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Uv Spectrophotometric Methods for Determination of Sofosbuvir in Pure Form and Pharmaceutical Dosage Forms in Presence of Its Alkaline Degradate

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Authors' contributions

This work was carried out in collaboration among all authors. Author NSS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SAAR and ZAN managed the analyses of the study. Author NSS managed the literature Searched. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Two spectrophotometric methods were developed and validated for the determination of sofosbuvir in presence of its alkaline degradate.
Study Design: Ratio difference and ratio derivative methods were assisted for determination of sofosbuvir in presence of its alkaline degradate, laboratory-prepared mixtures and in tablet dosage forms.
Place and Duration of Study: Pharmaceutical Analytical Chemistry Department, Faculty of Pharmacy (Girls), Al - Azhar University, between December 2019 and January 2020.
Methodology: Two analytical methods were achieved and validated for the quantitative determination of Sofosbuvir in presence of its alkaline degradate. The first method was ratio difference (RD) method, where the UV absorption spectra of different concentrations of sofosbuvir were divided by the spectrum of a certain concentration (15 µg mL^{-1}) as a devisor of its alkaline degradate to get the ratio difference spectra. Afterwards, the peak amplitudes difference between 253.7 and 243.5 nm were measured. The second method was the ratio derivative (^{1}DR) method, where the first derivative of the ratio spectra (^{1}DR) was obtained and its amplitude was measured

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at 247 and 268 nm. Good linearity was obtained over the concentration range of 3-15 µg mL^{-1} for the proposed methods. The proposed procedures were adopted for the selective determination of intact Sofosbuvir in presence of up to 80% of its degradation product. Sofosbuvir was exposed to different conditions as alkaline, acidic and oxidative degradation.

Results: The proposed methods were developed and validated with good linearity range of 3-15 µg mL^{-1} for both methods, and also with good accuracy and precision. And the obtained results were statistically compared to those obtained by the reported method.

Conclusion: Sofosbuvir was successfully determined by the proposed ratio difference and ratio derivative methods in bulk powder, laboratory prepared mixtures and tablet dosage form with good accuracy and precision. The methods were validated according to ICH guidelines. The results obtained were compared with those of the reported method and were found to be in good agreement.

Keywords: Sofosubvir; ratio difference; first-derivative of ratio spectra spectrophotometry.

1. INTRODUCTION

Sofosbuvir is nucleotide analog inhibitor, which specifically inhibits hepatitis C virus (HCV) infection NS5B (non-structural protein 5b) RNA-dependent RNA polymerase. following intracellular metabolism to form the pharmacologically active uridine analog triphosphate (GS-461203), sofosbuvir incorporates into HCV RNA by the NS5B polymerase and acts as a chain terminator [1].

Sofosubvir, chemically described as (*S*)-isopropyl-2-(*S*)-(2*R*, 3R, 4R, 5R)-5-(2,4-dioxo-3,4dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3hydroxy4methyltetrahydrofuran 2yl)methoxy)zphenoxy) phosphoryle amino) propanoate [2], Fig. 1. Sofosbuvir has been determined by several spectrophometric methods [3-6]. It also was determined in combination with other drugs by UPLC-ESI-MS/MS methods [7-10], LC – MS/MS method [11], RP- HPLC [12-16]. Moreover, some stability HPLC indicating methods were reported for its analysis of sofosbuvir [17-21]. The aim of this work was to develop a simple, rapid and sensitive methods for the determination of sofosbuvir in presence of its alkaline, acidic and oxidative degradates as well as in its tablet dosage forms.

2. EXPERIMENTAL

2.1 Instrumentation

Shimadzu UV-Vis. 1601PC Spectrophotometer, (Tokyo, Japan), equipped with 10 mm matched quartz cells.

- Hot plate, Torrey pines scientific (USA).
- pH meter combined plus electrode (Adwa model AD1030 pH mv), (UK).
- UV lamp with short wavelength 254nm (Desega-Germany).

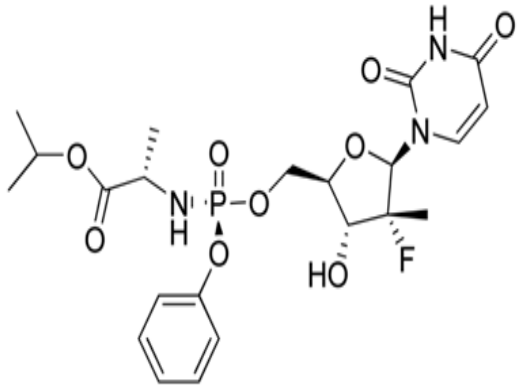


Fig. 1. Structure of sofosbuvir

2.2 Materials and Reagents

2.2.1 Pure and market samples

Pure Sofosbuvir after lab. analysis certified to contain 98.2% was kindly supplied by Marcyrl Company for Pharmaceutical Industry, El Obour City, Egypt, (B.N. OP-G-LD/12/17/099). MPIVIROPACK® tablets; B. N. 1830486, each tablet claimed to contain 400 mg Sofosbuvir, product of Marcyrl Company for Pharmaceutical Industry, El Obour City, Egypt.

2.2.2 Degraded samples

Ten mg of pure sofosbuvir was accurately weighed and refluxed separately with 25 mL of 5N NaOH for 3 h, or with 25 mL 5N HCL for 7 h. For oxidative degradation 10 mg of pure drug was set aside with 25 mL of 3% H_2O_2 for one week at room temperature. The alkaline and acidic hydrolysed solutions were cooled and neutralized to about pH 7 with 5N HCL or 5N NaOH and evaporated to dryness under vacuum. Also oxidative degradate was exposed to dryness at room temperature. The obtained residues were extracted three times each with 25 mL methanol then filtered separately into 100 mL volumetric flask and diluted to the volume with methanol to obtain a stock solution claimed to contain degradates derived from 0.1 mg mL^{-1} intact drug which was used for RD and ^{1}DR methods.

2.3 Chemicals and Reagents

All reagents used throughout the procedure were of analytical grade, solvents were of spectroscopic grade and fresh distilled water was used throughout the procedure. Methanol; HPLC grade (Sigma-Aldrich, USA). Sodium hydroxide, 3% H_2O_2 aqueous solution (El-Nasr Co., Egypt).

2.4 Standard Solutions

Stock solution of the drug (1 mg mL^{-1}) was prepared by dissolving 100 mg in 100 mL methanol. In which this solution was stable for 1 week in refrigerator or at room temperature. The working standard solution of 0.1 mg mL^{-1} was obtained by further dilution with methanol.

2.5 Procedures

2.5.1 Spectral characteristics

The zero order absorption spectra of sofosbuvir and its alkaline degradate (15 µg mL^{-1}) were recorded against methanol as blank over the range of 200- 400 nm. The stored data were subjected to different manipulations to obtain ratio difference and derivative ratio methods.

2.5.2 Linearity

Aliquots equivalent to (0.03 – 0.15 mg) sofosbuvir (0.1 mg mL^{-1}) and its degradate (derived from 0.1 mg mL^{-1} intact drug) were accurately transferred from their standard stock solutions into two separate series of 10- mL volumetric flasks then completed to volume with methanol. The spectra of the prepared standard solutions are scanned from 200 - 400 nm and stored in the computer.

2.5.3 Ratio difference (RD) method

Upon dividing the absorption spectrum of sofosbuvir by the spectrum of its alkaline degradate. The best result was obtained by using (15 µg mL^{-1}) as a devisor, The difference in peak amplitudes at the ratio spectra was measured at 253.7 and 243.5 nm. Calibration graph was obtained by plotting. The measured ΔP values versus the final concentrations in µg mL^{-1} and hence the regression equation was calculated.

2.5.4 First derivative of ratio spectra (^{1}DR) method

The above procedures detailed under ratio difference method were followed then made derevatization for the stored ratio spectra at Δλ = 8 nm and (scaling factor) SF=20. The amplitude values at 247 and 268 nm were measured. To obtain regression equation; the measured amplitude values were plotted against concentrations in µg mL^{-1}.

2.5.5 Accuracy and precision

Different concentrations within linearity range of sofosbuvir (6, 9, 12 µg mL^{-1}) were analyzed by the above methods within the same day or three successive days by adopting the procedure detailed under “2.5.2 Linearity”. Accuracy was calculated as recovery percent (R %) and precision as percent relative standard deviation (RSD %).

2.5.6 Selectivity

Aliquots of intact sofosbuvir solution containing (0.03- 0.12 mg) were introduced into a series of 10- mL volumetric flasks containing (0.12 - 0.03 mg) of the alkaline degradete of sofosbuvir and then diluted to the volume with methanol. The

obtained solutions were analyzed by the two proposed methods as previously described under "2.5.2 Linearity". The intact drug concentrations were calculated from the corresponding regression parameters.

2.5.7 Analysis of pharmaceutical sample

An accurately weighed quantity of the powder equivalent to 100 mg of the intact drug obtained from ten tablets of Mpiviropack® containing 400 mg of sofosbuvir in which introduced into 100 - mL volumetric flask, diluted to volume with methanol and filtered. The working solution (0.1 mg mL^{-1}) was obtained by further dilution to be analyzed by the proposed methods. The drug concentrations were calculated from the appropriate regression parameters.

3. RESULTS AND DISCUSSION

Two different analytical methods were developed aiming for the selective quantitation of sofosbuvir in its bulk powder, in pharmaceutical preparations or in presence of its alkaline degradation product. These methods are ratio difference (RD) and spectrophotometric first-derivative of ratio spectra (^{1}DR).

3.1 Degradation

Upon subjecting of sofosbuvir to different forced degradation conditions. Complete degradation was attained upon refluxing the drug with 5N NaOH and 5N HCL for 3h and 7 h respectively at 100°C. Also, oxidative degradation was carried out by keeping of the drug with 3% H_2O_2 for one week at room temperature. The degradation was confirmed with IR using KBr disc and Mass as follow:

The pure drug showed appearance of broad band of (OH) group at 3352 cm-1, sharp band of two (NH) group at 3248 cm-1, band of aromatic (CH) group at 3089 cm-1 and band of ester (COO) group at 1716 cm-1 in its IR spectrum, Fig. (2-a). While the alkaline degradate showed appearance of broad band of phosphoric (OH) group at 3421 cm-1 in its IR spectrum and disappearance of CH aromatic band at 3009 cm-1. EI mass showed molecular ion peak at m/z = 453 with high intensity (19%); Fig. (3-a), this indicate decreasing in molecular ion peak by 77 unit. this means loss of phenyl group. From IR and EI mass analyses, It was concluded that the proposed degradate formed by removal of phenyl group to afford the free phosphoryle group, Figs. (2-b), (3-b). The acidic degradate showed disappearance of the band of C=O group of ester moiety at 1720 cm-1 and appearance of broad band of phosphoric (OH) group at 3421 cm-1 in its IR spectrum, EI mass showed molecular ion peak at m/z = 416 with high intensity (25%), this indicate decreasing in molecular ion peak equal 113 unit. It was concluded that the proposed degradate compound formed by removal of isopropyl alaninate moiety to afford the free phosphate group, Figs. (2-c), (3-c). The appearance of ketonic band of (CO) at 1727 cm-1 group confirms the oxidation of (OH) group and EI mass showed molecular ion peak at m/z = 527.15 with high intensity (32%), this indicate decreasing in molecular ion peak by 2 units Figs. (2-d), (3-d). Thus a degradation pathway was illustrated in Scheme 1.

3.2 Method Development

Only the alkaline degradate was used for stability indicating UV- spectrophotometric analysis as both acid and oxidative degradates didn't give good results.

The zero-order absorption spectra of sofosbuvir and its alkaline degradate (Fig. 4) shows severe overlap, which does not permit direct determination of sofosbuvir in presence of its alkaline degradate.

3.2.1 Ratio difference (RD) method

The zero-order absorption spectra of sofosbuvir and its alkaline degradate (Fig. 4) shows severe overlap, which does not permit direct determination of sofosbuvir in presence of its alkaline degradate. In this method, the absorption spectra of sofosbuvir were divided by a suitable absorption spectrum of its degradation product as a divisor to get the ratio spectra. The difference in peak amplitudes between two selected wavelengths in the ratio spectra was found to be proportional with the concentration of the drug without interference from its degradation product (Fig. 5). The method comprises two critical steps, the first is the choice of the divisor; the selected divisor should compromise between minimal noise and maximum sensitivity. The divisor concentrations of 15 µg mL^{-1} gave the best results. The second critical step is the choice of the wavelengths at which measurements are to be recorded. The difference between amplitudes in the ratio spectra at 253.7 and 243.5 nm were selected for determination to determine the intact drug in presence of its alkaline degradate.

3.2.2 First derivative of ratio spectra (^{1}DR) method

In this method, the absorption spectra of sofosbuvir were divided by a suitable absorption spectrum of its degradation product as a divisor to get the ratio spectra. By application of the first-derivative to these ratio spectra, sofosbuvir can be quantitatively determined at 247 and 268 nm without any interference from its degradation product (Fig. 6). The first derivative corresponding to each ratio spectrum was recorded, using Δλ = 8 nm and SF: 20. Careful choice of the divisor and the working wavelength were of great importance. The best result obtained by using concentration of 15 µg mL^{-1} of alkaline degradates as a divisor.

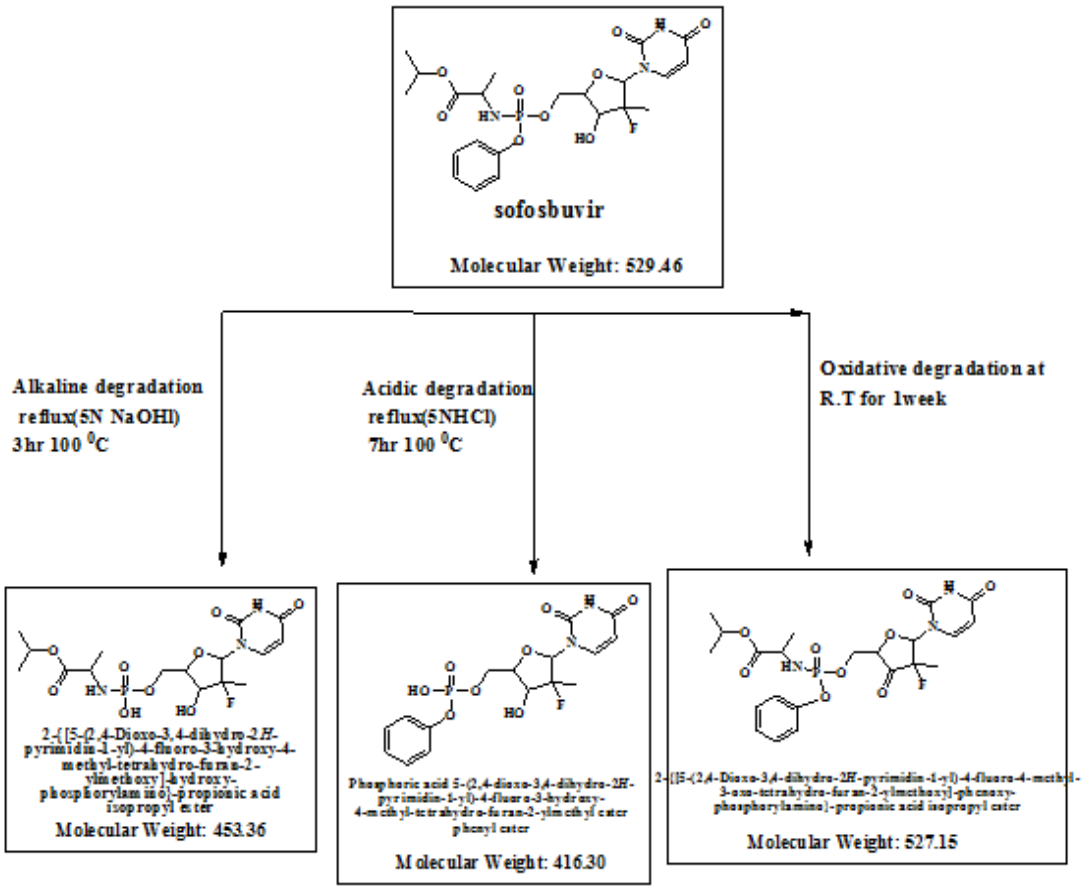
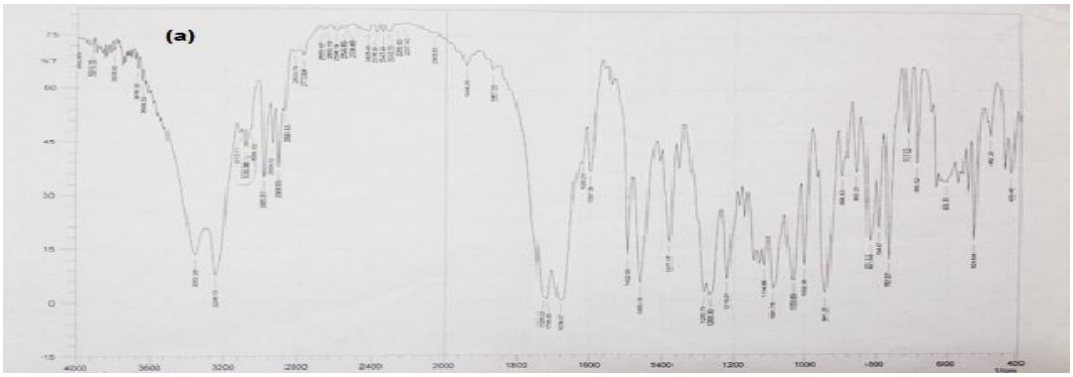


Chart 1. Suggested degradation pathway of Sofosbuvir



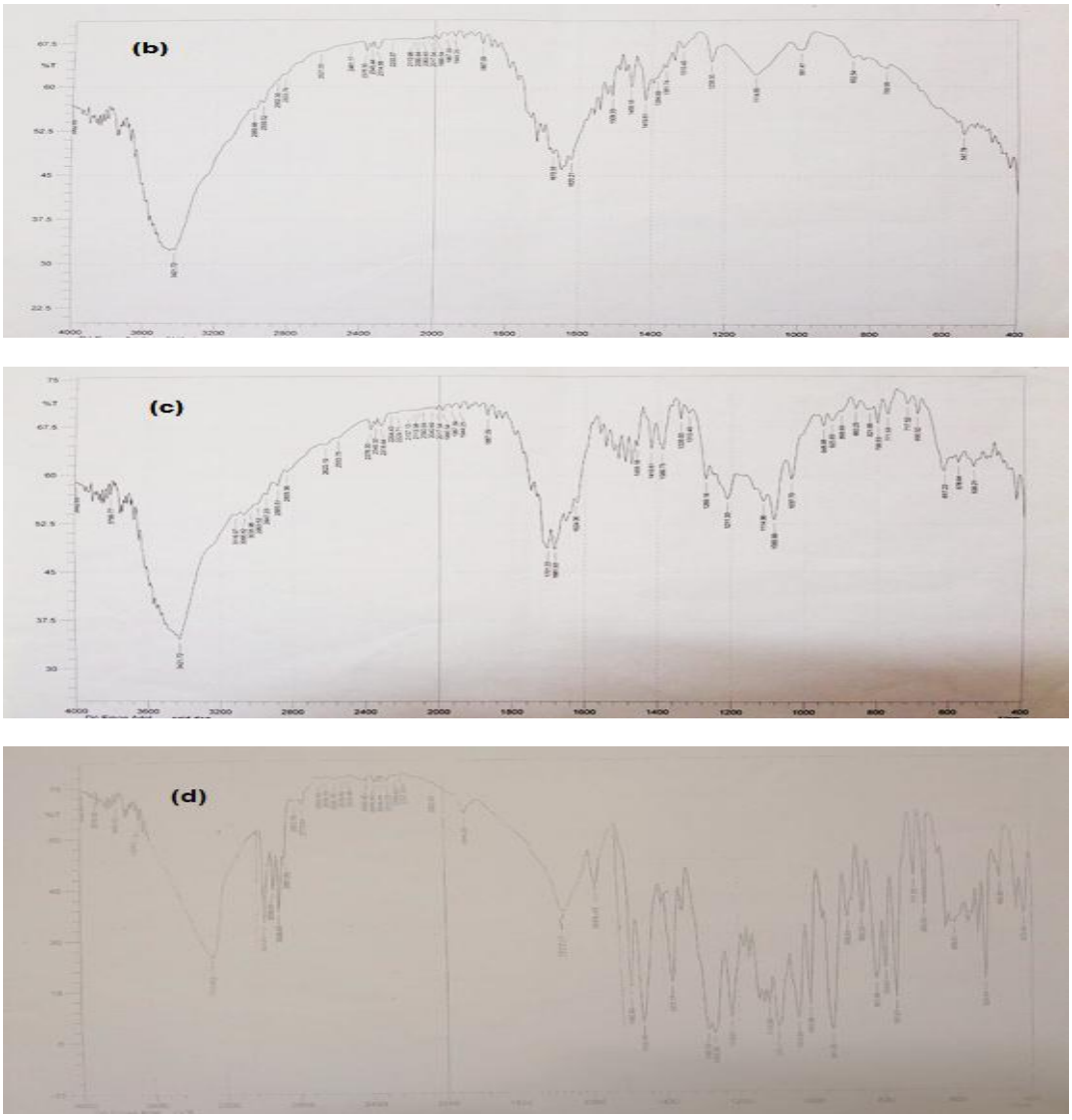
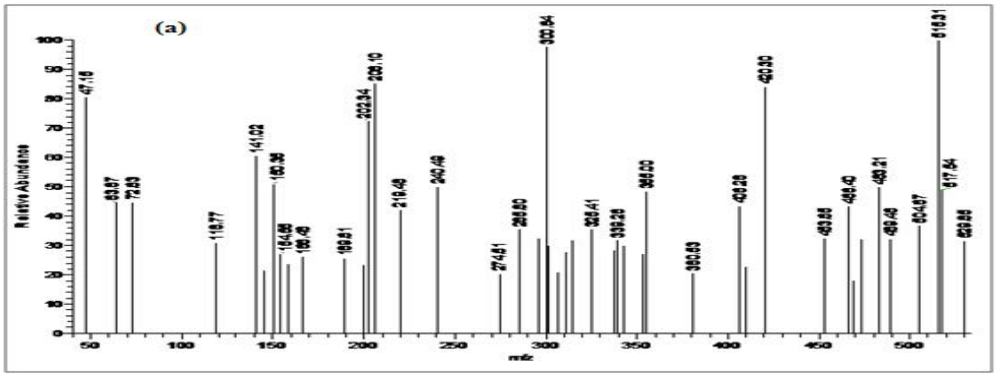


Fig. 2. IR Spectra of intact Sofosubuvir (a), Its alkaline degradate (b), its acidic degradate (c) and its oxidative degradate (d) on KBr disc



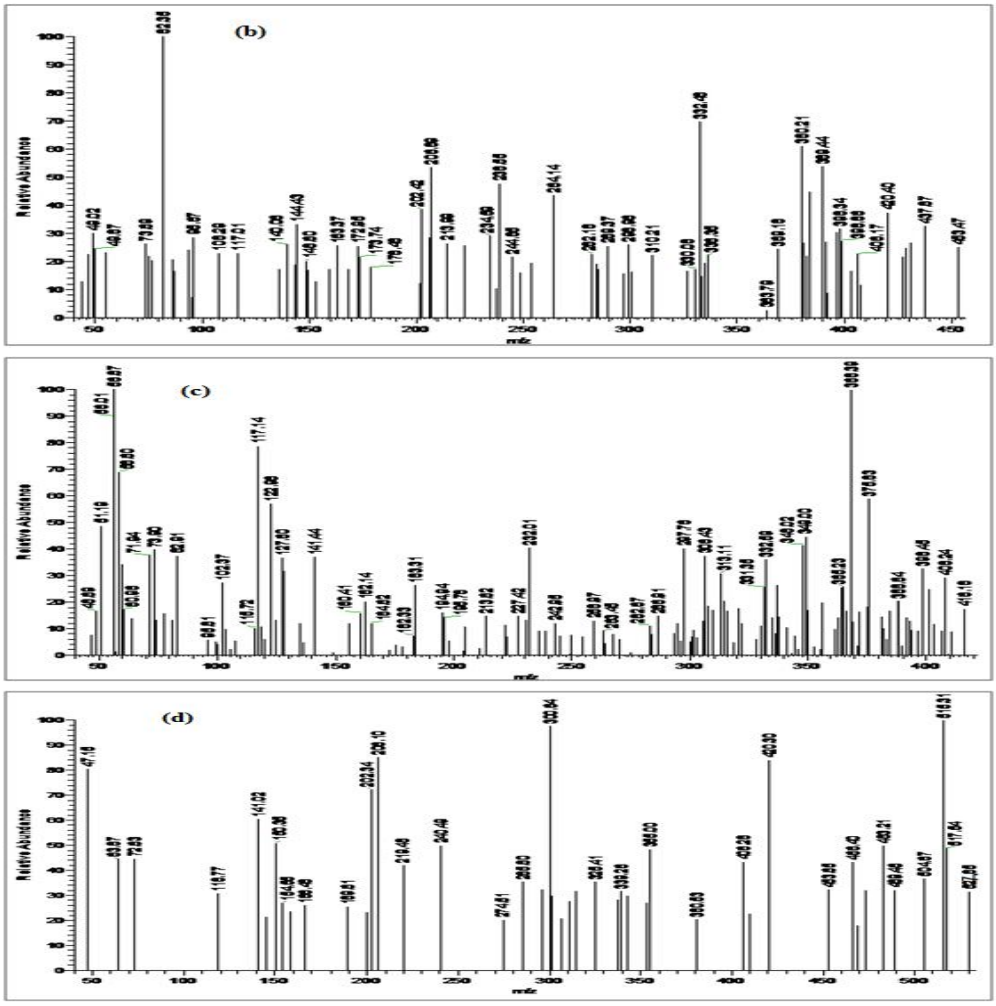


Fig. 3. Mass spectra of intact Sofosubuvir (a), Its alkaline degradate (b), its acidic degradate (c) and its oxidative degradate (d)

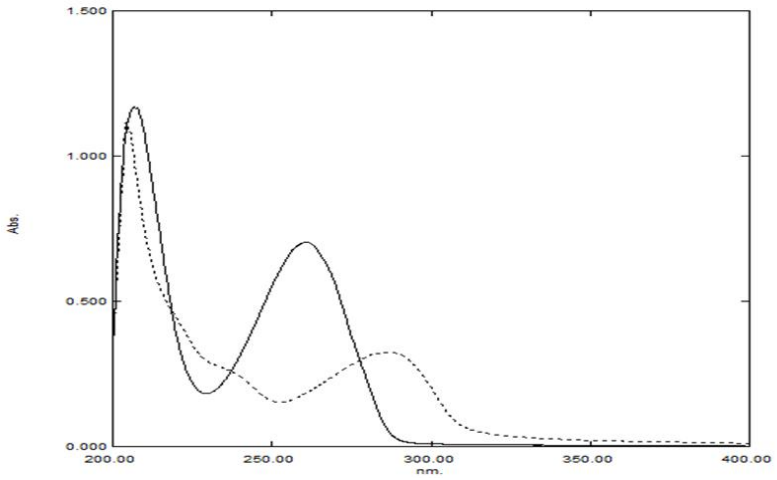


Fig. 4. Absorption spectra of sofosbuvir 15 µg mL^{-1} (—) and its alkaline degradate 15 µg mL^{-1} (…)

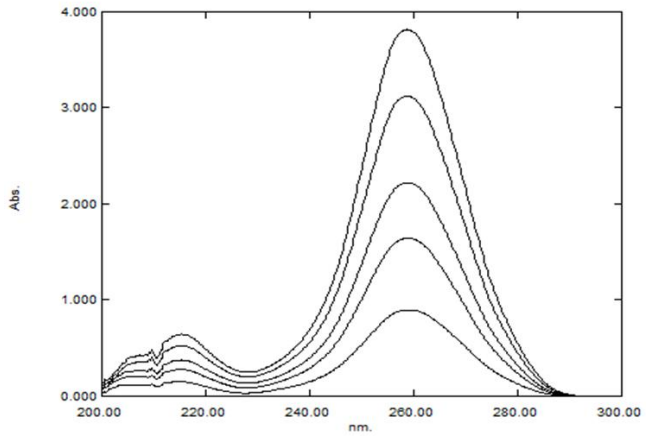


Fig. 5. Ratio spectra of sofosbuvir at various concentrations using 15 µg mL^{-1} of alkaline degradate as a divisor

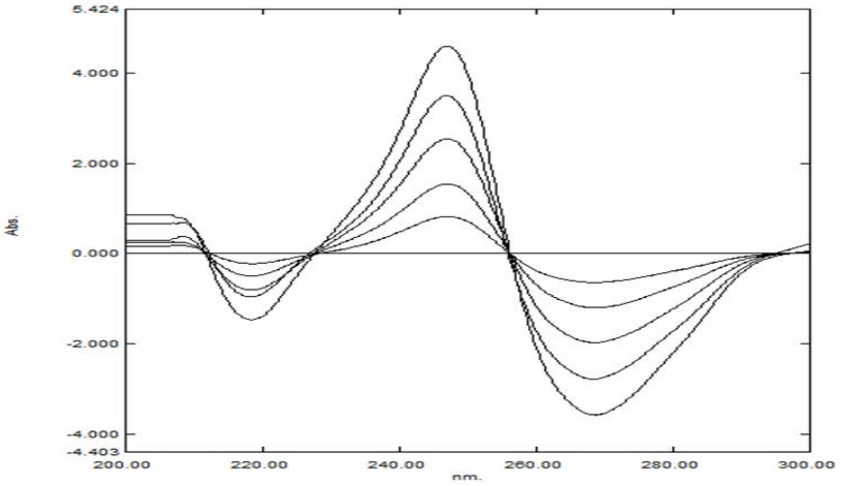


Fig. 6. First derivative of the ratio spectra of sofosbuvir at various concentrations using 15 µg mL^{-1} of alkaline degradate as a divisor

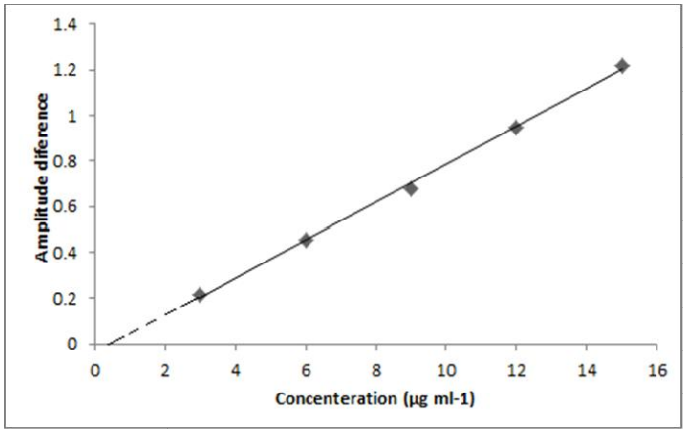


Fig. 7. Calibration curve of the difference in amplitude of ratio spectra at 253.7 and 243.5 nm to the corresponding concentration of sofosbuvir

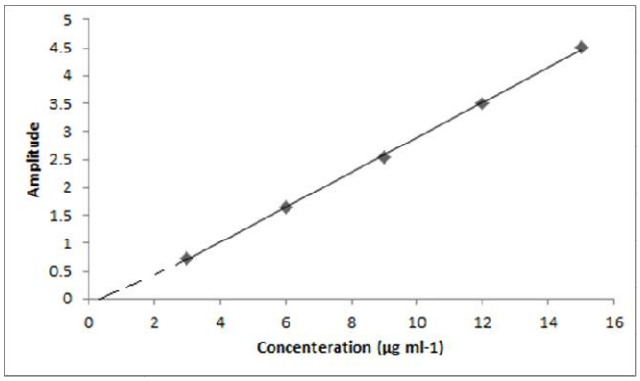


Fig. 8. Calibration curve of the first derivative of smoothed ratio spectra of sofosbuvir at 247 nm

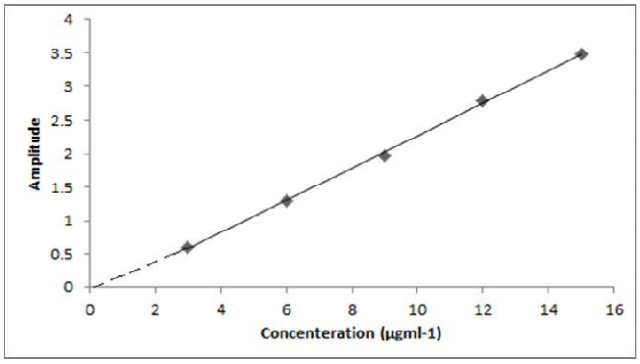


Fig. 9. Calibration curve of the first derivative of smoothed ratio spectra of sofosbuvir at 268 nm

Table 1. Regression analysis and validation parameters for the determination of sofosbuvir by the proposed methods

Parameter	Ratio difference method	Derivative ratio method	
λ_{max} (nm)	253.7-243.5	247	268
Linearity range(µgmL^{-1})	3-15	3-15	3-15
Regression equation			
Slope± SD(S_Y)	0.08333±0.0021	0.2476±0.005	0.3306±.005
Intercept± SD(S_X)	-0.0466±0.021	-0.1839±.057	-0.4121±0.058
SD of residual(S_{YX})	0.626	0.009	0.009
Correlation coefficient (r^2)	0.999	0.9991	0.9995
LOD	0.183	0.042	0.618
LOQ	0.55	0.129	1.87

3.3 Method Validation

The methods were validated as per ICH guidelines [22].

3.3.1 Linearity

Good Linearity was obtained between the amplitude and the corresponding drug concentration over the range of 3–15 µg mL^{-1} using the two studied methods, as shown in Figs. 7 - 9 the regression parameters were computed where values of r^2 ranged between (0.9991-.0.999) indicating good linearity and presented in Table 1.

3.3.2 LOD and LOQ

LOD and LOQ were determined according to ICH using the standard deviation of multiple blank samples and the slope of the calibration curve; Table 1.

Table 2. Intraday and interday accuracy and precision for the determination of sofosbuvir by the proposed UV- methods

Procedures	Taken µg mL^{-1}		Intraday			Interday		
			Found*+SD µg mL^{-1}	Accuracy R%	Precision RSD%	Found*+SD µg mL^{-1}	Accuracy R%	Precision RSD%
RD method	6		6.03±0.05	100.59	0.83	5.99±0.02	99.88	0.33
	9		9.03±0.10	100.23	1.11	9.03±0.1	100.23	1.11
	12		11.98±0.1	99.85	0.83	12.04±0.03	100.16	0.25
^{1}DR method		6	6.00±0.02	100.10	0.33	5.99±0.05	99.89	0.83
	247nm	9	8.97±0.06	99.73	0.67	9.03±0.07	100.36	0.78
		12	11.96±0.05	99.96	0.42	12.05±0.08	100.44	0.66
		6	5.95±0.01	99.27	0.17	6.03±0.01	100.60	0.17
	268nm	9	9.00±0.06	100.48	0.67	9.00±0.17	100.07	1.89
		12	12.02±0.06	100.21	0.50	11.99±0.12	99.94	1.00

Table 3. Determination of sofosbuvir in mixtures with its degradation product by the proposed UV- methods

Intact µg mL^{-1}	Degradate µg mL^{-1}	% of degradate	Ratio difference method (RD)	Derivative ratio method (^{1}DR)	
			% of intact	% of intact	
				247 nm	268 nm
3	12	80	99.66	99.33	100.33
5	10	66	100.20	100.20	99.60
7	8	53	98.42	99.57	99.71
9	6	40	98.66	100.22	99.77
12	3	20	100.83	99.10	99.75
Mean % ± SD			99.55±1.017	99.68±0.508	99.83±0.28

Table 4. Application of standard addition technique for the determination of sofosbuvir in Mpiviropack® tablets by the proposed ratio difference and derivative ratio methods

Preparation	Claimed taken (μg mL^{-1})	Pure added (μg mL^{-1})	Ratio difference method (RD)		Derivative ratio method (^{1}DR)			
					247 nm		268 nm	
			Mean % ± SD	Recovery% of pure added	Mean % ± SD	Recovery % of pure added	Mean % ± SD	Recovery % of pure added
Mpiviropack®	5	1		98.00		101.00		98.00
400 mg tablet	5	2	101.66±0.85	100.50	99.51±1.07	99.00	99.89±0.82	101.00
	5	3		99.33		100.33		99.30
	5	5		99.60		99.66		101.66
	5	6		100.16		100.40		97.80
Mean %± SD			99.51±0.96		100.08±0.77		99.55±1.73	

Table 5. Results obtained by the proposed methods compared with reported method [3] for determination of sofosbuvir in pharmaceutical dosage form

Parameters	Mpiviropack® tablets			
	Ratio difference method (RD)	Derivative ratio method (^{1}DR)		Reported method[3]
		247 nm	268 nm	
Linearity range (μgmL^{-1})	3-15	3-15	3-15	2-60
N	5	5	5	5
Mean%	101.66	99.51	99.89	99.87
SD	0.85	1.07	0.82	1.60
Variance	0.72	1.144	0.67	2.56
t	2.21(2.36)	0.42(2.36)	0.022(2.36)	---
F	3.56(6.59)	2.26(6.59)	3.82(6.59)	---

3.3.3 Accuracy and precision

They were achieved by triplicate analysis of three different concentrations (6, 9, 12 µg mL^{-1}) covering the linearity range within one day for intraday and three different days for interday analysis. Accuracy was calculated as recovery percent (R %) and precision as percent relative standard deviation (RSD %). (RD) method showed accuracy amounted to be 99.85% -100.59% and intraday precision RSD % ranged from 0.24% to 1.10. While upon using (^{1}DR) method, intraday and interdays accuracy were found to be ranged from 99.73% to 100.44%, whereas, precision RSD % from 0.33 to 0.83. at 247 nm, while at 268 nm intraday and interdays accuracy were found to be ranged from 99.27% to 100.60%, whereas, precision RSD % from 0.16 to 1.88; Table 2.

3.3.4 Selectivity

The selectivity of the proposed methods was validated by applying them to laboratory prepared mixtures of the intact drug together with its alkaline degradate. Good recoveries of intact sofosbuvir were obtained when they were applied for the determination of Sofosbuvir in presence of up to 80% of the alkaline degradate; Table 3.

3.3.5 Robustness

To check the robustness of the proposed methods studying the effect of different sources of the solvent. It was found that, using of methanol (sigma-Aldrich, USA) and (El-Nasr Co., Egypt) gave RSD% of 1.47%, confirming accuracy and precision.

3.4 Application to Tablet Dosage Form

The proposed RD and ^{1}DR methods were validated also by applying the study for the determination of Mpiviropack® tablets. The results revealed good mean recoveries 101.66±0.85for ratio difference method and 99.51±1.07, 99.89±0.82 for derivative ratio method at 247,268 nm respectively, as shown in Table 4. Standard addition technique was used to assist the recovery of the proposed methods, showing mean recoveries of added ± SD of 99.51+0.96, 100.08+0.77 and 99.55+1.75 for the two proposed methods, respectively; Table 4.

Statistical analysis of the results obtained by the suggested methods compared with the reference method [3] revealed no significant difference with respect to accuracy and precision within a probability of 95% confidence limit [23]; Table 5.

4. CONCLUSION

Sofosbuvir can be selectively determined by the proposed UV-spectroscopic ratio difference [RD] and derivative ratio [^{1}DR] methods in presence of its alkaline degradation product. The methods were validated and found to be simple, accurate, precise and selective. The two methods proved their ability to be used for stability indication of the drug. Therefore, they can be conveniently adopted for estimation, stability studies and routine quality control analysis of sofosbuvir.

COMPETING INTRESTS

Authors have declared that no competing interests exist.

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