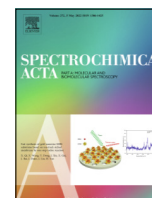**ELSEVIER**

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Application of different spectrophotometric methods for quantitative analysis of direct acting antiviral drugs simeprevir and sofosbuvir
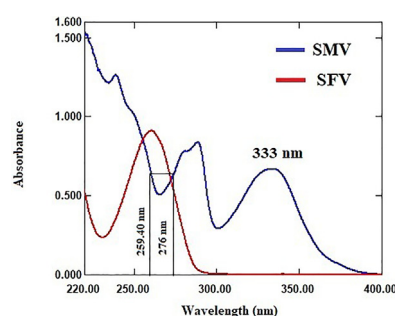
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HIGHLIGHTS

- Simeprevir and sofosbuvir are direct-acting antiviral drugs.
- Two simple spectrophotometric methods.
- Successful quantitative analysis of simeprevir and sofosbuvir.

GRAPHICAL ABSTRACT

ARTICLE INFO

ABSTRACT

Simeprevir and sofosbuvir are direct-acting antiviral drugs approved for the treatment of chronic HCV infection. Reports demonstrate the similarities between HCV and SARS-CoV-2 in terms of structure and replication mechanism. Therefore, it is suggested that a combination of simeprevir and sofosbuvir may be considered for COVID-19 patients. To date, no spectrophotometric methods have been published for quantitative analysis of simeprevir and sofosbuvir in combination. In this work, two simple spectrophotometric methods allowed quantitative analysis of the studied drugs in the mixed form. The zero-order direct method allowed quantitative analysis of simeprevir at 333 nm, with sofosbuvir showing zero absorbance values. The dual wavelength method allowed quantitative analysis of sofosbuvir by measuring the difference in absorbance values at 259.40 and 276 nm, where the difference in absorbance values of simeprevir was zero. With the applied methods, the investigated drugs in the mixtures and tablets prepared in the laboratory were successfully analyzed quantitatively with acceptable results.

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1. Introduction

Simeprevir (SMV), Fig. 1, is a direct acting antiviral which has been FDA approved in 2013 for the treatment of HCV genotype 1 in combination with ribavirin and PEG-interferon. Sofosbuvir (SFV), Fig. 2, is a direct acting antiviral which has been FDA approved in 2013 for the treatment of HCV genotype 1,2,3 and 4 in combination with ribavirin or PEG-interferon [1]. In November 2014, FDA approved the combination of simeprevir and sofosbuvir for the treatment of chronic HCV genotype-1 patients. It was the first ribavirin- and interferon-free regimen therapy [2]. The WHO guidelines for the screening, care and treatment of patients with HCV recommend simeprevir and sofosbuvir as combined treatment for HCV genotype 1 and 4 [3].

Recently, with the global outbreak of the COVID-19 and the similarities between HCV and SARS-CoV-2 in structure and replication, several studies have considered the activity of anti-HCV drugs such as simeprevir and sofosbuvir, remdesivir, daclatasvir, against SARS-CoV-2 [4–8].

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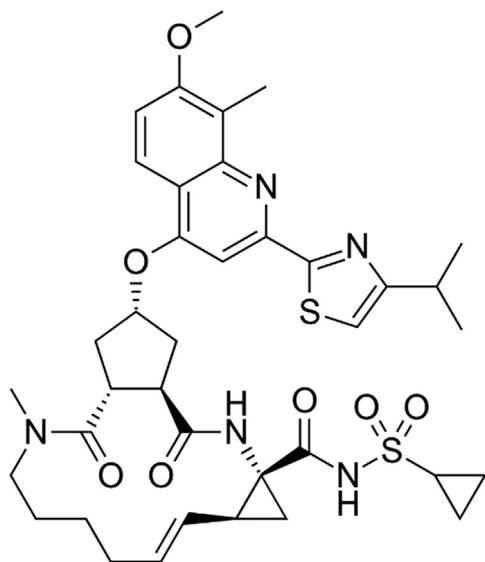 *E-mail address:* ahmed.hussienabdelazim@hotmail.com (A.H. Abdelazim).
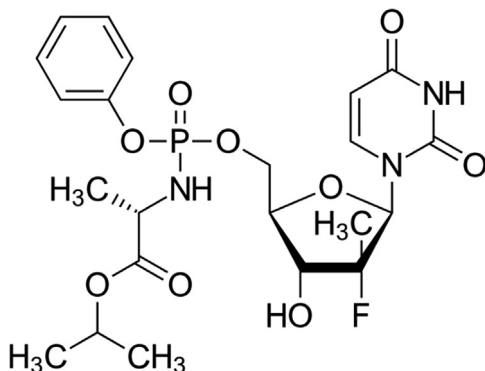
Fig. 1. Structural formula of SMV.

Fig. 2. Structural formula of SFV.

The literature review of SMV [9–13] and SFV [14–18] revealed few published methods for determination of the drugs individually. There were only two published methods for the simultaneous determination goal [19,20].

In this paper, we developed two simple spectrophotometric methods for determination of SMV and SFV in the laboratory prepared mixtures and tablets. Zero order method enabled quantification of SMV at 333 nm where SFV had zero absorbance values. The dual wavelength method was applied for quantification of SFV by measuring the difference in absorbance values at 259.40 and 276 nm at which the difference in absorbance values of SMV was equal zero.

2. Experimental

2.1. Materials and solvent

Pure SMV & SFV powders were supplied by Benchmark Health Company, Cairo, Egypt. Merospevir® tablets (150 mg SMV per tablet) and Mpiviropack® tablets (400 mg SFV per tablet) were purchased from local drugstore. Laboratory prepared tablets (150 mg SMV, 400 mg SFV, 20 mg talc powder, 15 mg maize starch and 7 mg magnesium stearate per tablet) were laboratory prepared. Ethanol, HPLC grade, was supplied by Sigma-Aldrich, Darmstadt,

Germany. Di methyl sulfoxide (DMSO), analytical grade, was supplied by El-Nasr pharmaceutical chemicals company, Cairo, Egypt.

2.2. Apparatus

UV–Visible 1800 Spectrophotometer (Shimadzu Corp., Tokyo, Japan), equipped with 10 mm × 3.5 mL quartz cuvette.

2.3. Standard solutions

SMV and SFV standard stock solutions (100 µg/mL) were freshly prepared by transferring 10 mg of each drug powder into two separate 100-mL volumetric flasks with 10 mL DMSO and 40 mL ethanol, shaken vigorously, and complete to the volume with ethanol.

2.4. Procedures

2.4.1. Linearity and calibration graphs

Two serial dilutions of SMV and SFV were separately prepared by transferring aliquots equivalent to (30–450 µg) and (30–440 µg) of SMV and SFV, respectively, from their standard solutions (100 µg/mL) into a two sets of 10-mL volumetric flasks and diluted to volume with ethanol. The absorption spectra of the prepared solutions were measured against ethanol as a blank in the wavelength range from 200 to 400 nm.

2.4.1.1. Zero-order method.
The absorption spectra of SMV were measured directly at 333 nm where there was no absorption interference from SFV. The absorbance values at 333 nm were plotted with SMV concentrations in µg/mL to develop the calibration plot. Regression equation was computed.

2.4.1.2. Dual wavelength method.
The difference between absorbance values of SFV spectra at 259.40 and 276 nm was plotted against SFV concentrations to develop the calibration plot. Regression equation was derived.

2.4.2. Analysis of synthetic mixtures

Synthetic mixtures of SMV and SFV were laboratory prepared by transferring different concentrations of SMV and SFV to a set of 10 mL volumetric flasks and adjusting the volume to the mark with ethanol. The mixtures were analyzed following the described procedure and the concentration of the drugs was calculated.

2.4.3. Analysis of SMV and SFV in their single pharmaceutical tablets

Ten Merospevir® and MPIVIROPACK® tablets were separately weighed and grinded to fine powder. A weighed powder equivalent to one tablet of Merospevir® and Mpiviropack® were thoroughly mixed and accurately transferred to 100-mL volumetric flask with 10 mL DMSO and 40 mL ethanol. The samples were shaken vigorously for 20 min, filtered, and adjusted to 100 mL with ethanol. Further dilution with ethanol was made to prepare five samples with different concentrations. The samples were analyzed following the described procedures and the concentration of the drugs was measured.

2.4.4. Analysis of SMV and SFV in their laboratory prepared tablets

Ten laboratory prepared tablets were weighed and grinded to fine powder. A weighed powder equivalent to one tablet was accurately transferred to 100-mL volumetric flask with 10 mL DMSO and 40 mL ethanol. The sample were shaken vigorously for 20 min, filtered, and adjusted to 100 mL with ethanol. Further dilution with ethanol was made to prepare five samples with different concentrations. The samples were analyzed following the procedure mentioned for each method under the linearity and calibration graphs and the concentration of each drug was computed.

3. Results and discussion

3.1. Spectral characteristics

The UV absorption spectra of both SMV and SFV, Fig. 3, SMV shows extended absorption peak at 333 nm without overlapping from SFV, which exhibits zero absorbance in the wavelength range from 400 to 300 nm. SMV could be directly determined in mixture with SFV using zero order absorption method. The absorbance values of zero order absorption spectra at 333 nm were directly proportional the concentration of SMV without any interference from SFV. On the other hand, SFV shows sever overlap spectra with SMV in the wavelength range from 300 to 200 nm, which hinders SFV direct determination. Therefore, it is recommended to overcome the contribution of SMV spectra by mathematical method to enable quantitative estimation of SFV.

Dual wavelength method is based on measuring the difference in absorbance values of the drug of interest at two wavelengths where the difference in absorbance values for any other overlapped drug in the mixture is equal zero. So, for determination of SFV in mixture with SMV by dual wavelength method, several pairs of wavelength at which the difference of absorbance values of SMV is equal zero were tested. Accepted linearity of SFV was obtained by measuring the difference in absorbance values at 259.40 and 276 nm. The difference in absorbance values at 259.40 and 276 nm is directly proportional to the SFV concentrations without contribution from SMV, Fig. 3.

3.2. Methods validation

The described methods were validated according to the guidelines of ICH [21]. Data listed in Table 1 represents the limits of detection (LOD) & limits of quantitation (LOQ), accuracy, precision, linearity, and regression parameters. The described methods succeeded to selectivity analyze SMV and SFV in the laboratory prepared mixtures as shown in Table 2.

3.3. Application of the methods

The described methods exhibited successful quantification of SMV and SFV in the synthetic mixtures (Table 2) and in single pharmaceutical tablets (Table 3) as well as in the laboratory prepared tablets (Table 4) without any interference from each other or tablet matrix.
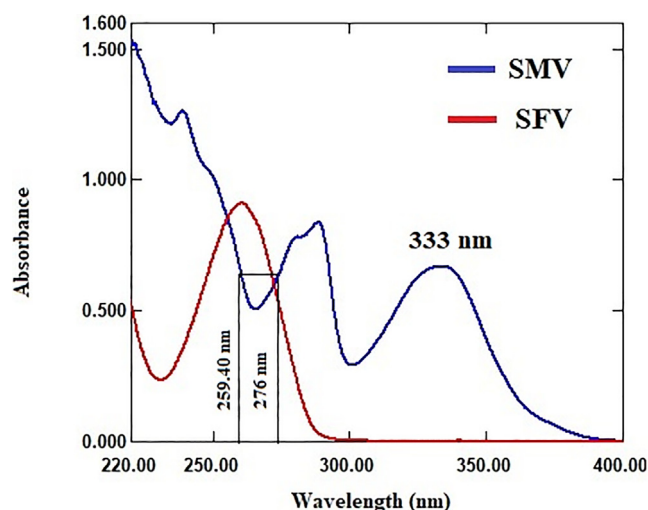

Fig. 3. Absorption spectra of SMV (25 μg/mL) and SFV (36 μg/mL).

Table 1
Regression and validation data for quantitative analysis of SMV and SFV by the proposed methods.

Parameters	SMV	SFV
Wavelength (nm)	333	259.40 & 276
Linearity range (μg/mL)	3–45	3–44
Slope	0.0263	0.0130
Intercept	0.0085	−0.0001
Coefficient of determination (r^2)	0.9997	0.9997
LOD (μg/mL)	0.888	0.803
LOQ (μg/mL)	2.692	2.434
Accuracy (%R)[a]	100.25	100.06
Repeatability precision (RSD)[b]	0.768	0.595
Intermediate precision (RSD)[b]	0.927	0.801

[a] Average of 9 determinations (3 concentrations repeated 3 times).
[b] RSD of 9 determinations (3 concentrations repeated 3 times).

Table 2
Application of the proposed methods for the determination of SMV and SFV in their laboratory prepared mixtures.

Taken (μg/mL)		%Recovery	
SMV	SFV	SMV	SFV
3	8	99.49	99.13
6	16	98.54	100.05
9	24	100.34	101.31
12	32	100.92	100.26
15	40	100.00	98.67
Mean ± RSD		99.86 ± 0.901	99.89 ± 1.031

Table 3
Application of the proposed methods for the determination of SMV and SFV in their single-ingredient tablets.

Taken (μg/mL)		%Recovery	
Merospevir® tablets	MPIVIROPACK® tablets	SMV	SFV
3	8	100.13	100.10
6	16	99.11	99.66
9	24	99.07	100.99
12	32	100.98	100.50
15	40	99.24	100.02
Mean ± RSD		99.71 ± 0.836	100.26 ± 0.508

Table 4
Application of the proposed methods for the determination of SMV and SFV in their laboratory prepared tablets.

Taken (μg/mL)		%Recovery	
SMV	SFV	SMV	SFV
3	8	100.51	101.06
6	16	100.76	99.90
9	24	100.34	99.71
12	32	99.97	100.14
15	40	99.49	100.98
Mean ± RSD		100.21 ± 0.494	100.36 ± 0.620

4. Conclusion

In this work, we developed the first spectrophotometric methods for quantitative analysis of SMV and SFV in their mixture. The applied methods were successfully determined the drugs under the study in the laboratory prepared mixtures and tablets without any interferences.

CRediT authorship contribution statement

Sherif Ramzy: Conceptualization, Methodology, Writing – original draft. **Ahmed H. Abdelazim:** Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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