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ORIGINAL RESEARCH PAPER

Spectrophotometric and spectrodensitometric quantification of a new antiviral combination

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Abstract
Accurate, simple, and selective spectrophotometric and spectrodensitometric methods were developed and adopted to quantify velpatasvir (VPS) and sofosbuvir (SFV) concurrently in their pure forms and tablets. The spectrophotometric technique was based on the first derivative of ratio spectra (^{1}DD) technique and developed to determine VPS and SFV simultaneously in table formulation. However, the spectrodensitometric technique was based on thin-layer chromatography (TLC) and densitometry and developed to determine VPS and SFV simultaneously in tablet dosage form. Chromatographic separation was performed using chloroform:methanol 9.5:0.5 (%, *v/v*) as the mobile phase on glass-coated TLC plates. Detection was achieved using a 265-nm deuterium lamp in absorbance mode. Both analytical methods were validated according to the International Conference on Harmonization (ICH)-Q2B guidelines. The linearity in the range of concentration ranges of 1–50 μg/mL and 5–80 μg/mL were obtained for VPS and SFV, respectively, using ^{1}DD spectrometric method. However, the linearity in the range of 5–50 and 10–70 μg/band for VPS and SFV, respectively, were recorded using TLC–densitometric method. Accuracy was recorded >100% for VPS and SFV using both methods. This is the first TLC–densitometry method that can separate and quantify the studied mixture of the drugs. The proposed analytical methods were found to be accurate, precise, selective, robust and sensitive for simultaneous analysis of VPS and SFV in tablet dosage forms.

Keywords First derivative of ratio spectra · Tablet dosage form · Sofosbuvir · Spectrophotometry · Spectrodensitometry · Velpatasvir

Introduction

Hepatitis C virus (HCV) is a well-known and dangerous virus that can cause a life-threatening disease. In the United States (US), HCV affects approximately three to five million people, while approximately 170 million people are infected globally. In its early stages, the disease caused by HCV has no symptoms; however, when it becomes chronic, it can lead to serious fatal complications, including hepatocellular carcinoma and death [1]. Velpatasvir (VPS), a direct-acting antiviral, is an effective agent that can be administered to HCV patients. VPS acts as a defective substrate for nonstructural protein 5A (NS5A), which plays a key role in the replication of HCV ribonucleic acid (RNA). Hence, VPS helps to prevent HCV-RNA replication [2]. Sofosbuvir (SFV), a nucleotide analog NS5B polymerase inhibitor that acts as a prodrug, is also used to treat HCV patients [2].

In 2016, US Food and Drug Administration (FDA) approved the combination of VPS and SFV for treating chronic HCV infection (genotypes 1, 2, 3, 4, 5, or 6) in adult patients [3]. VPS has been reported as practically insoluble in aqueous buffers having pH >5.0, slightly soluble in aqueous buffers of pH 2.0 and soluble in

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aqueous buffers of pH 1.2. However, SFV has been reported as slightly soluble in water and aqueous buffers of pH 2.0–7.7 [3, 4]. The bioavailability of VPS (25–30%) has been reported as low while it has been reported higher for SFV (92%) after oral administration of tablets [3]. These two drugs have been investigated using different chromatographic and spectrophotometric techniques [4–8]. The forced degradation of SFV was studied by liquid chromatography–mass spectrometry (LC–MS) technique [9]. The concurrent analysis of VPS and SFV in commercial tablets was studied extensively using reversed-phase high-performance liquid chromatography (HPLC) techniques [10–21]. HPLC technique was also applied for the concurrent analysis of VPS and SFV in human plasma [14]. Some LC–MS techniques have also been reported for the concurrent analysis of VPS and SFV in human plasma [22, 23]. Few ultra-performance liquid chromatography techniques were also applied for the concurrent analysis of VPS and SFV in tablet dosage forms [7, 24]. Capillary electrophoresis technique has also been reported for the concurrent analysis of VPS and SFV in formulations [25]. A single spectrodensitometric technique has been reported for the concurrent determination of VPS and SFV in tablets [26]. However, the most reported methods of analysis are based on chromatographic techniques that are more expensive and complicated than spectrophotometric and spectrodensitometric techniques. Spectrodensitometric techniques offer several advantages over other analytical techniques such as HPLC and these advantages include cost, economy, sample preparation and sample analysis etc. [26–28]. Thus, there remains an urgent need for accurate, simple and cost-effective methods for the simultaneous estimation of VPS and SFV. Hence, the goal of the present study was to establish accurate, simple and cost-effective analytical methodologies for the concurrent quantification of VPS and SFV either in their pure forms or tablets.

Experimental

Chemicals and materials

Chromatography grade water was utilized in all experiments and it was obtained from Milli-Q Water Purification Unit. VPS (purity of 100.23%) was obtained from BioVision (Milpitas, CA, USA). SFV (PSI-7977; purity of 99.98%) was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). Methanol was procured from Sigma-Aldrich (St. Louis, MO, USA). All other reagents or chemicals used were of analytical/pharmaceutical grades.

Instrumentation

A double-beam spectrophotometer (JASCO, Kyoto, Japan) containing matched quartz cuvettes with path lengths of 1 cm was used. The spectrophotometer was attached with an IBM-compatible computer with an HP 680 inkjet printer (Hewlett Packard, USA). A CAMAG thin-layer chromatography (TLC) scanner (3 S/N 130319) operated with winCATS software and a CAMAG Linomat 5 Autosampler (Muttenz, Switzerland) with a CAMAG microsyringe (100 μL) were used.

Pharmaceutical formulations

The pharmaceutical formulations were Epclusa® extended-release tablets (Gilead Sciences International, Cambridge, UK). The claimed amounts of VPS and SFV in Epclusa® tablet were 100 mg and 400 mg/tablet, respectively.

Standard and working solutions

Spectrophotometric method

Stock standard solution of VPS was obtained by accurately weighing 100 mg of pure VPS into a 100-mL volumetric flask. The powder was dissolved, and the volume was brought to 100 mL using methanol. Stock standard solution of SFV (2.5 mg/mL) was prepared in the same manner but with 25 mg of pure SFV in a 25-mL volumetric flask.

Working standard solutions for the studied drugs were prepared by diluting 5 mL of the VPS or SFV stock solution in a 50-mL volumetric flask and diluting with methanol to obtain standard working solutions with concentrations 100 and 250 μg/mL for VPS and SFV, respectively.

TLC–densitometric method

Standard stock solutions of VPS and SFV were obtained by dissolving 25 mg of VPS or SFV into 20 mL methanol. Both the drugs were dissolved separately under vortex mixing. The final volume of each drug was maintained with methanol in order to obtain stock solution of 1.0 mg/mL for both drugs.

Validation of the proposed methods

The above methods were validated according to ICH-Q2B guidelines [29].

Linearity

First derivative of ratio spectra (^{1}DD) method VPS (10–500 μg/mL) and SFV (50–800 μg/mL) working standards were prepared separately using methanol as a solvent. The ^{1}DD

technique was applied to determine VPS (1–50 μg/mL) by measuring the peak amplitudes at 290 nm using SFV (80 μg/mL) as a divisor. The proposed ^{1}DD technique was also applied to quantify SFV (5–80 μg/mL) by obtaining the peak amplitudes at 243 nm using VPS (50 μg/mL) as a divisor.

TLC–densitometric method Aliquiots of VPS (5–50 μL) and SFV (10–70 μL) stock standards (1 mg/mL) were applied to glass-coated TLC plates (silica gel 60 F_{254}; 20 cm × 20 cm, 0.25 mm) in the form of bands using an |autosampler (CAMAG Linomat 5) and a 100-μL microsyringe (Hamilton) to obtain the concentrations in the range of 5–50 μg/band for VPS and 10–70 μg/band for SFV. The bands were applied at spacings of 10 mm. The air-dried plates were developed and pre-saturated for at least 1 h using mobile phase [chloroform:methanol, 9.5:0.5 (*% v/v*)] at ambient temperature. The developed plates were air-dried and the spots were identified using a deuterium lamp at λ_{max} of 265 nm in reflectance/absorbance mode. p-Anisaldehyde was used as a visializing agent to identify the spots. Calibration curves for VPS and SFV were prepared separately by plotting the measured peak areas and concentration. Linear regression analysis was performed to generate regression equations and various stattistical parameters.

Accuracy

Accuracy was evaluated as the percent of recovery (% recovery) [29]. Accuracy was evaluated at three different concentrations of VPS (i.e., 5, 10 and 40 μg/mL for the spectrophotometric technique and 10, 20 and 30 μg/band for the TLC–densitometric technique) and SFV (5, 20 and 40 μg/mL for the spectrophotometric technique and 20, 30 and 40 μg/band for the TLC–densitometric technique).

Precision

Precision of both analytical methodologies were estimated as the repeatability and intermediate variation [29]. Three different concentrations of VPS (i.e., 5, 10 and 40 μg/mL for the spectrophotometric technique and 10, 20 and 30 μg/band for the TLC–densitometric technique) and SFV (5, 20 and 40 μg/mL for the spectrophotometric technique and 20, 30 and 40 μg/band for the TLC–densitometric technique) were analyzed for repeatability (intra-day precision) and intermediate precision (inter-day variation) using the two proposed analytical methods. The results were documented as the percent of the coefficient of variation (%CV).

Specificity and selectivity

Specificity was determined by comparing the ultraviolet, ^{1}DD spectra and TLC–densitometric results recorded for VPS, SFV and commonly used excipients with the blank (a solution of excipients in methanol without drugs) [29]. The selected excipients were those indicated in the manufacturer's monograph of the Epclusa® extended-release tablets. Selectivity was verified by the quantification of mixtures prepared in the laboratory containing different amounts of VPS and SFV.

Detection and quantification limits

The limit of detection (LOD) is the lowest detectable amount of drug which could be distinguished from the background, while the limit of quantification (LOQ) is the lowest accurate and precise amount of the target drug [29]. LOD and LOQ of the proposed analytical methodologies were obtained using standard deviation (σ) technique by applying Eqs. (1) and (2), respectively:

$$\mathrm{LOD} = 3.3 \times \frac{\sigma}{S} \quad (1)$$

$$\mathrm{LOD} = 10 \times \frac{\sigma}{S} \quad (2)$$

Where, S is the slope of the calibration curve.

Robustness

Robustness can be checked by evaluating the effect of minute changes in assay conditions on method validity. For the ^{1}DD method, robustness was evaluated by making minute changes in the degree of smoothing of the derivative ratio spectra. For the TLC–densitometric method, robustness was checked by making minute changes to the solvent mixture used in densitometric separation.

Analysis of VPS and SFV in Epclusa® extended-release tablets

Ten Epclusa® extended-release tablets were weighed and the average weight was obtained. The tablets were then crushed into a fine powder and mixed well. Tablet powder equivalent to 100 mg of VPS and 400 mg of SFV was then transferred to a 250-mL beaker and an amount of 40 mL of methanol was added followed by stirring for approximately 20 min. The solution was then filtered into a 100-mL volumetric flask. The residue was washed twice with approximately 10 mL methanol and the volume was brought to the mark using methanol followed by extensive mixing. The necessary dilutions were performed for both methods. The contents of VPS and SFV were determined using the proposed analytical methods. The concentration of each drug was calculated from the calibration curve of each drug.

Standard addition was performed by spiking various known amounts of VPS and SFV in the tablets. The samples were then re-analyzed using the proposed analytical methods.

Comparison with reference published methods

The results obtained using the proposed analytical techniques for quantifying VPS and SFV were statistically compared with those obtained by reported reference methods in order to evaluate the accuracy and precision of the proposed techniques [9].

Results and discussion

Spectrophotometric method

Molecular absorption spectroscopy was used to quantify the active pharmaceutical ingredients (APIs). However, the use of molecular absorption spectra in drug analysis by the aid of Beer–Lambert's law is hindered especially when the studied drugs have severe overlap in the zero order curves as in the case of the present study. Therefore, different alternatives were used like the use of first derivative of ratio spectra method in this study. The DD^1 absorption spectra of VPS and SFV recorded in this work are presented in Fig. 1. The overlap between the VPS and SFV spectra was addressed by applying the ^{1}DD method (Figs. 2 and 3). This technique depends on the derivatization of the ratio spectra to remove the interference between the components of the binary mixture. The most important merit of this method is the presence of many maxima and minima, which allow us to determine the concentrations of the target drugs in the presence of excipients and other interfering materials [30].

TLC–densitometric method

Without prior separation, the simultaneous determination of VPS and SFV was performed using a coupled TLC–densitometric method. Different solvent systems were used to separate the two drugs. Satisfactory results were obtained by applying the experimental conditions mentioned previously with chloroform:methanol (9.5:0.5; % *v/v*) as the mobile phase. The values of retention factor (Rf) for SFV and VPS were obtained as 0.19 and 0.29, respectively (Fig. 4).

Method validation

Linearity

Spectrophotometric method To determine the VPS concentration in the range of 1–50 μg/mL, a linear standard curve was plotted between the peak heights for the ^{1}DD spectra at 290 nm using SFV (80 μg/mL) as a divisor (Fig. 2). To determine SFV concentration in the range of 5–80 μg/mL, the peak heights in the ^{1}DD-spectra at 243 nm were used with VPS (50 μg/mL) as the divisor (Fig. 3). The linear regression equations for VPS and SFV were expressed as Eqs. (3) and (4), respectively:

$$^1DD_{SFV} = 0.0344C + 0.1099 \quad (r^2 = 0.9990) \tag{3}$$

$$^1DD_{SFV} = 0.0045C + 0.005 \quad (r^2 = 0.9999) \tag{4}$$

Where, ^{1}DD is the peak height, C is the corresponding concentration and r^2 is the correlation coefficient.

TLC–densitometric method The TLC–separation technique was also used to determine VPS and SFV concentrations. Linearity was established by plotting the measured peak area versus the corresponding concentrations at 265 nm over the concentration ranges of 5–50 and 10–70 μg/band for VPS and SFV, respectively (Fig. 5). The respective regression equations for VPS and SFV were expressed as Eqs. (5) and (6), respectively:

$$PA_{VPS} = 1.4514C + 2330.9 \quad (r^2 = 0.9994) \tag{5}$$

$$PA_{SFV} = 3.888C + 446.29 \quad (r^2 = 0.9995) \tag{6}$$

Where, PA is the area under the peak.

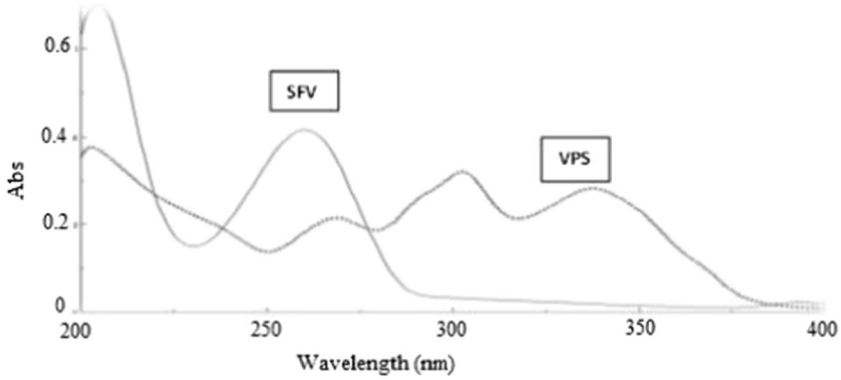

Fig. 1 Absorption spectra of SFV (20 μg/mL) and VPS (5 μg/mL)

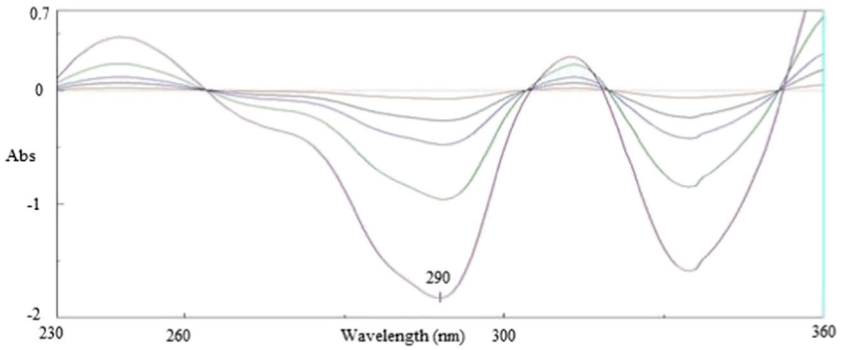

Fig. 2 DD^1 spectra of VPS in the concentration range of 1–50 μg/mL

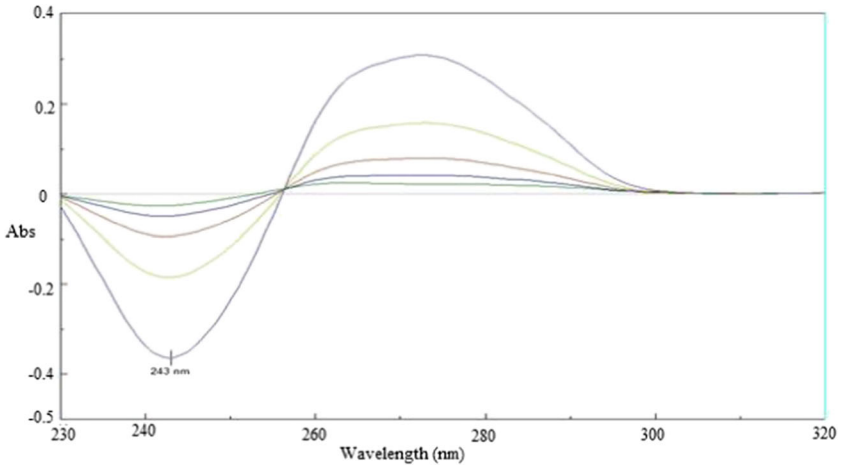

Fig. 3 DD^1 spectra of SFV in the concentration range of 5–80 μg/mL

Accuracy

Accuracy was obtained by the evaluation of various quality control (QC) samples at three different concentrations of VPS and SFV within the specified linearity ranges for each analyte. The recovery and %CV were calculated and results are tabulated in Table 1. The results of recovery and %CV were found to be satisfactory for both analytical methods. These results suggested that both analytical methods were accurate for concurrent analysis of VPS and SFV.

Precision

To determine the repeatability and intermediate precisions, three different concentrations of VPS or SFV were analyzed on the same day and three successive days, respectively. The %CV values for the repeatability and intermediate precisions were recorded as less than 2% for both the analytical methods. These results suggested the precision of the proposed analytical methods (Table 1).

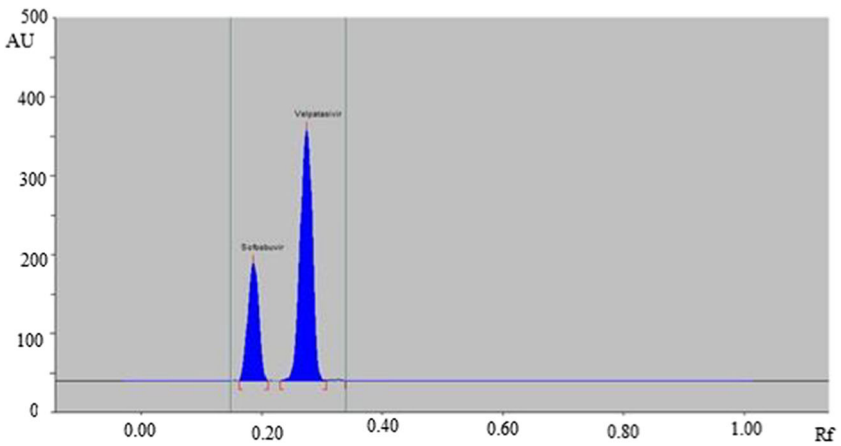

Fig. 4 TLC–densitometric separation patterns of VPS and SFV

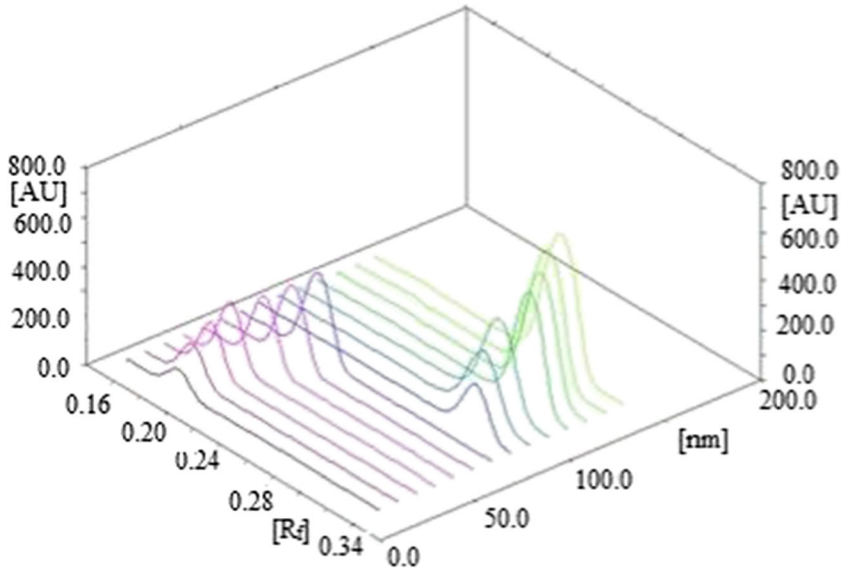

Fig. 5 Three-dimensional separation patterns for VPS and SFV in their linearity ranges

Specificity and selectivity

None of the examined excipients interfered with the analytes, indicating that the proposed methods are specific. Hence, the adopted methods are suitable for application in the assays for the two investigated HCV agents in commercial products since no detectable interference from the formulation matrix was found.

To check selectivity, the proposed analytical methods were applied in the quantification of VPS and SFV in the mixtures prepared in the laboratory. Good results with acceptable %CV values were obtained, confirming the selectivity of the proposed analytical methods (Table 2).

LOD and LOQ

The acceptability of the proposed methods was evaluated based on the LOD and LOQ values. The LOD and LOQ values confirmed that the proposed methods are sufficient and acceptable (Table 1).

Table 1 Validation parameters for the determination of VPS and SFV by the proposed methods

	VPS		SFV	
	[1]DD method	TLC–densitometry	[1]DD method	TLC–densitometry
Linearity				
Range	1–50 μg/mL	5–50 μg/band	5–80 μg/mL	10–70 μg/band
Slope	0.0344	1.4514	0.0045	3.888
Intercept	0.1099	2330.9	0.005	446.29
r^2	0.9990	0.9994	0.9999	0.9995
Accuracy				
Mean ± SD*	100.20 ± 1.545	100.25 ± 1.290	101.88 ± 1.312	100.94 ± 1.143
Variance	2.387	1.664	1.721	1.306
%CV	1.542	1.287	1.301	1.132
Precision				
Intra-day precision	101.94 ± 0.982	100.14 ± 0.742	99.74 ± 0.978	101.24 ± 0.548
Inter-day precision	101.21 ± 0.942	99.21 ± 0.175	100.84 ± 0.231	98.978 ± 1.012
Robustness				
Mean ± SD*	99.88 ± 0.811	101.23 ± 0.811	100.74 ± 0.741	99.74 ± 0.547
LOD	0.310 μg/mL	3.122 μg/band	2.500 μg/mL	8.412 μg/band
LOQ	0.982 μg/mL	4.970 μg/band	4.986 μg/mL	9.992 μg/band

* Standard deviation, average of three determinations

Table 2 Application of the proposed methods to the determination of VPS and SFV in laboratory-prepared mixtures

	VPS		SFV	
	[1]DD method	TLC–densitometry	[1]DDmethod	TLC–densitometry
Claimed concentration of VPS/SFV mixture	5 μg/mL	5 μg/band	20 μg/mL	20 μg/band
Mean ± SD*	99.47 ± 0.799	98.21 ± 1.211	102.38 ± 0.874	101.54 ± 1.391
%CV	0.803	1.233	0.854	1.370
Claimed concentration of VPS/SFV mixture	10 μg/mL	10 μg/band	40 μg/mL	40 μg/band
Mean ± SD*	101.24 ± 0.584	98.74 ± 0.814	98.54 ± 0.845	100.87 ± 0.943
%CV	0.577	0.824	0.858	0.935
Claimed concentration of VPS/SFV mixture	15 μg/mL	15 μg/band	60 μg/mL	60 μg/band
Mean ± SD*	101.97 ± 0.974	99.72 ± 0.247	101.24 ± 0.581	102.94 ± 1.412
%CV	0.955	0.248	0.574	1.372

* Standard deviation, average of three determinations

Table 3 Determination of VPS and SFV in Epclusa® extended-release tablets and standard addition recovery evaluation using the proposed methods

Epclusa® extended-release tablets labeled to contain 100 mg VPS and 400 mg SFV	Content uniformity		Standard addition	
	VPS		SFV	
	[1]DD method	TLC–densitometry	[1]DD method	TLC–densitometry
Mean ± SD*	99.54 ± 0.874	102.84 ± 1.024	100.11 ± 0.541	99.01 ± 1.134
%CV	0.878	0.996	0.540	1.145

* Standard deviation, average of three determinations

Robustness

Slight changes in the determination conditions resulted in effects smaller than 1%CV, confirming the robustness of the both analytical methods (Table 1).

Quantification of VPS and SFV in Epclusa® extended-release tablets

VPS and SFV in Epclusa® extended-release tablets were quantified to ensure content uniformity using the proposed methods (Table 3). The marketed combination showed

Table 4 Comparison of the proposed methods and a reference method for the determination of VPS and SFV in bulk samples

Item	VPS			SFV		
	[1]DD method	TLC–densitometry	Reference method	[1]DD method	TLC–densitometry	Reference method
Mean ± SD	100.20 ± 1.545	100.25 ± 1.290	101.48 ± 0.988	101.88 ± 1.312	100.94 ± 1.143	102.17 ± 1.219
%CV	1.542	1.287	0.974	1.301	1.132	1.193
n	5	5	5	5	5	5
Variance	2.388	1.664	0.977	1.720	1.306	1.485
F-value (6.39)	2.44	1.703	–	1.158	1.158	–
Student's *t* test (2.3)	1.542	1.690	–	1.609	1.652	–

Values between brackets are the theoretical values of *t* and *F* at $P = 0.05$

acceptable recoveries within the acceptable content uniformity limits. The standard addition technique was also applied by spiking various known amounts of VPS and SFV into the tablets in order to ensure the reliability and applicability of both analytical techniques. The results indicated satisfying recoveries of spiked drugs using the proposed methods (Table 3).

Comparison with reference method

The developed analytical techniques of the present study were compared statistically with previously reported technique [9]. The results of this comparison are tabulated in Table 4. The results indicated insignificant differences between the proposed methods and the reference method, confirming the accuracy and precision of the developed methods.

Conclusions

Based on the results presented herein, we can conclude that the proposed methods are accurate, selective, precise and simple approaches for simultaneously quantifying VPS and SFV over the specified linearity ranges in either bulk or tablet form. The developed methods can easily be adopted in laboratories without HPLC capabilities. The proposed analytical methods could be successfully applied for the routine analysis of all pharmaceutical products containing VPS and SFV as the active ingredients.

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Compliance with ethical standards

Conflict of interest The authors report that they have no competing interest associated with this manuscript.

References

1. Te HS, Jensen DM (2010) Epidemiology of hepatitis B and C viruses: a global overview. Clin Liver Dis 14:1–21
2. Li DK, Chung RT (2019) Overview of direct-acting antiviral drugs and drug resistance of hepatitis C virus. Methods Mol Biol 1911:3–32
3. El-Gamal RM, Abdel-Gawad SA, Belal FF, Moustapha ME et al (2018) RSC Adv 8:32909–32915
4. Abdel-Gawad SAN (2016) Simple chromatographic and spectrophotometric determination of sofosbuvir in pure and tablet forms. Eur J Chem 7:375–379
5. Miraghaei S, Mohammadi B, Babaei A, Keshavarz S, Bahrami G (2017) Development and validation of a new HPLC-DAD method for quantification of sofosbuvir in human serum and its comparison with LC–MS/MS technique: application to a bioequivalence study. J Chromatogr B 1063:118–122
6. Reema S, Dhara P, Khushboo P, Dhananjay M (2018) Development and validation of three vovel UV spectrophotometric methods for determination of newly discovered combination for the treatment of hepatitis C and their comparison using ANOVA. Int J Pharm Drug Anal 6:391–399
7. Kamal AH, Mabrouk MM, Bebawy LI, Mekky MA (2019) Spectrophotometric and robust UPLC methods for simultaneous determination of velpatasvir and sofosbuvir in their tablet. Microchem J 149:E103996
8. Rezk MR, Monir HH, Marzouk HM (2019) Spectrophotometric assessment of the brand new antiviral combination: sofosbuvir and in their pure forms and pharmaceutical formulation. Spectrochim Acta Part A 213:159–166
9. Nebsen M, Elzanfaly ES (2016) Stability-indicating method and LC–MS-MS characterization of forced degradation products of sofosbuvir. J Chromatogr Sci 54:1631–1640
10. Bandla J, Ganapaty S (2017) Stability indicating RP-HPLC method development and validation for the simultaneous determination of sofosbuvir and velpatasvir in tablet dosage forms. Indian J Pharm Biol Res 5:10–16
11. Nalla S, Seshagiri Rao J (2017) A stability indicating RP-HPLC method for simultaneous estimation of velpatasvir and sofosbuvir in combined tablet dosage forms. World J Pharm Pharm Sci 6:1596–1611
12. Jyothi U, Umadevi P (2017) Analytical method development and validation for the simultaneous estimation of sofosbuvir and velpatasvir drug product by RP-HPLC method. Indo Am J Pharm Res 7:401–409
13. Kumaraswamy G, Pranay K, Rajkumar M, Lalitha R (2017) Novel stability indicating RP-HPLC method simultaneous determination of sofosbuvir and velpatasvir in bulk and combined tablet dosage forms. Innov Int J Med Pharm Sci 2:81–85
14. Phani RSC, Prasad KRS, Mallu UR (2017) A bioanalytical method development and validation for simultaneous determination of velpatasvir and sofosbuvir in spiked human plasma. Asian J Chem 29:2565–2569
15. Harshalatha P, Chandrasekhar KB, Chandrasekhar MV (2018) A novel stability-indicating method development and validation for the simultaneous estimation of velpatasvir & sofosbuvir in bulk and its pharmaceutical formulations. Int J Res Pharm Sci 9:566–571
16. Mamatha J, Devanna N (2018) Simultaneous RP-HPLC method development and its validation for estimation of sofosbuvir and velpatasvir in their combined dosage form. Rasayan J Chem 11:392–400
17. Rani JS, Devanna N (2018) Development and validation of RP-HPLC method for the simultaneous estimation of sofosbuvir, velpatasvir and voxilaprevir in bulk and tablet dosage forms. Rasayan J Chem 11:452–459
18. Nekkala K, Shanmukha Kumar JV, Ramachandran D (2018) Analytical method development and validation for the simultaneous estimation of sofosbuvir and velpatasvir drug product by reverse phase high performance liquid chromatography method. Asian J Pharm Clin Res 11:164–168
19. Sreehitha V, Sireesha R, Sivagami B, Pavan Kumar V, Chandrasekar R, Babu MN (2018) Simultaneous estimation of sofosbuvir and velpatasvir tablets by RP-HPLC method. Int J Res Dev Pharm Life Sci 7:3092–3099
20. Vanaja B, Vageesh NM, Kistaya C, Urukundu V (2018) RP-HPLC method development and validation for simultaneous estimation of sofosbuvir and velpatasvir in pure and pharmaceutical dosage form. Innovat Int J Med Pharm Sci 3:45–48
21. Chinababu D (2019) A novel stress indicating RP-HPLC method development and validation for the simultaneous estimation of

velpatasvir and sofosbuvir in bulk and its tablet dosage form. J Pharm Res Int 26:1–10
22. El-Kadi EF, Aboelwafa AA (2018) Rapid bioanalytical LC-MS/MS method for the simultaneous determination of sofosbuvir and velpatasvir in human plasma-application to a pharmacokinetic study in Egyptian volunteers. J Chromatogr B 1102-1103:116–124
23. Narenderan ST, Babu B, Gokal T, Meyyanathan SN (2019) A novel simultaneous estimation of sofosbuvir and velpatasvir in human plasma by liquid chromatography tandem-mass spectrometry after protein precipitation method. Curr Pharm Anal 15:710–715
24. Susmitha AG, Rajitha G (2018) Development and validation of stability indicating UPLC method for simultaneous estimation of sofosbuvir and velpatasvir in tablet dosage form. Int J Pharm Sci Res 9:4764–4769
25. Attia KAM, El-Abasawi NM, El-Olemy A, Abdelazim AH (2018) Application of different analytical techniques for determination of velpatsvir and sofosbuvir in the pharmaceutical preparation. J Liq Chromatogr Relat Technol 41:467–473
26. Saraya RE, Elhenawee M, Saleh H (2019) Development of a high-performance thin-layer chromatographic method for the simultaneous determination of newly co-formulated antiviral drugs sofosbuvir and velpatasvir in their pure forms and tablet dosage form. J Plan Chromatogr 32:141–147
27. Mohamed AMI, Mohamed NA, Ali ABH (2019) Simultaneous determination of three 5-HT3 receptors antagonists accompanied by stability study using thin-layer chromatography-densitometry. J Planar Chromatogr 32:285–294
28. Raj D, Kielisz P (2019) High-performance thin-layer chromatographic-densitometric determination of the major triterpene saponins and their aglycones from *Centella asiatica*. J Planar Chromatogr 32:25–30
29. Guideline ICH (2005) Validation of analytical procedures: text and methodology Q2 (R1). International Conference on Harmonization, Geneva, pp 11–12
30. Salinas F, Nevado JB, Mansilla AE (1990) A new spectrophotometric method for quantitative multicomponent analysis resolution of mixtures of salicylic and salicyluric acids. Talanta 37:347–351