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FORMULATION DEVELOPMENT AND EVALUATION OF BILAYER TABLETS OF SOFOSBUVIR FOR EFFECTIVE TREATMENT OF CHRONIC HEPATITIS C USING NATURAL POLYMERS

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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). The purpose of this work is to develop bilayer tablets of Sofosbuvir by using natural polymers, 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 lifesaving drug was very much necessary for this disease. Hence a new approach is tried that gives one immediate release dose and a sustained release dose in single dosage form call bilayer tablets. Immediate release layer delivers the initial dose, it contains superdisintegrant which increase drug release rate whereas sustained release layer gastro retentive by using natural polymers and releases drug at sustained manner for prolonged period. A direct compression method was used to formulate 8 batches. Superdisintegrants like sodium starch glycolate, croscarmellose sodium, crospovidone sodium was used for immediate release layer and xanthan gum, gaur gum, karaya gum, PVP K30 like natural polymers were used in gastro retentive sustained release layer. The powders were evaluated for their flow properties and the finished tablets were evaluated for their physical parameters. A simple visible spectrophotometric method was employed for the estimation of Sofosbuvir at 264 nm and Beer's law is obeyed in the concentration range of 5-25 µg/ml. The prepared gastro retentive tablets were evaluated for general appearance, thickness and diameter, drug content, hardness, friability, uniformity of weight and in vitro dissolution studies. Successful formulation was developed having disintegration Time 100±4 sec and drug release was sustained up to 12 hrs. A biphasic drug release can be obtained by using bilayer tabletting technology which involved compression of immediate and sustained release layer together. Bilayered floating tablets with release characteristics offer critical advantages such as, site specificity with improved absorption and efficacy. This technology can be inculcated to various medicaments which have stomach as the major site of absorption.

Keywords: Bilayer floating tablets, Sofosbuvir, Biphasic drug release, Bioavailability, Xanthan gum, Gaur gum, Karaya gum

INTRODUCTION:

The new drug delivery system with better efficacy and safety with reduced dosing frequency and improved patient compliance is the current area of research by formulation development scientists. Tablet being most preferred dosage form for its ease of manufacturing and patient convenience is always a first

choice of dosage form. The single layer tablets lead to frequent dosing and unpredicted drug plasma level for drugs with shorter half-life [1, 2]. Number of diseases require immediate release of drug for instant effect to manage the panic attack at its presentation and then drug concentration has to be requirement for such a disease conditions, the multilayered tablet concept has been utilized [3]. Such a tablet has a fast releasing layer and may contain bi- or triple layers to sustain the drug release. Bilayer tablets present a better choice where one layer provide immediate dose which is then maintains the plasma drug level by its controlled release layer of the tablet. One of the novel approaches in the area of oral sustained release drug delivery is gastro retentive drug delivery system [4]. GRDDS can be retained in the stomach and assist in improving the oral sustained delivery of drugs that have an absorption window in a particular region of the gastrointestinal tract. These systems help in continuously releasing the drug before it reaches the absorption window, thus ensuring optimal bioavailability [5]. Sofosbuvir is chemically known as (S)-Isopropyl 2-((S)-(((2R, 3R, 4R, 5R)-5-(2, 4-dioxo-3, 4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl) methoxy)-(phenoxy) phosphoryl amino) propanoate. It has a molecular formula of $C_{22}H_{29}FN_3O_9P$ and a molecular weight of 529.45. Sofosbuvir is a white to off-white powder with a solubility of ≥ 2 mg/mL across the pH range of 2-7.7 at 37°C. The partition coefficient (log P) for Sofosbuvir is 1.62 and the pKa is 9.3 [6]. Sofosbuvir is a pangenotypic inhibitor of the HCV NS5B RNA-dependent RNA polymerase, which is essential for viral replication [7]. Sofosbuvir is a nucleotide prodrug that undergoes intracellular activation to form GS-461203 (active triphosphate, not detected in plasma), and ultimately the inactive, renally eliminated metabolite GS-331007 [8]. The pharmacologically active uridine analog triphosphate (GS-461203) can be incorporated by HCV NS5B and acts as a chain terminator. In a biochemical assay, GS-461203 inhibits the polymerase activity of the recombinant NS5B from HCV genotype 1b, 2a, 3a and 4a with an IC50 value ranging from 0.7 to 2.6 μm. GS-461203 is neither an inhibitor of human DNA and RNA polymerases nor an inhibitor of mitochondrial RNA polymerase [9]. In recent years natural polymers have been widely used because of their effectiveness, toxic nature, easy availability, biocompatible and cheap over synthetic polymers. Natural polysaccharides and dried mucilage have been reported as an emulsifying, suspending agent, binding agent, disintegrating agent, and as a sustained-release matrix. They have been utilized in variety of formulations like mucoadhesive, gastro retentive, colon specific drug delivery system etc., [10-12]. Guar gum is obtained from endospermic seeds of *Cyamopsis tetragonolobus* belonging to family Leguminosae. Guar gum occurs as nearly odorless, white to yellowish-white powder with a bland taste. Chemically guar gum is polysaccharides composed of galactose and mannose. It is made up of a linear chain of β-D-mannopyranose joined by β-(1–4) linkage with α-D-galactopyranosyl units attached by 1, 6- links. Karaya gum is dried gummy exudates of *Sterculia urens* belonging to family Sterculiaceae. It

is branched heteropolysaccharides consist of D-galactouronic acid and D-glucoronic acid. It is used as thickening agent in pharmaceutical preparations. Xanthan gum is a high molecular weight extra cellular polysaccharide produced by the fermentation of the gram-negative bacterium *Xanthomonas compestris*. The primary structure of this naturally produced cellulose derivative contains a cellulosic backbone (β-D-glucose residues) and a trisaccharide side chain of β-D-mannose-β-D-glucuronicacid-α-D-mannose attached with alternate glucose residues of the main chain. Xanthan gum showed a higher ability to retard the drug release. It is also used as a thickening agent [13]. Hepatitis C virus (HCV) infection is a major cause of end-stage liver disease and hepatocellular carcinoma (HCC). There have been rapid advances in treatment with the development of oral direct-acting antivirals (DAAs) such as sofosbuvir (NS5B nucleotide analogue), daclatasvir and ledipasvir (NS5A inhibitors). Sofosbuvir (SFB) is an oral nucleotide analogue inhibitor of non-structural 5B polymerase that has been approved for treatment of hepatitis C virus genotypes 1 to 4 (HCV). It is a pro nucleotide analogue, prodrug metabolized to the active antiviral agent 2'-deoxy-2'-α-fluoro-β-*C*-methyluridine-5'-triphosphate. The triphosphate serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis. The active substance in sofosbuvir, blocks the action of an enzyme called 'NS5B RNA-dependent RNA polymerase' in the hepatitis C virus, which is essential for the virus to multiply. This stops the hepatitis C virus from multiplying and infecting new cells. NS5B is one of the non-structural proteins essential for viral RNA replication, and has been found to be a valuable target for directly acting antiviral agents (DAAs) [14]. In the present study, a bilayer tablet for bimodal drug release in which one layer of immediate release and second layer of sustained release of sofosbuvir was designed by using natural polymers.

EXPERIMENTAL

Materials and methods

Materials

Sofosbuvir was obtained as free gift sample from M/s Hetero Drugs Pvt. Ltd. Hyderabad, xanthan gum, gaur gum; karaya gum was procured from local market of Bhopal, MP, India. PVP K30, sodium starch glycolate, croscarmellose sodium, crospovidone was obtained from Mapromax, Life sciences Pvt. Ltd. Dehradun. Microcrystalline cellulose, magnesium stearate and talc were obtained from Loba Chemical Pvt Ltd (Mumbai, India). Hydrochloric acid was obtained from S. D. Fine Chem. Ltd., Mumbai. All other chemical were purchased from Hi Media, Mumbai. Double distilled water was prepared freshly and used

whenever required. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

Procedure for the determination of λ max

The λmax of Sofosbuvir was determined by analyzing the drug solution in double beam ultraviolet spectrophotometer. Accurately weighed 10 mg of drug was dissolved in 10 ml of 7.2 pH buffer solution in 10 ml of volumetric flask. The resulted solution was 1000µg/ml of strength and from this solution 1 ml solution was pipette out and transfer into 10 ml capacity of volumetric flask and volume was made upto 10 ml with 1.2 pH solution. This solution was scan at wavelength 400-200 nm on UV spectrophotometer. The higher absorption peak was obtained at 264 nm which was the λmax of drug.

Formulation of immediate release (IR) layer

Fast dissolving tablets of Sofosbuvir were prepared by direct compression method after incorporating different super disintegrates such as, croscarmellose sodium (Ac-Di-Sol), crospovidone and sodium starch glycolate in different concentrations. The ingredients given below were weighed and mixed in geometric progression in a dry and clean mortar. Then the ingredients were passed through mesh #60. Magnesium stearate as lubricant and talc as glidant were added in a final step and mixed, this blend was subjected to analysis of pre-compression parameters which included angle of repose, bulk density, tap density, carr's index and hausner's ratio. The Blend was compressed on 8 mm (diameter) fat punches on a 'Rimek mini press 16 station rotary compression machine. Nine formulations of sofosbuvir granules were prepared and each formulation contained one of the three disintegrate in different concentration. Each tablets weighing 150mg, were obtained. Composition of tablets is mentioned in Table 1.

Table 1 Composition of Sofosbuvir Fast Dissolving Tablets

Ingredients(mg)	Formulation code								
	IF1	IF 2	IF 3	IF 4	IF 5	IF 6	IF 7	IF 8	IF 9
Sofosbuvir	100	100	100	100	100	100	100	100	100
Sodium starch glycolate	10	15	20	-	-	-	-	-	-
Croscarmellose sodium	-	-	-	10	15	20	-	-	-
Crospovidone	-	-	-	-	-	-	10	15	20
Microcrystalline cellulose	25	20	15	25	20	15	25	20	15
Talc	5	5	5	5	5	5	5	5	5
Magnesium stearate	10	10	10	10	10	10	10	10	10
Total weight	150	150	150	150	150	150	150	150	150

Method for preparation of sofosbuvir gastroretentive tablets

Direct compression was followed to manufacture the gastroretentive tablets of sofosbuvir. Eight different formulations (F1, F2, F3, F4, F5, F6, F7, & F8) were prepared by direct compression. All the polymers selected, drug and excipients were passed through sieve no. 40 before using into formulation. The amount and ratio of drug and polymers were weighed as per given in table 2 and all the formulation were used for further evaluations parameters.

Table 2: Various formulations of sofosbuvir gastro retentive tablets

Excipients (mg)	F1	F2	F3	F4	F5	F6	F7	F8
Sofosbuvir	300	300	300	300	300	300	300	300
Xanthan gum	90	120	-	-	-	-	30	40
Gaur gum	-	-	90	120	-	-	30	40
Karaya gum	-	-	-	-	90	120	30	40
PVP K30	15	15	15	15	15	15	15	15
Talc	5	5	5	5	5	5	5	5
Mg. Stearate	10	10	10	10	10	10	10	10
Lactose	80	50	80	50	80	50	80	50
Total Weight	500	500	500	500	500	500	500	500

Formulation development of bilayer tablet

Optimized formulation IF-7 of Instant release layer and optimized formulation of F-7 for control release used for formulation of Bi-layer tablet.

In vitro disintegration time of immediate release tablets

The disintegration time for all immediate release formulations was carried out using tablet disintegration test apparatus. Six tablets were placed individually in each tube of disintegration test apparatus and discs were placed. The medium, 0.1 N HCl was maintained at a temperature of 37° ± 2°C and time taken for the entire tablet to disintegrate completely was noted.

In vitro dissolution studies

In vitro drug release of the sample was carried out using USP- type II dissolution apparatus (Paddle type). The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.50ºc and rpm of 75. One sofosbuvir tablet was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 12 hours. Sample measuring 5 ml were withdrawn after every 1 hour up to 10 hours using 10ml pipette. The fresh dissolution medium (370C) was replaced every

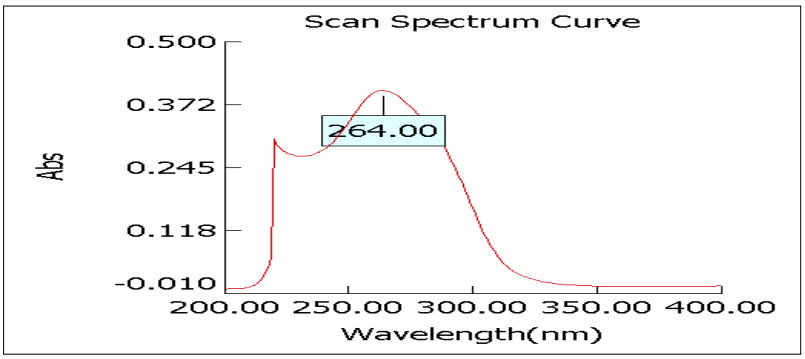
time with the same quantity of the sample. From this take 0.5 ml and dilute up to 10 ml with 0.1 N HCl and take the absorbance at 264nm using spectroscopy.

Stability studies

The stability of sofosbuvir bilayer GR tablets to assess their stability with respect to their physical appearance, drug content and release characteristics after storing at 25°C/60% RH and 40°C/75% RH in properly closed HDPE bottles along with 1 g desiccant for 3 months.

RESULTS AND DISCUSSIONS

λ max of sofosbuvir was found to be 264 nm by using U.V. spectrophotometer (Labindia-3000+) in linearity range 5-25 µg/ml Fig.1.



The powdered blends of different formulations of immediate release tablets and sustained release tablets were evaluated for angle of repose, bulk density (BD), tapped density (TBD) and compressibility index. The results of immediate release tablets and SRGR tablets are summarized in Table 3& 4. The results of SR tablets of BD and TBD ranged from 0.478 to 0.486 and 0.532 to 0.538 respectively. The range of compressibility index was found to be 18.045 to 20.677. The results of angle of repose (<35) indicate good flow properties of the powdered blend. The formulation of immediate release tablet prepared by using the superdisintegrants exhibited the LBD, TBD, compressibility index and Hausner's ratio of within the range, which shows good flow properties of the powdered blend.

Table 3 Results of pre-compression parameters of powder blend of immediate release

F. code	Parameters			
	Loose Bulk density(gm/ml)	Tapped bulk density(gm/ml)	Carr's Index (%)	Hausner's Ratio
IF1	0.422	0.532	20.677	1.261
IF2	0.425	0.535	20.561	1.259
IF3	0.432	0.536	19.403	1.241
IF4	0.425	0.534	20.412	1.256
IF5	0.431	0.538	19.888	1.248
IF6	0.436	0.536	18.657	1.229
IF7	0.432	0.532	18.797	1.231
IF8	0.436	0.532	18.045	1.220
IF9	0.432	0.536	19.403	1.241

Table 4 Result of pre-compression properties of sustained release GR tablets

F. code	Bulk density(gm/ml)	Tapped density(gm/ml)	Compressibility index	Hausner's Ratio
F1	0.485	0.580	16.379	1.196
F2	0.486	0.585	16.923	1.204
F3	0.482	0.586	17.747	1.216
F4	0.478	0.588	18.707	1.230
F5	0.482	0.583	17.324	1.210
F6	0.481	0.582	17.354	1.210
F7	0.482	0.583	17.324	1.210
F8	0.486	0.584	16.781	1.202

The prepared tablets were evaluated for different physico-chemical properties and the results are summarized in Table 5 & 6. The tablets were white, circular in shape and were found to be uniform with respect to weight variation, hardness; thickness, friability and content uniformity of different batch of tablets were found within acceptable range and the distribution of drug in all the formulations was uniform.

Table 5 Results of post-compression parameters of immediate release

F. Code	Hardness test (kg/cm^2)	Friability (%)	Weight variation (%)	Thickness (mm)	Drug content (%)	Disintegration Time (sec.) (n=3) Mean ± SD
IF1	3.2	0.489	155	2.32	99.52	110±3
IF2	3.1	0.498	152	2.42	98.89	95±4
IF3	3.2	0.758	153	2.35	99.36	86±5
IF4	3.4	0.658	150	2.33	98.98	115±6
IF5	3.4	0.698	152	2.35	99.56	100±5
IF6	3.5	0.458	155	2.34	98.89	95±6
IF7	3.2	0.568	148	2.21	99.98	100±4
IF8	3.3	0.478	142	2.25	99.65	73±3
IF9	3.4	0.658	152	2.32	99.56	98±2

Table 6 Results of post compression properties of sustained release GR tablets

F. code	Thickness (mm)	Hardness (kg/cm^2)	Weight variation (mg)	Friability (%)	Drug content (%)
F1	3.5	5.2	498	0.858	98.89
F2	3.4	5.3	495	0.658	99.85
F3	3.5	5.1	498	0.489	98.89
F4	3.6	5.4	502	0.558	99.56
F5	5.5	5.3	505	0.658	99.28
F6	3.4	5.4	504	0.856	99.56
F7	3.4	5.2	503	0.658	99.23
F8	3.4	5.1	502	0.758	99.12

The prepared bilayer tablets were evaluated for different physico-chemical properties and the results are summarized in Table 7. The tablets were found to be uniform with respect to weight variation and hardness (6.2 kg/cm^2). The thickness (5.36mm) and friability (0.856%) of optimized batch of tablets were found within acceptable range. Content uniformity of formulations was found to be 98.12 %, where the distribution of drug in all the formulations was uniform.

Table 7 Post-compressional parameters of bilayer tablets

Formulation	Hardness test (kg/cm^2)	Friability (%)	Weight variation	Thickness (mm)	Drug content
SIS-1	6.2±0.1	0.856	Passes	5.36	98.12

The Instant layer of sofosbuvir release Approx 98.85±1.41 percent drug within 15 minutes and control layer of sofosbuvir shows release up to 12 Hours Approx 99.12percent. The release of bilayer tablet.

CONCLUSION

Bilayer tablets of sofosbuvir containing sustained release layer and immediate release layer were successfully formulated. All the formulation batches tested for physical parameters like weight variation, hardness, friability and drug content, all were found to be within the USP limits. The optimized formulations were found to be stable at all the stability conditions. During stability studies, no significant variation (1–4%) in drug release was observed, indicating that formulation batch SIS-1 was stable over the chosen condition for 3 months. The optimized formulation SIS-1showed better drug release profile. Combination of xanthan gum, gaur gum, karaya gum is an interesting polymer mixture for the preparation of SR tablet because of good bioadhesive property, non toxicity and low cost and good binding capacity.

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