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Approach for Quantification of Nanogram Amounts of Hepatitis-C Drugs, Sofosbuvir, and Daclatasvir based on Cerium (IV) Oxidation

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Abstract

Sofosbuvir (SOFO) and Daclatasvir (DK) are known as hepatitis-C drugs. Through oxidation of these drugs in a sulfuric acid medium by an excess of Cerium (IV) and the subsequent determination of unreacted Cerium (IV), this research developed spectrophotometric methods for assessing the effectiveness of these hepatitis-C drugs. As an indicator of excessive Cerium (IV), we measured the decrease in the color of four chromotropic acid azo dyes, Chromotrope 2B (method A), Arsenazo I (method B), Spadns (method C), and Sulfonazo III (method D) at 510, 499, 505, and 570 nm, respectively. According to Beer's plots, there was a significant relationship between 0.1-2.1 and 0.1-5.1 µg mL^{-1} with molar absorptivity values of $1.7x10^5$, $2.7x10^5$, $1.4x10^5$, and $1.7x10^5$ for Sofosbuvir and $1.2x10^5$, $7.0x10^4$, $6.2x10^4$, and $9.0x10^4$ for Daclatasvir utilizing methods Chromotrope 2B (method A), Arsenazo I (method B), Spadns (method C), and Sulfonazo III (method D), respectively. To assess the efficacy of the suggested process, pure and pharmaceutical solutions containing Sofosbuvir and Daclatasvir were tested.

Keywords: Chromotropic azo dyes; Cerium (IV); Sofosbuvir; Daclatasvir

1. Introduction

Sofosbuvir (SOFO) is a nucleotide analog prescribed with other medications to treat the hepatitis C virus. Since 2013, it has been commercialized. The cure rate of regimens containing Sofosbuvir was higher than that of earlier therapies, while side effects were less common, and a 2- to 4-fold shorter medication period. Specifically, it is Isopropyl (2S) -2-[(2R,3R,4R,5R)-5-(2,4-dioxopyrimidin-1-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl] methoxyphenyl-phosphoryl amino] propanoate. This treatment allows the treatment of most patients without the use of pegylated interferon (pegIFN), an injectable drug that has serious side effects. PegIFN was an important component of prior combinations of drugs used for the treatment of HCV. A diagram of SOFO's structure is shown in Fig. 1. The sofosbuvir crystalline solid has a white to off-white color and is somewhat soluble in water [1]; it is soluble in ethanol completely [2].

Daclatasvir dihydrochloride (Fig.2) is used as a treatment for hepatitis-C virus (HCV) infections [3, 4]. It is a methyl((1S)-1-(((2S)-2-((2S)-2-(((methoxy carbonyl)amino)-3-methylbutanoyl)-2-pyrrolidinyl)-1H-imidazol-5-yl)-4-biphenylyl)-1H-imidazol-2-yl)-1 pyrrolidinyl) carbonyl)-2-methylpropyl) carbamate dihydrochloride. It is a non-hygroscopic powder that is white to yellow in color. In water, dimethyl sulfoxide, and methanol, it is freely soluble; in ethanol, it is soluble (95%); and in dichloromethane, tetrahydrofuran, acetonitrile, acetone, and ethyl acetate, it is practically insoluble [4].

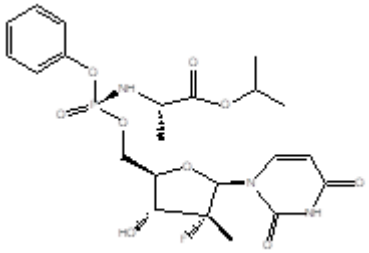

Fig. 1. The structural formula of Sofosbuvir

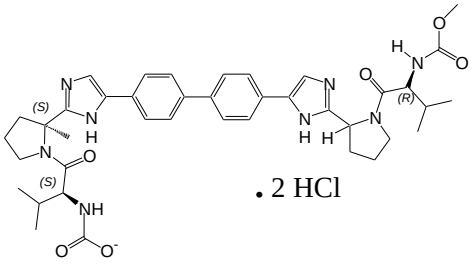

Fig. 2. The structural formula of Daclatasvir

There are a few spectrophotometric publications investigating Sofosbuvir and Daclatasvir in pure and

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dose forms [5-10]. HPLC procedures [11-18] were extraordinarily costly and difficult to perform. Quality control laboratories and clinical laboratories utilize visible spectrophotometry as one of the most convenient analytical tools [18-21]. A strong oxidant like cerium (IV) can be used to perform spectrophotometric analysis on pharmaceutical compounds [22-24]. A wide range of pharmaceutical formulations is determined by using chromotropic azo dyes [25, 26].

The purpose of this study is to develop a simple, sensitive, and rapid spectrophotometric method for quantitatively estimating cited drugs in a nano concentration in both pure and dose forms utilizing a lab tool with high sensitivity and accuracy that is readily available. Using chromotropic acid azo dyes as chromogenic agents, the developed methods describe the use of Ce (IV) for measuring Sofosbuvir (SOFO) and Daclatasvir (DK) spectrophotometrically. A summary of the spectrophotometric work that has been previously published on DK and SOFO is shown in table 1

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Table 1. Previous spectrophotometric methods for the determination of Sofosbuvir and Daclatasvir

Method	Solvents	λ_{max}, nm	Linear range µg mL^{-1}	Molar Absorptivity, L $mol^{-1}cm^{-1}$	LOD, µg mL^{-1}	Ref.
Sofosbuvir						
Cerium (IV) by Sulfonazo III	Sulfuric acid & water	570	0.1-2.1	$1.7x10^5$	0.013	C.S
Cerium (IV) by ammonium nitrate & indigo carmine	Sulfuric acid, methanol & water	610	0.2-3.0	2.354×10^4	0.06	[5]
Cerium (IV) by Alizarin red S	Sulfuric acid, methanol& water	360	0.2-4.0	1.933×10^4	0.06	[5]
UV	Methanol	272	10.0-80.0	-	1.7	[2]
UV	Methanol	261	12.0-40.0	-	-	[6]
Daclatasvir						
Cerium (IV) by chromotrope 2B	Sulfuric acid & water	570	0.1-5.1	$1.2\ x10^5$	0.024	C.S.
Cerium (IV) by ammonium nitrate & indigo carmine	Sulfuric acid&water	610	0.5-4.5	1.786×10^4	0.15	[5]
Cerium (IV) by Alizarin red S	Sulfuric acid&water	360	0.5-5.0	2.015×10^4	0.15	[5]
UV	Methanol	316	2.5-25.0	-	0.69	[2]
UV	Methanol	317	4.0-12.0	-	-	[6]
Bromophenol Blue,	Methanol, 1,2-dichloroethane	420	5.0-60.0	$1.08x10^4$	1.22	[8]
Bromothymol Blue,	Methanol, 1,2-dichloroethane	416	5.0-70.0	$0.84x10^4$	1.41	[8]
Bromocresol Green	Methanol, 1,2-dichloroethane	415	5.0-60.0	$1.19x10^4$	1.25	[8]

. Based on the table, we found that both drugs were tested with UV and visible spectrophotometry. To improve molar absorption and reduce the detection limit, this study is being conducted

2. Experimental

2.1. Materials and Methods

Jenway 6105 UV/Vis spectrophotometer with an optical path length of one cm was used for all spectrum measurements. Throughout the investigation, a SciTech SA 210 digital balance was used for weighing. Heating was done by using a TECCHIN water bath.

This investigation was conducted with analytical-grade chemicals. All experiments were conducted using bi-distilled water. Quality standards (Daclatasvir (99.3%), Sofosbuvir (99.9%), Mpiviropack® (patch number 1931182 with a 400 mg per tablet), and Daclaviroccyrl® (patch number (1932492 with a 60 mg/tablet) were obtained from Marcyrl Pharma (Egypt). BDH Limited, Poole (England) supplied the arsenazo I, sulfonazo III, and spadns. Alfa Aesar Gmbh and CoKG (Germany) provided chromotrope 2B. Intrade gmbh (Germany) provided cerium sulfate tetrahydrate.

In the least amount of solvent (ethanol and bi-

distilled water, respectively), stock solutions were prepared of SOFO and DK, which weighed 529 mg and 812 mg, respectively. Following this, 0.1 mol L^{-1} stock solutions of both components were prepared in a 100 mL volumetric flask.

In the least amount of H_2SO_4 and with heating, 404.3 mg of cerium sulfate tetrahydrate was dissolved in 0.1 mol L^{-1}. After transferring the later solution into a 100 mL volumetric flask, the same solvent was used to complete the experiment. Using H_2SO_4 as a diluent, other concentrations were prepared.

To prepare 10.0 mmol L^{-1} C2B, Arz (I), Sulf (III) standard stock solutions, 256.7, 274.1, 285.2, and 388.0 mg, respectively, were dissolved in bi-distilled water and transferred into volumetric flasks of 50 mL.

As part of the preparation of the pharmaceutical dose solution, ten Mpiviropack® and Daclavirocyrl® tablets were accurately weighed and ground using a mortar. One tablet's weight was calculated using the average of ten tablets. In the least volume of ethanol and bi-distilled water, respectively, 400 mg of the ground tablet powder equivalent to SOFO and 60 mg of DK were dissolved. To ensure that the entire active ingredient was dissolved in the powder, the filtrate was washed several times with ethanol and water respectively.

2.2. General Procedures

After heating 1.0 mL 1.0x10^{-3} mol L^{-1} Cerium (IV) solution for 25 minutes at 100°C, and cooling for 3 minutes, different solutions containing 0.0-10.0 µg mL^{-1} drug were added to the above solution in a 10 mL test tube. Chromotropic acid dyes were added at a constant concentration. By adding 1.0 mol L^{-1} sulfuric acid to each tube, the volume of the contents was quantitatively transferred to a 10 mL flask.

3. Results and Discussion

Sofosbuvir and Daclatasvir were determined using cerium (IV) sulfate, a powerful oxidizing agent. Specifically, these methods entail oxidizing selected drugs with excess Ce (IV) sulfate in an acidic medium and then measuring the difference in reagent absorbance. Two steps are involved in the proposed methods. As a first step, SOFO or Dk must undergo oxidation under conditions of known Ce (IV) excess in an acidic medium. To determine residual Ce (IV), a fixed amount of chromotropic acid azo dye is reacted with the residual Ce (IV). This can be done using C2B (method A), Arz (method B), Spd (method C), as well as Sulf III (method D) as follows.

1. SOFO or DK + excess Ce (IV)→ oxidation product of drug (OPD) (colorless) + Ce (IV) (yellow) + Ce (III) (colorless)
2. Residual Ce (IV) chromotropic acid azo dye (CTA) (colored) → Ce (III) +oxidation product CTA (colorless) + CTA excess (colored).

At maximum wavelengths of four chromotropic acid azo dyes, Chromotrope 2B (method A), Arsenazo I (method B), Spadns (method C), and Sulfonazo III (method D), CTA excess is measured. To determine the maximum absorbance wavelength at which the measurement will be conducted, chromotropic acid azo dye, drug, OPD with Ce (IV) absorption spectra were constructed in the range 300-700 nm., Fig. 3.

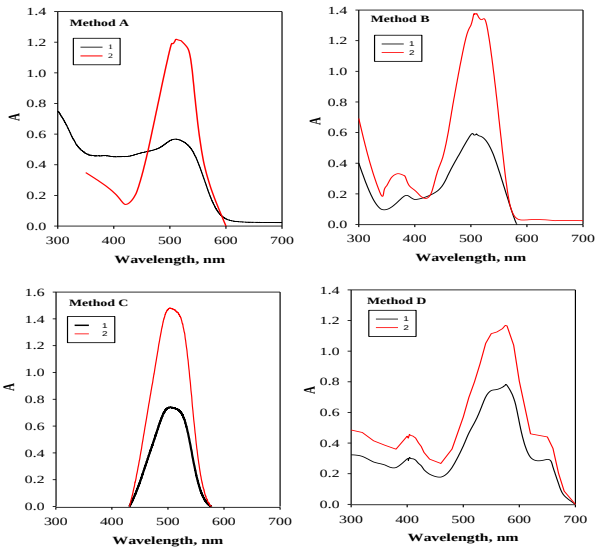

Fig. 3. Spectra of chromotropic azo (1), dye product of oxidation with 1x10^{-4} mol L^{-1} Ce (IV) and 1x 10^{-5} mol L^{-1} drug in the presence of 1.0 mol L^{-1} H_2SO_4 (2).

It was noted that neither the absorption spectrum of the drug nor the absorption spectrum of OPD with Ce (IV) exhibits any absorption maxima in the visible range. Chromotrope 2B (C2B) (method A), Arsenazo I (Arz I) (method B), spadns (Spd) (method C), and sulfonazo III (Sulf III) (method D) were all found to have maximum absorption at 510, 499, 505, and 570 nm, respectively.

3.1. Optimization of the Method

Different volumes (0.25-4.0 mL) of 1.0x10-3 mol L^{-1} cerium (IV) sulfate tetrahydrate were used to examine the effect of cerium (IV) sulfate tetrahydrate on the absorbance of the colored products. For both drugs, it was found that the maximum and constant absorbance was achieved with 1.0 mL of 1.0x10^{-3} mol L^{-1} Ce (IV) solution.

Various acids (sulfuric, hydrochloric, nitric, phosphoric, and acetic acids) were evaluated in different quantities. After a series of experiments, the most suitable acid to use with Ce (IV) as an acidic medium was 1.0 mol L^{-1} sulfuric acid in a total volume of 10 mL. Several amounts of 1.0 mol L^{-1} sulfuric acid were tested, whereas levels of oxidant and drug did not change. The data indicated that, in the presence of the drugs examined, practically the same absorbance values were achieved at 10 mL of sulfuric acid (1.0 mol L^{-1}). Following this, 10.0 mL of sulfuric acid (1.0 mol L^{-1}) was appropriate for later research.

A study was done to determine the effect of dye concentration C2B (method A), Arz I (method B), Spd (method C), and Sulf III (method D) on the intensity of the color developed to obtain the optimal dye concentration that reduces the residual cerium (IV). Various volumes (0.1-3.0 mL) of the studied dyes ($1.0x10^{-4}$ mol L^{-1}) were used to study the effects of dye concentration. The maximum color intensity of the unbleached color of dyes was found in the case of SOFO at 0.25 mL ($1.0x10^{-4}$ mol L^{-1}) for all chromotropic acid dyes. For DK, the volumes are 2.2, 2.2, 2.5, and 1.6 mL from C2B, Arz I, Sulf III, and Spd, respectively. Up to 24 hours, the color was stable.

Further experiments were performed to investigate the influence of the sequence of reactants on color development by measuring absorbance after optimizing all other experimental variables. In both cases, the optimum sequence of addition was (drug-cerium (IV)-H_2SO_4-dye). Other sequences of addition resulted in lower absorbances under the same experimental conditions.

In a water bath, a series of sample and blank solutions was heated at different temperatures ranging from 25 to 100 °C to study the effect of temperature. Increasing the temperature accelerates oxidation. 100 °C is the best temperature for oxidation.

To determine the effect of different mixing times on completing oxidation of SOFO or DK and reducing excess oxidant, absorbance measurements were taken on sample solutions compared to blank solutions prepared similarly at various intervals of time 2.0-30 minutes. Contact times at 25.0 minutes led to reproducible and constant absorbance values.

To bleach the dye color completely by residual cerium (IV), 5.0 minutes of standing time was required after the oxidation process. A stable absorbance of the unreacted dye was observed for at least 24 hours.

3.2. Assessment of Method Validation

3.2.1. Validity of Beer´s law, linearity, LOD, and LOQ

According to SOFO, the calibration graphs in the ranges 1-2.1 µg mL^{-1} were linear, Fig. 4. The LODs for methods A-D are 0.0138, 0.0183, 0.018and 0.013 µg mL^{-1}, while the corresponding LOQs are 0.046, 0.061, 0.060 and 0.045 µg mL^{-1}, respectively. Based on the application of methods A, B, C, and D, the molar absorption coefficient was $1.7x10^5$, $2.7x10^5$, $1.4x10^5$, and 1.7 $x10^5$ L mol^{-1} cm^{-1}, respectively. As shown in Table 2, the sensitivities for methods A, B, C, and D, are 3.2, 1.9, 3.8, and 3.1 ng cm^{-2}, respectively.

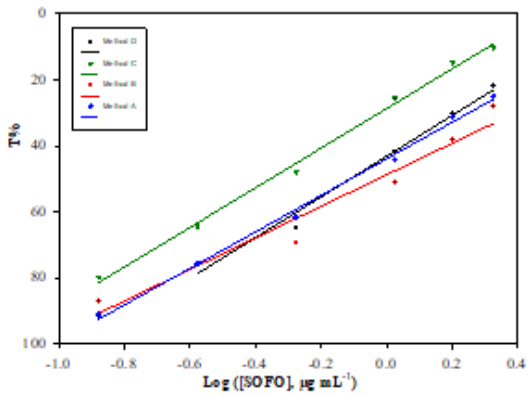

Fig. 4. SOFO calibration curves at 510, 505, 510, and 570 nm, for C2B (method A), Arz I (method B), Spd (method C), and Sulf III (method D), respectively.

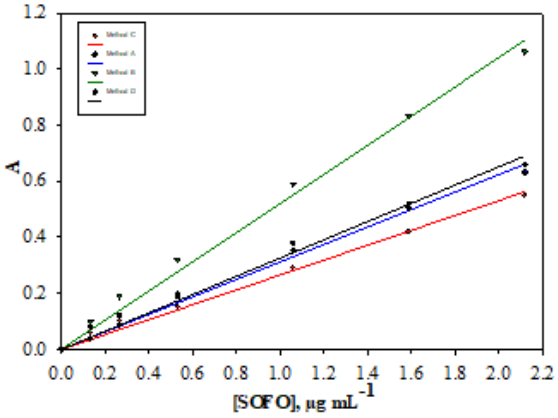

Fig 5. Ringbom's plots for SOFO using C2B (method A), Arz I (method B), Spd (method C), and Sulf III (method D).

Using methods A-D, the calibration graphs in figure 6 were linear between 0.1 and 5.1 µg mL^{-1} for DK. For the methods A, B, C, and D, the LOD was 0.0247, 0.022, 0.021, and 0.0247 µg mL^{-1}, respectively, table (2). Based upon the results of methods A, B, C, and D, we determined that the LOQ values were 0.08, 0.08, 0.07, and 0.08 µg mL^{-1}. Methods A, B, C, and D provide molar absorptivity values of 12.0 $x10^4$, 7.0 $x10^4$, $6.2x10^4$ and 9.0 $x10^4$ L mol^{-1} cm^{-1}. Table 3 gives the sensitivity values for methods A, B, C, and D, which are 0.080, 0.080, 0.070, and 0.080 µg cm^{-2}, respectively.

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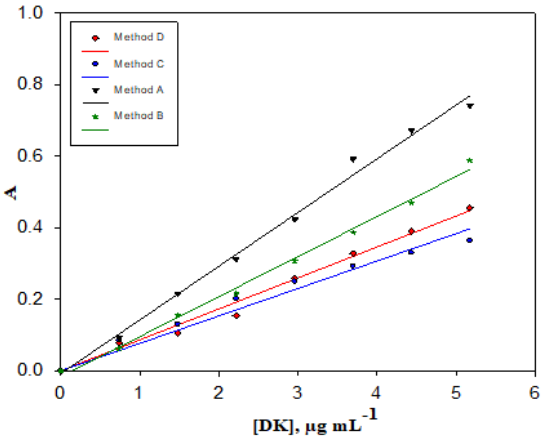

Fig. 6. DK calibration curves at 510, 505, 510, and 570 nm, for C2B (method A), Arz I (method B), Spd (method C), and Sulf III (method D), respectively.

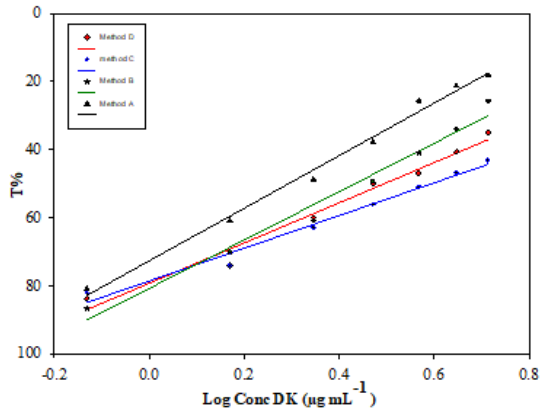

Fig. 7. Ringbom's plots for DK using C2B (method A), Arz I (method B), Spd (method C), and Sulf III (method D).

Table 2. Optical and regression characteristics of SOFO

	Method A (C2B)	**Method B (Arz I)**	**Method C (Spd)**	**Method D (Sulf III)**
Beer's law linear range, µg mL^{-1}	0.10-2.10	0.10-2.10	0.10-2.10	0.10-2.10
Ringbom's range, µg mL^{-1}	0.40-2.10	0.40-1.80	0.30-2.10	0.40-2.10
Molar absorptivity cofficient, L mol^{-1} cm^{-1}	$1.7x10^5$	$2.7x10^5$	$1.4x10^5$	$1.7x10^5$
Sandel sensitivity, ng cm^{-2}	3.2	1.9	3.8	3.1
LOD, µg mL^{-1}	0.0138	0.0183	0.018	0.013
LOQ, µg mL^{-1}	0.046	0.061	0.060	0.045
Data for Regression equation				
Slope	0.312	0.520	0.265	0.3258
Intercept	0.000	0.000	0.000	0.000
Correlation coefficient, r^2	0.9973	0.9982	0.9971	0.9981
Standard deviation	0.0046	0.0061	0.006	0.0045
λ_{max} (nm)	510	505	510	570
Calculated *t*-value	1.9	1.82	1,79	1.88
Calculated *F*-value	2.5	1.99	2.44	1.99

With a 95% confidence level and five degrees of freedom, theoretical values for t and F are 2.57 and 5.05, respectively.

3.2.2. Inter-day and Intra-Day Precision

Using a replicate set of calibration samples (n = 3 at each concentration) as a standard deviation, the intra- and inter-day variations were less than 15 %. The accuracy is reported as a percentage of recovery, while precision is expressed as a percentage of standard deviation, table 4. By using the four developed methods, the method of estimating the levels of the two drugs shows an excellent measure of accuracy and precision. A successful application of the proposed methods has been demonstrated for SOFO and DK in their pharmaceutical doses.

Table 3. Optical and regression characteristics for DK

	Method A (C2B)	Method B (Arz I)	Method C (Spd)	Method D (Sulf III)
Beer's law linear range, µg mL^{-1}	0.10-5.10	0.10-5.10	0.10-5.10	0.10-5.10
Ringbom's range, µg mL^{-1}	1.19-4.7	1.19-4.6	1.19-4.7	1.7-4.6
Molar absorptivity cofficient, L mol^{-1} cm^{-1}	1.20 $x10^5$	9.0 $x10^4$	6.2$x10^4$	7.0 $x10^4$
Sandel sensitivity, ng cm^{-2}	6.66	9.01	13.1	11.6
LOD, µg mL^{-1}	0.024	0.024	0.021	0.024
LOQ, µg mL^{-1}	0.080	0.080	0.070	0.080
Data for Regression equation				
Slope (b)	0.150	0.111	0.076	0.086
Intercept (a)	-0.033	-0.016	-0.00	-0.00
Correlation coefficient	0.9932	0.9947	0.9972	0.9981
Standard deviation	0.008	0.008	0.007	0.008
λ_{max} (nm)	510	505	510	570
Calculated *t*-value	1.4	1.59	1.73	1.66
Calculated *F*-value	1.32	1.61	1.44	1,73

With a 95% confidence level and five degrees of freedom, theoretical values for t and F are 2.57 and 5.05, respectively.

Table 4. Predicting DK and SOFO intra- and inter-days using methods A-D

		Intra-day data			Inter-day data	
		Taken	Found	Recovery ±SD	Found	Recovery ±SD
		µg mL^{-1}		%	µg mL^{-1}	%
DK results						
	Method A (C2B)	1.32	1.30	98.48±0.75	1.29	97.73±0.52
		3.23	3.12	96.59±1.02	3.14	97.21±0.42
	Method B (Arz I)	132	1.29	97.72±0.72	1.30	98.48±0.0.63
		3.23	3.21	99.38±0.70	3.17	98.14±0.79
	Method C (Spd)	1.32	1.34	101.15±0.48	1.31	99.24±0.98
		3.23	3.32	102.78±0.66	3.32	102.78 ±0.87
	Method D (Sulf III)	1.32	1.35	102.72±1.05	1.27	96.21±0.88
		3.23	3.28	101.54 ±0.53	3.13	96.90±0.53
SOFO Results						
	Method A (C2B)	0.78	0.76	97.43±0.80	0.75	96.15±0.84
		1.73	1.71	98.84±0.77	1.70	98.26±0.92
	Method B (Arz I)	0.78	0.77	98.71±053	0.76	97.43±0.73
		1.73	1.73	100.00±0.42	1.74	100.57±0.0.49
	Method C (Spd)	0.78	0.76	97.43±0.74	0.77	98.84±0.34
		1.73	1.70	98.26±0.75	1.75	101.15±1.01
	Method D (Sulf III)	0.78	0.76	97.43±0.93	0.76	97.43±0.40
		1.73	1.74	100.57±0.47	1.71	98.84±0.66

SD = Standard deviation

3.3. Pharmaceutics Analysis

By examining the formulations of SOFO and DK, the applicability of the proposed method was assessed.

3.3.1. An Investigation of Sofo in Pharmaceutical Formulations

Our lab analyzed Mpiviropack® tablets to determine the amount of SOFO they contained. Solutions of Mpiviropack® tablet containing 0.78,

1.73, and 2.13 µg mL^{-1} of SOFO were tested using the above mentioned chromotropic acid dyes, the recovery percentages were 97.58, 98.77, and 98.11% for the three concentrations, respectively, using C2B, 96.81, 96.33, and 98.77% for Arz (I), 98.92, 101.28 and 99.57% using Spd and 99.13, 99.31 and 99.83% using Sulf (III), Table 5

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Table 5. Assessing the accuracy and precision of SOFO's proposed chromotropic dye procedures

Strategy	Taken µg mL^{-1}	Recovery ±SD%	
		Pure SOFO	Mpiviropack® tablet, 150 mg/tablet
Method A, C2B	0.78	99.61±0.17	97.58 ± 0.23
	1.73	98.42 ±0.47	97.77 ± 0.18
	2.13	99.11±0.21	98.11 ± 0.42
Method B, Arz (I)	0.78	97.39±0.10	96.81±0.14
	1.73	103.2 ±0.74	96.33 ±0.13
	2.13	95.83±0.35	98.77±0.18
Method C, Spd	0.78	104.92±0.11	98.92±0.33
	1.73	102.88 ±0.37	101.28 ±0.82
	2.13	97.99±0.87	99.57±0.66
Method D, Sulf (III)	0.78	96.74±0.62	99.13±0.29
	1.73	97.68 ±0.55	99.31 ±0.77
	2.13	104.83±0.36	99.83±0.22

Table 6. Assessing the accuracy and precision of DK's proposed chromotropic dye procedures

Reagent	Taken (µg ml^{-1})	Recovery ±SD%	
		Pure DK	Daclavirocyrl ® tablet, 60 mg/ tablet
Method A, C2B	1.32	103.20±0.58	99.11±0.82
	3.23	95.44±0.98	104.30 ±0.73
	4.68	106.22±0.28	99.21±0.99
Method C, Arz (I)	1.32	95.24±0.89	95.28±0.44
	3.23	105.40±0.54	101.32 ±0.35
	4.68	96.33±0.57	101.20±0.50
Method C, Spd	1.32	109.87±0.97	101.12±0.35
	3.23	107.77±0.88	103.32 ±0.22
	4.68	101.23±0.42	97.38±0.29
Method D, Sulf (III)	1.32	100.90±0.93	100.52±0.85
	3.23	99.52±0.57	99.81 ±0.72
	4.68	102.15±0.25	106.22±0.57

3.3.2. An Investigation of Dk in Pharmaceutical Formulations

Daclavirocyrl® tablet was analyzed for its Dk content. The measurements were carried out according to the general procedure. Samples containing 1.32, 3.23, and 4.68 µg mL^{-1} of DK were analyzed using chromotropic dyes. The recovery percentages were 99.11, 104.30, and 99.21% using C2B, 95.28, 101.32, and 101.20% using Arz (I), 101.12, 103.32, and 97.38% using Spd and 100.52, 99.81, and 106.22% using Sulf (III), Table 6.

3.4. Analyzing The Data Statistically

Validation of the developed methods was performed in accordance with the reference procedures [6]. For comparing accuracy and precision, Student's t-test was applied along with the variance ratio F-test. A good correlation could be found during experimentation since the developed methods followed Beer's law. Based on the statistical calculations, the t- and F- values were below the critical value, demonstrating no significant difference between the proposed and reference methods, Table 2, 3. The proposed methods yielded higher accuracy with a high recovery rate in comparison with the reference method. This means that they can be used routinely in most quality control laboratories.

4. Conclusions

By oxidizing SOFO and DK with Ce (IV) in an acidic medium, then determining the unreacted Ce (IV), the proposed method was developed. Comparable to other reported visible spectrophotometric methods, they have the advantage of being more sensitive, allowing the determination of nanogram amounts, as well as being simpler, reproducible, precise, and accurate. In summary, the recovery values for this method are 95.24- 106.22%,

RSD% 0.34 - 1.02%, suggesting that it can be used as a routine analytical and quality control procedure for evaluating the analysis of raw materials and pharmaceutics.

5. Conflicts of interest

It is declared that the authors have no financial competing interests.

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7. References

[1] Bandla, J.; Ganapathy, S. "Development and validation of a stability-indicating method for the simultaneous estimation of sofosbuvir and ledipasvir by RP-HPLC", Indian J Pharm Sci. 2018, 80(6), 1170-1176.

[2] Eissa, M.S; Fayed, A.S.; Hegazy, M.A.; Kamel, E.B. "Spectrophotometric analysis for simultaneous determination of the new antiviral drug combination: Daclatasvir/Sofosbuvir in their pure form and pharmaceutical preparation", Research J. Pharm. and Tech. 2020, 13(12), 5939-5946.

[3] World Health Organization, "World Health Organization model list of essential medicines: 21st list 2019. Geneva:hdl:10665/325771. WHO/MVP/EMP/IAU/2019.06. License: CC BY-NC-SA 3.0 IGO.

[4] Procedure under Article 20 of Regulation (EC) No 726/2004 resulting from pharmacovigilance data, EMA/103226/2017.

[5] Saad, M.Z.; Amer, A.; Elgendy, K.; Elgendy, B. "Spectrophotometric methods for determination of sofosbuvir and daclatasvir in pure and dosage forms", Int J App Pharm. 2021, 13(6), 112-119.

[6] Ashok, C.V.; Sailaja, B.B.V.; Praveen, K.A. "Method development and validation of an ultraviolet-visible spectroscopic method for the estimation of hepatitis-c drugs - daclatasvir and sofosbuvir in active pharmaceutical ingredient form". Asian J Pharm Clin Res. 2016, 9(3), 61-66.

[7] Hassan, W.S; Elmasry, M.S.; Elsayed, H.M; Zidan, D.W. "Comparative study of six sequential spectrophotometric methods for quantification and separation of ribavirin, sofosbuvir and daclatasvir: An application on Laboratory prepared mixture, pharmaceutical preparations, spiked human urine, spiked human plasma, and dissolution test". Spectrochim Acta A Mol Biomol Spectrosc. 2018, 5(202), 159-173.

[8] Gautam, CH. V. S; Harika, N.; Balaji, V.; Srinivas, P. V. "Method Development And Validation Of Daclatasvir In Bulk & Pharmaceutical Dosage Form By Uv-Visible Spectrophotometry". IAJPS, 2018, 05(03), 1980-1985,

[9] Keyvan, K.; Sohrabi, M.R.; Motiee, F. "Improved spectral resolution for the rapid simultaneous spectrophotometric determination of sofosbuvir and daclatasvir as anti-hepatitis C virus drugs in pharmaceutical formulation and biological fluid using continuous wavelet and derivative transform". Spectrochim Acta A Mol Biomol Spectrosc. 2021, 251, 1386-1425.

[10] Rathod, S.M.; Patel, P.U. "Development and validation for simultaneous estimation of sofosbuvir and daclatasvir dihydrochloride in pharmaceutical dosage form by ratio derivative and dual wavelength methods". IJPQA, 2020, 11(1), 25-31.

[11] Youssef, A. A.; Magdy, N.; Hussein, L.A.; El-Kosasy, A.M. Validated RP-HPLC Method for Simultaneous Determination of Ribavirin, Sofosbuvir and Daclatasvir in Human Plasma: A Treatment Protocol Administered to HCV Patients in Egypt. J Chromatogr Sci. 2019, 57(7), 636-643.

[12] Nekkala, K.; Kumar, J.V.S.; Ramachandran, D. "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. 2018, 11(2), 164-168.

[13] Hassouna, M.E.M.; Abd Elrahman, M.M.; Abd Elfatah, M. "Assay and dissolution methods development and validation for simultaneous determination of sofosbuvir and ledipasvir by RP-HPLC method in tablet dosage forms", J Forensic Sci and Criminal Inves. 2017, 1(3), 1-11.

[14] Al-Tannak, N.F.; Hemdan, A.; Eissa, M.S. "Development of a robust UPLC method for simultaneous determination of a novel combination of sofosbuvir and daclatasvir in human plasm". International Journal of Analytical Chemistry. 2018, 1-9.

[15] Jyothi, U.; Umadevi, P. "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, 7(08), 401-409.

[16] Saroja, J.; Anantha, P.V.; Rammohan, Y.; Divya, D.; Santhosh K.P. "Concurrent estimation of sofosbuvir and velpatasvir in raw and tablets using stability indicating RP-HPLC method". Rasayan J. Chem. 2018, 11(3), 1058-1066.

[17] Fayed, A.S.; Hegazy, M.A; Kamel, A.B.; Eissa, M.S. "HPLC-UV and TLC-Densitometry Methods for Simultaneous Determination of Sofosbuvir and Daclatasvir: Application to Darvoni®Tablet". J. Chrom. Sci.2021.

[18] Kumari, K.P.; Sankar, D.G. "UPLC method for simultaneous estimation of ledipasvir and sofosbuvir in bulk and dosage forms and their stress degradation studies". J Bioanal Biomed. 2019, 11(1), 136-141.

[19] Mohamed, S.H.; Issa, Y.M.; Salim A.I. "Simultaneous quantification of simeprevir sodium: a hepatitis C protease inhibitor in binary and ternary mixtures with sofosbuvir and/or ledipasvir utilizing direct and H-point standard addition strategies". Spectrochim. Acta A: Mol. Biomol. Spectrosc. 2019, 210, 290-297.
[20] Mohamed, S.H.; Issa, Y.M.; Salim, A.I. "An eco-concerned development of a fast, precise and economical spectrophotometric assay for the antiviral drug simeprevir based on ion-pair formation". BRIAC. 2021, 11(5), 13474-13489.
[21] Attia, K.A.M.; El-Abasawi, N.M.; El-Olemy, A.; Serag, A. "Different spectrophotometric methods applied for the analysis of simeprevir in the presence of its oxidative degradation product: A comparative study". Spectrochim. Acta A: Mol. Biomol. Spectrosc. 2018, 190, 1-9.
[22] Krebs, A.; Starczewska, B.; Puzanowska-Tarasiewicz, H.; Sledz J. "Spectrophotometric determination of olanzapine by its oxidation with N-bromosuccinimide and cerium (IV) sulfate". Anal Sci. 2006, 22(6), 829-33.
[23] El Sheikh, R.; Hassan, W.S.; Gouda, A.A.; Al Owairdhi, AA. "Validated and sensitive spectrophotometric methods for estimation of rizatriptan benzoate in pharmaceutical formulations using cerium (IV) Sulphate". Res J Pharm Technol. 2019, 12, 2123-30.
[24] Abdellatef, H.E.; El-Henawee, M.M.; El-Sayed, H.M.; Ayad, M.M. "Spectrophotometric and spectrofluorimetric methods for analysis of acyclovir and acebutolol hydrochloride". Spectrochim Acta A Mol Biomol Spectrosc. 2006, 65(3-4), 997-999.
[25] Shoukry, A.F.; Abdel Ghani, N.T.; Issa, Y.M.; Wahdan, O.A. "Spectrophotometric determination of dipyridamole and chlorpheniramine maleate using some chromotropic acid azo dyes". Analytical letters. 2001, 34(10), 1689-1701.
[26] Abou-Attia, F.M.; Issa, Y.M.; El-Reis, M.A.; Aly, F.A.; Abd El-Moety, M. "New application of chromotropic acid for the determination of some sulphonamides in their pharmaceutical preparations". Sci. Pharm, 2002, 70, 49-55.