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REGULAR ARTICLE

Study on Prescription Technology of Sofosbuvir/ledipasvir Tablet

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Abstract: Clone the Harvoni™(Sofosbuvir/Ledipasvir) tablet manufactured by Gilead Sciences, Inc. and screen out the best formula of prescription, to make the pesticide effect of prepared tablet was equal to the original tablet. Methods: Based on the prescription of Harvoni™ manufactured by Gilead Sciences, Inc., the compatibility test of the excipient with the active ingredient was carried out. The stability of self-made samples and reference preparations were investigated, which includes high temperature, high humidity and light conditions. According to the above test, the most reasonable prescription process was chosen. Results: The determined prescription process contained sofosbuvir (400mg), ledipasvir (90mg), copovidone (97.5mg), croscarmellose sodium (50mg), microcrystalline cellulose (115mg), lactose monohydrate (230mg), magnesium stearate (7.5mg), gum arabic(10mg), opadry film coating powder, and purified water. Conclusion: The determined prescription process was stable and the pesticide effect of prepared tablet was equal to the original tablet, so the prescription design was reasonable.

Key word: Harvoni™, Prescription Technology, Sofosbuvir.

1. Introduction

Harvoni™ was developed by Gilead Sciences, Inc., which is a fixed-dose combination tablet containing sofosbuvir and ledipasvir. Sofosbuvir is a nucleotide analog inhibitor of HCV NS5B polymerase and ledipasvir is an HCV NS5A inhibitor. Each tablet contains 400 mg sofosbuvir and 90 mg ledipasvir[1-2].

Sofosbuvir was first approved by the United States on December 6, 2013, the IUPAC

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name is (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) phosphorylamino) propanoate.

Ledipasvir has not been approved for clinical use alone, and the IUPAC name is Methyl [(2*S*)-1-{(6*S*)-6-[5-(9,9-difluoro-7-{2-[(1*R*, 3*S*, 4*S*)-2-{(2*S*)-2-[(methoxycarbonyl)amino]-3-ethyl-butanoyl}-2-azabicyclo[2.2.1]hept-3-yl]-1*H*-benzimidazol-6-yl}-9*H*-fluoren-2-yl)-1*H*-imidazol -2-yl]-5- azaspiro [2.4] hept-5-yl}-3-methyl-1-oxobutan-2-yl] carbamate.

Harvoni™ was recognized by FDA as a breakthrough therapeutic drug, it is the first fixed-dose combination oral tablet to be preferred approved to treat genotype 1 of hepatitis C without requiring interferon injections. Harvoni™ can be used alone or combine with other oral anti-HCV agents, such as ribavirin.

Currently, the popularity rate of Harvoni™ is very low for its high price, while the number of patients with genotype 1 of hepatitis C is very huge. To meet the needs of patients, increase their choice of medication and reduce the cost of the drug, we should study the combination tablet (Sofosbuvir/Ledipasvir) and make it commercialization, marketization. This not only brings good news to patients, but also can generate enormous social and economic benefits.

2. Reagents and Instruments

2.1 Reagents

Ledipasvir, sofosbuvir, microcrystalline cellulose, croscarmellose sodium, lactose monohydrate, magnesium stearate, copovidone, gum arabic, coating powder.

2.2 Instruments

Laboratory hopper mixer, dry granulator, tablet machine, high efficiency coating machine, high performance liquid chromatography, dissolution tester, electronic balance.

3. Ingredients Screening and Stability Testing

This research was to clone the original drug Harvoni ™ (Sofosbuvir/ Ledipasvir). By searching the information on the FDA website, we can know the composition of original drug: croscarmellose sodium, micro sodium cellulose, lactose monohydrate, magnesium stearate, copovidone, gum arabic and coating powder.

The compatibility between excipients and drugs in solid preparations has a very important guiding role in screening prescriptions and process [3]. Through compatibility experiments, we can select the appropriate excipients to ensure the stability of drugs effectively.

According to **Table 1**, the ingredients were mixed with the active ingredient. Placed them under high temperature condition (60 °C), high humidity condition (92.5% RH) and

illumination condition (4500lx) for 0 days, 5 days, 10 days, and observed the characters and changes of the relevant substances. The results were shown in **Table 1**

Table 1. Compatibility Experiment

Material	Detectionwavelength	Impurities	0th day	Illumination		High Humidity (92.5% RH)		High Temperature (60 °C)	
				5th day	10th	5th	10th	5th	10th
blank excipient [a]	260 nm	appearence	off-white	off-white	off-white	off-white	off-white	off-white	off-white
		Impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		Impurity Π (%)	NA	NA	NA	NA	NA	NA	NA
		any other impurity (%)	NA	NA	NA	NA	NA	NA	NA
		total impurity（%）	NA	NA	NA	NA	NA	NA	NA
	330 nm	impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	NA	NA	NA	NA	NA	NA	NA
		any other impurity（%）	NA	NA	NA	NA	NA	NA	NA
		total impurity（%）	NA	NA	NA	NA	NA	NA	NA
Ledipasvir	330 nm	appearence	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow
		impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.17	0.25	0.06	0.06	0.06	0.06
		any other impurity（%）	0.03	0.03	0.02	0.03	0.03	0.03	0.03
		total impurity（%）	0.15	0.28	0.34	0.15	0.15	0.17	0.16
Sofosbuvir	260 nm	appearence	off-white	off-white	off-white	off-white	off-white	off-white	off-white
		impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		impurity Π (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		any other impurity（%）	0.01	0.01	0.03	0.01	0.03	0.01	0.03
		total impurity（%）	0.02	0.02	0.04	0.03	0.03	0.02	0.05
Ledipasvir+ Copovidone(1:5)	330 nm	appearence	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow
		impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.25	0.29	0.06	0.06	0.06	0.06
		any other impurity（%）	0.04	0.04	0.03	0.05	0.04	0.05	0.04

		total impurity（%）	0.16	0.38	0.43	0.17	0.16	0.17	0.16
Ledipasvir+blank excipient(1:5)	330 nm	appearence	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow
		impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.43	0.58	0.06	0.07	0.06	0.07
		any other impurity（%）	0.04	0.04	0.15	0.04	0.03	0.04	0.06
		total impurity（%）	0.16	0.73	0.95	0.16	0.16	0.19	0.19
Sofosbuvir+blank excipient(1:5)	260 nm	appearence	off-white	off-white	off-white	off-white	off-white	off-white	off-white
		impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		impurity Π (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		any other impurity（%）	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		total impurity（%）	0.03	0.02	0.03	0.02	0.02	0.03	0.06
Ledipasvir+Sofosbuvir+Microcrystalline cellulose (1: 4: 25)	260 nm	appearence	off-white	off-white	off-white	off-white	off-white	off-white	off-white
		impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		impurity Π (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		any other impurity（%）	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		total impurity（%）	0.03	0.02	0.03	0.02	0.02	0.03	0.06
	330 nm	impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.42	0.7	0.06	0.06	0.06	0.06
		any other impurity（%）	0.04	0.04	0.04	0.04	0.03	0.04	0.03
		total impurity（%）	0.15	0.42	0.84	0.16	0.15	0.16	0.15
Ledipasvir+Sofosbuvir+Lactose monohydrate (1: 4: 25)	260 nm	appearence	off-white	off-white	off-white	off-white	off-white	off-white	off-white
		impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		impurity Π (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		any other impurity（%）	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		total impurity（%）	0.03	0.02	0.03	0.02	0.02	0.03	0.06
	330 nm	impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.49	0.58	0.06	0.06	0.06	0.06
		any other impurity（%）	0.04	0.04	0.05	0.04	0.04	0.03	0.03
		total impurity（%）	0.16	0.34	0.7	0.16	0.15	0.15	0.15
Ledipa	260	appearence	off-wh	off-wh	off-wh	off-wh	off-wh	off-wh	off-wh

svir+ Sofosbuvir+ croscarmellose sodium (1: 4: 25)	nm		ite	ite	ite	ite	ite	ite	ite
		impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		impurity Π (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		any other impurity（%）	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		total impurity（%）	0.03	0.02	0.03	0.02	0.02	0.03	0.05
	330 nm	impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.48	0.5	0.06	0.06	0.06	0.06
		any other impurity（%）	0.04	0.03	0.04	0.04	0.03	0.03	0.03
		total impurity（%）	0.16	0.59	0.66	0.16	0.15	0.15	0.15
ledipasvir+ sofosbuvir+gum Arabic (1:4:0.25)	260 nm	appearence	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow
		impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		impurity Π (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		any other impurity（%）	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		total impurity（%）	0.03	0.02	0.03	0.02	0.02	0.03	0.06
	330 nm	impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.35	0.51	0.06	0.07	0.06	0.07
		any other impurity（%）	0.04	0.03	0.04	0.04	0.05	0.04	0.04
		total impurity（%）	0.16	0.44	0.81	0.16	0.16	0.15	0.16
ledipasvir+ sofosbuvir+ magnesium stearate (1: 4: 0.25)	260 nm	appearence	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow	light yellow
		impurity I (%)	NA	NA	NA	NA	NA	NA	NA
		impurity Π (%)	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		any other impurity（%）	0.01	0.01	0.01	0.01	0.01	0.01	0.01
		total impurity（%）	0.03	0.02	0.03	0.02	0.02	0.03	0.06
	330 nm	impurity A（%）	NA	NA	NA	NA	NA	NA	NA
		impurity B（%）	0.06	0.31	0.44	0.06	0.06	0.06	0.06
		any other impurity（%）	0.04	0.04	0.03	0.03	0.03	0.04	0.03
		total impurity（%）	0.14	0.41	0.55	0.15	0.15	0.16	0.15

a blank excipient: copovidone, croscarmellose sodium, microcrystalline cellulose, lactose monohydrate, magnesium stearate, gum arabic, opadry film coating powder.

Table 1 showed that:

1. After 10 days of influence factors experiment, ledipasvir was sensitive to light and the content of impurities increased slightly.

2. Under light condition, the content of impurities in ledipasvir or ledipasvir with excipients increased slightly.

3. The content of impurities in sofosbuvir or sofosbuvir with excipients was not significantly different with the 0^{th} during the influence factors experiment, including high temperature condition (60 °C), high humidity condition (92.5% RH) and illumination condition (4500 lx).

4. No impurities were produced in the blank excipient, indicating that the above ingredients were well compatible with sofosbuvir and ledipasvir, and can be used for the prescription screening of the sofosbuvir/ledipasvir tablet.

Conclusion: The selected excipients can be used for the development of this prescription process. During the development process, we should pay attention to the effects of lighting, humidity and temperature on the products.

4. Selection of Process

In the preparation of tablets, there are dry granulation, wet granulation, powder direct granulation, and fluidized bed granulation. According to the above experiments, we can know that, under high temperature and high humidity conditions, the impurity content of ledipasvir increased slightly. While in the wet granulation process will contain high humidity, which may affected ledipasvir's stability. Sofosbuvir has the character of low bulk density, poor fluidity, and large viscosity. Due to its physical limitations, the process of powder direct granulation and fluidized bed granulation cannot be chosen [4]. Dry granulation process has the properties of smaller equipment, less energy consumption, shorter production cycle of tablets, and does not affect the stability of drugs. In summary, the dry granulation process was selected. During the granulation process, the particles between 20 ~ 60 mesh were selected.

5. Prescription Screening and Optimization

(1) Screening of Disintegrants

According to the relevant information of the original drug, we can know that the disintegrant of the original drug was croscarmellose sodium. Croscarmellose sodium is a commonly used disintegrant in tablet, it has good compressibility, strong disintegration ability, and the disintegration time of the prepared tablets will not change with the increase of storage time. The conventional dosage of croscarmellose sodium is 0.5%-5%, and different dosage of disintegrant can influence the tablets including the molding, hardness, surface

gloss and dissolution. Therefore, it was necessary to screen out the appropriate amount of disintegrant. The disintegration agent was added by internal addition, the amount of disintegrating agent was 0%, 3%, 5%, respectively. Compare the appearance and dissolution. The results were shown in **Table 2** and **Table 3**.

Table 2. The Appearance of Product in Different Amount of Disintegrants.

No.	Disintegrant	Dosage (w/w) %	Appearance
1	Croscarmellose sodium	0	Surface has spots, not smooth
2		3.0	smooth
3		5.0	smooth

Table 3. The Dissolution of Product in Different Amount of Disintegrants.

Time	Ledipasvir				Sofosbuvir			
min	0%	3%	5%	original drug	0%	3%	5%	original drug
10	0.9	42.64	51.1	59.09	1.47	56.48	51.13	89.16
15	1.33	58.86	65.9	78.85	1.98	72.16	65.9	96.12
30	2.38	85.74	89.7	95.88	3.3	93.41	89.67	100.55
45	3.34	97.77	98.8	99.19	4.4	100.8	98.8	100.83

Table 2 and **Table 3** showed that it was necessary to add disintegrants in the tablet. Without the disintegrant agent, the appearance of tablet will had spots and was not smooth, and the dissolution speed was very slow. When added 3.0% amount of disintegrant agent, the appearance of the tablet was smooth and the dissolution speed improved significantly. When the amount of disintegrant agent was 5.0%, the appearance of the tablet was smooth too and the dissolution speed was faster than 3.0%, but it was still lower than the original drug.

(2) Study on the Way of Adding Disintegrant

The disintegrant can be added in three ways: internal addition, external addition, internal and external addition. The addition way of disintegrating agent has a great influence on disintegration, which affects the dissolution. So it is necessary to study on the way of adding disintegrant agent.

Combined with the above experiments, we added 5% amount of croscarmellose sodium. Then we studied on the way of adding disintegrant agent, which included internal addition, external addition, internal and external addition (50%, respectively). And the difference of

dissolution result was shown in **Table 4.**

Table 4. The Dissolution Result of Difference Addition Way

Dissolution	Ledipasvir				Sofosbuvir			
min	internal addition	internal and external addition	external addition	original drug	internal addition	internal and external addition	external addition	original drug
10	51.13	72.25	44.08	59.09	51.13	83.02	57.42	89.16
15	65.9	85.33	56.51	78.85	65.9	92.13	69.9	96.12
30	89.67	96.87	75.39	95.88	89.76	98.09	85.45	100.55
45	98.8	99.94	86.78	99.19	98.8	99.39	93.55	100.83

Table 4 showed that if the addition way was internal and external addition, the dissolution result was most close to the original drug. So we chose the amount of croscarmellose sodium at 5.0%, and the addition way was internal and external addition.

(3) Select the Filling Agent and Determine The Proportion

Due to the poor compressible property of sofosbuvir and ledipasvir, it is hard to press tablet. Microcrystalline cellulose has good compressible property, which can improve the properties of the material significantly, make it suitable in dry granulation and is helpful to suppress the tablets with sufficient hardness and good fragility. So it is required to add microcrystalline cellulose in the tablet. Compared with microcrystalline cellulose, lactose monohydrate has twice good compressibility. After dry granulation, it still can provide good compression performance in the process of pressing. Therefore, microcrystalline cellulose and lactose monohydrate were selected as filling agents.

Table 5. The Dissolution of Different Proportions of Filling Agents

Time	Ledipasvir				sofosbuvir dissolution			
min	microcrystalline cellulose: lactose monohydrate (1:1)	microcrystalline cellulose: lactose monohydrate (2:1)	microcrystalline cellulose: lactose monohydrate (1:2)	original drug	microcrystalline cellulose: lactose monohydrate (1:1)	microcrystalline cellulose: lactose monohydrate (2:1)	microcrystalline cellulose: lactose monohydrate (1:2)	original drug
10	63.73	65.01	68.91	59.09	72.5	75.32	79.58	89.16
15	76.28	78.55	81.5	78.85	81.9	85.68	88.94	96.12
30	91.97	94.09	96.51	95.88	90.89	94.72	97.09	100.55
45	98.19	100.95	100.81	99.19	93.74	97.89	98.49	100.83
F2	72.9	73.61	63.96	-	45.11	51.54	59.63	-

The proportions of microcrystalline cellulose and lactose monohydrate were investigated by the ratio of 1:2, 1:1 and 2:1. And the dissolution was tested, the result were shown in **Table 5**.

Table 5 showed that the proportion of microcrystalline cellulose had no obvious effect on the dissolution of ledipasvir, but affects the dissolution of Sofosbuvir slightly. After the proportion of microcrystalline cellulose increased, the molding pressure increased. Due to the proportion of filling agents had no significant difference, the proportion of microcrystalline cellulose and lactose monohydrate was set at 2:1.

(4) Selection of Anti-adhesive Agent

In dry granulator, magnesium stearate is often used to reduce the adhesion between the granules and the metal pressure roller under high pressure [5]. However, when 1% by weight of magnesium stearate is added to the granulation formulation, adhesion is reduced. But it still retains static electricity. Magnesium stearate may cause side effects of tablet softening also limits the amount of its use. The combination of talcum powder or silicon dioxide with the combination of magnesium stearate can improve the lubrication effect [6]. And the introduction of silicon dioxide can reduce the electrostatic interaction between particles and increase the fluidity of the particles. Therefore, it is necessary to investigate the effect of silicon dioxide on the removal of static electricity and the anti-adhesion effect with synergistic use of magnesium stearate. The results were shown in **Table 6**.

Table 6. Different amounts of anti-adhesive and glidant inspection results

No.	Magnesium Stearate (w/w)%	Gum Arabic	Granulation State	Tablet Situation
1	1.0	0.0	material close to the pressure wheel, there was static electricity	non-viscous rush and other phenomena, good condition
2	1.0	1.0	weak static electricity, the material was plate	in good condition
3	0.0	0.0	more particles of fine powder, poor mobility, astringent axis	the weight of tablet is different
4	0.0	0.5	there was static electricity, the material was plate, adhesion pressure roller	in good condition
5	0.8	1.0	static electricity was weak, the material was plate, good	in good condition

Table 6 showed that the use of magnesium stearate together with the gum arabic can reduce the particle adhesion of the pressure roller and improve the fluidity of the particles. In order to ensure the good liquidity of products, the proposed amount of gum arabic was 1.0% and mgnesium stearate was 0.75%.

6. Conclusions

In summary, through a series of experiments, the prescription was determined and shown in **Table7**.

Table7. The prescription of Sofosbuvir /Ledipasvir tablet

Drug	Content (mg/tablet)	Proportion (%)
ledipasvir	90	9
sofosbuvir	400	40
lactose monohydrate	230	23
microcrystalline cellulose	115	11.5
copovidone	97.5	9.75
croscarmellose sodium	50	5.0
microsilica	10	1.0
magnesium stearate	7.5	0.75
total	1000	100

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