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A STUDY ON HPLC METHOD DEVELOPMENT AND VALIDATION OVER SOFOSBUVIR AND SIMEPREVIR TABLET DOSAGE FORM

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ABSTRACT

The simultaneous quantitative analysis of Sofosbuvir and Simeprevir in tablet dosage form has been developed and validated using high-performance liquid chromatography (HPLC). Specificity, precision, accuracy, and reproducibility of the method made it suitable for routine quality control testing in industries. Simeprevir and Sofosbuvir had retention times of 2.512 minutes and 3.116 minutes, respectively. Sofosbuvir and Simeprevir were found to have a good level of precision with %RSD values of 0.2 and 0.7, respectively. For Sofosbuvir, the %Recovery values were 99.44%, while for Simeprevir, they were 99.96%, indicating excellent accuracy. A regression equation was used to determine the LOD (limit of detection) and LOQ (limit of quantification) of the method. A LOD of 0.38 and a LOQ of 1.17 were determined for Sofosbuvir, whereas 0.11 and 0.34 were determined for Simeprevir. A linear regression equation was established for Sofosbuvir and Simeprevir as y = 11806x + 1985.4 and y = 9492.1x + 514.5, respectively, ensuring linearity and providing a reliable method of quantification. ICH guidelines were followed for validating the method, including linearity, accuracy, precision, LODs, LOQs, robustness, and reproducibility. This study confirmed that the method was reliable and suitable for routine analysis based on the validation results. This method is also simple and cost-effective due to its decreased retention times and reduced run times. As a result, it is a highly practical testing method for quality control in industrial settings. As a result, the HPLC method proposed provides a robust and efficient method for simultaneously analyzing Sofosbuvir and Simeprevir tablets. A valuable tool in the pharmaceutical industry for quality control, it is simple, specific, precise, accurate, and cost-effective.

KEYWORDS: Sofosbuvir, Simeprevir, validation, HPLC, Quality control.

INTRODUCTION

A quality assurance and quality control program are essential to ensuring that drugs are available to consumers in safe and effective formulations.[1] In order for analytical data to be of high quality, the methods used to generate them must be of high quality. It is therefore very important that rugged and robust analytical methods be developed in order to meet regulatory requirements for drugs and their formulations.[2] In general, quality and safety of a drug are assured through effective monitoring and control of assays and impurities. Assay of pharmaceutical products plays an important role in efficacy of the drug in patients.

When developing methods for different drugs, a wide variety of challenges are encountered, based on their natures and properties. Chemical reactions produce a variety of physico-chemical phenomena that can be studied with different methods.[3]

A number of new methods are becoming increasingly popular, including nuclear magnetic resonance (NMR) and para magnetic resonance (PMR). Combined with gas chromatography, mass spectroscopy (MS) can be a powerful tool. Drug development is constantly growing.[4] The quality of these products must be controlled in a new way. For the treatment of chronic hepatitis C virus (HCV) infections with genotypes 1-6, as well as co-infections with HIV and HCV, sofosbuvir is used in combination with other antiviral medications. It exhibits pH-dependent aqueous solubility and is white to off-white powder. Among its solubility properties[5], it is soluble in methanol, insoluble in ethanol, insoluble in acetone, insoluble in isopropanol, and insoluble in isopropyl acetate.

In addition to being an antiviral agent, simeprevir is also an inhibitor of the enzyme NS3/4A, a necessary enzyme for reproducing HCV. A macrocyclic, noncovalent

inhibitor with competitive properties[6], Simeprevir is unlike Boceprevir and Telaprevir. Through noncovalent binding to the protein target, the macromolecular cyclic portion of the molecule improves affinity and selectivity characteristics. Methanol, ethyl alcohol, acetone, ethyl acetate, and acetonitrile used for the solubility, which is a white to off-white powder.[7,8]

With the increasing number of drugs on the market and their combinations, quality control methods are needed to ensure the safety of the drugs. In order to achieve the best accurate and robust results, the methods must take as little time as possible to develop. As a result of this concept, the present study aims to develop novel analytical methods to evaluate Sofosbuvir, Simeprevir simultaneously in bulk and tablet dosages. The aim of this study is to develop an accurate, precise, sensitive, selective, reproducible, and rapid analytical technique.

Chromatography

Chromatography (chrome meaning 'color', graphein means 'writing') is a set of laboratory techniques for separating mixtures. Through differential partitioning between the mobile and stationary phases, the desired analyte is separated from other molecules in a solution dissolved in a "mobile phase" by passing it through a stationary phase.[9]

There are two types of chromatography: preparative and analytical. Separation of the components of a mixture for further use is the purpose of preparative chromatography. Chromatography used in analytical analysis measures the relative proportions of analytes in mixtures with smaller amounts of material.

High Performance Liquid chromatography (HPLC)

Using liquid chromatography, ions or molecules dissolved in a solvent can be separated from each other. A variety of chemical separation techniques were used during the 1970's, including open-column chromatography[10], paper chromatography, and thin layer chromatography (TLC). In spite of this, these chromatographic techniques were inadequate for quantifying compounds and resolving similar compounds. The development of column packing materials led to rapid improvements in high pressure liquid chromatography.

Normal Phase- HPLC

Analytes in the mixture interact differently with the stationary phase in NP-HPLC because of their polar interactions. Adsorption sites on the stationary phase surface are competed for by the analyte molecules and the mobile phase molecules. Mobile phase interactions with stationary phase are stronger when they are stronger, which decreases the difference between stationary phase interactions and analyte interactions, which results in a lower retention of analytes.

A small amount of polar modifier (e.g., methanol, ethanol) is added to non-polar solvents in NP-HPLC (e.g., hexane, heptanes, etc.). Porous oxides such as silica (SiO_2) and alumina (Al_2O_3) are typically used as packing materials.

NP-HPLC can also use chemically modified stationary phases. A typical packing material with decreased surface polarity is silica modified with trimethoxy glycidoxypropyl silanes (commonly known as diol-phase). NP-HPLC makes use of non-polar solvents and thus is preferred for highly hydrophobic compounds (which may interact very strongly with non-polar mobile phases) which are insoluble in aqueous or polar solvents.

Reverse Phase-HPLC

In terms of chromatography, RP-HPLC is by far the most popular method. The RP-HPLC is used almost 90% of the time to analyze low-molecular-weight samples. Compared with other separation methods[11], the dispersive forces used in this separation mode are the weakest in terms of intermolecular interactions, so the background interaction energy is very low. A porous rigid material with hydrophobic surfaces is used as an adsorbent in this mode of chromatography.[12] In RP-HPLC, most packing materials are porous silica that has been chemically modified.

MATERIALS AND METHODS

Sofosbuvir and Simeprevir pure drugs (API), Combination Sofosbuvir and Simeprevir tablets (Synthetic Mixture), Distilled water, Acetonitrile, Phosphate buffer, Methanol, Potassium dihydrogen ortho phosphate buffer, Ortho-phosphoric acid. All the above chemicals and solvents are from Rankem.

Reagents and solutions

Accurately weighed 15mg of Simeprevir 40mg of Sofosbuvir and transferred to 50ml volumetric flask. $3/4^{th}$ of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (800µg/ml of Sofosbuvir and 300µg/ml Simeprevir).

1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (80µg/ml of Sofosbuvir and 30µg/ml of Simeprevir).

Sample preparation

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (4000µg/ml of Sofosbuvir and 1500µg/ml of Simeprevir).

0.2ml of filtered sample stock solution was transferred to 10ml volumetric flask and made up with diluent. (80µg/ml of Sofosbuvir and 30µg/ml of Simeprevir).

Buffer Preparation

0.01N Na_2HPO_4 Buffer: Take 1.41gm of Sodium hydrogen phosphate accurately was transferred in 1000ml beaker and Add 900ml of hplc grade water and sonicated for 25 min, further the volume was made up with 1grade water upto mark and filtered by HPLC filters and ph is adjusted by adding 1ml 0.1%OPA to the above solution (Ph- 4.5).

Assay of Sofosbuvir and Simeprevir

Tablet bearing the label claims Sofosbuvir 400mg, Simeprevir 150mg. Assay was performed with the above formulation. Average % Assay for Sofosbuvir and Simeprevir obtained was 99.23and 99.53% respectively.

Sample preparation

5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 1 tablet was taken and finely powdered with motor and pestle and then the weight equivalent to 1 tablet was taken and transferred into 100 mL volumetric flask, 25mL of diluent added and sonicated for 50 min, further the volume made up with diluent and filtered and The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined and Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45-µm nylon filter. (4000 µg/ml or ppm of Sofosbuvir, 1500µg/ml or ppm of Simeprevir) From the filtered solution 0.2ml was pipette out into a 10 ml volumetric flask and made upto 10ml with diluents. (80 µg/ml or ppm of Sofosbuvir, 30µg/ml or ppm of Simeprevir) and injected into hplc system to study the efficacy of the samples through Chromotograms.

VALIDATION

Linearity and Range[13]

The Linearity of detector response is established by plotting a graph to concentration versus area of 15mg of Simeprevir & 40mg of Sofosbuvir and determining the correlation coefficient. A series of solution of Simeprevir & Sofosbuvir in the concentration ranging from 20, 40, 60, 80, 100 and 120 µg/ml of Sofosbuvir and also 7.5, 15, 22.5, 30, 37.5, and 45 µg/ml of Simeprevir) were prepared and injected into the HPLC system.

Accuracy

Standard preparation

Accurately weighed 15mg of Simeprevir 40mg of Sofosbuvir and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (800µg/ml of Sofosbuvir and 300µg/ml Simeprevir).

Sample Preparation

5 tablets were weighed and the average weight of each tablet was calculated, then the weight equivalent to 1 tablet was transferred into a 100 ml volumetric flask, 50ml of diluents was added and sonicated for 25 min, further the volume was made up with diluent and filtered by HPLC filters (4000µg/ml of Sofosbuvir and 1500µg/ml of Simeprevir).

Preparation of Spiked solution

0.5ml, 1.0ml, and 1.5 ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent. (50, 100 and 150%).

Robustness

Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there was no recognized change in the result and are within range as per ICH Guide lines. Robustness conditions like Flow minus (0.8ml/min), Flow plus (1.0ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD & LOQ

0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.3ml (LOD) and 0.9ml (LOQ) each of Simeprevire, Sofosbuvir, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents.

RESULTS AND DISCUSSION

During the method development process, various mobile phase ratios, buffers, and conditions were adjusted to optimize the elution of Sofosbuvir and Simeprevir peaks. Initially, using a mobile phase of 0.1% OPA and MeCN in a 50:50 ratio, only Sofosbuvir peak was eluted while Simeprevir peak was not eluted and the baseline was not satisfactory. Further adjustments were made using a mobile phase of 0.01N Na_2HPO_4 and MeCN in a 30:70 ratio, resulting in the elution of both Sofosbuvir and Simeprevir peaks. However, the baseline was still not ideal. Finally, by using a mobile phase consisting of 60% 0.01N Na2HPO4 and 40% methanol, both peaks were eluted with good resolution. The optimized method met the criteria set by ICH guidelines, with satisfactory plate count, tailing factor, and resolution. Sofosbuvir and Simeprevir were eluted at retention times of 2.512 min and 3.118 min, respectively. No interfering peaks were observed in blank and placebo samples at the retention times of the drugs, indicating method specificity. Linearity of the method was established by injecting six linear concentrations of Sofosbuvir and Simeprevir. The obtained average areas were used to calculate linearity equations, and high correlation coefficients (0.999) were obtained for both drugs. Precision of the method was evaluated by calculating the average area, standard deviation, and %RSD from multiple injections of working standard and sample solutions. The obtained %RSD values for both Sofosbuvir and Simeprevir were

within the acceptable limit of 2%, indicating good system precision. Accuracy of the method was assessed by performing a standard addition method at three levels. Triplicate injections were given for each level, and the mean %Recovery was calculated as 99.44% for Sofosbuvir and 99.96% for Simeprevir, indicating accurate and reliable results. Robustness of the method was evaluated by testing various conditions such as flow rate variations, mobile phase composition changes, and temperature adjustments. The system suitability parameters were not significantly affected, and all parameters remained within acceptable limits[14-16], demonstrating the robustness of the method. Regarding pH adjustments in the mobile phase for acid and base degradation studies, the retention time of the drugs was found to be unaffected due to neutralization of acid samples with 2N base solution and base samples with 2N acid solution.[17]

Table: 1 Assay Data of Sofosbuvir and Simeprevir.

S. No.	Sofosbuvir			Simeprevir		
	Std Area	Sample area	% of Assay	Std area	Sample area	% of Assay
1	951492	951188	99.38	285813	288952	100.81
2	943481	951671	99.43	284412	283417	98.88
3	962531	950008	99.25	286796	283875	99.04
4	962544	949537	99.21	283513	284988	99.43
5	948820	948639	99.11	288917	284476	99.25
6	951017	947383	98.98	286885	286014	99.79
Avg	953314	949738	99.23	285672	285287	99.53
Stdev	7689.3	1594.1	0.17	1931.3	2009.9	0.7
%RSD	0.8	0.2	0.2	0.7	0.7	0.7

Table: 2 Degradation data of Sofosbuvir and Simeprevir

S. No.	Degradation Condition	% Drug Degraded	
		Sofosbuvir	Simeprevir
1	Acid	2.28	2.11
2	Alkali	3.91	4.71
3	Oxidation	5.10	6.39
4	Thermal	2.69	2.74
5	UV	1.12	1.99
6	Water	1.12	0.74

Validation of Sofosbuvir and Simeprevir

Table:3 Validation parameters on Sofosbuvir and Simeprevir

Parameters		Sofosbuvir	Simeprevir	Limit
Linearity Range(µg/ml)		20_120µg/ml	7.5-45µg/ml	R< 1
Regression coefficient		0.999	0.999	
Slope(m)		11806	9492.1	
Intercept(c)		1985.4	514.5	
Regression equation (Y=mx+c)		Y =11806x + 1985.4.	y = 9492.1x + 514.5.	
Assay (% mean assay)		99.23%	99.53%	90-110%
Specificity		Specific	Specific	No interference of any peak
System precision %RSD		0.8	0.7	NMT 2.0%
Method precision %RSD		0.2	0.7	NMT 2.0%
Accuracy% recovery		99.44%	99.96%	98-102%
LOD		0.38	0.11	NMT 3
LOQ		1.17	0.34	NMT 10
Robustness	**FM**	0.6	0.4	%RSD NMT 2.0
	FP	0.9	0.4	
	MM	1.0	0.3	
	MP	0.8	0.6	
	TM	1.0	0.2	
	TP	0.2	0.9	
Degradation		**% Drug Degraded**		NMT 20%

	Acid	2.28	2.11	
	Alkali	3.91	4.71	
	Oxidation	5.10	6.39	
	Thermal	2.69	2.74	
	UV	1.12	1.99	
	Water	1.12	0.74	

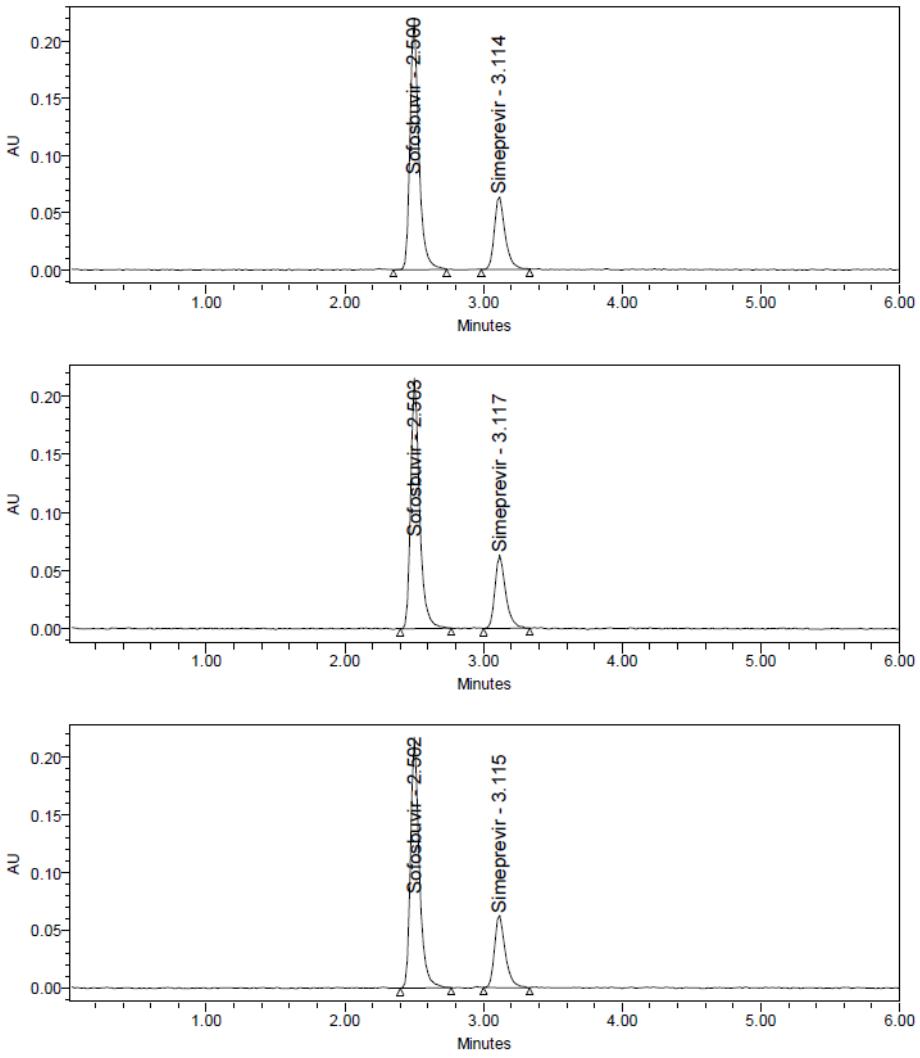

Fig. 1: HPLC Chromatogram of Sofosbuvir and Simeprevir.

CONCLUSION

Using this HPLC method, we are able to simultaneously analyze Sofosbuvir and Simeprevir in Tablet dosage forms with simple, precise, accurate, and reproducible quantitative results. According to ICH guidelines, this method was validated for linearity, accuracy, precision, limits of detection (LOD) and quantification (LOQ), robustness, and reproducibility. Since retention times and run times were decreased, the method developed can be adopted for regular quality assurance tests in industries, as it is simple and economical.

REFERENCES

1. Mastanamma SK. Development and validation of stability indicating RP HPLC method for simultaneous estimation of sofosbuvir and ledipasvir in bulk and their combined dosage form. Future J of Pharm Sci., 2018; 4(2): 116–123.
2. Sandhya Rani J. A new RP HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasir in pharmaceutical dosage form. Int J Eng Technol Sci & Res., 2017; 4(11): 145–152.
3. Swathi P. RP HPLC method development and validation for estimation of sofosbuvir in pure and tablet dosage forms. Asian J Pharm Tech., 2017; 7(3): 153–156.
4. Zaman B, Siddique F. RP HPLC method for simultaneous determination of sofosbuvir and ledipasvir in tablet dosage form and its application

to in vitro dissolution studies. Chromatographia, 2016; 79(23-24): 1605–1613.
5. https://en.wikipedia.org/wiki/Sofosbuvir
6. The Merck Index. 12 th ed., 1996; 67: 877[2].
7. Budavari S. The Merck Index: An Encyclopedia of Chemicals, Drugs and Biologicals. Merck Research Laboratories Division of Merck and Co., Inc. Whitehouse Station. Merck Index: 13th ed., 2001; 67–68.
8. United States Pharmacopoeia, 2006; USP22-NF17: 1710-1712.
9. Uppalapati. Jyothi, Parimi.Umadevi, Analytical method development and validation for the simultaneous estimation of Sofosbuvir and Velpatasvir drug product by RP-HPLC method, Indo American Journal of Pharmaceutical Research, 2017; 401-409.
10. Bakht Zaman, Faisal Siddique, Waseem Hassan, RP-HPLC Method for Simultaneous Determination of Sofosbuvir and Ledipasvir in Tablet Dosage Form and Its Application to In Vitro Dissolution Studies, December, 2016; 79(23–24): 1605–1613.
11. Nagaraj T, Vardhan S.V.M, Ravikumar D, and Ramachandra, A new RP-HPLC method for the Simultaneous Assay of Sofosbuvir and Ledipasvir in combined dosage form. International Journal of Chemtech Research, 2017; 10(7): 761-769.
12. Surya Prakash Y. Rai, Yural Prajapati, Pragnesh Patni, development and Validation of RP-HPLC and UV Spectroscopic methods for Simultaneous Estimation of Sofosbuvir and Ledipasvir in their combined tablet dosage forms, An International journal of Pharmaceutical Sciences, Apr-Jun 2017; 8(2).
13. ICH Q2B, Validation of Analytical Procedures: Methodology, ICH Harmonized Tripartite Guideline, 1996; 1-8.
14. J.E.F. Reynolds (Ed.), Martindale: The Extra Pharmacopoeia, 32nd edn, The Pharmaceutical Press, London, 1999; 155.
15. Singh K. HPLC method for estimation of drug release of sofosbuvir in pharmaceutical formulations. World J Pharm Pharm Sci., 2017; 6(8): 2249–2258.
16. Vajendla R, Subramanyam CVS Estimation and validation of sofosbuvir in bulk and tablet dosage form by RP HPLC. Int J Pharm., 2016; 6(2): 121–127.
17. Shaikh SN, Dhabade MP Development and validation of RP HPLC method for quantitative analysis of sofosbuvir in pure and pharmaceutical dosage forms. Int. J Pharm Sci & Res., 2019; 10(1): 367–372.