“Formulation Development of Sofosbuvir Film Coated Tablet”

A Project Report to be submitted in the Department of Pharmacy for the Partial Fulfillment of the Degree of Masters of Pharmacy.

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I, Jobayda Jannat, ID: 2015-1-79-020, hereby declare that the dissertation entitled **"Formulation Development of Sofosbuvir Film Coated Tablet''** submitted to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the degree of Masters of Pharmacy is a bonafide record of original research work carried out by me under the supervision and guidance of Nazia Hoque, Assistant professor, Dept. of Pharmacy, East West University Dhaka. The contents of this dissertation, in full or in parts, have not been submitted to any other institute or University for the award of any degree or Diploma of Fellowship.

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DEDICATION

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List of Abbreviations

AV:	Acceptance Value
BU:	Blending Uniformity
Cmax:	Maximum Plasma Concentration
CPP:	Critical Process Parameter
CQA:	Critical Quality Attribute
CU:	Content Uniformity
DOE:	Design of Experiments
DS:	Drug Substance
ffc:	flow function coefficient
ICH:	International Conference on Harmonization
IR:	Immediate Release
LOD:	Loss on Drying
MCC:	Microcrystalline Cellulose
N/A:	Not applicable
ND:	Not detected
NLT:	Not Less Than
NMT:	Not More Than
No.:	Number
Nrev:	Number of revolutions
PK:	Pharmacokinetic
PSD:	Particle Size Distribution
QbD:	Quality by Design
QTPP:	Quality Target Product Profile
RSD:	Relative Standard Deviation
RT:	Room Temperature
Tmax:	Time for achieving Maximum Plasma Concentration
RLD	Reference Listed Drug

Abstract

Formulation Development is an important part of Drug Design and Development. Bioavailability and Bioequivalence are totally dependent on Formulation Development. Now-a-days Formulation Development is done by following QbD (Quality by Design). Here formulation development has also been done by following QbD. The purpose of this work is to develop a formulation of Sofosbuvir, which is an Anti-viral drug. It is used to treat Hepatitis C. As it is a fatal disease which damages the liver to an extent that one may die suffering from this disease. So development of a life saving drug was very much necessary for this disease. After treatment with Sovaldi (Innovator), a large amount of patients' lives were saved and it turned out to be a miraculous drug. So this formulation was done to develop a drug to save the lives of the people of Bangladesh who are suffering from Hepatitis C at a much cheaper rate than the Innovator and also with an aim to give effect at an extent to Innovator as much as possible. Here a formulation of Sofosbuvir 400 mg Tablet was done where all the parameters (Hardness, water content, LOD, Disintegration Time, Dissolution and Assay) were found within expected limit. The found results are Hardness 28.8 Kp – 45.5 Kp (Limit: 20.0 Kp – 50.0 Kp), Water Content 2.24 % – 3.48 % (Limit: 1.00 % - 4.00 %), LOD 2.23 % - 3.35% (Limit: 1.00% - 4.00 %), Disintegration Time 0.67 minutes – 3.12 minutes (Limit: NMT 30 minutes), Dissolution 82 % - 102 % (Limit: NLT 75% (Q) in 45 minutes) and Assay 388.96 mg – 408.91 mg (Limit: 360.00 mg – 440 mg) in different stability conditions including initial condition.

Keywords: QbD, Sofosbuvir, Formulation Development, Sovaldi, Hepatitis C, Bioavailability, Hardness, water content, LOD, Disintegration Time, Dissolution, Assay

Chapter One

Introduction

1.1 Preface:

A drug is defined as a substance used for diagnosis, prevention and treatment of disease. A Dosage Form of a drug is a product suited for administration to the patient by various routes for diagnosis or treatment of disease. Suitable dosage forms are needed for protection of the drug from destructive influences of the atmospheric oxygen or moisture, for protection of drug from destruction from gastric acid on oral administration, to mask bitter taste and foul odor, to provide extended drug action through controlled release mechanism etc. (Dosage_form, 2013)

There are several types of Dosage Forms. Like:

- Solid Dosage Form
  - Tablets
  - Capsules
  - Pellets
  - Pills
  - Troches
  - Lozenges
- Liquid Dosage Form
  - Solution
  - Suspension
  - Emulsion
  - Injection
- Dosage Form for External Administration
  - Lotion
  - Ointment
  - Paste
  - Suppositories
  - Sprays
  - Inhalants

Following agents are generally used with drug for suitable solid dosage form:

- **Diluent/Filler** (Pharmainfo.net, 2014)**:** They are inactive ingredients that are added to tablets and capsules in addition to the active drug. Diluents are fillers used to increase the bulk volume of a tablet. Diluents are very important in the pharmaceutical industry. It helps

to adjust the weight of a tablet. Purpose of using diluents is: increase bulkiness, to provide improved cohesion, to enhance flow, to allow direct compression manufacturing. Diluents should be inert, nontoxic, biocompatible, acceptable, nonhygroscopic, stable, and commercially available in acceptable grades, color compatible, no deleterious effect on bioavailability of drugs. Some very common diluents in tablets include starch, cellulose derivatives, Lactose, Mannitol, Sorbitol, Microcrystalline Cellulose.

- **Binder** (Pharmainfo.net, 2014)**:** It is used for binding drug with other excipients. Binder is one of an important excipient to be added in tablet formulation. In simpler words, binders or adhesives are the substances that promote cohesiveness. It is utilized for converting powder into granules through a process known as Granulation. Granulation is the unit operation by which small powdery particles are agglomerated into larger entities called granules. Ex.: Acacia, Gelatin, Maize Starch, Methyl Cellulose, Hydroxypropyl Methyl Cellulose, Hydroxypropyl Cellulose, Povidone, Pregelatinzed Starch.

- **Disintegrant** (Pharmainfo.net, 2014)**:** It is used for breaking down the tablet into fine granules for absorption purpose. Bioavailability of a drug depends in absorption of the drug, which is affected by solubility of the drug in gastrointestinal fluid and permeability of the drug across gastrointestinal membrane. The drugs solubility mainly depends on physical - chemical characteristics of the drug. However, the rate of drug dissolution is greatly influenced by disintegration of the tablet. Disintegrants, an important excipient of the tablet formulation, are always added to tablet to induce breakup of tablet when it comes in contact with aqueous fluid and this process of desegregation of constituent particles before the drug dissolution occurs, is known as disintegration process and excipients which induce this process are known as disintegrants. Mechanism of tablet disintegration:
  - By capillary action
  - By swelling
  - Because of heat of wetting
  - Due to disintegrating particles
  - Due to deformation
  - Due to release of gases
  - Due to enzymatic action

Ex.: Croscarmellose Sodium, Sodium Starch Glycolate, Starch, Pregelatinzed Starch, Crospovidone.

- **Lubrication:** It is used for lubricating the blend so that sticking cannot occur. Ex.: Magnesium Stearate.
- **Glidant:** It is used for improving flow property. Ex.: Purified Talc.
- **Anti-adherent:** It is used to avoid adherence of blend to die or punch. Ex.: Colloidal Anhydrous Silica.

This report presents a summary of the pharmaceutical development of the dosage form of Sofosbuvir 400 mg film coated tablet. It emphasizes a science and risk-based approach to product and process development, and presents findings as a knowledge-based report, where relevant and supporting data have been summarized in appropriate tables or illustrations. The scientific approach used begins with identification of the desired dosage form and performance attributes through the target product profile. From this target product profile, an initial list of critical quality attributes was developed. A risk assessment was undertaken to identify the variables and unit operations which are most likely to impact the critical quality attributes. This was then used to focus development activities on potential high risk areas. A risk assessment, starting with the physico-chemical characteristics of the API, led to the identification of a viable formulation and manufacturing approach. Formulation development involved the use of prior knowledge and structured experimentation to investigate the relationship between formulation component levels, API attributes and the drug product quality attributes. Development of the manufacturing process focused on the unit operations posing greatest potential risk to drug product quality. Using prior knowledge, models, extrapolation and risk assessment processes, the material attributes and process parameters, which could have an impact upon final product quality, were identified. For each unit operation experimentation was undertaken to define the relationship between the input attributes, process parameters, output attributes and final drug product quality. The intermediate critical quality attributes, operating conditions and a control strategy were defined to mitigate risk and ensure final product quality.

Sofosbuvir is a novel nucleotide prodrug. In human hepatocytes, Sofosbuvir is converted to an active uridine triphosphate form, which acts as an inhibitor of the hepatitis C virus (HCV) non-structural (NS) 5B ribonucleic acid (RNA) polymerase. The proposed indication for Sofosbuvir is

for use in combination with other medicinal products for the treatment of chronic hepatitis c (CHC) in adults. (Drugs.com)

1.2 Hepatitis C and its Treatment **(Sovaldi assessment report, EMA, 2013)**

Hepatitis C is the most common single cause of Liver transplantation. HCV is divided into six major genotypes and numerous subtypes, which are based on phylogenetic relationship. Genotype 1 is the most common genotype in Europe, comprising approximately 70% of the infections. Genotype 3 is the second most common, followed by genotype 2. Genotype 4 is predominant in Egypt, the nation in the world with the highest documented HCV prevalence. Genotype 5 and 6 are uncommon in Europe and US, but are common in South Africa and South-East Asia. HCV genotypes do not clearly impact the rate of disease progression. Treatment response, however, differs between genotypes.

The goal of Anti viral therapy against HCV is to reach sustained Virological response (SVR), which is traditionally defined as the absence of quantifiable virus in plasma at least 24 weeks after the end of therapy. However, most relapses occur within 4 weeks of treatment discontinuation.

Presently licensed treatment options for HCV all include Peginterferon (PEG) and Ribavirin (RBV). For the treatment of genotype 1 infection, the addition of either one of the NS 3/4A protease inhibitors telaprevir or boceprevir, approved in 2011, is presently considered standard-of-care. But response rates are low, eg: in patients with prior non-response to interferon based therapy or with cirrhosis. For genotypes other than 1, there is no direct acting antiviral presently approved.

In summary, the evolving field of hepatitis C therapeutics is similar to that of Antiretroviral therapy in the following aspects:

- Combination therapy is anticipated in all cases
- Agents with different mechanisms of action or lack of cross-resistance consistently show additive antiviral effects

- Failure of antiviral therapy is in many cases associated with selection of drug resistant viral variants which may impact future therapeutic option. Furthermore, in hepatitis C, there are naturally occurring viral polymorphisms that impact the activity of some agents.
- Consequently individual viral drug susceptibility will need to be taken into account when selecting an appropriate combination regimen.

1.3 General Information on RLD, Sovaldi (Dailymed.com, 2014)

Innovator Brand: Sovaldi

Figure 1: Image of Innovator Sovaldi

Manufacturer: Gilead Sciences.

Active Ingredient: Sofosbuvir INN 400 mg

Inactive Ingredient

Core:

1. Mannitol
2. Microcrystalline Cellulose
3. Croscarmellose Sodium
4. Magnesium Stearate
5. Purified Talc
6. Colloidal Anhydrous Silica

Coating:

1. Polyvinyl Alcohol
2. Titanium Dioxide
3. Polyethylene Glycol

4. Talc
5. Ferric Oxide Yellow

Therapeutic Category: Anti-viral

Available Dosage Form: Tablet

Proposed Strength: 400 mg

Indication: Indicated for the treatment of Chronic Hepatitis C (CHC) Infection.

Mechanism of Action: Sofosbuvir is a NS5B polymerase inhibitor.

Side Effects (Drugs.com, 2014)

Sovaldi is used in combination with other medications, usually ribavirin with or without peginterferon alfa. Ribavirin can cause birth defects or death in an unborn baby.If the patients is pregnant Sofosbuvir with Ribavirin should not be used, or if a female is pregnant the man sexual partner should not use this drug. Should use at least 2 effective forms of non- hormonal birth control while using these medicines together and for at least 6 months after treatment ends.

Precaution (Drugs.com, 2014)

One should not use Sofosbuvir he or she is allergic to sofosbuvir. To make sure Sofosbuvir is safe for you, tell your doctor if you have:

- a history of hepatitis B;
- liver problems other than hepatitis, or if you have had a liver transplant;
- kidney disease (or if you are on dialysis);
- HIV (human immunodeficiency virus); or
- If one has recently used a heart rhythm medicine called amiodarone (Cordarone, Pacerone).

Sofosbuvir Dosing Information (Drugs.com, 2014)

Usual Adult Dose for Chronic Hepatitis C: 400 mg orally once a day

Recommended Regimens:

-Genotype 1 or 4 chronic hepatitis C (CHC): Sofosbuvir, peginterferon alfa, and ribavirin

-Genotype 2 CHC: Sofosbuvir and ribavirin

-Genotype 3 CHC: Sofosbuvir and ribavirin

-Hepatocellular carcinoma awaiting liver transplantation: Sofosbuvir and ribavirin

Duration of Therapy:

-Genotype 1 or 4 CHC: 12 weeks

-Genotype 2 CHC: 12 weeks

-Genotype 3 CHC: 24 weeks

-Hepatocellular carcinoma awaiting liver transplantation: Up to 48 weeks or until time of liver transplantation (whichever occurs first)

Pharmacokinetics of Sofosbuvir (API) (Dailymed.com, 2014)

The pharmacokinetics of Sofosbuvir is approximately dose proportional over the recommended dose range.

Absorption

Following oral administration of Sofosbuvir, it was absorbed with a peak plasma concentration observed at ~0.5–2 hour post-dose, regardless of dose level.

Distribution

Sofosbuvir is approximately 61–65% bound to human plasma proteins and the binding is independent of drug concentration over the range of 1 microgram/mL to 20 microgram/mL.

Metabolism

Sofosbuvir is extensively metabolized in the liver to form the pharmacologically active nucleoside analog triphosphate. The metabolic activation pathway involves sequential hydrolysis of the carboxyl ester moiety catalyzed by human cathepsin A or carboxylesterase 1 and phosphoramidate cleavage by histidine triad nucleotide-binding protein 1 followed by phosphorylation by the pyrimidine nucleotide biosynthesis pathway. Dephosphorylation results in the formation of nucleoside metabolite GS-331007 that cannot be efficiently rephosphorylated and lacks anti-HCV activity in vitro.

Excretion

Following a single 400 mg oral dose of sofosbuvir, mean total recovery of the dose was greater than 92%, consisting of approximately 80%, 14%, and 2.5% recovered in urine, feces, and expired air, respectively. The majority of the sofosbuvir dose recovered in urine was GS-331007 (78%) while 3.5% was recovered as sofosbuvir.

Pharmacodynamics of Sofosbuvir (Dailymed.com, 2014)

Cardiac Electrophysiology

The effect of sofosbuvir 400 and 1200 mg (three times the recommended dosage) on QTc interval was evaluated in a randomized, single-dose, placebo- and active-controlled (moxifloxacin 400 mg) four period crossover thorough QT trials in 59 healthy subjects. At a dosage three times the maximum recommended dosage, SOVALDI does not prolong QTc to any clinically relevant extent.

1.4 Components of Drug Product

Drug Substance (Sofosbuvir)

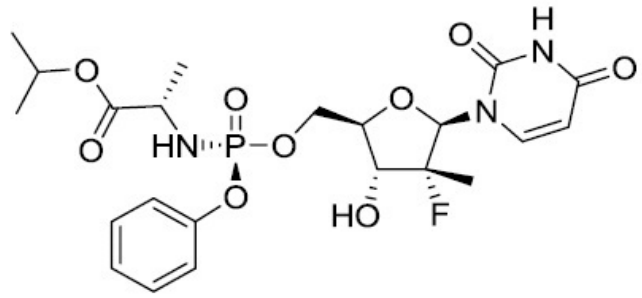
Physical Properties (Pubchem.com, 2015)

The following physical description is for Sofosbuvir:

Appearance	:	White to off white powder
Particle size	:	D_{50}: 20-60 µm, D_{90}: 60-120 µm
Polymorphic Form	:	Form-I
Solubility	:	Freely soluble in Ethanol, Methanol and Acetone, soluble in 2-Propanol, slightly soluble in water and insoluble in Heptane.
Hygroscopicity	:	Sofosbuvir is not hygroscopic
Photosensitivity	:	Sofosbuvir is not photosensitive
Flowability	:	Poor Flow property

Chemical Properties (Pubchem.com, 2015)

Chemical Structure :



Chemical Formula	:	$C_{22}H_{29}FN_3O_9P$
Molecular Weight	:	529.45 g/mol
PKa	:	9.3
Moisture Content	:	NMT 0.5%
IUPAC Name	:	(S)-Isopropyl 2-(((S)-(((2R,3R,4R,5R)-5-(2,4-Dioxo-3,4-

Di-Hydropyrimidine-1(2H)-yl)-4-fluro-3-hydroxy-4-methyletrahydrofyran-2-yl)methoxy(phenoxy) phosphoryl) amino) propanoate.

Polymorphism : There are six available polymorphic form of Sofosbuvir. They are:

- Form I
- Form II
- Form III
- Form IV
- Form V
- Form VI

Isomerism : Original synthetic pathway of Sofosbuvir yielded a diasteriomeric mixture. Separation of the mixture yielded SP – 4 isomer, which had a 10 fold better activity. Hence, for formulation development of Sofosbuvir Tablet, this sepcific Isomer was sought.

The available polymorphs of Sofosbuvir were subjected to stability study by being stored under opened conditions at 40/75 conditions for 30 days. Among these 6 forms, polymorphic forms I and VI were found to be more stable than the others. Furthermore, polymorphic form VI was found to be most stable among all the six polymorphs. The innovator used Polymorph VI for European market and Form I for African market.

Sofosbuvir is chiral and possess six stereogenic centres which are all controlled by the synthetic process and the specifications of raw materials. The absolute and relative configuration of these chiral centres was established by single crystal X-ray crystallography. Eight polymorphic form of Sofosbuvir have been observed and the manufacturing process consistently produces Sofosbuvir as the most thermodynamically stable polymorphic form, containing a small amount of metastable

form which were determined to be pharmaceutically equivalent. Other polymorphic forms are excluded by the manufacturing process and their absence is confirmed by DSC. (Darryl *et al*, 2013)

Biological Properties

Partition Coefficient (LogP)	:	1.62 (Pubchem.com, 2015)
Biopharmaceutical Classification	:	Sofosbuvir belongs to the class III of BCS, exhibiting high solubility and low permeability. (Assessment Report, EMA, 2013)

1.5 Aim of the Study

The aim of the study is to develop a formulation for Sofosbuvir 400 mg Film Coated Tablet which will be close to Reference Listed Drug (RLD) as much as possible to get proper effect of the drug and to make it bioavailable to treat the fatal disease Chronic Hepatitis C.

Chapter Two

Literature Review

2.1 Synthetic Procedure of Sofosbuvir

N-Benzoyl Sofosbuvir (6 g) was added to 70% w/w aqueous acetic acid (90 mL) and the contents were stirred at 90-95° C. After completion of the reaction, which was monitored by qualitative HPLC, the reaction mass was cooled to ambient temperature, diluted with water and filtered through a Hyflo filter. Thereafter, obtained filtrate was extracted with ethyl acetate which was further washed with ˜4% w/w aqueous hydrochloric acid followed by ˜9% w/w aqueous sodium carbonate solution. Finally, the ethyl acetate layer was washed with water and dried. The dried layer was concentrated under reduced pressure at 60-65° C. Thereafter, the concentrated mass was dissolved in a mixture of 5% isopropanol in methylene dichloride and isopropyl ether was added to precipitate the product. After stirring at 0-5° C. for 2 hours, the product was filtered, washed with methylene dichloride/isopropyl ether mixture, which was recrystallized with methylene dichloride/isopropyl ether mixture to yield sofosbuvir as white crystals (3 g). (Kaushik *et al*. 2016)

2.2 Treatment of Hepatitis C with Sofosbuvir

Due to its global pervasiveness and chronicity, the hepatitis C virus (HCV) is a major health problem that claims around half a million lives annually. In recent years, the pharmaceutical industry has witnessed a surge in the development of new therapies for the treatment of hepatitis C. One such drug, sofosbuvir, marketed by Gilead Sciences, was recently approved for clinical use in several countries. In combination with other antiviral agents, sofosbuvir has shown remarkable efficacy for a broad range of viral genotypes, along with high tolerability. The clinical success of sofosbuvir demands efficient approaches for the synthesis of this pharmaceutical. Marketed as a single isomer, sofosbuvir presents several interesting synthetic challenges, including fluorination chemistry, nucleotide synthesis, and regio- and stereoselective phosphoramidation. This review provides a brief pharmacological background of sofosbuvir including its mode of action, followed by an in-depth analysis of the current synthetic approaches to sofosbuvir and its close analogues. (Barth *et al*. 2015)

2.3 Targeted Treatment of Hepatitis C of Patients having Fibrosis

Targeted treatment recommendations in chronic hepatitis C tailored to diagnostic methods of fibrosis. It is examined different policy scenarios for giving individuals with chronic hepatitis C infection access to direct-acting antiviral (DAA) treatments. The authors used a Markov model to estimate the 5-year reduction in the incidence of cirrhosis, liver complications and liver deaths compared to no treatment under three different rules for access: providing therapy only to patients

with F3 fibrosis scores, providing therapy only to patients with severe F2 fibrosis scores, and providing universal therapy. (Hellard *et al.* 2016)

2.4 Preparation of Amorphous Sofosbuvir

A process for the preparation of amorphous solid dispersion of Sofosbuvir and a pharmaceutically acceptable carrier Sofosbuvir form-M2. Within the context of the present invention, crystalline Sofosbuvir form-M2 may be characterized by a PXRD pattern having characteristic peaks at about 8.09, 12.42, 19.39, 19.98 and 20.84 (+) 0.2° 2-theta. Within the context of the present invention, crystalline Sofosbuvir form-M2 may be further characterized by a PXRD pattern having characteristic peaks at about 8.09, 10.38, 12.09, 12.42, 13.47, 16.21, 16.80, 17.22, 18.00, 18.67, 19.39, 19.98, 20.17, 20.84, 21.41, 21.77, 22.02, 23.03, 23.32, 24.38, 24.94, 25.31, 25.55, 26.88, 27.15, 28.16, 28.58, 29.04, 29.59, 31.28, 31.97, 32.33, 32.74, 33.12, 33.45, 34.71, 35.20, 35.90, 36.47, 36.77, 37.25, 37.87, 38.22, 39.14, 39.39, 40.59, 40.99, 41.27, 42.02, 42.44, 43.59, 44.46, 45.08, 46.10, 46.56, 47.03, 47.33, 47.68 and 48.90 (+) 0.2° 2-theta. (Jetti *et al*. 2016)

2.5 Preparation of Crystalline Sofosbuvir

Crystalline form of Sofosbuvir characterized by data selected from one or more of the following: (i) an X-ray powder diffraction pattern having peaks at 12.4, 13.5, 16.2, 25.3, and 27.2 degrees two theta ± 0.2 degrees two theta; (ii) an X-ray powder diffraction pattern as depicted in Figure 8 and (iii) combinations of an X-ray powder diffraction pattern having peaks at 12.4, 13.5, 16.2, 25.3, and 27.2 degrees two theta ± 0.2 degrees two theta and an X-ray powder diffraction pattern as depicted in Figure 8; or characterized by data selected from one or more of the following: (iv) an X-ray powder diffraction pattern having peaks at: 12.4, 16.2, 17.2, 25.0 and 25.3 degrees two theta ± 0.1 degrees two theta; (v) an X-ray powder diffraction pattern as depicted in Figure 1 or Figure 7: (vi) an X-ray powder diffraction pattern having peaks at: 12.4, 16.2, 17.2, 25.0 and 25.3 degrees two theta ± 0.1 degrees two theta and absence of peaks at: 10.9 and 14.2 degrees two theta ± 0.2 degrees two theta, and combinations of (iv)-(vi). (Albrecht *et al*. 2015)

2.6 Pharmaceutical Composition Containing Sofosbuvir

This invention is a novel pharmaceutical composition comprising Sofosbuvir and ribavirin and at least one pharmaceutically acceptable excipient for use in the treatment of hepatitis C virus infections, chronic hepatitis C (CHC), hepatocellular carcinoma or patients with end-stage liver disease awaiting liver transplantation. A pharmaceutical composition comprising Sofosbuvir and

ribavirin and at least one pharmaceutically acceptable excipient. The pharmaceutical composition according to claim 1, wherein sofosbuvir in an amount of between 50 and 1500 mg and ribavirin in an amount of between 50 and 2000 mg. The pharmaceutical composition according to claim 2, wherein the weight ratio of Sofosbuvir to ribavirin is in the range of 1:1, 1:2, 2:1, 2:3, 1:3, 3:1, 3:2, 1:4, 4:1, 1:5, 5:1, 1:6 or 6:1 (w/w) and preferably it is in the range of 1:1 to 1:6 (w/w). The pharmaceutical composition, wherein said composition is administrated once a day or twice a day or three times a day and dosage regimen preferably is twice a day for 6 weeks to 52 weeks. (Cifter *et al*. 2015)

2.7 Process of preparing formulation of Sofosbuvir

Disclosed herein do a composition and unit dosage form for the treatment of hepatitis C virus (HCV) infection comprise GS-7977 and at least one pharmaceutically acceptable excipient, as well as methods for making said composition and unit dosage form. Also disclosed herein is a method of treating a subject, preferably a human, infected with hepatitis C virus, and said method comprising administering to the subject for a time period an effective amount of GS-7977 and an effective amount of ribavirin. In one aspect, the method comprises administering to the subject an interferon-free treatment regimen comprising an effective amount of GS-7977 and an effective amount of ribavirin. In a particular aspect, the method is sufficient to produce an undetectable amount of HCV RNA in the subject for at least 12 weeks after the end of the time period. (Darryl *et al*. 2013)

2.8 Preparation of Sofosbuvir Formulation Containing Different Weight

A pharmaceutical formulation containing the active ingredient Sofosbuvir, characterized in that the concentration of the active ingredient is 40% by weight or higher. The pharmaceutical formulation according to any one of the preceding claims, characterized in that it comprises other pharmaceutically acceptable materials, including 20 to 45% by weight of the filler, 5 to 12% by weight of the disintegrant, 4 to 15% by weight of a glidant and a lubricant. (Dohnal, 2016)

2.9 Method for Treating Hepatitis C

Disclosed are methods for treating hepatitis C in a human patient in need thereof that entails administering to the patient of about 350 mg of Sofosbuvir and another anti-HCV compound. (Yang, 2014)

2.10 Composition of Sofosbuvir Formulation

A solid composition comprising Sofosbuvir and at least one pharmaceutically acceptable matrix compound wherein at least 99 weight-% of the Sofosbuvir comprised in the composition are present in amorphous form, at least 99 weight-% of the solid composition consist of the Sofosbuvir and the at least one matrix compound, and wherein the solid composition contains the Sofosbuvir in an amount of at least 55 weight-% based on the combined weight of the Sofosbuvir and the at least one matrix compound. (Martin, 2015)

2.11 Preparation of Sofosbuvir with Specific Polymorphic Form

A crystalline form of Sofosbuvir having an X-ray powder diffraction pattern comprising no reflection at 2-theta angles in the range of from 2.0 to 7.8 when measured at a temperature of 15 to 25 °C with Cu-Kalpha1,2 radiation having a wavelength of 0.15419 nm°. (Martin, 2016)

2.12 Preparation of Sofosbuvir Salts

The present disclosure relates to processes for the preparation of Sofosbuvir or of its pharmaceutically acceptable salts. The present disclosure also provides intermediates useful in the synthesis of Sofosbuvir. (Kaushik *et al*. 2016)

2.13 Process of Preparing Stable Composition of Sofosbuvir

The present invention relates to stable pharmaceutical compositions of Sofosbuvir or a pharmaceutically acceptable salt thereof comprising at least one pharmaceutically acceptable excipient, in the form of immediate release tablets, to a process for the manufacture of said stable pharmaceutical compositions and to uniform pharmaceutical batches of said immediate release tablets. (Arroyo, 2016)

2.14 Modified Release Dosage Form of Sofosbuvir

This invention is a novel modified release pharmaceutical composition comprising Sofosbuvir and ribavirin and at least one pharmaceutically acceptable excipient for use in the treatment of hepatitis C virus infections, chronic hepatitis C (CHC), hepatocellular carcinoma or patients with end-stage liver disease awaiting liver transplantation. (Cifter *et al*. 2015)

2.15 Preparation of Novel Nucleotide Analogs

Aspects of the present application relate to novel nucleotide analogs, their use in the preparation of nucleoside phosphoramidates, (2R)-2-deoxy-2-fluoro-2-C- methyl-D-ribofuranose compounds, their use in the preparation of nucleoside phosphoramidates, stereoselective preparation of Sofosbuvir, crystalline polymorph, cocrystal of Sofosbuvir, processes for their preparation, amorphous solid dispersion of Sofosbuvir and processes for the preparation of amorphous Sofosbuvir. (Rao *et al*. 2016)

2.16 Preparation of Analog of Sofosbuvir

The present invention provides a novel process for preparation N-[(2,3,4,5,6-Pentafluorophenoxy)phenoxyphosphinyl]-L-alanine 1-methylethyl ester (formula 2) and resolving the formula 2 in the presence base to form N-[(S)-(2,3,4,5,6-Pentafluorophenoxy)phenoxyphosphinyl]-L-alanine 1-methylethyl ester (formula 2'). (Singh *et al*. 2016)

2.17 Pharmaceutical Composition of Sofosbuvir

Disclosed here are pharmaceutical compositions comprise Compound I, having the formula and an effective amount of Sofosbuvir wherein the Sofosbuvir is substantially crystalline. Also disclosed are methods of use for the pharmaceutical composition.(Gorman *et al*. 2015)

2.18 Method of Treating Hepatitis C

Disclosed are methods for treating hepatitis C in a human patient in need thereof that entails administering to the patient of about 350 mg of Sofosbuvir and another anti-HCV compound. (Yang. 2014)

2.19 Methods of Treating Hepatitis C with Sofosbuvir & Ledipasvir

Disclosed herein is a method of treating a subject infected with hepatitis C virus, said method comprising administering to the subject for a time period an effective amount of Sofosbuvir, an effective amount of ribavirin and an effective amount of ledipasvir. In one aspect, the method comprises administering to the subject an interferon-free treatment regimen comprising an effective amount of Sofosbuvir, an effective amount of ribavirin and an effective amount of ledipasvir. In a particular aspect, the method is sufficient to produce an undetectable amount of HCV RNA in the subject for at least 12 weeks after the end of the time period. (Ding *et al*. 2014)

2.20 Preparation of Crystalline Nucleoside Phosphoramidate

Provide new crystalline compounds containing nucleoside phosphoramidate and a cocrystal former. Particularly, the present invention relates to Sofosbuvir Piperazine cocrystals. (Gangavaram *et al*. 2016)

2.21 Therapeutic Combination of Sofosbuvir, Feldaprevir Ledipasvir and Ribavirin

The present invention relates to therapeutic combinations comprising faldaprevir, Sofosbuvir, ledipasvir and, optionally, ribavirin, and methods of using such therapeutic combinations for treating HCV infection in a patient. (Afdhal, 2015)

2.22 Method for Determination of Hepatitis B Virus (HCV)

Methods and compositions for the efficient and accurate determination of susceptibility of a hepatitis C virus (HCV) or HCV population to interferon (IFN), ribavirin (RBV), nucleoside inhibitor (NI), 2'C-methyl adenosine (2'CMeA), Sofosbuvir (SOF), or non-nucleoside inhibitor A or B (NNI-A or NNI-B) are provided. The methods may involve determining the genotype of the HCV or the phenotype of the HCV with respect to IFN, RBV, NI-1, 2'CMeA, SOF, NNI-A, or NNI-B susceptibility. The methods may further include the selection of a suitable treatment based on the genotype or phenotype determined. (Reevers, 2014)

2.23 Interferon Free Treatment of Hepatitis C

The present invention features interferon-free therapies for the treatment of HCV. Preferably, the treatment is over a shorter duration of treatment, such as no more than 12 weeks. In one aspect, the treatment comprises administering at least two direct acting antiviral agents and ribavirin to a subject with HCV infection, wherein the treatment lasts for 12 weeks and does not include administration of interferon, and said at least two direct acting antiviral agents comprise (a) Compound 1 or a pharmaceutically acceptable salt thereof and (b) Compound 2 or a pharmaceutically acceptable salt thereof. (Awni *et al*. 2016)

2.24 Process & Preparation of Intermediate Compounds of preparing Anti-viral Compounds

The invention is related to anti-viral compounds, compositions containing such compounds, and therapeutic methods that include the administration of such compounds, as well as to processes and intermediates useful for preparing such compounds. (Bacon *et al*. 2015)

2.25 Treatment of Hepatitis C with Specific Compound

The disclosure is related to compounds having a polycyclic core and at least one 2,6-dimethyltetrahydro-2H-pyran-4-yl, 4- methyltetrahydro-2H-pyran-4-yl, or tetrahydro-2H-pyran-3-yl capping group, which compounds are provided for use in pharmaceutical compositions and methods for treating hepatitis C (HCV). (Bacon *et al*. 2014)

2.26 Mechanism of Hepatitis C Virus

Hepatitis C virus (HCV) is the major causative agent of chronic non-A, non-B hepatitis. The life cycle of HCV is largely unknown because a reliable culture system has not yet been established. HCV presumably binds to specific receptor(s) and enters cells through endocytosis, as do other members of Flaviviridae. The viral genome is translated into a precursor polyprotein after uncoating, and viral RNA is synthesized by a virus-encoded polymerase complex. Progeny viral particles are released into the luminal side of the endoplasmic reticulum and secreted from the cell after passage through the Golgi apparatus. Understanding the mechanisms of HCV infection is essential to the development of effective new therapies for chronic HCV infection. Several host membrane proteins have been identified as receptor candidates for HCV. Recent advances using pseudotype virus systems have provided information surrounding the initial steps of HCV infection. An HCV RNA replicon system has been useful for elucidating the replication mechanism of HCV. In this review, we summarize our current understanding of the mechanisms of HCV infection and discuss potential antiviral strategies against HCV infection. (Moriishi *et al*. 2003)

Chapter Three

Materials & Methods

3.1 Formulation Part

3.1.1 Quality Target Product Profile

The proposed indication for Sofosbuvir is for use in combination with other medicinal products for the treatment of chronic hepatitis c (CHC) in adults. The intent is to develop a rapid onset therapy which will treat the chronic hepatitis c (CHC). The pharmaceutical target profile for Sofosbuvir is a safe efficacious convenient dosage form, preferably a tablet that will facilitate patient compliance. The tablet should be of an appropriate size, with a single tablet per dose. The manufacturing process for the tablet should be robust and reproducible, and should result in a product that meets the appropriate drug product critical quality attributes, for example identity, assay, appearance, dissolution as well as related substance and Uniformity of Dosage Unit. The drug product should be packaged in a container closure system that will provide adequate protection from moisture vapour, protection through distribution and use as well as convenience of use for the patient.

A Target Product Profile is presented in the **Table 1** below. From the profile, the initial Critical Quality Attributes which were used to define satisfactory quality were identified.

Table 1: Quality Target Product Profile (QTPP) for Sofosbuvir 400 mg Film Coated Tablets

QTPP Elements	Target	Justification
Dosage Form	Film Coated Tablet	Pharmaceutical equivalence requirement: same dosage form
Dosage Design	Immediate release film coated tablet	Immediate release design needed to meet label claims
Route of administration	Oral	Pharmaceutical equivalence requirement: same route of administration
Dosage Strength	400 mg	Pharmaceutical equivalence requirement: same strength
Pharmacokinetics	Immediate release enabling Tmax in 30 to 120 minutes	Bioequivalence requirement: Needed to ensure rapid onset and efficacy

Stability		At least 24 months shelf-life at room temperature	
Drug product quality attributes	Appearance	Acceptable for patient	Pharmaceutical equivalence requirement: Must meet the same compendia or other applicable (quality) standards (i.e., identity, assay, purity, and quality).
	Identification	Positive for Sofosbuvir	
	Assay	400 mg ± 10 %	
	Uniformity of dosage unit	Must meet the requirement	
	Dissolution	NLT 75 % (Q) dissolved in 30 minutes	
	Water Content	Suitable for product compressibility	
	Hardness	Suitable for product compressibility and stability	
Packaging System		Packaging system qualified as suitable for this drug product	Needed to achieve the target shelf life and to ensure tablet integrity during transportation.
Alternative methods of administration		none	

3.1.2 Critical Quality Attributes

Table 2 summarizes the quality attributes of generic Sofosbuvir tablets and indicates which attributes were classified as drug product critical quality attributes (CQAs). For this product, assay, uniformity of dosage unit and dissolution are identified as the subset of CQAs that have the potential to be impacted by the formulation and/or process variables and, therefore, will be investigated and discussed in detail in subsequent formulation and process development studies.

On the other hand, CQAs including identity, residual solvents and microbial limits which are unlikely to be impacted by formulation and/or process variables will not be discussed in detail in the pharmaceutical development report. However, these CQAs are still target elements of the QTPP and are ensured through a good pharmaceutical quality system and the control strategy.

Table 2. Critical Quality Attributes (CQAs) of Generic Sofosbuvir 400 mg Film Coated Tablets

Quality Attributes of the Drug Product		**Target**	**Is this a CQA?**	**Justification**
Physical Attributes	Appearance	Color and shape acceptable to the patient. No visual defects observed.	No	Color, shape and appearance are not directly linked to safety and efficacy. Therefore they are not critical. The target is set to ensure patient acceptability
	Odor	No unpleasant odor	No	In general, a noticeable odor is not directly linked to safety and efficacy, but odor can affect patient acceptability. For this product, neither the drug substance nor the excipients have any unpleasant odor.
	Size	Acceptable to patient	No	For comparable ease of swallowing as well as patient acceptance and compliance with treatment regimens, the target for tablet dimensions is set such as it will be acceptable for patient.
	Score configuration	One Score	No	Tablet will not have any deleterious effect upon on one score.
	Friability	NMT 1.0% w/w	No	Friability is a routine test as per compendial requirements for tablets. A target of NMT 1.0% w/w of mean weight loss assures a low impact on patient safety and efficacy and minimizes customer complaints.
Identification		Positive for Sofosbuvir	Yes	Though identification is critical for safety and efficacy, this CQA can be effectively controlled by the quality management system and will be monitored at drug product release. Formulation and process variables do not impact identity. Therefore, this CQA will not be discussed during formulation and process development.

Assay	(90 – 110)% w/w of label claim (400 mg)	Yes	Assay variability will affect safety and efficacy. Process variables may affect the assay of the drug product. Thus, assay will be evaluated throughout product and process development.
Uniformity of dosage unit	Must meet the requirement	Yes	Variability in uniformity of dosage unit will affect safety and efficacy. Both formulation and process variables impact uniformity of dosage unit, so this CQA will be evaluated throughout product and process development.
Dissolution	Not less than 75% (Q) dissolve in 30 minutes	Yes	Failure to meet the dissolution specification can impact bioavailability. Both formulation and process variables affect the dissolution profile. This CQA will be investigated throughout formulation and process development.
Water Content	Suitable for Compressibility	Yes	Generally, water content may affect degradation and microbial growth of the drug product and can be a potential CQA. In this case, Sofosbuvir is sensitive to hydrolysis and moisture will impact stability.
Microbial Limits	Must meet the requirement	No	Non-compliance with microbial limits will impact patient safety. However, in this case, the risk of microbial growth is very low. Therefore, this CQA will not be discussed in detail during formulation and process development.
Related Substance	Any other impurity: NMT 0.20 %	Yes	Degradation products can impact safety and must be controlled based on compendial/ICH requirements. Both

	Total impurities: NMT 1.00 %		formulation and process variables can impact degradation products. Therefore, degradation products will be assessed during product and process development.

3.1.3 Risk Assessment of Drug Substance Attributes

A risk assessment of the drug substance attributes was performed to evaluate the impact that each attribute could have on the drug product CQAs. The outcome of the assessment and the accompanying justification is provided as a summary in the pharmaceutical development report. The relative risk that each attribute presents was ranked as high, medium or low. The high risk attributes warranted further investigation whereas the low risk attributes required no further investigation. The medium risk is considered acceptable based on current knowledge. Further investigation for medium risk may be needed in order to reduce the risk. The same relative risk ranking system was used throughout pharmaceutical development and is summarized in Table 3.

Low	Broadly acceptable risk. No further investigation is needed.
Medium	Risk is acceptable. Further investigation may be needed in order to reduce the risk.
High	Risk is unacceptable. Further investigation is needed to reduce the risk.

Based upon the physicochemical and biological properties of the drug substance, the initial risk assessment of drug substance attributes on drug product CQAs is shown in Table 3.

Table 3. Initial risk assessment of the drug substance attributes

Drug Product CQAs	**Drug Substance Attributes**				
	Particle Size	**Hygroscopicity**	**Solubility**	**Moisture Content**	**Flow Properties**
Assay	Medium	Low	Low	Low	Medium
Uniformity of dosage unit	High	Low	Low	Low	High
Dissolution	High	Low	High	Low	Low
Related Substances	Low	Low	Low	High	Low

The justification for the assigned level of risk is provided in Table 4.

Table 4. Justification for the initial risk assessment of the drug substance attributes

Drug Substance Attributes	Drug Products CQAs	Justification
Particle Size	Assay	A small particle size adversely impact blend flowability. In extreme cases, poor flowability may cause an assay failure. The risk is medium.
	Uniformity of dosage unit	Particle size distribution has a direct impact on drug substance flowability and ultimately on uniformity of dosage unit. The risk is high.
	Dissolution	PSD have a great effect on dissolution. The risk is High.
	Related Substances	PSD has no such impact on Related Substance. The risk is low.
Hygroscopicity	Assay	Drug substance is non-hygroscopic. The risk is Low.
	Uniformity of dosage unit	
	Dissolution	
	Related Substances	
Solubility	Assay	Solubility does not affect tablet assay & uniformity of dosage unit. Thus, the risk is low.
	Uniformity of dosage unit	
	Dissolution	Solubility has significant impact on dissolution. The risk is High. The formulation and manufacturing process will be designed to mitigate this risk.
	Related Substances	Related Substance has no impact on Solubility. The risk is low.
Moisture Content	Assay	Moisture is controlled in the drug substance specification. Thus, it is unlikely to impact assay, uniformity of dosage unit and dissolution. The risk is
	Uniformity of dosage unit	

	Dissolution	low.
	Related Substances	Related Substance is dependent on Moisture Content. That is why the risk is high.
Flow Properties	Assay	The drug substance has poor flow properties. Flow does have some impact on assay. The risk is medium.
	Uniformity of dosage unit	Flow properties have impact on tablet weight uniformity. The risk is high which will be mitigated by manufacturing process. Therefore, the risk is High.
	Dissolution	The flowability of the drug substance is not related to its dissolution. Therefore, the risk is low.
	Related Substances	The flowability is not dependant on Related Substance. So the risk is low.

3.1.4 Excipients

The characterization of pharmaceutical excipients using a material science approach has helped to design drug formulations to obtain a desired set of performance properties. For tablets, a better understanding of the compression properties of the material alone and in combination with other potential components helps in developing desirable formulations as well as acceptable products. When formulating tablets, the choice of excipients is extremely critical. It must fulfill certain requirements such as compressibility, good binding functionality, flowability and acceptable moisture content. Moreover, it is essential to have a well designed particle size distribution for favorable mixing conditions with drug.

The excipients used in RLD are as follows: Mannitol, Microcrystalline Cellulose, Croscarmellose Sodium, Magnesium Stearate, Purified Talc, Colloidal Anhydrous Silica, Polyvinyl Alcohol Titanium Dioxide, Polyethylene Glycol, Talc, Ferric Oxide Yellow.

Only difference from RLD is Opadry II White for coating. A summary of the excipient-drug substance compatibility studies and the selection of each excipient grade are provided in the following section.

3.1.4.1 Excipient Compatibility Studies

Drug / Excipient compatibility was assessed through HPLC analysis of binary mixtures of drug to excipient, at a 1:1 ratio in the solid state, stored at 30°C/65% RH and 40°C/75% RH (open and closed conditions) for 1 month. No significant interaction was seen between Sofosbuvir and Excipients at 30°C/65% RH and 40°C/75% RH. Subsequent assurance of compatibility was provided by long-term stability data for formulations used in the pilot batch study and the ongoing prototype stability studies using the formulation proposed for commercialization. Common excipients functioning as filler, binder, disintegrant, Glidant and lubricant were evaluated in the excipient compatibility study. Table 5 summarizes the results.

Table 5. Excipient compatibility (binary mixtures) at 40°C/75% RH

Mixture	Assay (% w/w)	Degradants (% w/w)
Microcrystalline Cellulose/DS (1:1)	99.6	ND
Mannitol/DS (1:1)	98.5	ND
Croscarmellose Sodium/ DS (1:1)	98.9	ND
Colloidal Anhydrous Silica/DS (1:1)	99.4	ND
Magnesium Stearate/ DS (1:1)	99.1	ND

Table 6. Excipient compatibility (binary mixtures) at 30°C/65% RH

Mixture	Assay(% w/w)	Degradants (% w/w)
Microcrystalline Cellulose/DS (1:1)	99.9	ND
Mannitol/DS (1:1)	99.5	ND
Croscarmellose Sodium/ DS (1:1)	99.9	ND
Colloidal Anhydrous Silica/DS (1:1)	99.4	ND
Magnesium Stearate/ DS (1:1)	99.1	ND

ND: Not Detected

Loss in assay or detection of degradants indicative of an incompatibility was not observed for the selected excipients. No loss in assay was observed in any of these mixtures at 40 °C/75% RH or at 30 °C/65% RH. There is no incompatibility with the selected excipients with Sofosbuvir.

3.1.4.2 Excipient Grade Selection

Based on the results of excipient compatibility studies, the excipient types of the RLD formulation were selected for the generic product development. The selection of excipient grade and supplier was based on previous formulation experience and knowledge about excipients that have been used successfully in approved products manufactured by direct compression. The level of excipients used in the formulation was studied in subsequent formulation development studies.

Mannitol: Mannitol having a good flow property is used in many Direct Compression products. Here Sofosbuvir has a poor flow property. So the flow was improved by using Mannitol.

Microcrystalline Cellulose (MCC): Microcrystalline cellulose is widely used as filler for both direct compression and dry granulation process though it is reported in the literature that MCC may physically bind or adsorb drug substance, no such physical interaction was evident in the formulation dissolution studies. As the drug substance has poor flow property, it is improved by using MCC 200. For Direct Compression, MCC 200 was selected.

Croscarmellose Sodium: Croscarmellose Sodium is used as disintegrant. Disintegration occurs by rapid uptake of water followed by rapid and enormous swelling when it comes in contact with water.

Colloidal Anhydrous Silica: Colloidal Anhydrous Silica is widely used in pharmaceuticals. Its small particle size and large specific surface area give it desirable flow characteristics that are exploited to improve the flow properties of powders or granules in a number of processes such as tableting. It was selected both as intra granular and extra granular excipient.

Magnesium Stearate: It is the most commonly used lubricant for tablets. Magnesium stearate was selected as an extra granular excipient.

3.1.5 Drug Product

3.1.5.1 Formulation Development

The target product profile was to develop an immediate release tablet dosage form for oral dosing. The formulation should provide an acceptable tablet size which is easy to swallow. The manufacturing process must be robust and reproducible and convenient for large scale. The drug product will have to meet the critical quality attributes of identity, assay, appearance, dissolution and uniformity of dosage unit while also delivering suitable stability in order to not constrain commercialization in markets.

Identity – the API must be of the required chemical structure and solid state form in order to deliver the desired efficacy and safety profile (ICH Q6A).

Assay- is related to dose delivery to the patient, thus to efficacy and needs to comply with appropriate limits for drug content (ICH Q6A).

Appearance- the appearance of the tablets must be acceptable such that the patient will comply with the dosing regimen (ICH Q6A)

Dissolution –dissolution needs to comply with the requirement for an immediate release tablet as dictated by the target product profile. This requirement relates to efficacy of the product.

Uniformity weight - is related to consistency of the dose delivered to the patient, thus to efficacy and needs to comply with USP and BP acceptance criteria for Uniformity of Dosage Units.

3.1.5.1.1 Initial Risk Assessment of the Formulation Variables

The results of the initial risk assessment of the formulation variables are presented in Table 7 & 8 and the justification for the risk assignment is presented in Table 9.

Table 7: Initial risk assessment of the Formulation Variables (Core)

DP CQAs	Drug Substance	Mannitol	Microcrystalline Cellulose 200	Croscarmellose Sodium	Colloidal Anhydrous Silica	Magnesium Stearate
Assay	High	Medium	Medium	Low	Medium	Low
Uniformity of Dosage Unit	High	Medium	Medium	Low	Medium	Low
Dissolution	High	Medium	Medium	High	Low	High
Related Substances	High	Low	Low	Low	Low	Low

Table 8: Initial risk assessment of the Formulation variables (Coating Layer)

DP CQAs	Opadry II White
Assay	Low
Uniformity of Dosage Unit	Low
Dissolution	Medium
Related substances	Medium

Table 9: Justification for the initial risk assessment of the formulation variables (Core)

Formulation Variables	Drug Products CQAs	Justification
Drug Substance	Assay	See Justifications provided in Table 4.
	Uniformity of dosage unit	
	Dissolution	

	Related Substance	
Mannitol	Assay	Mannitol can impact the flow properties of the blend. This, in turn, can impact tablet uniformity of dosage unit. The risk is medium. Occasionally, poor Uniformity of dosage unit can also adversely impact assay. The risk is medium.
	Uniformity of dosage unit	
	Dissolution	Mannitol can impact dissolution via tablet hardness. However, hardness can be controlled during compression. The risk is medium.
	Related Substance	Mannitol is compatible with Sofosbuvir. So the risk is low.
Microcrystalline Cellulose	Assay	Microcrystalline Cellulose can impact the flow properties of the blend. This, in turn, can impact tablet uniformity of dosage unit. The risk is medium. Occasionally, poor Uniformity of dosage unit can also adversely impact assay. The risk is medium.
	Uniformity of dosage unit	
	Dissolution	Microcrystalline Cellulose can impact dissolution via tablet hardness. However, hardness can be controlled during compression. The risk is medium.
	Related Substance	Microcrystalline Cellulose is Compatible with Sofosbuvir. The risk is low.
Croscarmellose Sodium	Assay	Since the level of Croscarmellose Sodium used is low and its impact on flow is minimal, it is unlikely to impact assay and uniformity of dosage unit. The risk is low.
	Uniformity of dosage unit	
	Dissolution	Croscarmellose Sodium level can impact the disintegration time and, ultimately, dissolution. Since achieving rapid disintegration is important for a drug product, the risk is high.

	Related Substance	Croscarmellose Sodium is Compatible with Sofosbuvir. For this reason, the risk is low.
Colloidal Anhydrous Silica	Assay	Though the level of Colloidal Anhydrous Silica level is low but its impact on flow of the granules is high. So it is likely impact on assay and Content Uniformity. So the risk is medium.
	Uniformity of dosage unit	
	Dissolution	The CAS has some disintegrating property. But low level of CAS used in the formulation is not expected to impact disintegration time which in turns on dissolution. So The risk is low.
	Related Substance	Colloidal Anhydrous Silica is Compatible with Sofosbuvir study. So the risk is low.
Magnesium Stearate	Assay	Though the level of magnesium Stearate used is low and it can impact on flow and unlikely to impact assay and uniformity of dosage unit. The risk is low.
	Uniformity of dosage unit	
	Dissolution	Over-lubrication due to excessive lubricant may retard dissolution. The risk is high.
	Related Substance	Magnesium Stearate is Compatible with Sofosbuvir. So the risk is low.

3.1.5.1.2 Drug Substance Particle Size Selection for Product Development

Drug substance with slightly soluble in water and particle size in the micrometer range, a larger drug substance particle size improves manufacturability because it has better flow. For having better flow property for this Direct Compression product a fixed PSD was selected. With an aim to identify the appropriate drug substance particle size distribution range, three different particle sizes were selected for formulation development. Ultimately, the goal was to test the formulations to finalize the drug substance particle size for commercialization.

The particle sizes are D_{10} : 7.132 µm, D_{50} : 27.187 µm and D_{90} : 88.625 µm. When d90 is 88.625 µm, it displays a better flowability. Poor material flow may produce tablets with variable weight and content variability due to an uneven distribution of the drug substance in the blend, uneven bulk

density and, eventually, uneven filling of die cavities on the tablet press. Direct compression of the blend was then performed. The blend uniformity (BU) and the tablet Uniformity of dosage unit was good. Therefore, direct compression was considered as an acceptable process for this formulation.

3.1.5.1.3 Process Selection

Direct compression was then selected for the drug substance to improve the flow property of blend. By controlling the size distribution and flow properties of the granules, the risk of non-uniformity can be reduced. Thus, direct compression was selected as the process for drug product development.

3.1.5.1.4 Formulation Development Study

The initial prototype formulation component levels were selected based on preformulation study & prior manufacturing platform knowledge, the properties of Sofosbuvir and acceptable compatibility with Sofosbuvir. The prototype formulation has been utilized in other drug products and resulted in acceptable large scale manufacturing process attributes. Microcrystalline Cellulose 200 and Croscarmellose Sodium are among the commonly used ingredients for direct compression formulations, as they have good compression properties. Microcrystalline Cellulose 200 is proven to be stable, safe, physiologically & pharmacologically inert for human body. Microcrystalline Cellulose 200 revolutionized tableting technology because of its unique compressibility and carrying capacity. It exhibits excellent properties as an excipient for solid dosage forms. It compacts well under minimum compression pressures, has high binding capability, and creates tablets that have optimum hardness, stable, yet disintegrate rapidly. Other advantages include low friability and inherent lubricity. These properties make Microcrystalline Cellulose particularly valuable as a filler and binder for formulations.

The initial Magnesium Stearate level was selected based on knowledge of this formulation and levels required to produce acceptable ejection forces.

Table 10: Tentative Composition of Generic Sofosbuvir 400 mg Film Coated Tablet

Ingredient	Function	Composition
Sofosbuvir	Active	32%
Excipients		
Mannitol	Filler, Binder	25-30%
Microcrystalline Cellulose 200	Filler, Binder	30-35%
Croscarmellose Sodium	Disintegrant	8-12%
Colloidal Anhydrous Silica	Glidant	0.1-0.5%
Magnesium Stearate	Glidant	0.5-1%

A 5.0 kg batch was manufactured using the direct compression process. The granules were made using the formulation shown in the table. The granules were then split into Five sub-lots and different amounts of magnesium stearate were added according to the composition shown in the table 11 keeping the other excipients amount constant. The final blend was compressed into tablets. The experimental results for tablet appearance, tooling appearance and hardness at fixed compression force (5KN) are presented.

Table 11: Effect of extragranular lubricants on tablet appearance, tooling appearance and hardness

Batch No	**Mixture Components**	**Response**		
	Extragranular Magesium Stearate Level (%w/w)	**Tablet Appearence**	**Tooling Appearence**	**Tablet Hardness**
1	0.10	Poor	Visible Indication of sticking on punches and binding in the die	20.00
2	0.20	Poor		18.00
3	0.50	Poor		18.00
4	0.75	Acceptable	Shiny appearance with no evidence of picking and sticking	15.00
5	1.00	Acceptable		15.00

Tablet and tooling appearance

With lower concentration of Magnesium Stearate and talc level significant compression related issue such as tablet picking, sticking and side wall striation were observed. However at higher

concentration of lubricant, tablets were elegant in appearance and showed no evidence of sticking and binding to the tablet tooling.

Tablet hardness

The tablet hardness is the inversion factor of the lubricant level. The hardness is decreased with the increased level of lubricant and hardness is increased with the decreased level of lubricants.

Dissolution and Uniformity of Dosage Unit

Magnesium Stearate is hydrophobic in nature and retard the dissolution of the Sofosbuvir to some extent with increasing the amount. But all the five batches showed dissolution within the specified range. The entire five batches showed dosage uniformity had a % RSD less than 3%.Therefore Magnesium Stearate did not show any significant impact on the tablet dissolution and Uniformity of Dosage within the ranges studied.

3.1.5.1.5 Formulation Development Conclusion

The formulation composition was finalized on the Formulation Development studies. The excipients were finalized in the study, it was concluded that a minimum level of Magnesium Stearate is required in the formulation to prevent picking and sticking. The finalized formulation for Generic Sofosbuvir 10 mg film coated tablet is presented in the table 12.

Table 12: The Formulation of Sofosbuvir 400 mg Film Coated Tablet

Ingredient	Function	Composition	
		mg/tab	%w/w
Sofosbuvir	Active	400	31.373%
Excipients			
Mannitol	Filler, Binder	344.166	27.000%
Microcrystalline Cellulose 200	Filler, Binder	409.666	32.130%
Croscarmellose Sodium	Disintegrant	102.000	8.000%
Colloidal Anhydrous Silica	Glidant	6.416	0.500%
Magnesium Stearate	Glidant	12.750	1.000%

3.1.5.1.6 Updated Risk Assessment of the Formulation Variables

Table 13: Updated Risk Assessment of the Formulation Variables

DP CQAs	Drug Substance	Mannitol	Microcrystalline Cellulose	Croscarmellose Sodium	Colloidal Anhydrous Silica	Magnesium Stearate
Assay	Low*	Low*	Low*	Low	Low*	Low
Uniformity of Dosage Unit	Low*	Low*	Low*	Low	Low*	Low
Dissolution	Low*	Low*	Low*	Low*	Low	Low*
Related Substances	Low*	Low	Low	Low	Low	Low

Acceptable ranges for the high risk formulation variables have been established and are included in the control strategy. Based on the results of the formulation development studies, the risk assessments of the formulation variables were updated.

*The level of risk was reduced from the initial risk assessment

Table 14: Justification for the reduced risks of the formulation variables

Drug Substance Attributes	Drug Products CQAs	Justification
Sofosbuvir PSD	Assay	All tablets showed acceptable assay. The risk is reduced from High to low as Microcrysatlline Cellulose 200 was used in the formulation and flow of this excipient is good enough.
	Uniformity of Dosage Unit	The flow of the drug substance is improved more by using fillers that have good flow ability. The risk is reduced from high to low.
	Dissolution	The risk is reduced from high to low by controlling the drug substance PSD and optimizing the superdisintegrant.

Mannitol	Assay	As the level of Mannitol used is high and the flow is good as the grade is direct compression grade so the risk is reduced from medium to low in case of assay and uniformity of dosage.
	Uniformity of Dosage Unit	
	Dissolution	
Microcrystalline Cellulose Level	Assay	Microcrystalline Cellulose 200 grade is used which is having a very good flow and suitable for direct compression The risk is reduced from medium to low.
	Uniformity of Dosage Unit	
	Dissolution	The size of Microcrystalline Cellulose 200 is acceptable range during milling process which confirms maximum dissolution. The risk is reduced from high to low.
Croscarmellose Sodium Level	Dissolution	The Croscarmellose Sodium was used in an optimum concentration. The risk is reduced from high to low.
Colloidal Anhydrous Silica	Assay	The risk is reduced form medium to low by optimizing the quantity of Colloidal Anhydrous Silica.
	Uniformity of Dosage Unit	
Magnesium Stearate level	Dissolution.	The risk is reduced form high to low by optimizing the amount of Magnesium Stearate.

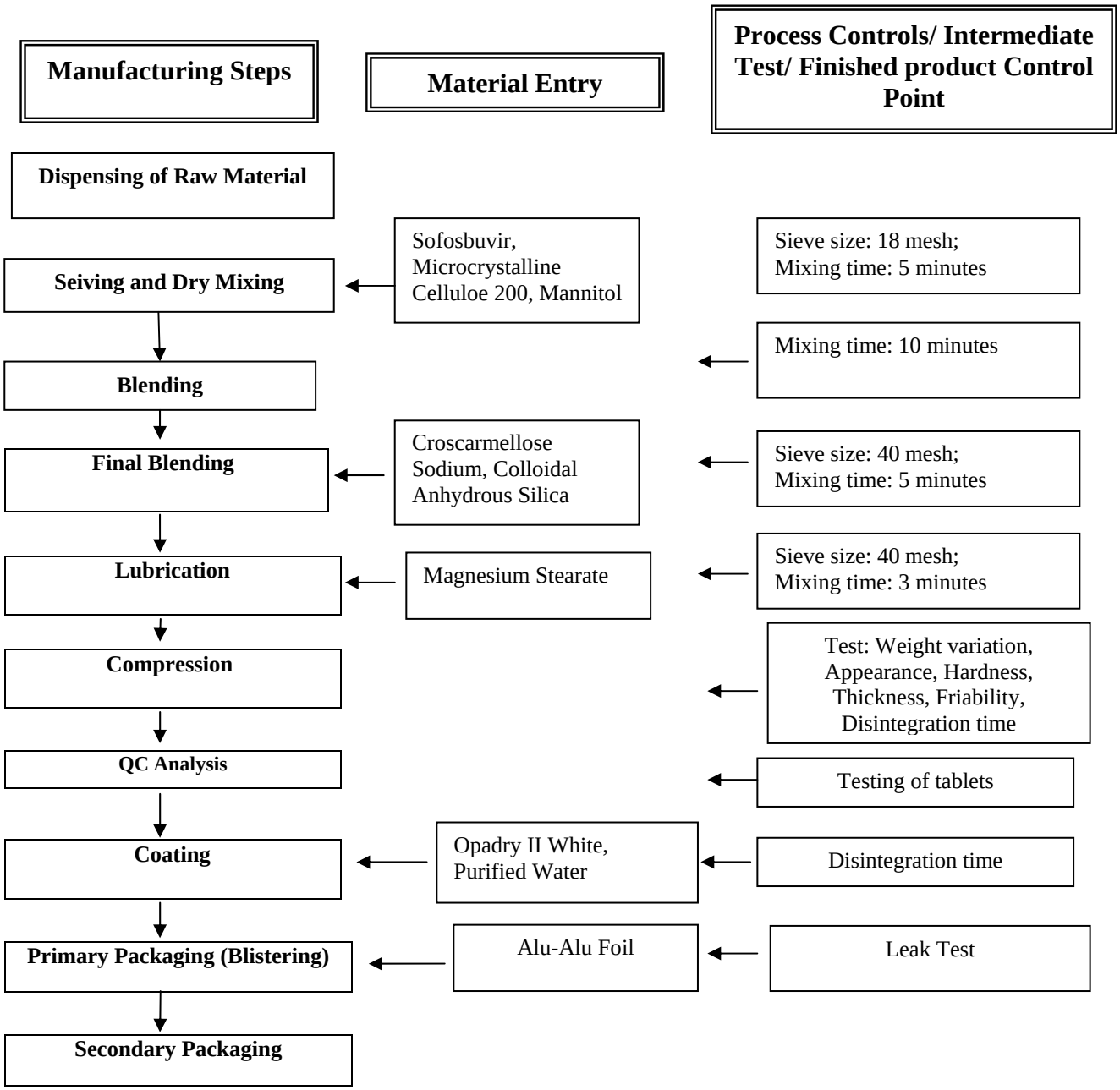
3.1.6 Overage

No overage of active has been given to the product.

3.1.7 Manufacturing process Development:

The formulation type chosen was an oral immediate release tablet, in consideration of the known pharmacokinetic characteristics of the molecule. The development of Sofosbuvir 400 mg tablet and the associated manufacturing process is used from prior knowledge of previous products and development projects. A direct compression process was chosen based on prior scientific knowledge of products with similar physical and chemical properties, and available technologies and equipment.

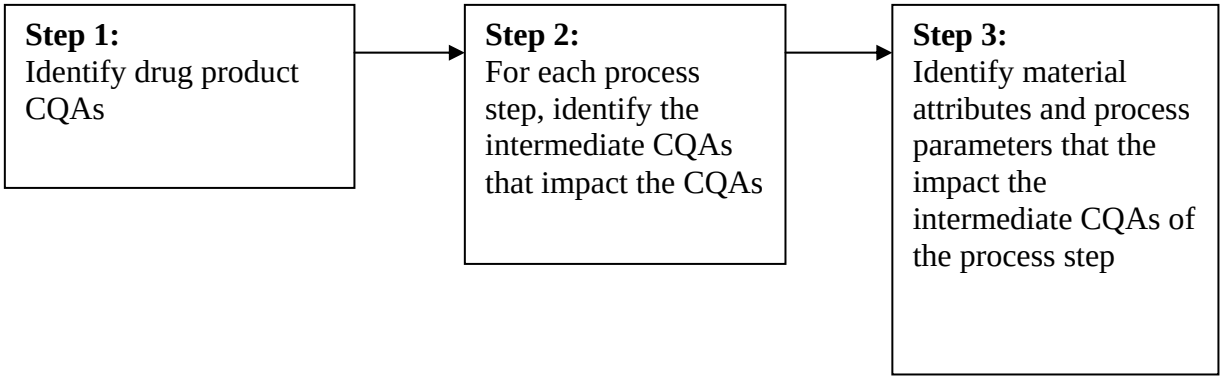
Figure 02: Flow Chart of Manufacturing Process



3.1.8 Initial Risk Assessment of the Drug Product Manufacturing Process

A risk assessment of the overall drug product manufacturing process was performed to identify the high risk steps that may affect the CQAs of the final drug product. Subsequently, the intermediate CQAs of the output material from each process step that impact the final drug product CQAs were identified. For each process step, a risk assessment was conducted to identify potentially high risk process variables which could impact the identified intermediate CQAs and, ultimately, the drug product CQAs. These variables were then investigated in order to better understand the manufacturing process and to develop a control strategy to reduce the risk of a failed batch. This method of identifying process variables for further study is illustrated in Figure 03 and is applied in each process step risk assessment.

Figure 03: Steps of Risk Assessment



The initial risk assessment of the overall manufacturing process is shown in Table 15 and justifications are provided in Table 16. Previous experience with these process steps was used to determine the degree of risk associated with each process step and its potential to impact the CQAs of the finished drug product.

Table 15: Initial risk assessment of the manufacturing process for Generic Sofosbuvir 400 mg tablet

Drug product CQAs	**Process Step**			
	Dry Mixing	**Final Blending and Lubrication**	**Compression**	**Coating**
Assay	Medium	Low	Medium	Low
Uniformity of Dosage Unit	High	Low	High	Low
Dissolution	Medium	High	High	Medium

Table 16: Justification for the initial risk assessment of the manufacturing process for the Generic Sofosbuvir 400 mg Film coated tablet

Drug Substance Attributes	**Drug Products CQAs**	**Justification**
Dry Mixing	Assay	Suboptimal dry mixing may cause variable flowability of the blend. The risk is medium.
	Uniformity of Dosage Unit	The PSD and cohesiveness of the drug substance adversely impact its flowability which, in turn, affects uniformity of dosage unit. The risk is high.
	Dissolution	Blending process variables may impact the distribution of actives in the blend which causes variation in dissolution result of the tablets. The risk is medium.

Final Blending and Lubrication	Assay	Lubrication has no impact on assay and Uniformity of Dosage Unit. So the risk is medium here.
	Uniformity of Dosage Unit	
	Dissolution	Over-lubrication due to excessive number of revolution may impact the disintegration and ultimate the dissolution of the tablets. The risk is high.
Compression	Assay	In extreme cases tablet weight variability can lead to out-of-specification assay results. The risk is medium.
	Uniformity of Dosage Unit	Compression process variable such as feed frame paddle speed and press speed can cause tablet weight variability which cause tablets to fall out of specification for uniformity of dosage unit. The risk is high.
	Dissolution	Compression Force directly impact disintegration as well as dissolution. High compression force can lead to high hardness of the tablet. So tablet disintegration time will be higher as well as dissolution will be less. The risk is high.
Coating	Assay	Coating operation has no such impact on Assay and Uniformity of Dosage Unit. It only gives a barrier to the core tablet. That is why the risk is low.
	Uniformity of Dosage Unit	
	Dissolution	If the percent weight gain of coating becomes higher then the rate of Dissolution is hampered. Dissolution rate can be decreased in this case. So, the risk is high.

3.1.8.1 Dry Mixing Process Development

Initial Risk Assessment of the Dry Mixing Process Variables

Table 17 presents the initial risk assessment for the Dry Mixing process step.

Table 17: Risk assessment of Dry Mixing variables

Process Step: Dry Mixing		
Output Material CQA: Blend Uniformity		
Variables	Risk Assessment	Justification and Initial Strategy
Input Material Attributes		
Sofosbuvir PSD	High	Sofosbuvir particle size has a great role in BU. The risk is high.
Sofosbuvir Flowability	High	Based on the preformulation study by identifying the bulk density and angle of repose it was found that the flow property is not good for Sofosbuvir which could impact the blend uniformity. The risk is high.
Diluent Flowability	Low	Although diluents Microcrystalline Cellulose (200) and Mannitol are used in large quantities both of them have good flowability. The risk is low.
Diluents PSD	High	Diluents (Microcrystalline Cellulose (200) and Mannitol) are used in large quantities and their PSD are likely to impact the BU.
Excipient Bulk Density **Process Step: Dry Mixing**	High	Diluent, Disintegrant and Lubricant used occupied large portion of the total batch size. Thus excipients bulk density is likely to affect the BU. The risk is high.
Output Material CQA: Blend Uniformity		
Variables	**Risk Assessment**	**Justification and Initial Strategy**
Process Parameter		
Blender Type	Low	Different blender types have different mixing dynamics. Double Cone Blender was selected based on the equipment availability. The risk is low. However, if

		blender type is changed during scale up the risk should be re-evaluated.
Order of addition	Medium	Order of addition may impact the ease of evenly dispersing ingredients charged in lower quantities. Materials were added in following order. 1) Sofosbuvir 2) Microsrystalline Cellulose (200) 3) Mannitol
Blender fill level	High	The mixer fill level depends on equipment capacity, blend bulk density and batch size. Since the blender fill level may affect mixing dynamics, the risk is high
Rotation Speed	Medium	Rotation speed is often fixed by equipment constraint. Different blenders have different rotation speeds. The risk is medium.

Summary of Dry Mixing Process Development

Based on the results of the dry mixing studies, the process parameter was optimized. The number of revolutions needed to achieve blend uniformity differed depending on the drugs particle size distribution. Within the range of 35-75%, the Blender fill level did not adversely impact blend uniformity.

Updated Risk Assessment of the Dry Mixing Process Variables

Table 18 presents the risk reduction for the Dry Mixing process as a result of the development studies. Only the process variables that were initially identified as high risk to the blend uniformity are shown.

Table 18: Updated Risk Assessment of the Dry Mixing Variables

Process Step: Dry Mixing		
Output Material CQA:	**Blend Uniformity**	
Variables	Risk Assessment	Justification of the reduced risk
Sofosbuvir PSD & Flowability	Low	The effect of particle size of both active and excipients has been mitigated by dry granulation method and the fill volume was kept 50% of the capacity. The risk is made low.
Excipients PSD & Flowability	Low	
Number of Revolution	Low	
Blender Fill level	Low	

3.1.8.2 Blending and Lubrication Process Development

Initial Risk Assessment of the Final Blending and Lubrication Process Variables

The initial risk assessment of the overall manufacturing process presented in Table 18 identified the risk of the final blending and lubrication step to impact tablet dissolution as high. The lubrication process variables that could potentially impact tablet dissolution were identified and their associated risk was evaluated. Table 19 presents the initial risk assessment of the final blending and lubrication step.

Table 19: Initial risk assessment of the final blending and lubrication

Process Step: Final Blending and Lubrication **Output material CQA: Tablet Dissolution**		
Variables	Risk Assessment	Justification and Initial strategy
Powder uniformity	Medium	The powder uniformity has impact on the dissolution. The risk is medium.
Powder Flowability	Low	The powder flowability should not impact on the dissolution of the tablet. The risk is low.
Powder Size distribution	High	The variability in the powder size distribution observed after dry mixing process showed impact of the dissolution. The risk is High.
Powder Bulk Density	Low	The Powder bulk density has little impact on tablet Dissolution. The risk is low.
Lubrication Variable		
Blender Type	Low	Due to differences in the operating principle, different types of blenders may impact blending efficiency. Based on the availability, Double Cone Blender is selected. The risk is medium. However if the blender type is changed during scale-up or commercialization, the risk should be re-evaluated.
Order of addition	Low	Powder and other excipients except the Magnesium Stearate and Colloidal Anhydrous Silica are blended together first followed by addition of Magnesium Stearate and Colloidal Anhydrous Silica. Order of addition is fixed and has a minimal impact on dissolution. The risk is low.

Rotation Speed	Medium	Rotation speed is fixed by equipment constraint. Different size blenders have different rotation speed. The rotation speed for the Double Cone Blender was fixed to 20. The risk to impact tablet dissolution is medium.
Number of revolution	High	Over Lubrication may result in retarded disintegration and dissolution. The risk is high.
Blender fill level	Medium	Blender fill level may affect mixing dynamics. It is fixed for these development studies. But could not change upon scale up. The risk is medium

A study was performed to investigate the effect of Magnesium Stearate specific surface area and number of revolutions during lubrication on tablet hardness, disintegration, and dissolution. For this study, a 4.00 kg blend was manufactured in a pilot scale blender (50 L). It was subdivided into five batches 800 g each. For each batch, the mixture and Colloidal Anhydrous Silica were blended for 100 revolutions in a double cone blender at 10 rpm prior to lubrication with Magnesium Stearate. Then, Magnesium Stearate was added and blended according to the experimental design as shown in Table 20. After lubrication, samples were pulled from the 3 locations to verify blend uniformity. The lubricated blend was then compressed using 10 kN of force to manufacture tablets. Ejection force was monitored. Compressed tablets were checked for appearance and the tablet press tooling (punches and dies) was evaluated for evidence of picking/sticking and binding. Additionally, tablets were subjected to friability, assay and uniformity of dosage testing.

Table 20: Effect of Extragranular Magnesium Stearate

Batch no.	Factors : Process Variables	Responses		
	N_{rev} Lubrication time	Hardness (kp)	Disintegration Time	Dissolution
1	16 (2 Minutes)	16.6	1.2 minutes	99%
2	16 (3 Minutes)	14.7	1.6 minutes	99%
3	16 (5 Minutes)	12.5	2.0 Minutes	97%
4	16 (10 Minutes)	10.4	2.2 minutes	95%
5	16 (12 Minutes)	8.2	2.5 minutes	92%

Form this study it was shown that tablet hardness is slightly decreased with the increase of lubrication time which in turn impact the disintegration time (increase with the increase of lubrication time). The dissolution of Sofosbuvir is slightly decreased with the increase of lubrication time.

Updated Risk Assessment of Final Blending and lubrication Process Variables.

Table 21 presents the risk reduction for the final blending and lubrication step as a result of the development studies. Only the process variables that were initially identified as high risk to the dissolution of the final drug product are shown.

Table 21: Updated risk assessment of the final blending and lubrication process variables

Process Step: Final Blending and Lubrication		
Output Material CQA: Tablet Dissolution		
Magnesium Stearate Specific Surface area	Low	Within the range 5.8 -10.4 m^2 Magnesium Stearate specific surface area does not adversely impact tablet dissolution. The risk is reduced form high to low and this material attribute will be controls in the control strategy.
Number of revolutions	Low	A proven acceptable range for a number of revolutions (30-60) was established for this scale based on elegant tablet appearance and rapid dissolution. The risk is reduced form high to low and number of revolution is controls in the control strategy.

3.1.8.3 Tablet Compression Process Development

Initial Risk Assessment of the Tablet Compression Process Variables

Based on the initial risk assessment of the overall manufacturing process shown in Table 18, the risk of the compression step to impact content uniformity and dissolution of the tablets was identified as high. Process variables that could potentially impact these two drug product CQAs were identified and their associated risk was evaluated. The results of the initial risk assessment of the compression process variables are summarized in Table 22.

Table 22: Initial risk assessment of the tablet compression process variable

Process Step: Tablet Compression Drug product CQAs: Uniformity of dosage unit, Dissolution			
Variables	Drug products CQAs	Risk Assessment	Justification and Initial Strategy
Blend Assay	Uniformity of dosage unit	Low	The blend assay varied between 97.57% and 101.52% during the lubrication process development. This low variability is unlikely to impact uniformity of dosage unit and dissolution. The risk is low.
	Dissolution	Low	
Blend uniformity	Uniformity of dosage unit	Low	The lubricated blend demonstrated acceptable BU during the lubrication process development. Therefore, the risk is low.
	Dissolution	Low	
Powder Size Distribution	Uniformity of dosage unit	High	The Powder demonstrated good flowability. But PSD of the different powders were different which might impact Uniformity of dosage unit. The risk is high.
	Dissolution	Medium	The formulation contains 8 % CCS and the variability in powder size distribution observed during showed no impact on

			dissolution. The risk is medium.
Blend Flowability	Uniformity of dosage unit	High	Blend flowability could impact powder flow from the hopper to the feed frame and, ultimately, to the die cavity. The risk is high.
	Dissolution	High	
Blend Compressibility and Compactibility	Uniformity of dosage unit	Low	Uniformity of dosage unit is unaffected by the blend compressibility and compactability. The risk is low.
	Dissolution	High	Suboptimal blend compressibility and compactability can affect tablet hardness. The Compressibility and compactability of the blend are directly related to the powder size. The variables may vary from batch-to-batch and may cause tablet hardness variation if the compression force is not adjusted. This may, in turn, impact dissolution. The risk is high.
Blend Bulk Density	Uniformity of dosage unit	High	The variability of Bulk Density has significant impact on Uniformity of dosage unit and dissolution. The risk is high.
	Dissolution	High	
Compression Variables			
Press Type and number of station used	Uniformity of dosage unit	Low	The risk is low here as it is not dependent much on it.
	Dissolution	Low	
Tooling Design	Uniformity of dosage unit	Low	Tooling design was selected to compress a tablet with appropriate size and shape. No picking was observed during the final blending and lubrication studies. The risk is low.
	Dissolution	Low	

Compression Force	Uniformity of dosage unit	Low	Uniformity of dosage unit is dominated by BU and flowability and is unrelated to main compression force. The risk is low.
	Dissolution	High	Suboptimal compression force may affect tablet hardness and friability and, ultimately, dissolution. The risk is high.
Press speed (dwell Time)	Uniformity of dosage unit	High	A faster than optimal press speed may cause inconsistent die filling and weight variability which may then impact uniformity of dosage unit and dissolution. For efficiency, the press speed will be set as fast as practically possible without adversely impacting tablet quality. The risk is high.
	Dissolution	High	
Compression run time	Uniformity of dosage unit	Medium	It is possible during long compression run times that the uniformity of content may drift. The risk is medium.
	Dissolution	Low	It is unlikely for compression run time to cause a drift that leads to a dissolution failure. The risk is low

Summary of the Tablet Compression process Development

A press speed in the range of 20- 60 rpm did not show any significant impact on the responses investigated. An acceptable range for compression force was identified. Force adjustments can be made to accommodate the acceptable variation in tablet hardness between batches.

Updated Risk Assessment of the tablet compression variables

The risks identified during the initial assessment of the compression step were reduced through development studies. The updated risk assessment is presented in Table 28

Table 23: Updated risk assessment of the tablet compression Variable

Process Step: Tablet Compression			
Drug product CQAs: Uniformity of the dosage unit, Dissolution			
Variables	Drug Products CQAs	Risk Assessment	Justification of the reduced risk
Blend compressibility and compactibility	Dissolution	Low	Compression force can be adjusted in order to achieve the target tablet hardness. The risk is reduced from high to low.
Main Compression Force	Dissolution	Low	Tablet hardness increases with compression force. Within the compression force range studied, the resulting tablet hardness did not adversely affect dissolution and > 75% dissolution at 30 min was achieved. The risk is reduced from high to low.
Press Speed(dwell time)	Uniformity of Dosage Unit	Low	A press speed of 20-60 rpm had no impact on uniformity of dosage unit or dissolution. Thus, the risk is reduced from high to low.
	Dissolution	Low	

3.1.8.4 Updated Risk Assessment of the Drug Product Manufacturing Process

During process development, the identified high risks for each process step were addressed. Experimental studies were defined and executed in order to establish additional scientific knowledge and understanding, to allow appropriate controls to be developed and implemented, and to reduce the risk to an acceptable level. After detailed experimentation, the initial manufacturing process risk assessment was updated in line

with the current process understanding. Table 24 presents how the application of the control strategy to the manufacturing process has reduced the identified risks. Table 25 provides the justification for the reduced risk following process development.

Table 24: Updated risk assessment of the manufacturing process for Generic Sofosbuvir 400 mg

Drug product CQAs	Process Step			
	Dry Mixing	Final Blending and Lubrication	Compression	Coating
Assay	Low	Low	Low	Low
Uniformity of Dosage Unit	Low	Low	Low	Low
Dissolution	Low	Low	Low	Low

Table 25: Justification for the updated risk assessment of the manufacturing process for Generic Sofosbuvir 400 mg

Process Step	**Drug Product CQAs**	**Justification for the reduced risk**
Dry Mixing	Assay	All development batches and the exhibit batch achieved acceptable assay, uniformity of dosage unit and dissolution. The risk is reduced from high to low for uniformity of dosage unit and from medium to low for assay and dissolution.
	Uniformity of Dosage Unit	
	Dissolution	
Final Blending and Lubrication	Dissolution	Within the range studied, number of revolutions and Magnesium Stearate specific surface area did not exhibit a significant impact on disintegration or dissolution of the tablets. The risk is reduced from high to low.

Compression	Assay	The development studies demonstrated that flowability of blend is such that it does not significantly impact the tablet weight variability, assay or uniformity of dosage unit. The risk is reduced from high to low for uniformity of dosage unit and from medium to low for assay.
	Content uniformity	
	Dissolution	The desired tablet hardness (12.0-20.0 kP) can be achieved by adjusting the compression force. The risk is reduced from high to low.

3.1.9 Coating Material and Coating Process Development:

The core tablet was coated with suitable coating agent. The coating materials is listed below

Table 26: List of Coating Materials

Name of the Materials	**Function**
1) Opadry II White	Film forming mix
2) Purified Water	Solvent

The reason behind the selection of the purified water is the suitability of coating material dispersion preparation. The optimization of the inprocess coating parameter is based on the following factors:

1) The batch size of the product
2) Knowledge about the coating related problem and remedial action like chipping, twinning, blistering etc.

The in-process coating parameters are listed below
(Name of the Coating Machine: Eco Coater)

Table 27: In process coating parameter

Parameter	Standard
Inlet Temperature	40-60^{o}C
Bed Temperature	35-45^{o}C
Pan RPM	6-10
Spray Air Pressure	4-5 bar

3.1.10 Experimental Batch:

An experimental batch at lab scale was done to check the 6 months stability studies based on the above studies.

Table 28: Formula

Sl. No	Raw Materials	Percentage (%)	Qty/unit (mg)
1	Sofosbuvir	31.373	400.000
2	Mannitol	27.000	344.250
3	Microcrystalline Cellulose (200)	32.127	409.625
4	Croscarmellose Sodium	8.000	102.000
5	Magnesium Stearate	1.000	12.750
6	Colloidal Anhydrous Silica	0.500	6.375

3.1.11 Container Closure System

The proposed generic drug product Sofosbuvir 400 mg is intended to be labeled for storage at dry place and keep away from light. The tablets are packaged with Alu-Alu Blister Foil. Each paper carton contains 1 Alu-Alu strip of 6 tablets. Packaging details are summarized in the table.

Table 29:

Item no	Description	Specification
1	Printed Aluminium Foil (TOP)	Hard tempered, heat sealable printed Aluminium foil with thickness 20 micron
2	Alu-Alu Bottom Foil	Alu-Alu Bottom Foil having thickness of 164 mm.
3	Printed Paper Carton	Dimension 29.5 x 22.5 x 16.5 mm3, 300 GSM Swedish Board, Matt Lamination.

3.1.12 Microbiological Attributes

An accelerated stability study of the exhibit batch demonstrated that the drug product has low water activity and is not capable of supporting microbial growth. Routine microbiological testing of Generic Sofosbuvir 400 mg film coated tablet is unnecessary due to the low water activity of the product and controls on incoming raw materials.

3.1.13 Compatibility

This section is not applicable because the drug product is a solid oral dosage form and there are no reconstitution diluents.

3.2 Analytical Part:

3.2.1 Methodology:

Description

Off white Caplet biconvex film coated tablet, One score on one side

Identification

The retention time of the major peak in the chromatogram of the sample solution corresponds to that in the chromatogram of the standard solution, as obtained in the Assay.

Average weight

Record average weight of at least 20 tablets by using specified balance and check the result against the specification.

Uniformity of weight

Record individual weight of tablets by using specified balance and check the result against the specification.

Water Content

Collect approximately 1 g of sample. Crush the sample with mortar and pastle. Measure moisture content (% w/w) with KF titrator taking approximately (70 - 100) mg of crushed sample.

Length X Width

Measure the Length X Width of tablets with slide calipers and confirm the result against the specification.

Thickness

Measure the Thickness of tablets with slide calipers and confirm the result against the specification.

Hardness

Place the tablet between the jaws (where applicable, consider the shape, break-line and the inscription). For each measurement, orient the tablet in the same way with respect to the direction of application of the force. Record the result and check against the specification.

Disintegration Time:

Determine the disintegration time (DT) of 6 tablets with disintegration tester with disc maintaining the temperature of specific medium at 35^0 C to 39^0 C and confirm the result against the specification.

Dissolution:

Reagents

- Orthophosphoric acid (AR grade)
- Sodium hydroxide (AR grade)
- Ethanol (HPLC Grade)
- Potassium dihydrogen orthophosphate (AR grade)
- Water (Milli-Q, Millipore)

Dissolution Parameter

Apparatus	: 2 (paddle)
RPM	: 75
Temperature	: 37^0C
Time	: 30 minutes
Medium	: 0.05 M Phosphate Buffer (pH – 6.8)

Diluent: 0.05 M Phosphate buffer, pH 6.8

Preparation of 0.05 M Phosphate buffer,pH 6.8

Dissolve 6.8 g of potassium dihydrogen orthophosphate in 1000mL of water, add 0.896g of sodium hydroxide, mix properly and, if necessary, adjust the adjust the pH 6.8 using either 1M sodium hydroxide or conc. orthophosphoric acid.

Dissolution Procedure

Place dissolution medium upto 900mL in the dissolution vessel. Assemble the apparatus and warm the media to $37^0C \pm 0.5^0C$. Weigh and place one tablet in each vessel, immerse into the media. The distance between the paddle and the bottom of the vessel to be 2.5 ± 0.2 cm and operate the apparatus at 75 RPM. After 30 minutes withdraw 25 mL of solution & filter through Whatman no.1 or equivalent grade filter paper.

Sample Solution

Dilute 5 mL of the filtrate obtained from dissolution procedure to 100 mL with dissolution media and mix well.

Preparation of Standard_1 (a)

Weigh accurately about 22 mg Sofosbuvir WS into a 50 mL volumetric flask. Add 2 mL of ethanol, hand shake for 5 minutes. Make up the volume with the 0.05 M Phosphate buffer, pH 6.8 and Sonicate for 5 minutes. Filter through Whatman No 1 or equivalent grade filter paper.

Further dilute 5 mL of this solution to 100 mL with buffer solution and mix well to have a concentration of 0.022 mg/mL.

Preparation of Standard_2 (a)

Weigh accurately about 22 mg Sofosbuvir WS into a 50 mL volumetric flask. Add 2 mL of ethanol, hand shake for 5 minutes. Make up the volume with the 0.05 M Phosphate buffer, pH 6.8 and Sonicate for 5 minutes. Filter through Whatman No 1 or equivalent grade filter paper.

Further dilute 5 mL of this solution to 100 mL with buffer solution and mix well to have a concentration of 0.022 mg/mL.

Calculate similarity factor using following formula:

$$\frac{\text{Average Absorbance of Sofosbuvir WS Obtained with standard solution \_1(a)}}{\text{Absorbance of Sofosbuvir WS Obtained with standard solution \_2(a)}} \times \frac{\text{Weight of Sofosbuvir WS in standard solution \_2(a) in mg}}{\text{Weight of Sofosbuvir WS in standard solution \_1(a) in mg}}$$

Note: If the similarity factor does not fall within 0.98 to 1.02, prepare fresh solution preparation in duplicate, re-measure the absorbance. If the similarity factor falls within the limit and continue the sequence for standard and sample.

Procedure

Measure the absorbance of the standard and sample solution at 260 nm using the dissolution media as blank.

Calculation:

$$\frac{\text{Dilution x Wt. of std (mg) x Abs. of smp. x Potency of std (\%) as it is x Av. tab. Wt (mg)}}{\text{Tablet wt. (mg) x Dilution x Abs. of std x Claim (mg)}}$$

= % of Sofosbuvir dissolved.

Assay:

0.1% Phosphoric acid buffer solution

Dissolve 1 mL of Phosphoric acid into 1000 mL of Water, volume upto the mark with water.

Mobile phase-A: Prepare a filtered and degassed mixture of 0.1% Phosphoric acid buffer solution

Mobile phase-B: Acetonitrile (Mark)

Diluent: Mixture the water and Acetonitrile in the ratio of 50:50 (v/v)

Chromatogrphic Condition

Column : C-18, 4.6 x 250 mm, 5µ [Phenomenex Luna is suitable]
Flow rate : 1.0 mL per minute,
Detector : 260 nm,
Injection volume : 10 µL,
Column temperature: Ambient
Run time : 18 minutes

Table 30: Gradient program

Time (min)	Mobile phase-A (%)	Mobile phase-B (%)
0	70	30
10	50	50
12	50	50
13	70	30
18	70	30

Stock Standard Solution

Transfer 80.0 mg of Sofosbuvir WS into a 100-mL volumetric flask, add 100 mL of Diluent, sonicate for 8 minutes, cool down to room temperature, volume up to the mark with Diluent and mix well.

Standard Solution

Dilute 10.0 mL of Sofosbuvir Stock Standard Solution to 25 mL with Diluent and mix well. Pass through a filter having a nominal pore size not greater than 0.45 µm, discarding the first 5 mL of filtrate and place the rest of the filtrate in an HPLC vial.

[Prepare standard solution in duplicate and designate as Standard solution _1 (a) and Standard solution _2 (a)]

Calculate similarity factor using following formula:

$$\frac{\text{Average peak area response of Sofosbuvir Obtained with standard solution \_1(a)}}{\text{Peak area of Sofosbuvir Obtained with standard solution \_2(a)}} \times \frac{\text{Weight of Sofosbuvir WS in standard solution \_2(a) in mg}}{\text{Weight of Sofosbuvir WS in standard solution \_1(a) in mg}}$$

Note: If the similarity factor does not fall within 0.98 to 1.02, prepare fresh solution preparation in duplicate, re-inject in single injection and calculate Similarity factor again as above. If the similarity factor falls within the limit, inject the re-prepared solution in replicate and continue the sequence for standard and sample.

Preparation of Sample Solution [For Blend Sample analysis]

Weigh and powder not less than 20 tablets, take about 1327 mg of powder (equivalent to 400 mg Sofosbuvir) into a 250-mL volumetric flask, add 150 mL of Diluent, shake by mechanical shaker with intermittent hand shaking for one hour. Volume up to the mark with the diluent and sonicate for 15 minutes, cool down to room temperature.Centrifuse a certain portion of mixture at 4000 rpm for 10 minutes.

Dilute 5.0 mL of filtrate of Stock Sample Solution to 25 mL with Diluent and mix well to have a concentration of 0.32 mg/mL of Sofosbuvir. Filter through a syringe filter.

Preparation of Sample Solution [For Finished product analysis]

Weigh 4 tablets and transfer (equivalent to 1600 mg Sofosbuvir) into a 1000-mL volumetric flask, add 350 mL of Diluent, shake by mechanical shaker with intermittent hand shaking for one hour. Volume up to the mark with the diluent and sonicate for 1 hour, cool down to room temperature.Centrifuse a certain portion of mixture at 4000 rpm for 10 minutes.

Dilute 5.0 mL of filtrate of Stock Sample Solution to 25 mL with Diluent and mix well to have a concentration of 0.32 mg/mL of Sofosbuvir. Filter through a syringe filter.

Table 31: Sequence of injection

Sample Name	No. of injection	Injection volume
Standard solution _1 (a)	6	10 µL
Standard solution _2 (a)	1	10 µL
Assay Sample	1	10 µL
Bracketing Std	1	10 µL

Calculation of Assay

Blend Sample analysis:

Calculate the quantity Sofosbuvir in mg per tablet by using the following equation:

$$= \frac{As}{Astd} \times \frac{Wstd \times 10\,mL}{100\,mL \times 25\,mL} \times \frac{250\,mL \times 25\,mL}{Ws \times 5\,mL} \times \frac{P}{100} \times Wa$$

Finished product analysis:

Calculate the quantity Sofosbuvir in mg per tablet by using the following equation:

$$= \frac{As}{Astd} \times \frac{Wstd \times 10\ mL}{100\ mL \times 25\ mL} \times \frac{1000\ mL \times 25\ mL}{Ws \times 5\ mL} \times \frac{P}{100} \times Wa$$

Where,

As = peak area of sample solution,

A_{std}= peak area of standard solution,

W_{std}= weight of Sofosbuvir WS (mg),

Ws = weight of sample (mg),

P = potency of standard as Sofosbuvir (%),

Wa = average weight of tablets (mg)

System suitability requirements:

The test is not valid unless,

1. The relative standard deviation for the peak area response of Sofosbuvir for replicate injections of Standard Preparation is not more than 2.00% respectively.
2. The similarity factor between Standard solution _1 (a) and Standard solution _2 (a) (separately prepared and injected) is 0.98 to 1.02.
3. Column efficiency: NLT 2000 theoretical plates, for each analyte in Standard solution.
4. Tailing factor: NMT 2.00 for the Sofosbuvir peak, for each analyte standard solution.
5. The relative standard deviation for the peak area response of Sofosbuvir and retention time of standard for bracketing standard injections, including replicate injections of standard preparation is not more than 2.00%

Chapter Four

Results & Discussion

4.1 Stability Study Results

Table 32: Stability Study Results in three different conditions for six months with initial condition and Limit

Storage period	Storage condition	Appearance	Hardness (Kp)	Water Content (%)	LOD (%)	DT (Minutes)	Dissolution (%)	Assay (mg /tab)
Initial Result	-	White to off white caplet deep biconvex tablet, with one score on one side	30 - 41.1	2.24	2.23	1.25 – 2.00	92 - 95	408.91
One Months 06.08.15	25°C/ 60%RH	White to off white caplet deep biconvex tablet, with one score on one side	30.2-39.2	3.01	2.81	1.00-1.50	99 - 102	403.04
	30°C/ 65%RH	White to off white caplet deep biconvex tablet, with one score on one side	28.8-39.7	3.24	3.35	1.00-1.67	82 - 93	404.53
	40°C/ 75%RH	White to off white caplet deep biconvex tablet, with one score on one side	32.6-38.0	3.14	2.88	0.67-1.00	88 - 94	403.54
Three Months 05.10.15	25°C/ 60%RH	White to off white caplet deep biconvex tablet, with one score on one side	30.9-41.2	3.07	2.99	1.00-2.00	95-101	400.81
	30°C/ 65%RH	White to off white caplet deep biconvex tablet, with one score on one side	29.4-45.5	3.48	2.94	1.00-1.50	93-100	388.96
	40°C/ 75%RH	White to off white caplet deep biconvex tablet, with one score on one side	29.9-35.0	2.31	2.58	0.83-1.17	94-100	390.70
Six Months 03.01.16	25°C/ 60%RH	White to off white caplet deep biconvex tablet, with one score on one side	30.2-41.1	2.76	2.56	1.18-2.17	96-101	397.22
	30°C/ 65%RH	White to off white caplet deep biconvex tablet, with one score on one side	29.0-33.1	2.88	2.90	1.50-2.68	96-99	396.88
	40°C/ 75%RH	White to off white caplet deep biconvex tablet, with one score on one side	29.2-38.4	2.53	2.46	1.42-3.12	97-102	398.12

Limit	-	White to off white caplet deep biconvex tablet, with one score on one side	20.0-50.0	1.00-4.00	1.00-4.00	NMT 30 minutes (BP)	NLT 75%(Q) in 45 minutes	360 - 440

4.2 Discussion:

Appearance: The limit for appearance was set "White to off white caplet deep biconvex tablet, with one score on one side". Almost in all the stability conditions, the appearance was found within limit and it was acceptable throughout the stability period.

Hardness: This parameter is checked to see if there are any changes in hardness due to absorbing moisture during stability period. For this product, Hardness was found within limit in all the conditions: 28.8 Kp – 45.5 Kp (Limit: 20.0 Kp – 50.0 Kp).

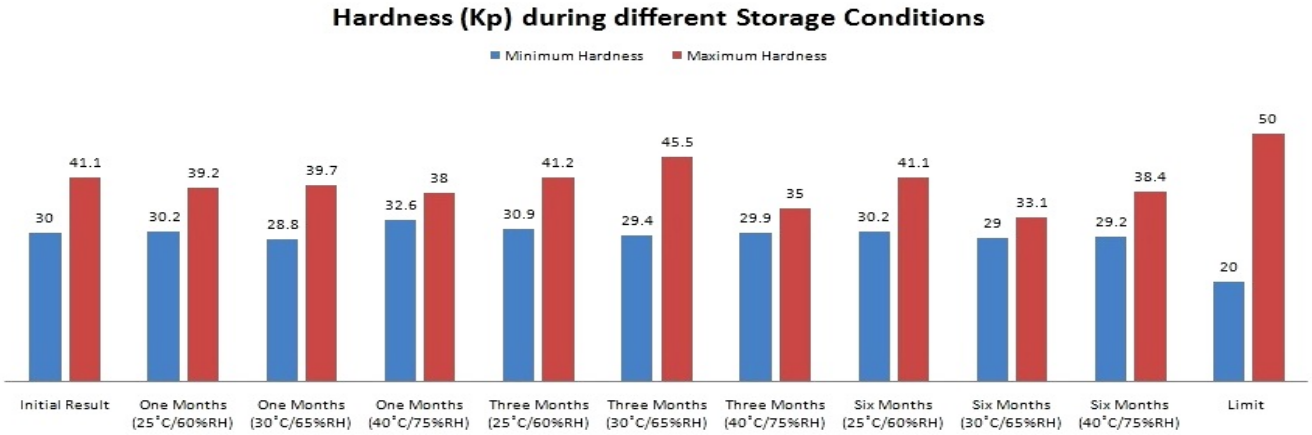


Figure 4: Hardness (Kp) during different Storage Conditions

Water Content: Water content increase can increase the chance of microbial growth. So this parameter is very much important for any formulation. For this product, water content that is KF is found within limit in all stability condition: 2.24 % – 3.48 % (Limit: 1.00 % - 4.00 %).

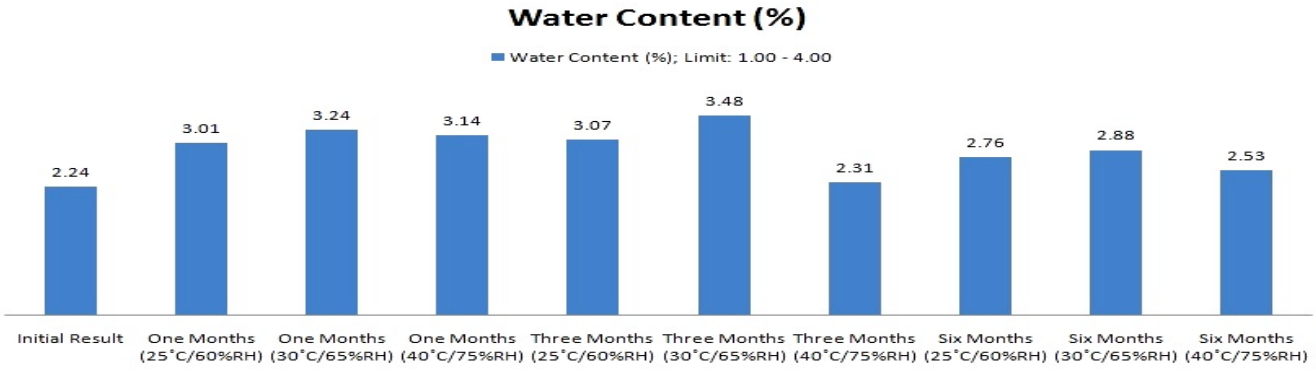


Figure 5: Water Content change in different Storage Conditions

LOD: Loss on Drying (LOD) also has the same role as Water Content. It plays a key role in Microbial Growth. LOD was also found within limit for this formulation: 2.23 % – 3.35 % (Limit: 1.00 % - 4.00 %).

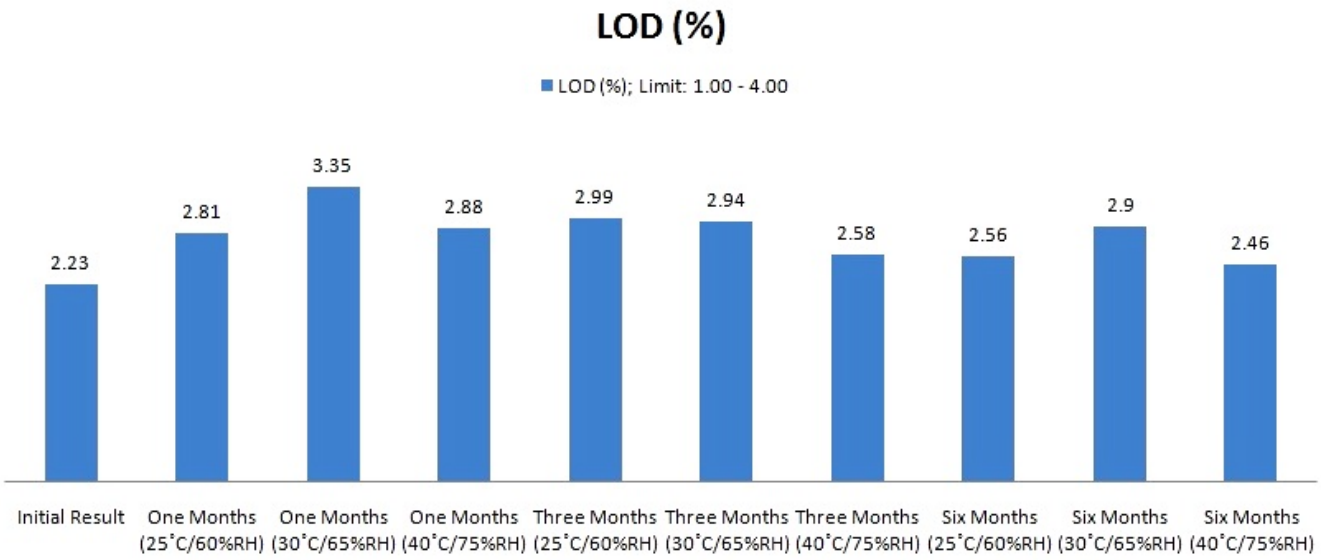


Figure 6: LOD change in different Storage Conditions

Disintegration Time (DT): DT supports Dissolution. Higher the DT lower the Dissolution. Here, DT found within acceptable limit: 0.67 minutes - 3.12 minutes (Limit: NMT 30 minutes).

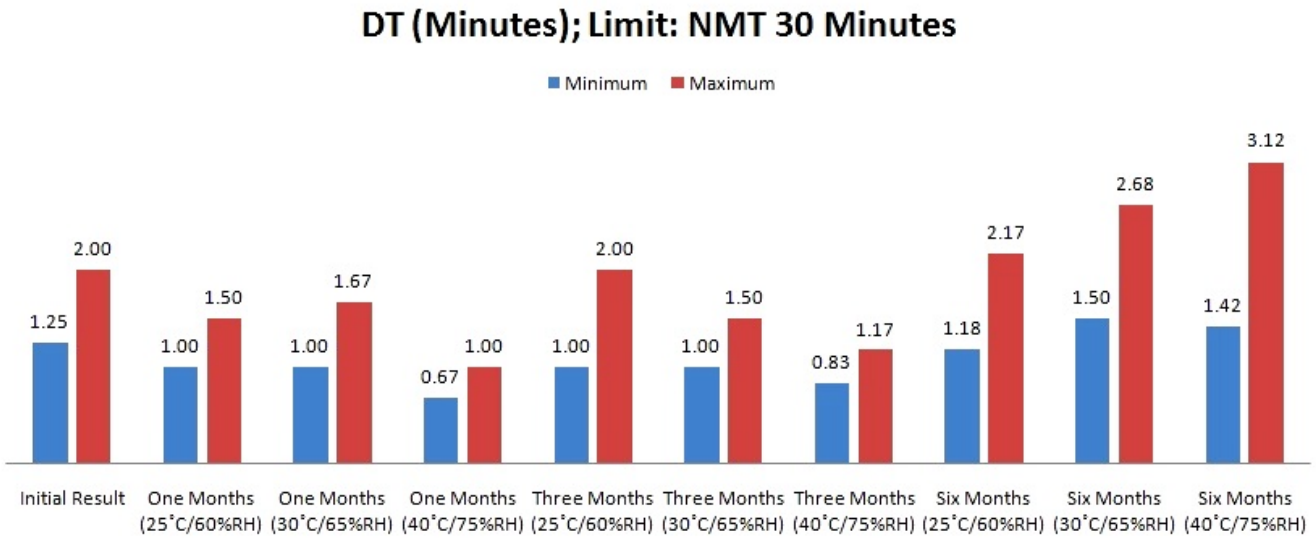


Figure 7: DT change in different Storage Conditions

Dissolution: The rate of drug release is known as Dissolution. It is very much critical factor for proper action of a drug and for Bioavailability. For this product, Dissolution was found absolutely alright throughout the stability Condition: 82 % - 102 % (Limit: NLT 75% (Q) in 45 minutes).

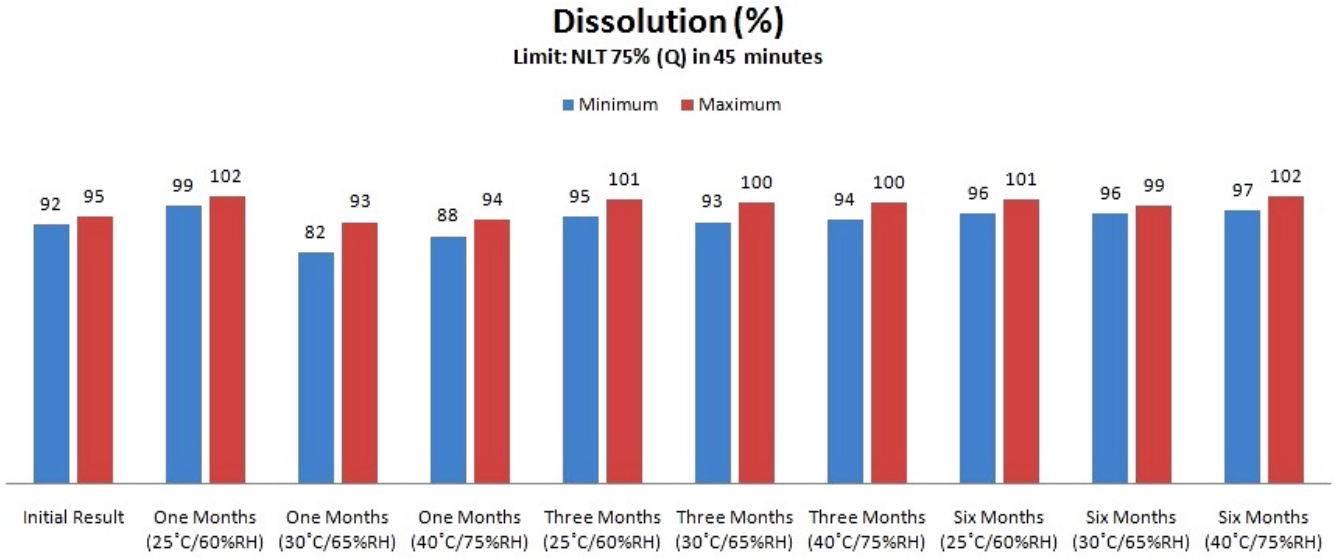


Figure 8: Dissolution range in different Storage Conditions

Assay: The content of drug per tablet is known as Assay or Potency of that particular drug. For this formulation Assay was found within range in all conditions: Assay 388.96 mg – 408.91 mg (Limit: 360.00 mg – 440 mg).

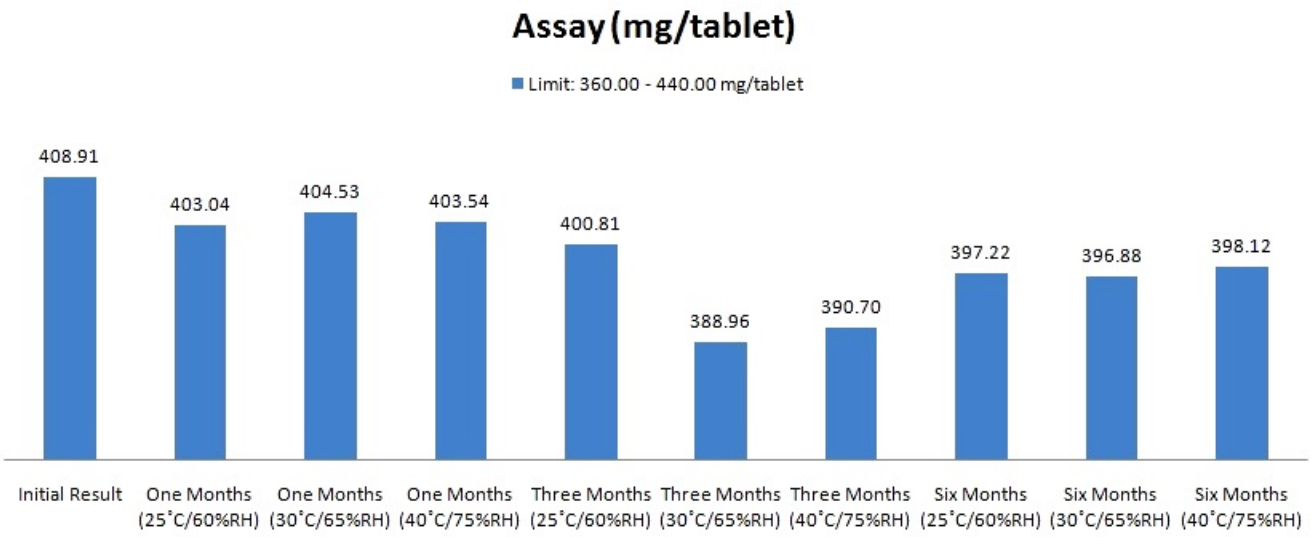


Figure 9: Assay variation in different Storage Conditions

4.3 Batch formula of the Sofosbuvir 400 mg Film Coated Tablet

The manufacturing formula for Sofosbuvir 400 mg Film Coated Tablet (Batch Size: 0.012 MU) is presented in the table 33

Table 33: Ingredients and Quantity per Batch

Name of the Ingredients	**Unit**	**Quantity Per Batch** **(Batch Size: 0.012 MU, Batch Weight: 15.300 kg)**
Active		
Sofosbuvir	Kg	4.800
Excipients		
Mannitol	Kg	4.130
Microcrystalline Cellulose 200	Kg	4.916
Croscarmellose Sodium	kg	1.224
Colloidal Anhydrous Silica	Kg	0.077

Magnesium Stearate	Kg	0.153
Coating Materials		
Opadry II White	Kg	0.780

Chapter Five

Conclusion

5.0 Conclusion:

As the six months stability study result gave satisfactory results for every parameter, we can conclude with that this product can be manufactured in large scale and can be supplied in the market. And our aim for developing a quality based product of Sofosbuvir is successful now. But with time as new drugs for treatment of Hepatitis C are being discovered day by day like, Sofosbuvir 400 mg in combination with Ledipasvir 90 mg Tablet, Daclatasvir 30 mg & 60 mg Tablet, Velpatasvir Tablet etc further works can be done with these molecules for better treatment. With the improvement of drug design and discovery, new drug molecules are synthesized with time being. Further works can be done with these new molecules and create new scope for treatment of patients.

Chapter Six

References

Afdhal Nezam H. 2015. Combination therapy for Treating HCV infection. WO/2015/166071A1.

Albrecht W., Aronhime J., Charasia B. 2012. Solid State Form of sofosbuvir. WO/2015/191945A2.

Arroyo H. 2015. Stable Pharmaceutical Composition of Sofosbuvir. WO/2015/132321A1.

Awni W. Bernstein B. 2016. Methods for Treating HCV. US20160317603.

Bacon Elizabeth M., Cottell Jeromg J., Link J. 2014. Antiviral Componds. WO/2014/100500A1.

Bacon Elizabeth M., Cottell Jeromg J., Link J. 2015. Antiviral Componds. 9079887.

Barth R., Rox A C., Olgan S. Synthetic Routes to Sofosbuvir, 44 PP 51-88.

Cifter U., Erdem Y., Ucar E. 2015. Modified Release Pharmaceutical Compositions of Sofosbuvir and Ribavirin. EP2959901A1.

Cifter U. Turkyilmaz A. Erdem Y. 2017. Pharmaceutical Combinations of Sofosbuvir and Ribavirin. EP2959891A1.

Darryl G., Charles J. Reynolds Miriam Michelle B. Gilead Pharmasset Llc, 2013.

Dailymed.com. [Online] https://dailymed.nlm.nih.gov/dailymed/drugInfo.cfm. Accessed on: 20.01.2017.

Drugs.com. [Online] https://www.drugs.com/. Accessed on: 20.01.2017

Ding X., Hyland Robert H., Pang P. 2014. Methods for Treating Hepatitis C Virus Infection. US20140249101.

Dohnal J. 2016. A Pharmaceutical Formulation of Sofosbuvir. WO/2016/206663A1.

Dosage form, 2013 [Online] http://medical-dictionary.thefreedictionary.com/dosageform. Accessed on : 21.01.17.

Gangavaram R., Mamilla B., Subbu R. 2016. Solid forms of nucleoside phosphoramidate. WO/2016/189443A2.

Gorman E., Mogalian E. Oliyai R. 2015. Combination Formulation of Two Antiviral Compounds. US20150064253.

Hellard M., Alisa P., Nick S. Targeted Direct-acting antiviral treatment for chronic hepatitis C: A financial reality on an obstacle to elimination, 2016.

ICH Q6A Guideline.

Jetti Ramakoteswara R., Gohanta A., Beeravelly S. Bhagava T. 2016. Polymorphic Forms of Sofosbuvir. WO/2016/038542A2.

Kaushik Vipin K. Ravi Vijaya K. Vahiti S, 2016. Process for the preparation of Sofosbuvir. WO2015097605A1.

Kaushik Vipin K. Ravi Vijaya K. Vahiti S, 2016. Process for the preparation of Sofosbuvir. US20160318966.

Martin N. 2015. Solid Composition comprising Sofosbuvir. WO/2015/150561A3.

Martin N. 2015. A Crystalline Form of Sofosbuvir. WO/2016/023906A1.

Moriishi K., Matssura Y. Mechanism of Hepatitis C Infection, 2003 Nov;14(6):285-97.

Pharmainfo.net, 2014 http //www.pharmainfo.net/reviews.

Rao P., Oroganti S., Sud A. 2016. Novel neucleotide analogs, process for the preparation of Sofosbuvir and its analog, novel forms of Sofosbuvir and Solid dispersion of Sofosbuvir. WO/2016/035006A1.

Reeves Jacqueline D. 2014. Methods for determining viral sensitivity to viral inhibitors. WO/2014/146048A2.

Singh G. Mechare K., Gulabra M. 2016. A process for the preparation of Sofosbuvir intermediataes and its polymprph. WO/2016/181313A1.

Sovaldi Assessment Report, EMA, 2013.

Yang Cheng Y. 2014. Hepatitis C treatments with Sofosbuvir. WO/2014/185995A1.

Yang Cheng Y. 2014.Hepatitis C treatment. US20140343008