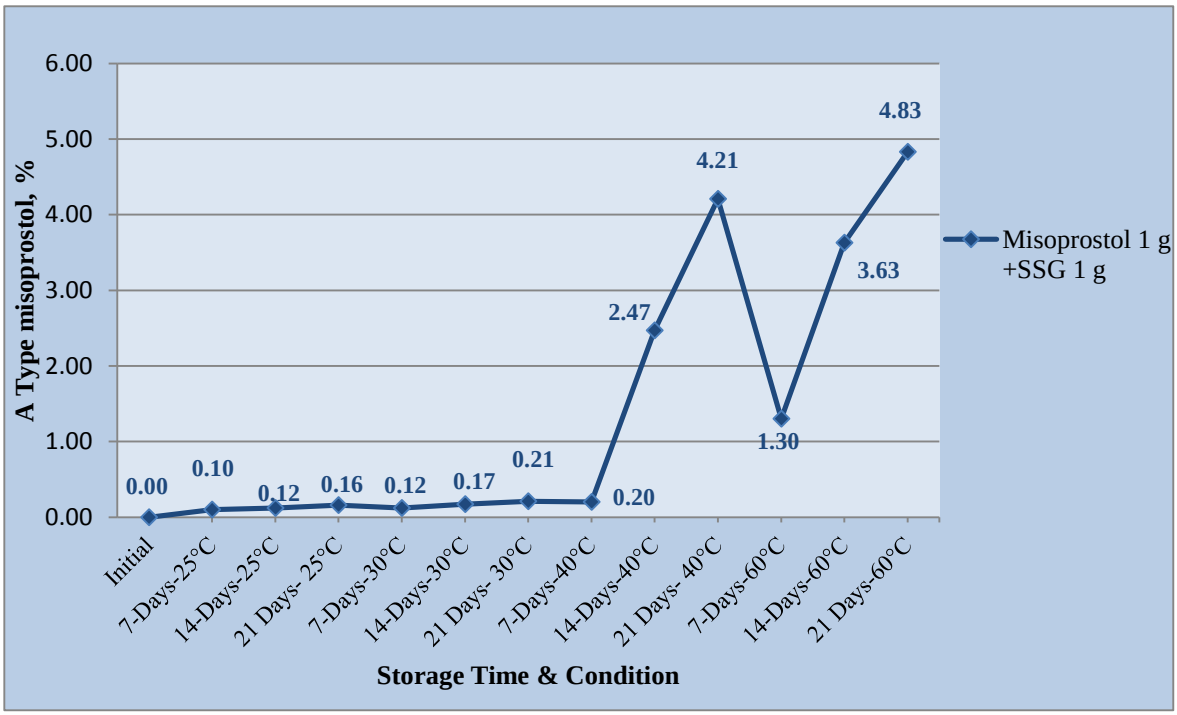


Fig 4.24: A-Type Misoprostol Results of Combination 03 (a) at diffetrent storage conditions

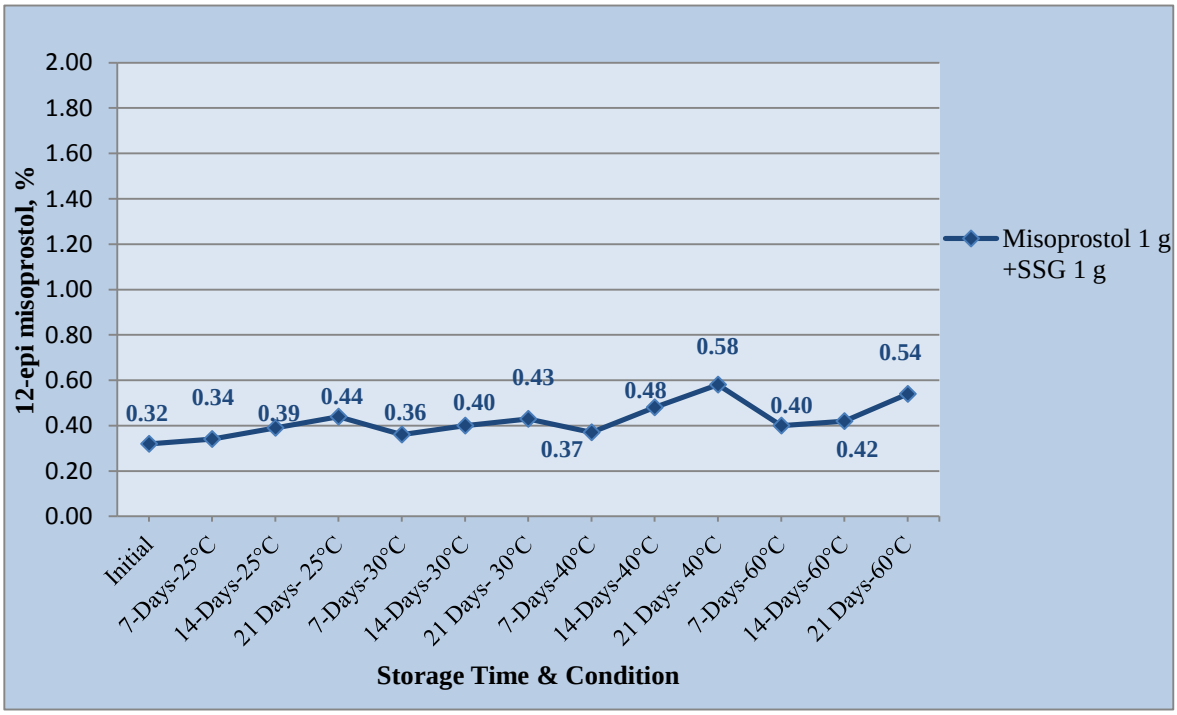


Fig 4.25: 12-epi Misoprostol Results of Combination 03 (a) at diffetrent storage conditions

4.1.3.5 Chemical Results of Combination 03 (b) (API : Sodium Starch Glycolate = 1 : 0.15)

Table 4.16: Assay & Related Substances of Combination 03 (b) at Different Storage Conditions

Materials Present	Storage Condition	Time Interval (Days)	Results						Remarks
			Assay	Related Substances					
				8-epi Misoprostol	B-Type impurity	A-Type impurity	12-epi Misoprostol	Total Impurity*	
Misoprostol Dispersion : Sodium Starch Glycolate (1 : 0.15)	N/A	Initial	99.8%	ND	ND	ND	0.34%	0.00%	OK
	25° C/ 60% RH	07	99.7%	0.04%	ND	0.08%	0.36%	0.12%	OK
		14	99.4%	0.06%	ND	0.09%	0.38%	0.15%	OK
		21	98.9%	0.09%	ND	0.12%	0.41%	0.21%	OK
	30° C/ 75% RH	07	99.0%	0.05%	ND	0.09%	0.35%	0.14%	OK
		14	98.7%	0.07%	ND	0.14%	0.39%	0.21%	OK
		21	98.3%	0.10%	ND	0.20%	0.45%	0.30%	OK
	40° C/ 75% RH	07	98.2%	0.06%	ND	0.20%	0.37%	0.26%	OK
		14	96.8%	0.38%	ND	1.23%	0.42%	1.61%	OK
		21	95.0%	0.68%	ND	2.62%	0.48%	3.30%	OK
	60° C Temp.	07	97.8%	0.16%	ND	0.89%	0.42%	1.05%	OK
		14	95.8%	0.32%	ND	1.22%	0.43%	1.54%	OK
		21	93.0%	1.02%	0.59%	3.24%	0.49%	4.85%	OK

* Excluding 12-epi Misoprostol.

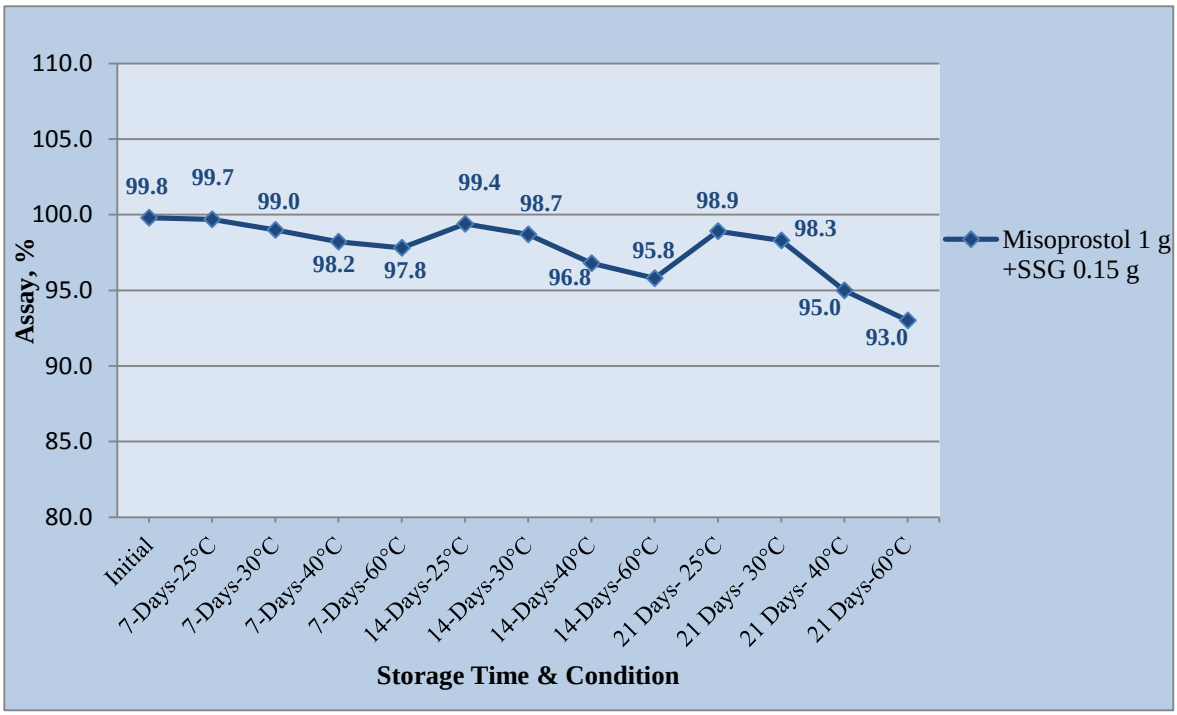


Fig 4.26: Assay Results of Combination 03 (b) at diffetrent storage conditions

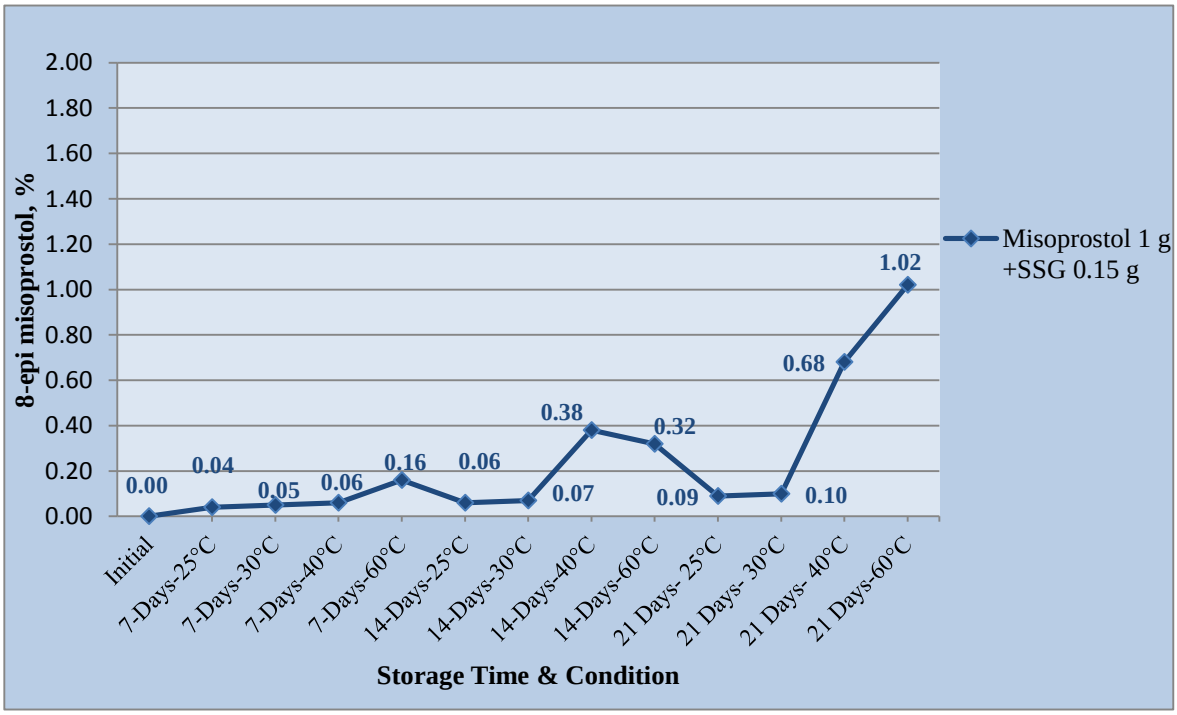


Fig 4.27: 8-epi Misoprostol Results of Combination 03 (b) at diffetrent storage conditions

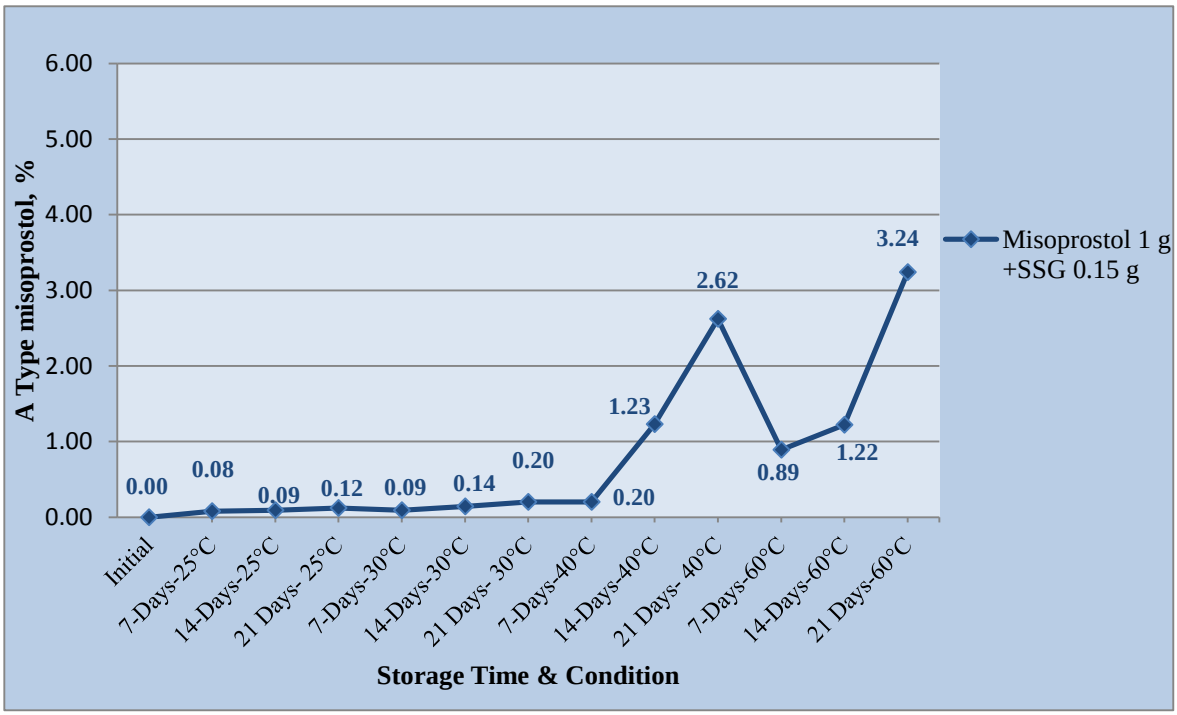


Fig 4.28: A-Type Misoprostol Results of Combination 03 (b) at diffetrent storage conditions

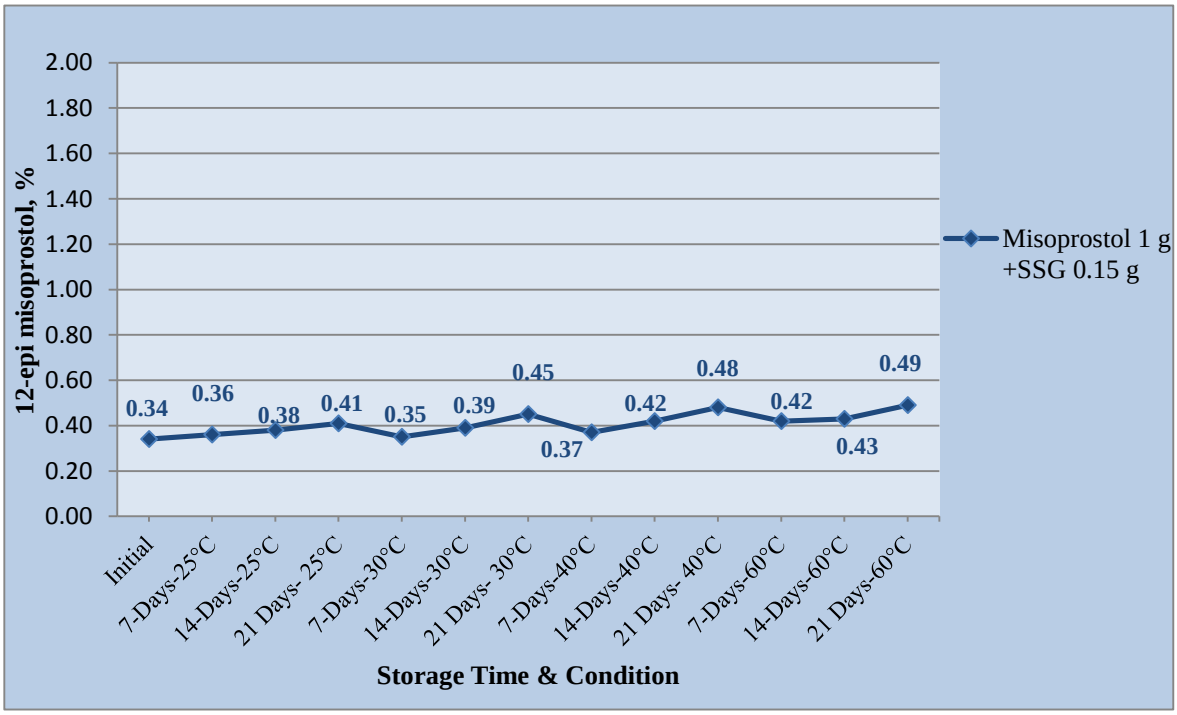


Fig 4.29: 12-epi Misoprostol Results of Combination 03 (b) at diffetrent storage conditions

4.1.3.6 Chemical Results of Combination 03 (c) (API : Sodium Starch Glycolate = 1 : 0.45)

Table 4.17: Assay & Related Substances of Combination 03 (c) at Different Storage Conditions

Materials Present	Storage Condition	Time Interval (Days)	Results						Remarks
			Assay	Related Substances					
				8-epi Misoprostol	B-Type impurity	A-Type impurity	12-epi Misoprostol	Total Impurity	
Misoprostol Dispersion : Sodium Starch Glycolate (1 : 0.45)	N/A	Initial	100.4%	0.01%	ND	ND	0.31%	0.01%	OK
	25° C/ 60% RH	07	99.6%	0.04%	ND	0.09%	0.34%	0.13%	OK
		14	98.8%	0.06%	ND	0.11%	0.38%	0.17%	OK
		21	99.1%	0.09%	ND	0.13%	0.40%	0.22%	OK
	30° C/ 75% RH	07	99.0%	0.05%	ND	0.13%	0.34%	0.18%	OK
		14	98.5%	0.07%	ND	0.16%	0.38%	0.23%	OK
		21	97.6%	0.10%	ND	0.20%	0.41%	0.30%	OK
	40° C/ 75% RH	07	98.3%	0.06%	ND	0.18%	0.38%	0.24%	OK
		14	97.1%	0.35%	ND	1.34%	0.41%	1.69%	OK
		21	95.7%	0.82%	ND	2.74%	0.45%	3.56%	OK
	60° C Temp.	07	98.9%	0.17%	ND	0.93%	0.40%	1.10%	OK
		14	97.1%	0.37%	ND	1.86%	0.40%	2.23%	OK
		21	93.4%	1.09%	0.64%	3.66%	0.50%	5.39%	OK

* Excluding 12-epi Misoprostol.

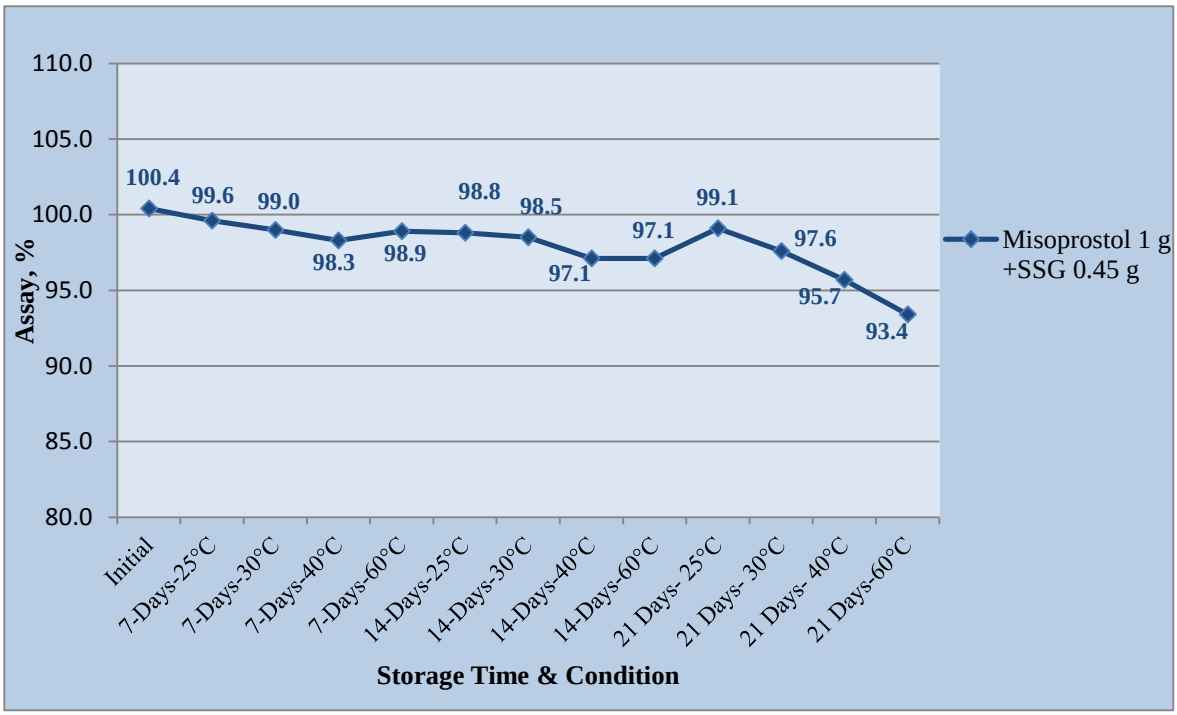


Fig 4.30: Assay Results of Combination 03 (c) at diffetrent storage conditions

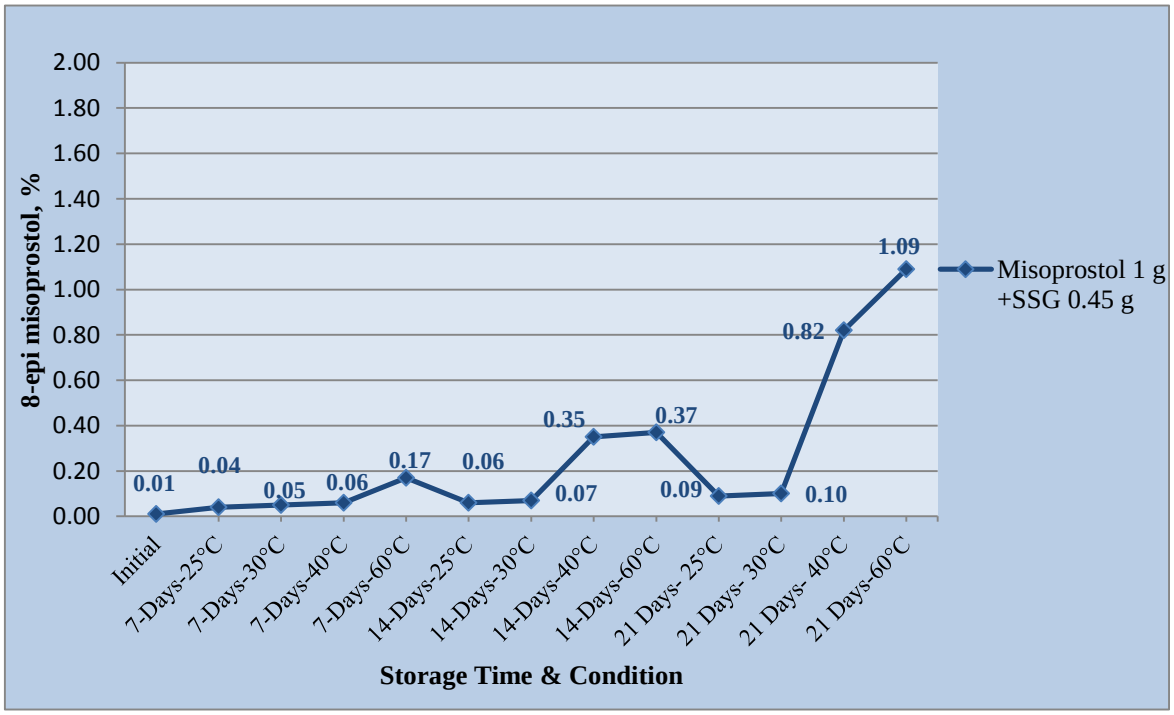


Fig 4.31: 8-epi Misoprostol Results of Combination 03 (c) at diffetrent storage conditions

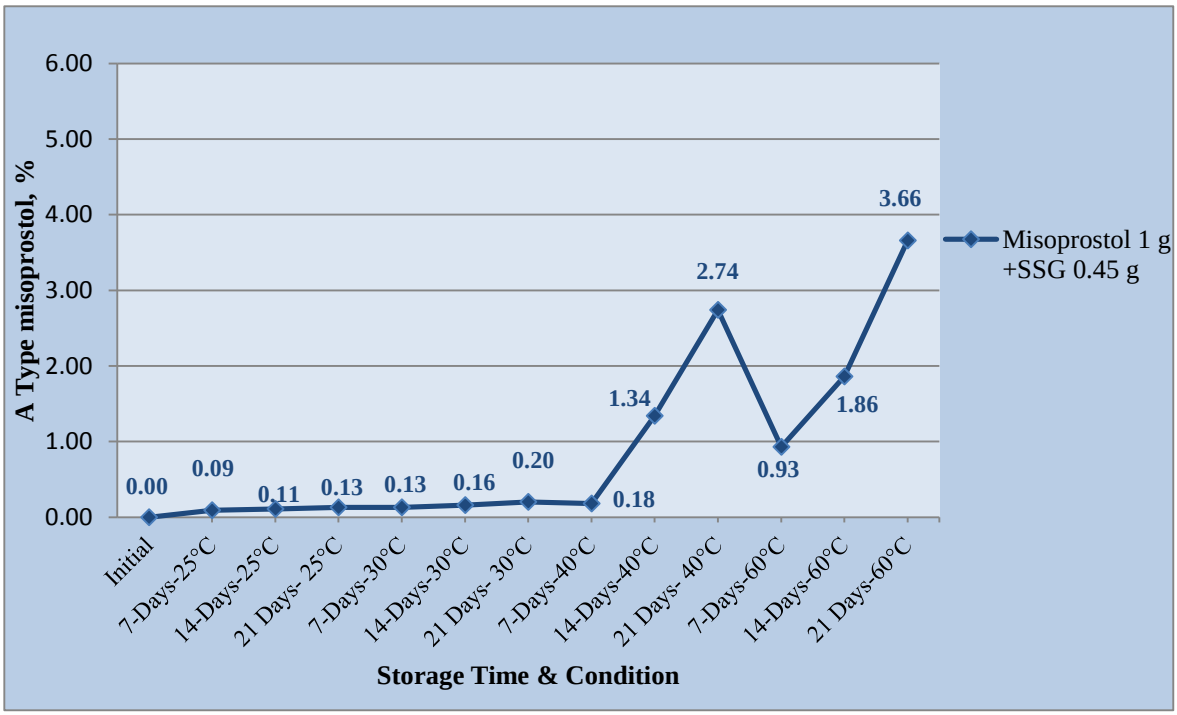


Fig 4.32: A-Type Misoprostol Results of Combination 03 (c) at diffetrent storage conditions

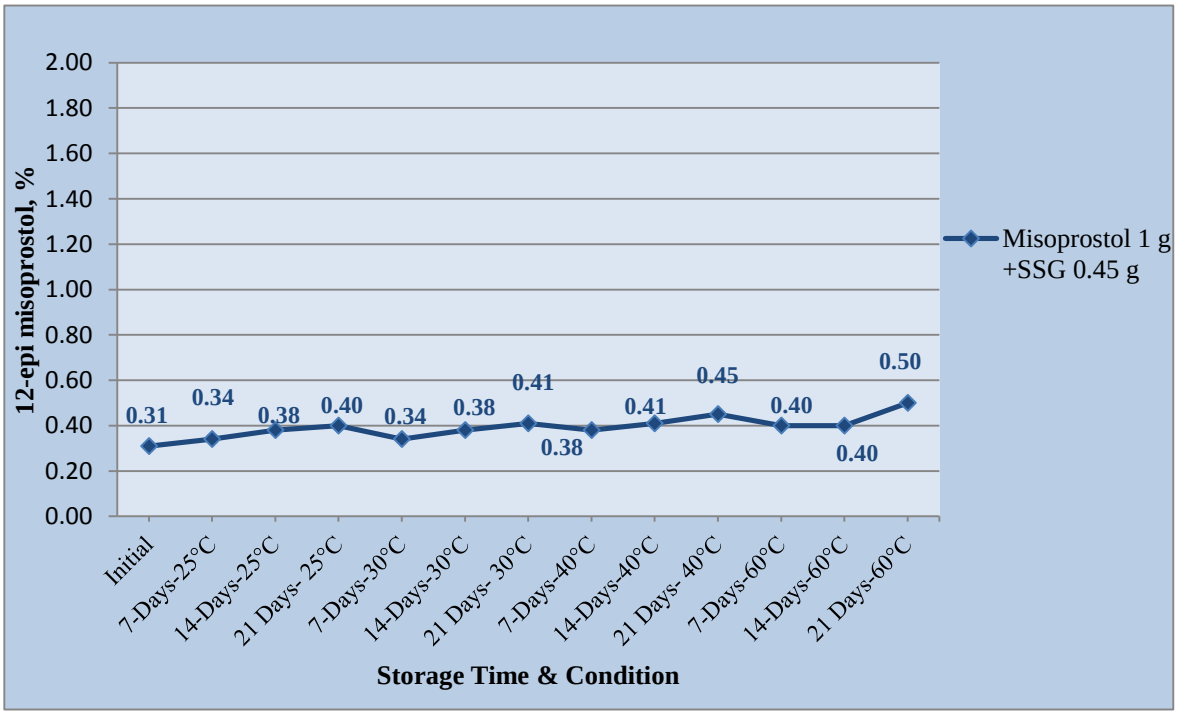


Fig 4.33: 12-epi Misoprostol Results of Combination 03 (c) at diffetrent storage conditions

4.1.3.7 Chemical Results of Combination 04 (API : Hydrogenated Castor Oil = 1 : 1)

Table 4.18: Assay & Related Substances of Combination 04 at Different Storage Conditions

Materials Present	Storage Condition	Time Interval (Days)	Results						Remarks
			Assay	Related Substances					
				8-epi Misoprostol	B-Type impurity	A-Type impurity	12-epi Misoprostol	Total Impurity*	
Misoprostol Dispersion : Hydrogena-ted Castor Oil (1 : 1)	N/A	Initial	100.6%	0.02%	ND	ND	0.49%	0.02%	OK
	25° C/ 60% RH	07	100.1%	0.07%	ND	0.11%	0.46%	0.18%	OK
		14	99.9%	0.08%	ND	0.12%	0.48%	0.20%	OK
		21	99.7%	0.09%	ND	0.13%	0.51%	0.22%	OK
	30° C/ 75% RH	07	100.1%	0.08%	ND	0.17%	0.43%	0.25%	OK
		14	99.6%	0.09%	ND	0.17%	0.51%	0.26%	OK
		21	99.3%	0.10%	ND	0.18%	0.57%	0.28%	OK
	40° C/ 75% RH	07	99.9%	0.10%	ND	0.21%	0.48%	0.31%	OK
		14	97.7%	0.41%	ND	1.32%	0.52%	1.73%	OK
		21	95.7%	0.85%	ND	2.70%	0.58%	3.55%	OK
	60° C Temp.	07	99.8%	0.21%	ND	0.68%	0.38%	0.89%	OK
		14	96.7%	0.42%	ND	1.56%	0.45%	1.98%	OK
		21	95.3%	0.92%	0.36%	2.82%	0.52%	4.10%	OK

* Excluding 12-epi Misoprostol.

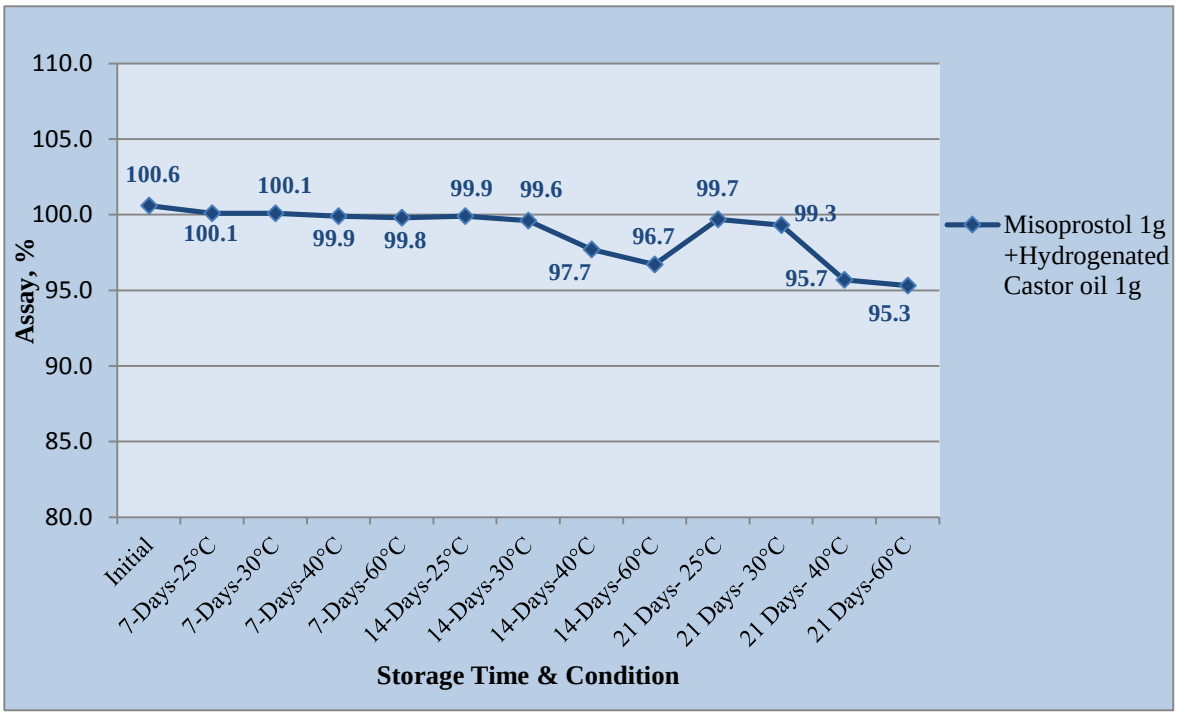


Fig 4.34: Assay Results of Combination 04 at diffetrent storage conditions

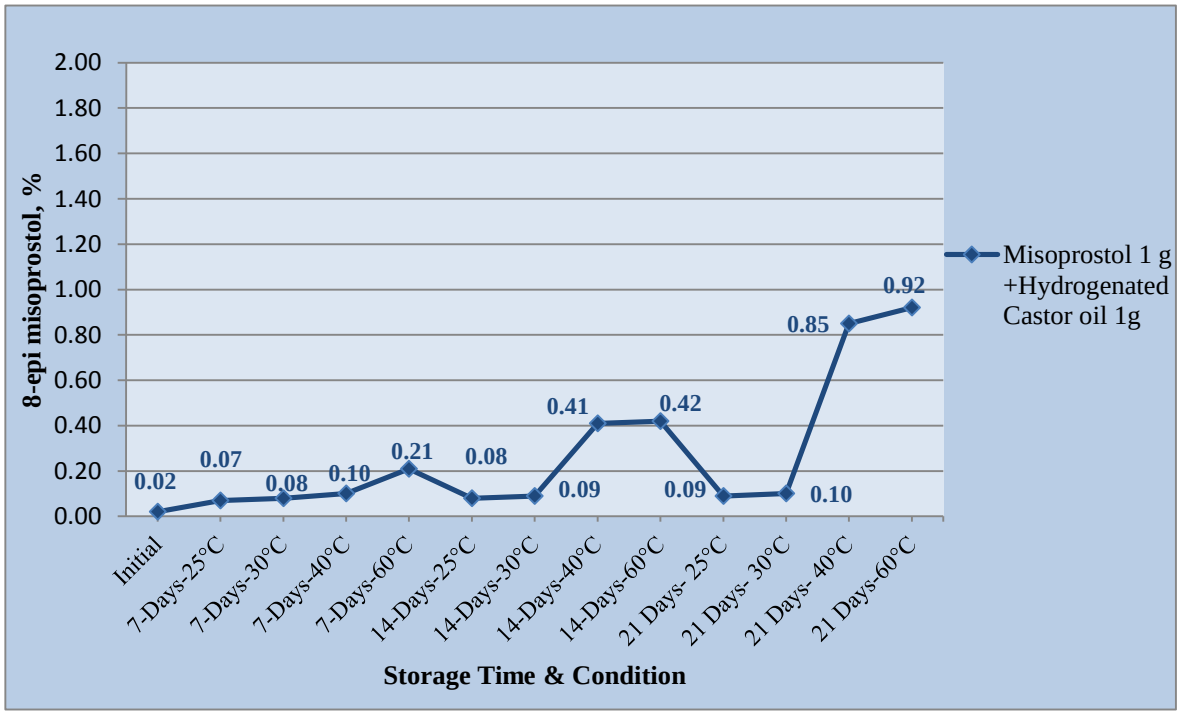


Fig 4.35: 8-epi Misoprostol Results of Combination 04 at diffetrent storage conditions

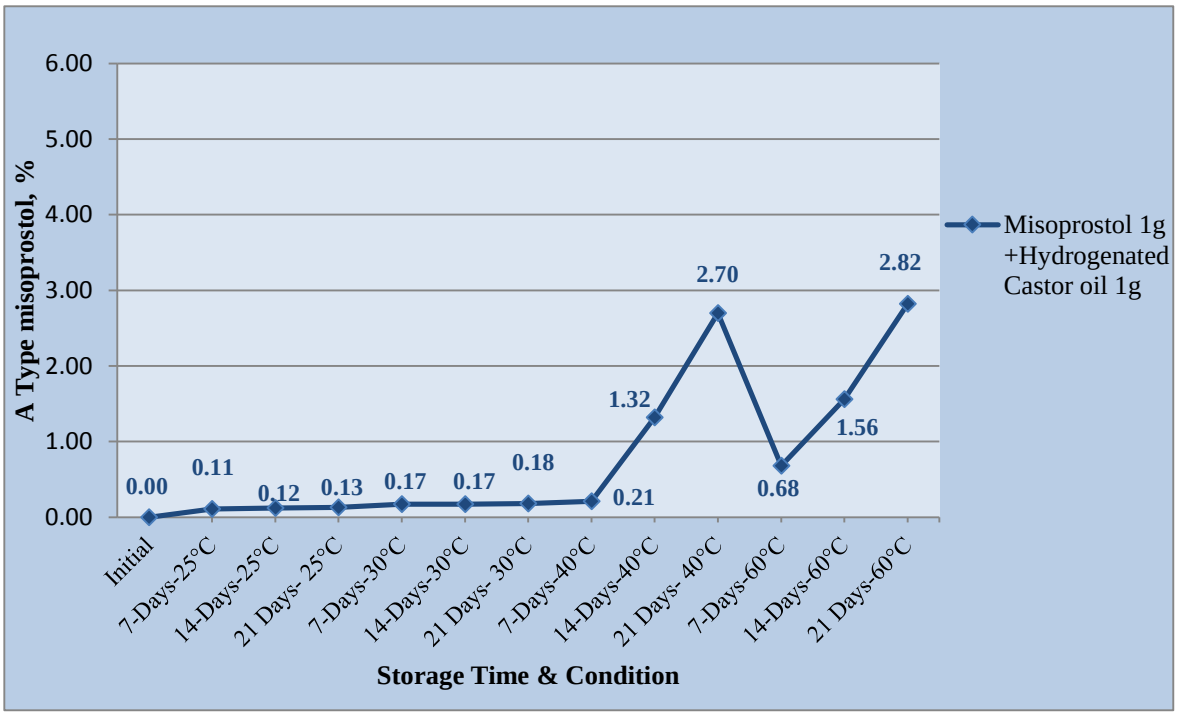


Fig 4.36: A-Type Misoprostol Results of Combination 04 at diffetrent storage conditions

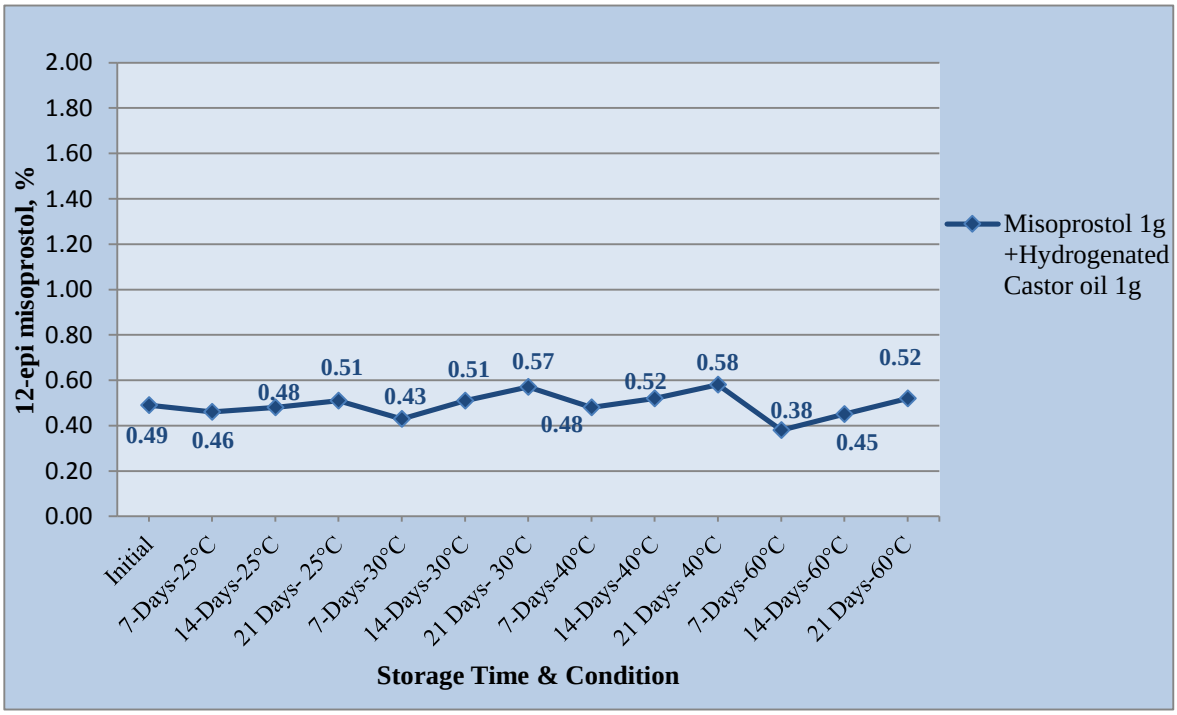


Fig 4.37: 12-epi Misoprostol Results of Combination 04 at diffetrent storage conditions

Chapter Five

DISCUSSION

5.1 DISCUSSION

5.1.1 Phase-One Trial

After evaluation of different formulations, outcomes are as below:

- Disintegration time was not changed in different trials, even though there were two different hardness ranges,
- No significant changes were obsereved in assay results with different formulations,
- Dissolution results were better,
  - with increased quantity of Sodium Starch Glycolate from innovator's,
  - with lower hardness (range: 120 N to 140 N) than innovator's (range: 160 N to 180 N).
  - with Microcrocrystalline Cellulose 113 in compared to Microcrocrystalline Cellulose 103.

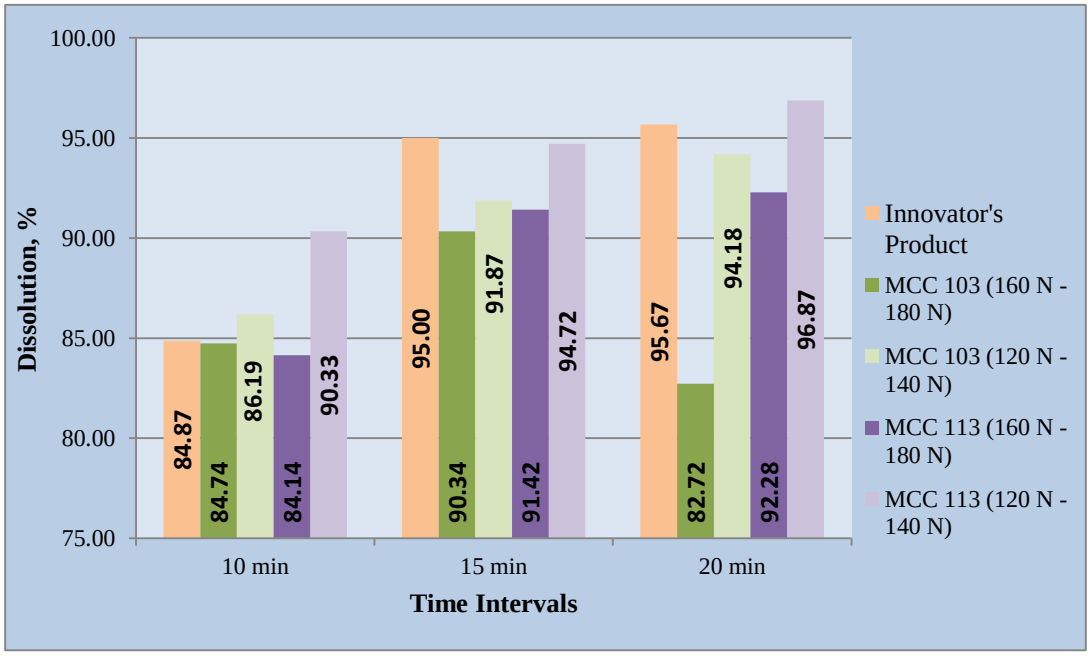


Fig 5.1: A plot showing dissolution results (% average value) of Innovators product and different formulations of Phase-One trial

5.1.2 Phase-Two Trial

After Phase-One Trial, two formulations were selected to proceed further in Phase-Two Trial. Compressed tablets with these formulas were evaluated and outcomes are noted below:

- Amount of disintegrant (Sodium Starch Glycolate) is kept in higher quantity (approx. 4.5%) than innovator's (approx. 1.5%),
- No significant changes were obsereved in assay results with different formulations,
- Hardness of compressed tablets was in lower range (120 N to 140 N), than innovator's (160N to 180 N) but no significant changes were observed in assay and disintegration results,
- Both grades of Microcrystalline Cellulose were used (Microcystalline Cellulose 103 and Microcystalline Cellulose 113) but dissolution results were better in the formulation with Microcystalline Cellulose 113.

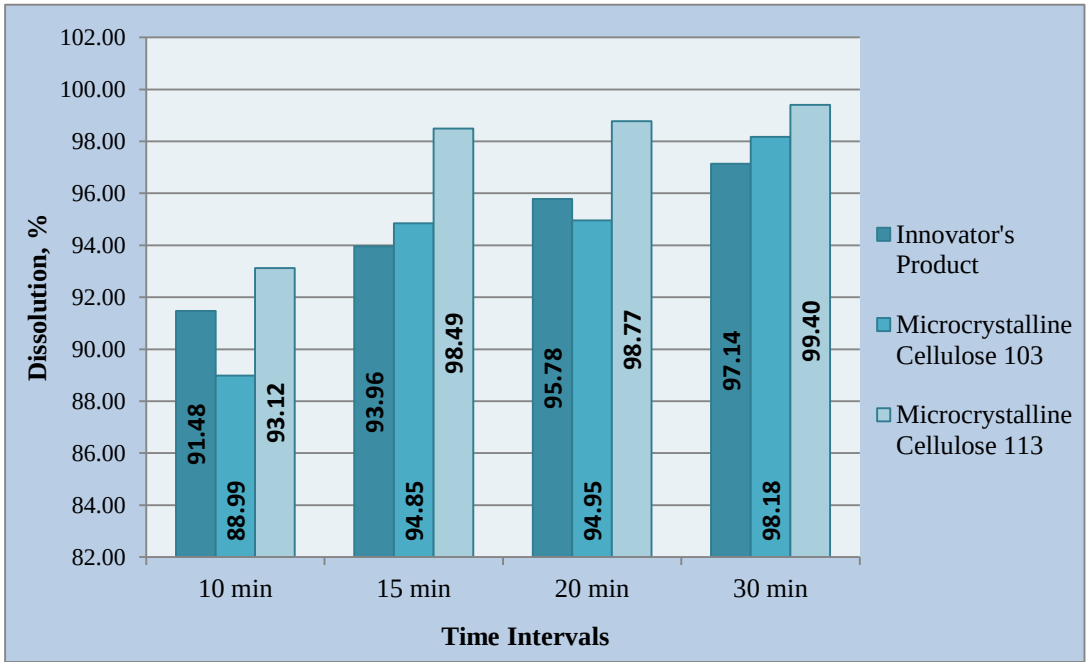


Fig 5.2: A plot showing dissolution results (% average value) of Innovators product and different formulations of Phase-Two trial

5.1.3 Phase-Three Trial

5.1.3.1 Assay Results

In case of trials with different grade of Microcrystalline Cellulose, there were no significant changes between two grades of the diluent over the time in different storage conditions, eventhough dissolution results varied.

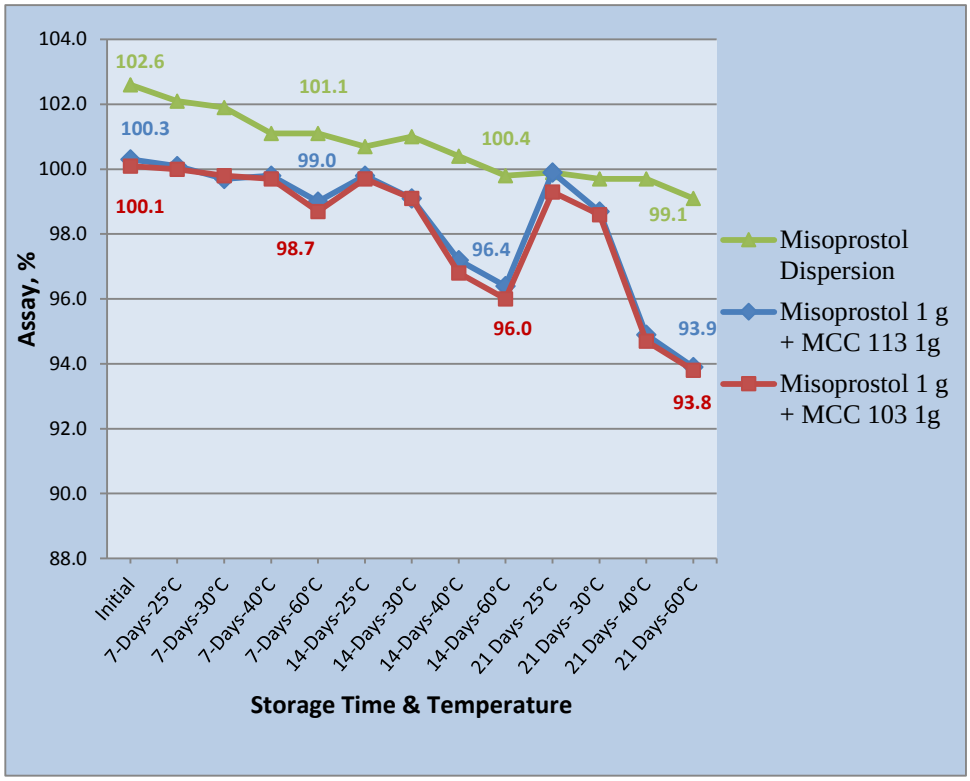


Fig 5.3: A plot showing assay results of Misoprostol Dispersion and mixes with different grades Microcystalline Cellulose

In case of Sodium Starch Glycolate, there was no such noticeable changes were observed in terms of potency of Misoprostol. Similar trend was found with Hydrogenated Castor Oil also.

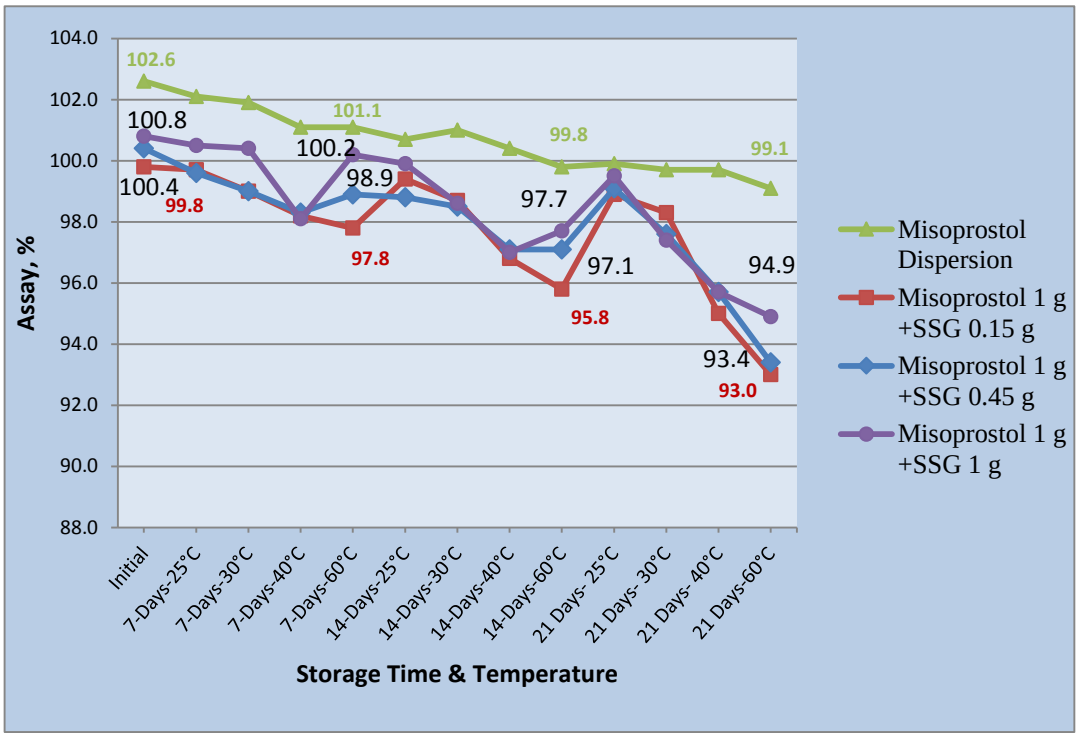


Fig 5.4: A plot showing assay results of Misoprostol Dispersion and mixes with different quantity of Sodium Starch Glycolate

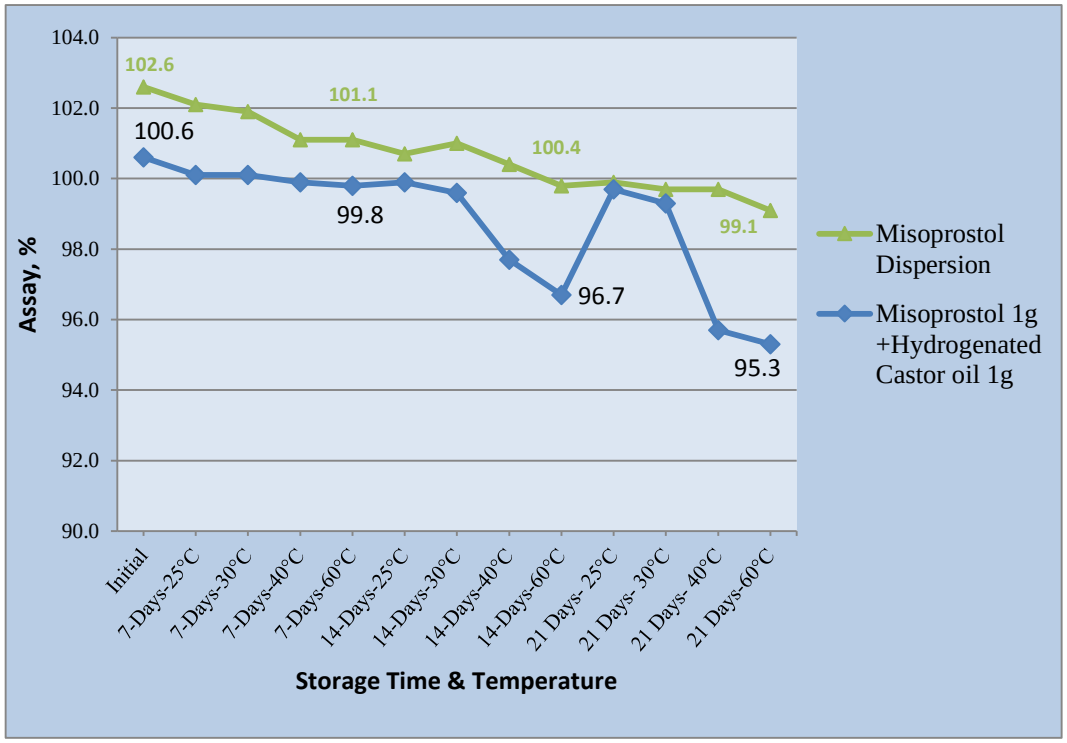


*Fig 5.5:*A plot showing assay results of Misoprostol Dispersion and Hydrogenated Castor Oil mix

5.1.3.2 Related Substances (Impurities)

5.1.3.2.1 8-epi Misoprostol

No significant change is observed in 8-epi Misoprostol generation in 25˚C and 30˚C temperature over the time. Impurity generation rate is higher with increase of temperature and days.

In case of comparison with both grades of Microcrystalline Cellulose, it was observed that Microcrystalline Cellulose 113 followed similar trend with Misorprostol Dispersion in 8-epi Misoprostol generation. This type of impurity generated at higher concentration in case of Microcrystalline Cellulose 103 over the time.

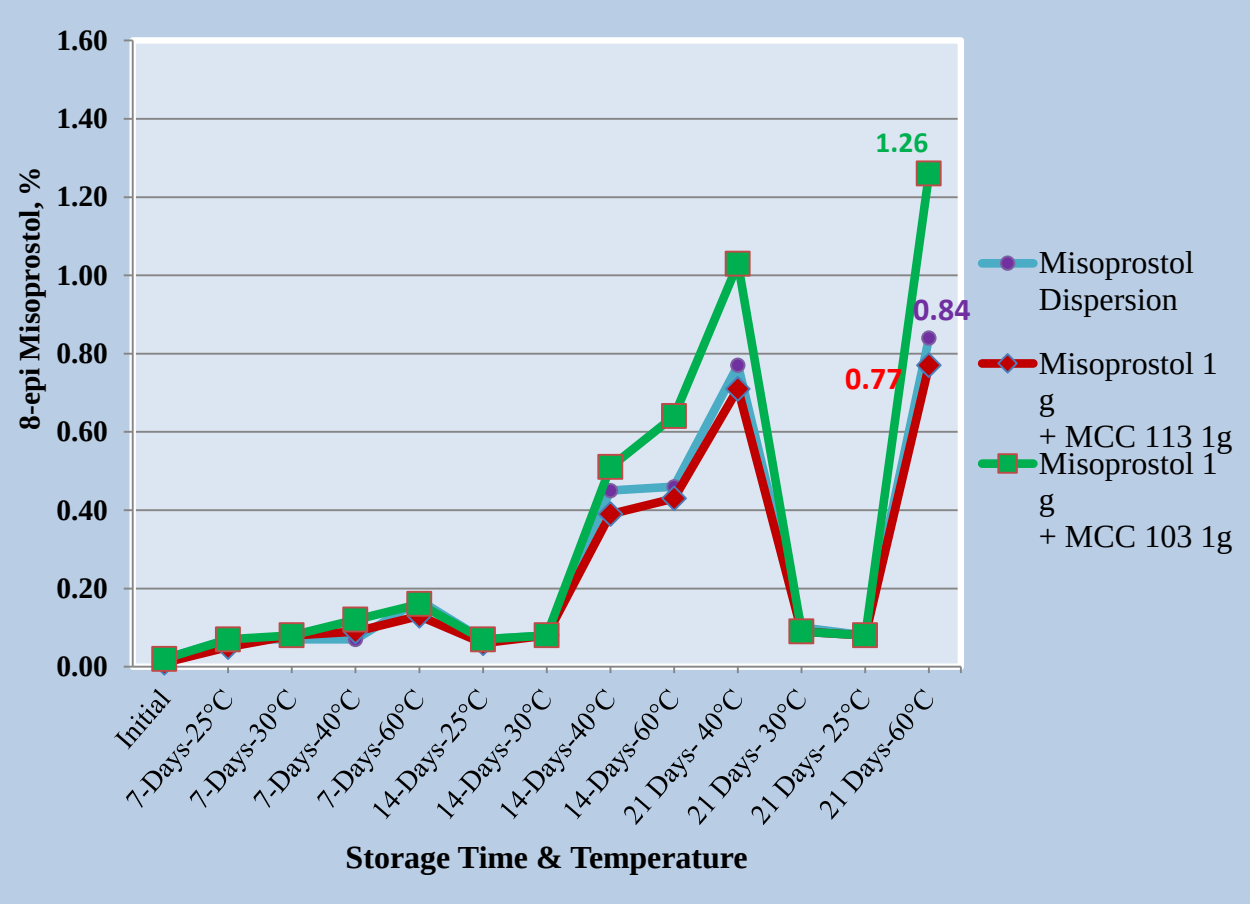


Fig 5.6: A plot showing 8-epi Misoprostol results of Misoprostol Dispersion and mixes with different grades Microcystalline Cellulose

There were no such variation in this impurity generation with different amount of Sodium Starch Glycolate in different combinations, but the combination with 1 : 1 ratio of API and Sodium Starch Glycolate has generated a little higher amount of this impurity over the time. Hydrogenated Castor Oil does not actually affect the generation of 8-epi Misoprostol in formulation.

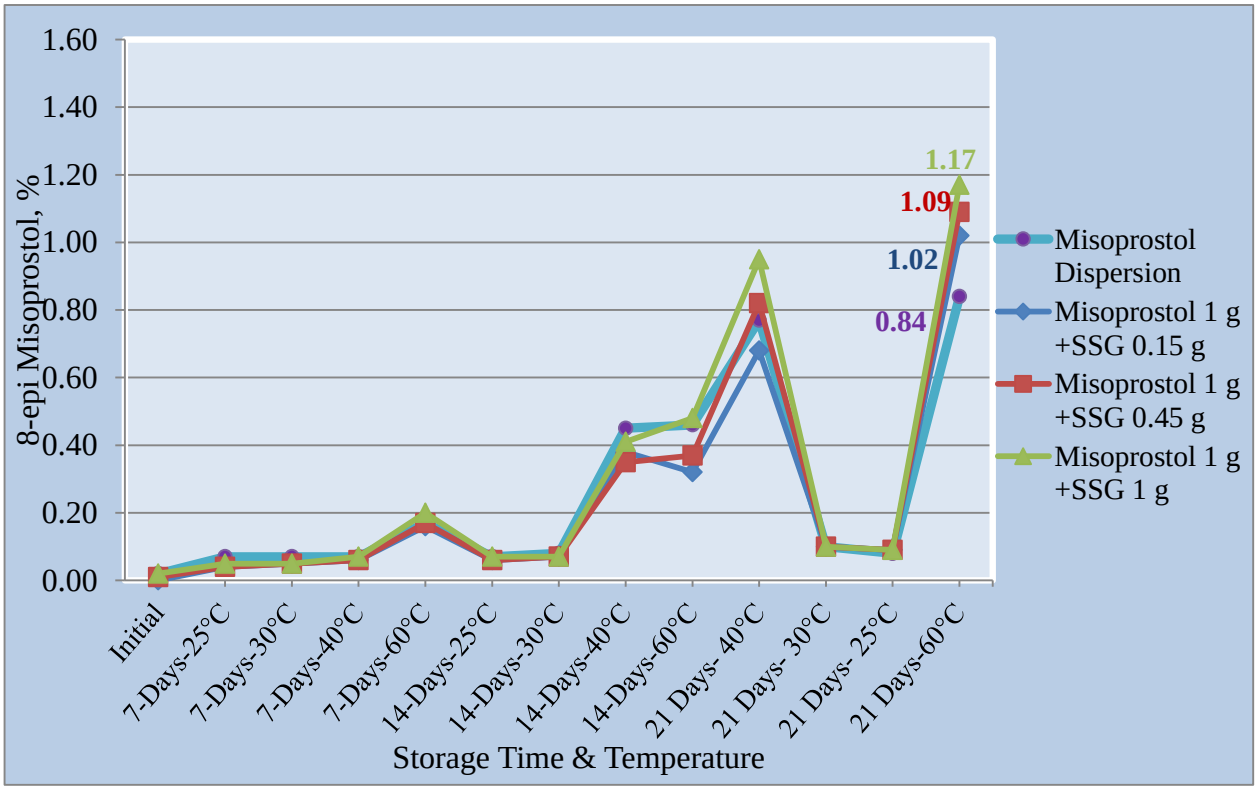


Fig 5.7: A plot showing 8-epi Misoprostol results of Misoprostol Dispersion and mixes with different amounts of Sodium Starch Glycolate

5.1.3.2.2 A- Type Impurity

A-type Misoprostol is a degradation impurity which is generated over the time in acidic/basic condition and in presence of moisture (Hall, 2014). When stored in lower temperature, there is no such variation in generation of this type of impurity in the samples. Amount is gradually increased with raise of temperature over the time. If Microcrystalline Cellulose is considere, it is observed that grade 103 caused higher impurity generation than grade 113 at different storage conditions. In case of three different ratio of Sodium Starch Glycolate, higher amount of this disintegrator in the mix leads to higher percentage of this impurity generation . This is because of

the presence of basic condition (due to sodium) and higher temperature causes release of water molecule from Misoprostol Dispersion as well as other excipients. Hydrogenated Castor Oil does not significantly affect the generation of Type-A Misoprostol in the formulation. Trend is similar to API dispersion itself (used as control).

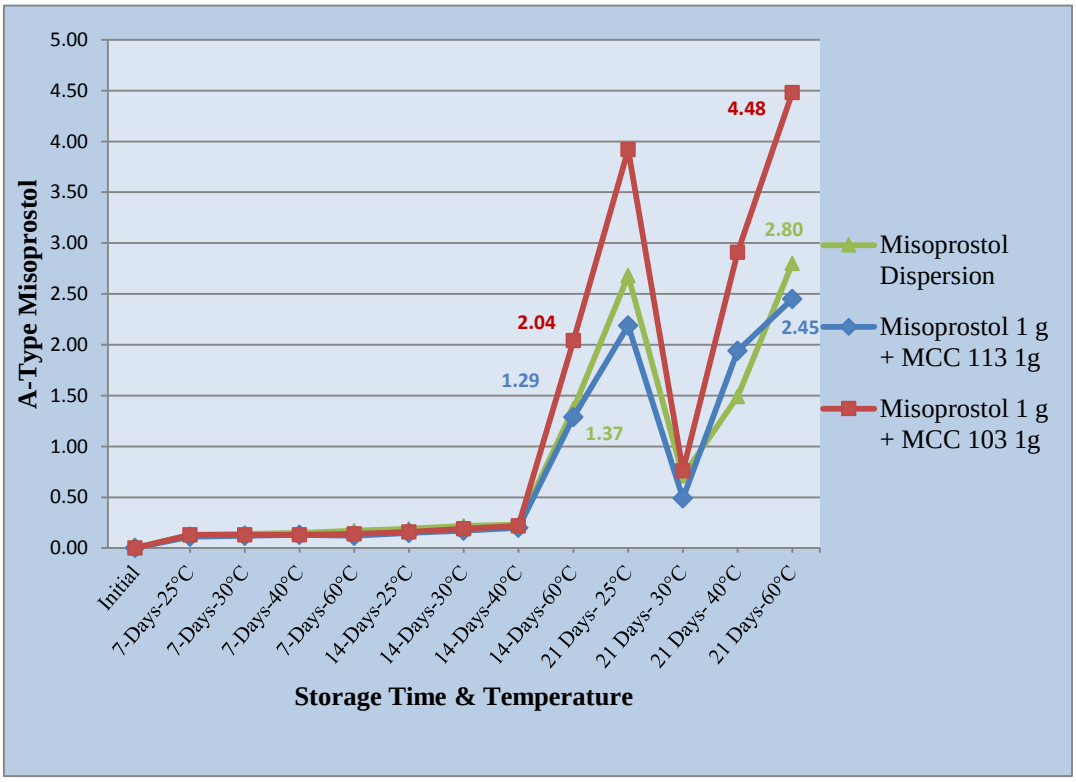


Fig 5.8: A plot showing A-Type Misoprostol results of Misoprostol Dispersion and mixes with different grades Microcystalline Cellulose

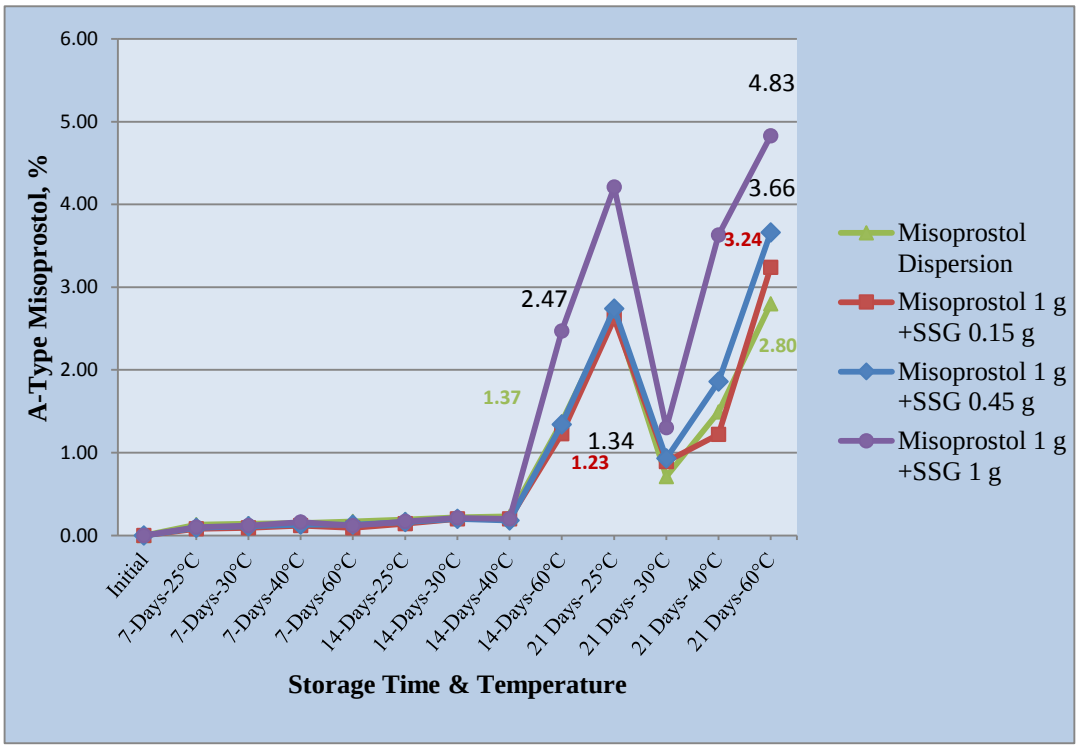


Fig 5.9: A plot showing A-Type Misoprostol results of Misoprostol Dispersion and mixes with different amounts of Sodium Starch Glycolate

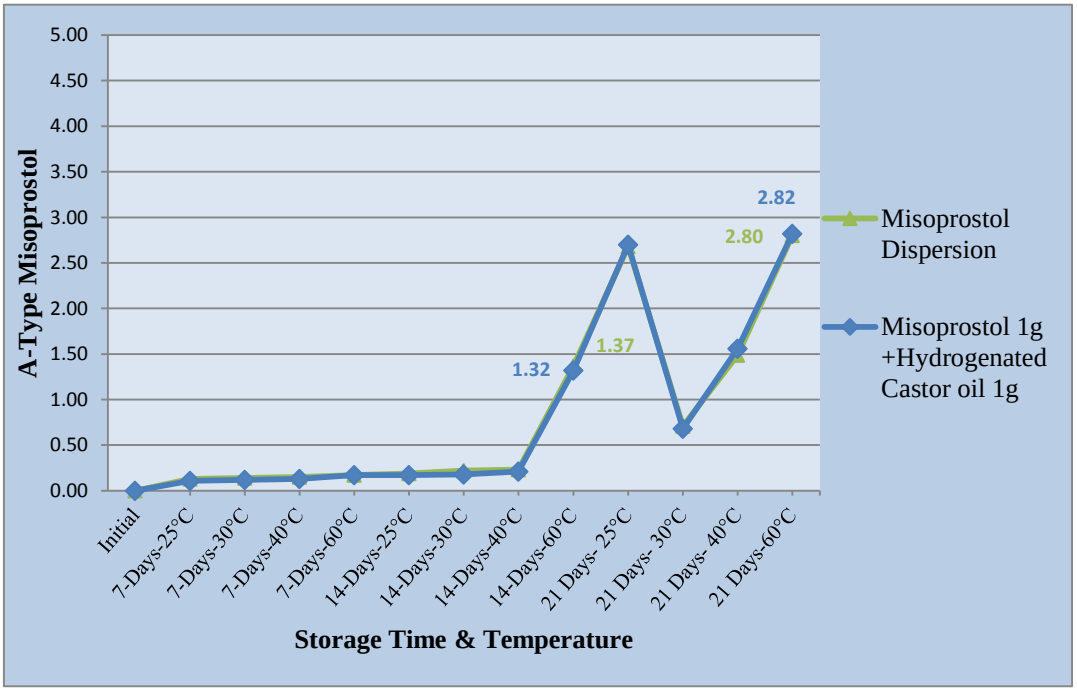


Fig 5.10: A plot showing A-Type Misoprostol results of Misoprostol Dispersion and Hydrogenated Castor Oil mix

5.1.3.2.3 B- Type Impurity

Type A Misoprostol is converted to Type B Misoprostol by isomerization (Hall, 2014). Heat is an important cause of this conversion, as well as the saturation point of Type A Misoprostol generation. That is why, B-type impurity was not generated in combinations at lower storage temperature. From the tabulated data in results section (section 4.1) it is observed that no Type B impurity was generated in API dispersion, API-HCO (Hydrogenated Castor Oil) mix and in API-SSG (Sodium Starch Glycolate) mix (at amount of 1.5% and 4.5% SSG), except after 21 days at 60˚C temperature. Microcrystalline Cellulose (MCC) also played a role in B-type Misoprostol generation. Binary mixture (1:1) of API dispersion with the following excipients caused impurity generation, as per below mentioned trend:

MCC 103 > SSG > MCC 113 (Higher > Lower)

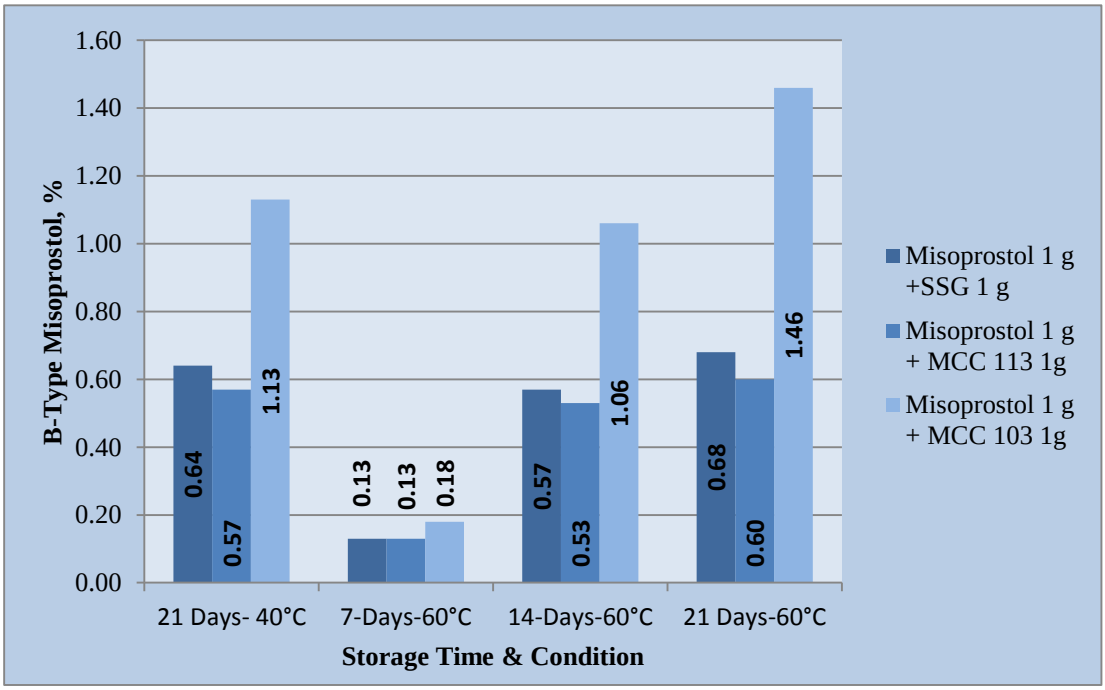


Fig 5.11: A plot showing B-Type Misoprostol results of Misoprostol Dispersion and different combinations with API and excipients

5.1.3.1.4 12-epi Misoprostol

This is a process impurity of Misoprostol generated during API roduction from Norprostol. (Misoprostol DMF, Piramal Healthcare, 2012)

Some observations were found regarding this type of impurity:

- Excipients and their amount do not vary the amount of this impurity.
- There is no such trend of generation of this impurity over the time under different storage conditions.

5.1.3.1.5 Total Impurities

Total impurity data is calculated excluding the amount of process impurities (12-epi Misoprostol), since this is not a degradation impurity and storage condition does not directly have impact on the generation of this type of impurity. Total impurities amount increases with increased amount of Sodium Starch Glycolate used in the mix for compatibility study. Microcrystalline Cellulose 103 has significantly higher ranges of impurities in comparison with Microcrystalline Cellulose 113. Main reason behind this is exposure to heat which causes water depletion as well as the nature of excipients. Since Sodium Starch Glycolate is basic in nature, higher amount of this excipient in the combinations leads to larger amount of impurity generation. On the other hand, Microcrystalline Cellulose 103 itself contains higher amount of water compared to Microcrystalline Cellulose 113. For this reason, MCC 103 has caused degradation in higher amount in comparison with MCC 113. Hydrogenated Castor Oil has no such impact on impurity generation. API dispersion itself also generates degradation impurities over the time in different storage condition.

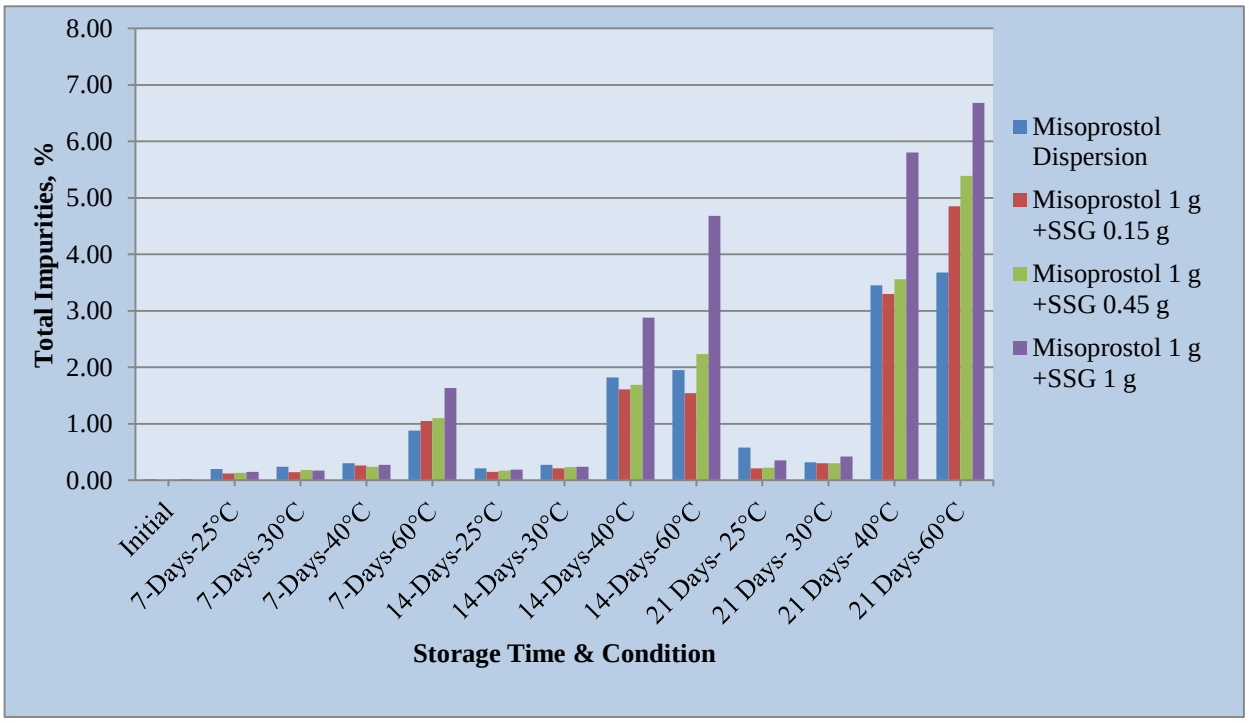


Fig 5.12: A plot showing Total Impurities results of Misoprostol Dispersion and Different Combinations with API and Sodium Starch Glycolate

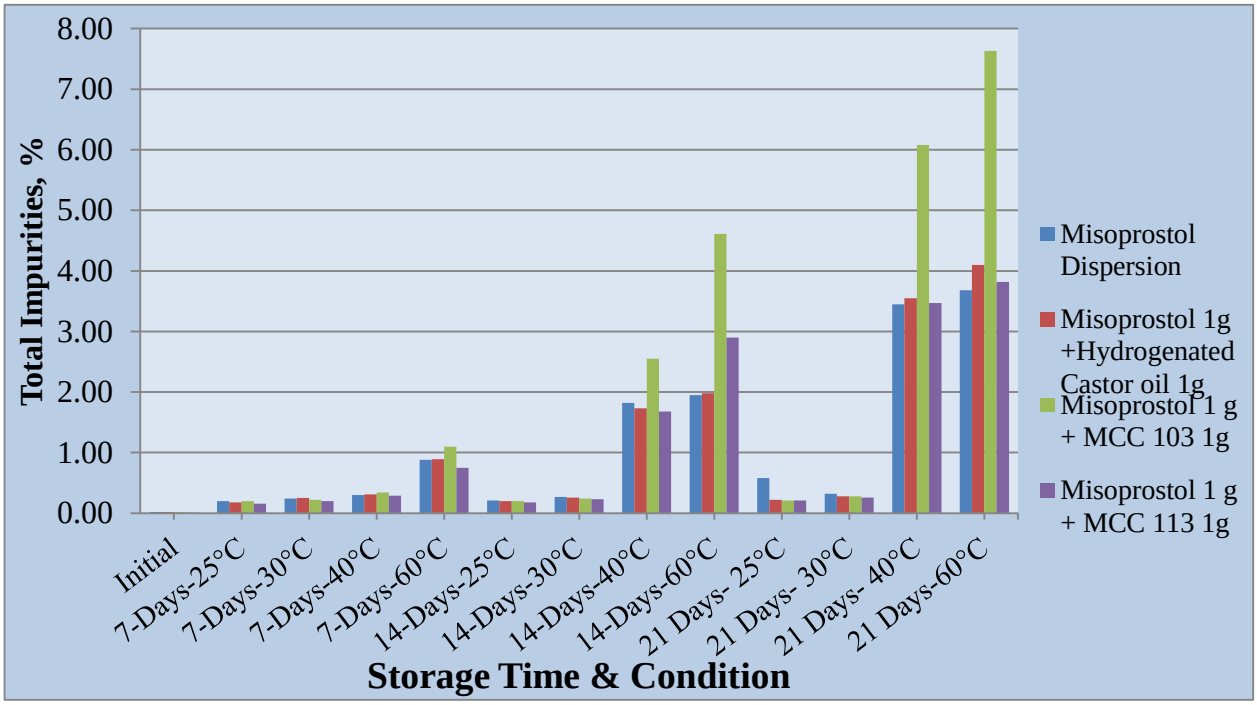


Fig 5.13: A plot showing Total Impurities results of Misoprostol Dispersion and Different Combinations with API and Excipients

Chapter Six

CONCLUSION

6.1 CONCLUSION

From the results and discussion of the three sets of trial, it can be concluded in a way that increased amount of disintegrant, Sodium Starch Glycolate, does not have any significant impact on potency and degradation of the product Misoprostol 200 mg Tablet, rather it increases dissolution rate. Similarly, a slight decrease in hardness of the finished product does not alter its physicochemical properties. The use of Microcrystalline Cellulose 113 instead of Microcrystalline Cellulose 103 is justified evaluating dissolution rate of the product, as well as, moisture content of the product, which directly has impact on API degradation [impurity generation) in the finished dosage form over the time. These slight changes in excipients and its amount, in comparison to innovator's product [Cytotec 200 Tablet), are justified with the order of trials with different generic formulations and evaluation of there physicochemical properties along with appropriate data. Amount of Hydrogenated Castor Oil does not need to be modified, since it has no such impact. In case of proposed generic formulation, if potency of the finished dosage form remains within specification, and degradation of the product can be controlled with the inbuilt quality by design of the product, the use of proposed grade and quantity of excipient can be established.

Chapter Seven

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7.1 REFERENCE

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