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Research Article

ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS ESTIMATION OF SOFOSBUVIR AND VELPATASVIR DRUG PRODUCT BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD

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ABSTRACT

Objectives: The purpose of the research is to develop a simple, precise, economical, accurate, reproducible, and sensitive method for the estimation of sofosbuvir and velpatasvir drug product by reverse phase high performance liquid chromatography method.

Methods: New analytical method was developed for the estimation of velpatasvir and sofosbuvir in drug product by liquid chromatography. The chromatographic separation was achieved on the C18 column (Luna 18 150*4.6 mm3.0 um) at ambient temperature. The separation achieved employing a mobile phase consists of 0.1%v/v formic acid in water: methanol:acetonitrile (35:40:25). The flow rate was 0.8 ml/min and ultraviolet detector at 269 nm. The average retention time for velpatasvir and sofosbuvir found to be 2.62 min and 3.72 min.

Results: The developed method was validated as per the ICH analytical method validation guidelines. All validation parameters were within the acceptable range. The assay methods were found to be linear from 80 to 240 µg/ml for sofosbuvir and 20–60 µg/ml for velpatasvir. The correlation coefficient was 0.9998 and 0.9992 for velpatasvir and sofosbuvir respectively. The mean percentage recovery for the developed method was found to be in the range of 98.4–100.4% for velpatasvir and 98.6–100.6% for sofosbuvir. The developed method was also found to be robust.

Conclusion: The developed method was found to be suitable for the routine quantitative analysis of velpatasvir and sofosbuvir in bulk and pharmaceutical dosage form. It was also concluded that developed method was accurate, precise, linear, reproducible, robust, and sensitive.

Keywords: Sofosbuvir, Velpatasvir, Isocratic, High performance liquid chromatography, C18, Formic acid, Methanol.

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INTRODUCTION

Sofosbuvir

The drug is used for the treatment of hepatitis C. It is only recommended for some combination of ribavirin, peginterferon-alfa, simeprevir, ledipasvir, or daclatasvir. Cure rates are 30–97% depending on the type of hepatitis C virus involved. Safety during pregnancy is unclear; while, some of the medications used in combination may result in harm to the baby. It is taken by mouth.

Molecular formula: C22H29FN3O9P.

Molecular weight: 529.45 g/mol.

Solubility: Soluble in Methanol, Acetonitrile, and water.

Pka: 9.3.

Mechanism of action: Sofosbuvir inhibits the hepatitis C NS5B protein. Sofosbuvir appears to have a high barrier to the development of resistance.

It is metabolized to the active antiviral agent GS-461203 (2'-deoxy-2'-α-fluoro-β-C-methyluridine-5'-triphosphate). GS-461203 serves as a defective substrate for the NS5B protein, which is the viral RNA polymerase, thus acts as an inhibitor of viral RNA synthesis. Although sofosbuvir has a 3' hydroxyl group to act as a nucleophile for an incoming NTP, a similar nucleotide analog, 2'-deoxy-2'-α-fluoro-β-C-methylcytidine, is proposed to act as a chain terminator because the 2' methyl group of the nucleotide analog causes a steric clash with an incoming NTP.

Half-Life: Sofosbuvir has a half-life of 0.4 h.

Route of elimination: Sofosbuvir, as a single agent, has very mild toxicity. The most common adverse reactions are a headache and fatigue.

Velpatasvir

Velpatasvir is an NS5A inhibitor which is used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

Molecular formula: C49H54N8O8.

Molecular Weight: 883.02 g·mol−1.

Solubility: Soluble in water, methanol, and acetonitrile.

Pka: 3.74.

Indication: Used together with sofosbuvir in the treatment of hepatitis C infection of all six major genotypes.

Mechanism of action: The substance blocks NS5A, a protein necessary for hepatitis C virus replication and assembly.

METHODS

Equipment

The chromatographic technique performed on a waters 2695 with 2487 detector and Empower 2 software, reversed phase C18 column (Luna C18 150*4.6,3 um) as stationary phase, ultrasonic cleaner, scaletech analytical balance, vacuum microfiltration unit with 0.45 µ membrane filter was used in the study.

Materials

Pharmaceutically pure sample of sofosbuvir and velpatasvir was obtained as gift samples from Fortune Pharma Training Institute, Sri Sai Nagar Colony, KPHB, and Hyderabad, India.

High performance liquid chromatography-grade Methanol and Acetonitrile were from qualigens reagents Pvt., Ltd. Formic acid (AR grade) was from sd fine chem.

Chromatographic conditions

The sample separation was achieved on a C18 (Luna C18 150*4.6, 3 um) column, aided by mobile phase mixture of 0.1%v/v formic acid in water: methanol:acetonitrile (35:40:25). The flow rate was 0.8 ml/min and ultraviolet detector at 269 nm, injection volume is 10 µl and ambient temperatures.

Preparation of mobile phase

Buffer preparation: Take accurately 1 ml of Formic acid in 1000 ml of water.

Mobile phase: Then add 35 volumes of buffer, 40 volumes of Methanol and 25 volumes of Acetonitrile mixed well and sonicated for 10 min.

Diluent: water: Acetonitrile: 50:50 v\v

Preparation of standard solution

Preparation of stock solution

A 400 mg of pure sofosbuvir and 100 mg velpatasvir were weighed and transferred into 100 ml of volumetric flask and dissolved in the diluent. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution containing 800 µg/ml of sofosbuvir and 200 µg/ml of velpatasvir.

Calibration standards

From the primary stock solution 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml of aliquots are pipette into 50 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 80 µg/ml, 120 µg/ml, 160 µg/ml, 200 µg/ml, and 240 µg/ml of sofosbuvir and 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, and 60 µg/ml of velpatasvir.

Sample solution

Accurately weighed 20 tablets were ground to obtain fine powder equivalent to 400 mg of sofosbuvir and 100 mg of velpatasvir sample were weighed and transferred to 100 ml of volumetric flask and dissolved in diluents. The flask was shaken and volume was made up to mark with diluent to give a primary stock solution. From the above solution, 2 ml of solution is pipetted out into a 50 ml volumetric flask and volume was made up to mark with diluent to give a solution containing 160 µg/ml of sofosbuvir and 40 µg/ml of velpatasvir.

Method validation

System suitability

The typical values for evaluating system suitability of a chromatographic procedure are relative standard deviation (RSD) <2%, tailing factor <1.5, and theoretical plates >1500. The retention time, peak area, theoretical plates, and tailing factor were evaluated for the system.

Linearity

Linearity was studied by analyzing five standard solutions covering the range of 80–240 µg/ml for sofosbuvir and 20–60µg/ml for velpatasvir. From the primary stock solution 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml of aliquots are pipetted into 50 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 80 µg/ml, 120 µg/ml, 160 µg/ml, 200 µg/ml, and 240 µg/ml of sofosbuvir and 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, and 60 µg/ml velpatasvir. A calibration curve with concentration versus peak areas was plotted by injecting the above-prepared solutions.

Limit of detection and limit of quantification

The limit of detection (LOD) and limit of quantification (LOQ) were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

LOD=3.3 δ/S.

LOQ=10 δ/S.

Where,

δ=The standard deviation of the response.
S=The slope of the calibration curve.

The slope S may be estimated from the calibration curve of the analyte.

Method precision

The precision of the method was checked by repeated preparations. The measurement of peak areas of repeated solutions (n=6) for 160 µg/ml of sofosbuvir and 40 µg/ml of velpatasvir.

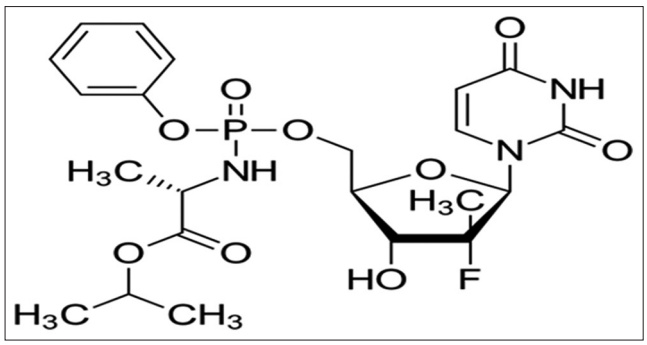

Fig. 1: Chemical structure of sofosbuvir

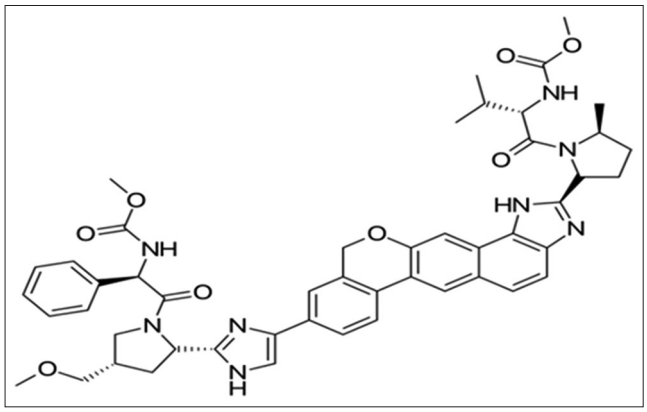

Fig. 2: Chemical structure of velpatasvir

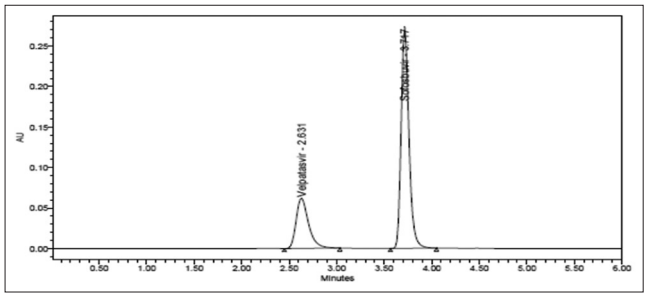

Fig. 3: Chromatogram of velpatasvir and sofosbuvir

Accuracy
The accuracy of the method was determined by calculating the recoveries of sofosbuvir and velpatasvir by analyzing solutions containing approximately 50%, 100%, and 150% of the working strength of sofosbuvir and velpatasvir.

Robustness
Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters such as flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied±2 nm and flow rate was varied±0.1 ml/min. The results are shown in Table 8.

RESULTS AND DISCUSSIONS

System suitability
The system suitability of the method was checked by repeated preparations for 160 µg/ml of sofosbuvir and 40 µg/ml of velpatasvir. The typical values for evaluating system suitability of a chromatographic procedure are RSD <2%, tailing factor <1.5, and theoretical plates >1500. The retention time, peak area, theoretical plates, and tailing factor were evaluated for the system, system suitability data of velpatasvir and sofosbuvir are shown in Table 1.

Linearity and range
Linearity was studied by analyzing five standard solutions covering the range of 80–240 µg/ml for sofosbuvir and 20–60 µg/ml for velpatasvir. From the primary stock solution 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, and 3.0 ml of aliquots are pipetted into 50 ml volumetric flasks and made up to the mark with the mobile phase to give a concentrations of 80 µg/ml, 120 µg/ml, 160 µg/ml, 200 µg/ml, and 240 µg/ml of sofosbuvir and 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml, and 60 µg/ml velpatasvir. A calibration curve with concentration versus peak areas was plotted by injecting the above-prepared solutions. Correlation coefficient values for velpatasvir and sofosbuvir are 0.9998 and 0.9992, respectively. The linear regression data for the calibration plot indicate a good linear relationship between peak area and concentration. The linearity data for velpatasvir and sofosbuvir are shown in Fig. 4.

Precision
The precision of the method was checked by repeated preparations. The measurement of peak areas of repeated solutions (n=6) for 160 µg/ml of sofosbuvir and 40 µg/ml of velpatasvir. The study was expressed as RSD of a set of results. The precision of the method (% RSD) of was found to be <1% showing good repeatability. The values of percentage RSD for velpatasvir and sofosbuvir are shown in Tables 2 and 3.

LOD and LOQ
The LOD and LOQ were separately determined based on standard deviation of the y-intercept and the slope of the calibration curve.

The LOD and LOQ of the proposed method were found to be 0.001 and 0.004 for velpatasvir and 0.01 and 0.03 for sofosbuvir.

Accuracy study
The accuracy of the method was determined by calculating the recoveries of sofosbuvir and velpatasvir by analyzing solutions

Table 1: System suitability data of velpatasvir and sofosbuvir

Parameter	Velpatasvir	Sofosbuvir	Acceptance criteria
Retention time	2.620	3.718	±10
Theoretical plates	2075	9822	>2000
Tailing factor	1.34	1.23	<1.50
RSD	0.63	0.42	<2.00

RSD: Relative standard deviation

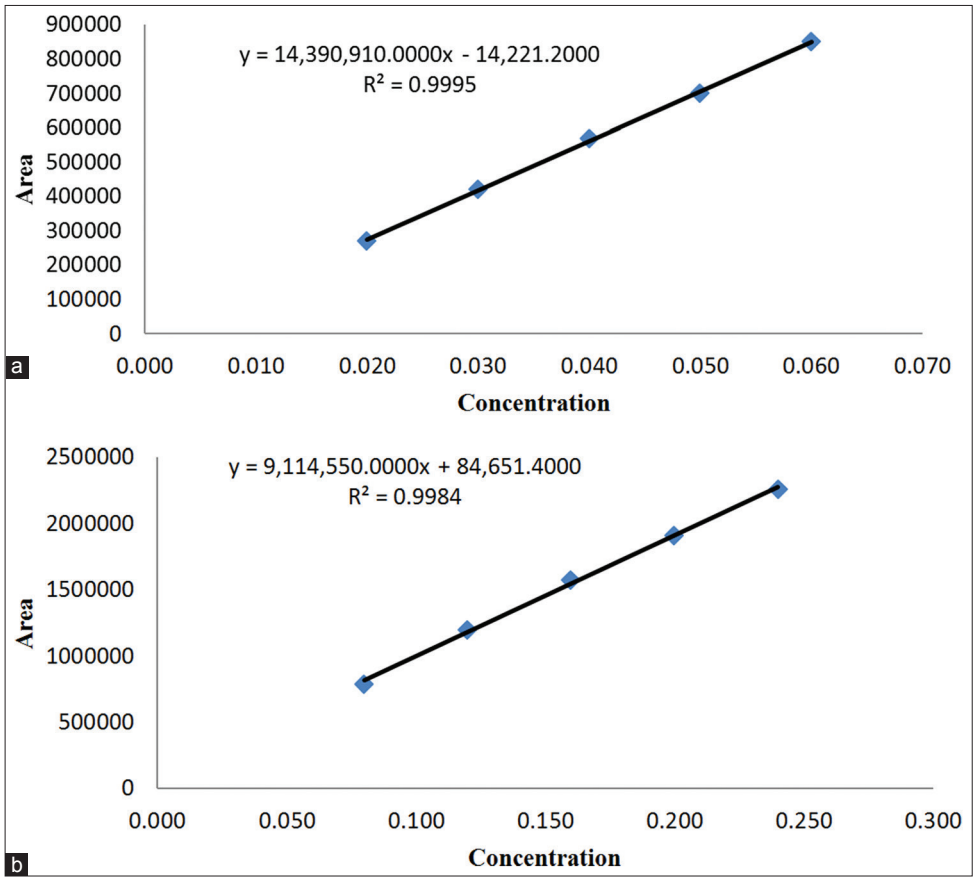

Fig. 4: Calibration curve: (a), Velpatasvir; (b), Sofosbuvir

Table 2: Method precision data for velpatasvir

Sample number	Retention time	Peak area	Percentage assay
1	2.590	567139	100.3
2	2.593	565935	99.5
3	2.589	566080	99.9
4	2.585	569675	100.0
5	2.586	565706	99.3
6	2.585	569999	99.8
Mean±SD	2.588±0.003	567422±1937	99.8±0.37
RSD	0.12	0.34	0.37

RSD: Relative standard deviation, SD: Standard deviation

Table 3: Method precision data for sofosbuvir

Sample number	Retention time	Peak area	Percentage assay
1	3.716	1576563	100.6
2	3.719	1570565	99.8
3	3.718	1577711	99.5
4	3.715	1579770	100.2
5	3.718	1573853	98.8
6	3.717	1583134	100.6
Mean±SD	3.717±0.001	1576933±4411	99.9±0.69
RSD	0.04	0.28	0.69

RSD: Relative standard deviation, SD: Standard deviation

Table 4: LOD and LOQ values calculated from calibration curve

Parameter	Sofosbuvir (mg)	Velpatasvir
LOD	0.010	0.001
LOQ	0.030	0.004

LOD: Limit of detection, LOQ: Limit of quantification

Table 5: Recovery data for velpatasvir

Accuracy level (%)	Injection	Sample area
50	1	100.4
	2	99.7
	3	98.4
100	1	100.3
	2	99.5
	3	99.9
150	1	99.1
	2	98.6
	3	100.3

Table 6: Recovery data for sofosbuvir

Accuracy level (%)	Injection	Sample area
50%	1	100.1
	2	100.2
	3	99.0
100%	1	100.6
	2	99.8
	3	99.5
150%	1	100.0
	2	98.6
	3	99.4

containing approximately 50%, 100%, and 150% of the working strength of sofosbuvir and velpatasvir. Recovery data for velpatasvir and sofosbuvir are shown in Tables 5 and 6.

Table 7: Robustness data for velpatasvir

Parameter	RT	Area
Decreased flow rate (0.7 ml/min)	2.906	654418
Increased flow rate (0.9 ml/min)	2.290	495734
Wavelength 268 nm	2.631	559851
270	2.628	564858

RT: Retention time

Table 8: Robustness data for sofosbuvir

Parameter	RT	Area
Decreased flow rate (0.8 ml/min)	4.221	1832255
Increased flow rate (1.2 ml/min)	3.302	1375616
Wavelength 268 nm	3.717	1552022
270	3.720	1559976

RT: Retention time

Robustness

Robustness is the measure of a method remain unaffected by small, deliberate changes in method parameters such as flow rate and detection wavelength on assay of the analyte of interest. Here the detection wavelength varied±2 nm and flow rate was varied±0.1 ml/min. The results are shown in Table 7 and Table 8.

CONCLUSION

From the experimental results and parameters it was concluded that, this newly developed method for the simultaneous estimation of sofosbuvir and velpatasvir was found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost-effective, and it can be effectively applied for routine analysis in research institutions, quality control department, and approved testing laboratories.

CONFLICTS OF INTERESTS

All authors declare no conflicts of interests.

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Nil.

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