SPECTROPHOTOMETRIC METHODS FOR DETERMINATION OF SOFOSBUVIR AND DACLATASVIR IN PURE AND DOSAGE FORMS

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ABSTRACT

Objective: Two simple, sensitive and accurate spectrophotometric methods have been developed for the determination of sofosbuvir (SOF) and daclatasvir (DAC) in pure forms and pharmaceutical formulations.

Methods: The proposed methods are based on the oxidation of SOF and DAC by a known excess of cerium(IV) ammonium nitrate in sulphuric acid medium followed by determination of unreacted cerium(IV) by adding a fixed amount of indigo carmine (IC) and alizarin red S (ARS) dyes followed by measuring the absorbance at 610 and 360 nm, respectively. The experimental conditions affecting the reaction were studied and optimized.

Results: The Beer’s law was obeyed in the concentration ranges of 0.2-3.0, 0.2-4.0 for SOF and 0.5-4.5 and 0.5-5.0 μg/ml for DAC using IC and ARS methods, respectively with a correlation coefficient 0.9991. The calculated molar absorptivity values are 2.354 × 10^4, 1.933 × 10^4 for SOF and 1.786 × 10^4 and 2.015 × 10^4 L/mol cm for DAC using IC and ARS methods, respectively. The limits of detection and quantification are also reported. Intra-day and inter-day precision and accuracy of the methods have been evaluated.

Conclusion: The methods were successfully applied to the assay of SOF and DAC in tablets and the results were statistically compared with those of the reference method by applying Student’s t-test and F-test. No interference was observed from the common tablet excipients. The accuracy and reliability of the methods were further ascertained by performing recovery studies using the standard addition method.

Keywords: Sofosbuvir, Daclatasvir, Spectrophotometry, Cerium (IV), Dyes, Method validation, Dosage forms

INTRODUCTION

Sofosbuvir, (S)-isopropyl 2-{[(2R,3R,4R,5R)-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)-4-fluoro-3-hydroxy-4-methyltetrahydrofuran-2-yl]methoxy} (phenoxo) phosphorylamino) propanoate (SOF) (fig. 1). It is a prodrug nucleotide analog used for the treatment of chronic hepatitis C, genotypes 1, 2, 3, 4, 5, and 6, usually in combination with other medications depending on the specific genotype. Sofosbuvir, is a medication used in combination with other medications for the treatment of hepatitis C [1, 2].

Daclatasvir dihydrochloride (DAC) is methyl [25]-1-{{25}-2-[4-{4-[(25)-1-{{25}-2-[{(methoxy carbonyl)amino]-3-methyl butanoyl]}}-2-pyrrolidinyl]-1H-imidazol-4-yl]-4-biphenyl yl]-1H-imidazo[2,1-b] carbamate dihydrochloride (fig. 1). DAC is an antiviral drug used to treat chronic [long-lasting] hepatitis C, a viral infection of the liver. DAC is an antiviral and acts directly against the hepatitis C virus [3].

A through literature search has revealed that only a few spectrophotometric methods have been developed for the determination of SOF and DAC in pure and dosage forms [19-26]. However, many of the above methods suffered from one or other disadvantage like poor sensitivity, require high cost solvents in addition to elaborate treatment, need tedious extraction procedures, rigid pH control, measurements done at shorter wavelengths, heating or cooling step, use of expensive chemical and/or complicated experimental set-up. In contrast, visible spectrophotometry is considered as the most convenient analytical technique in most quality control and clinical laboratories. Spectrophotometric technique, because of simplicity and low cost, sensitivity and good analytical selectivity, significant accuracy and precision and broad availability and applicability for pharmaceutical analysis.

Cerium(IV) has been widely used as an effective analytical reagent in spectrophotometric methods for the determination of many pharmaceutical compounds [27-35]. Cerium(IV) is a strong oxidant, and it has not been applied for the assay of SOF and DAC in pure forms and tablets.

This paper describes for the first time the application of acidic ammonium cerium(IV) nitrate to the spectrophotometric determination...
of SOF and DAC using Indigo Carmine (IC) and Alizarin Red S (ARS) as chromogenic agents. The proposed methods have the advantages of simplicity, sensitivity and rapidity besides being accurate precise and validated spectrophotometric method for the estimation of SOF and DAC in pure and dosage forms and can be adopted by the pharmaceutical laboratories for industrial quality control.

MATERIALS AND METHODS

Apparatus

All absorption spectra were made using Shimadzu UV/Vis, double beam spectrophotometer (Japan) equipped with a 10 mm quartz cell was used for absorbance measurements.

Materials and reagents

All chemicals and reagents used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily. Bidistilled water was used throughout the investigation.

Pure drugs and pharmaceutical formulations

Pharmaceutical grade SOF was kindly supplied by SEDICO, Cairo, Egypt. The commercial pharmaceutical formulations (Hopforhep or DAC were transferred into 100 ml calibrated flask and dissolved accurately weighed quantity of the powder equivalent to 10 mg SOF or DAC were transferred into 100 ml calibrated flask and dissolved in 25 ml methanol. The content of the flask was shaken and sonicated for about 10 min, mixed well and then filtered using Whatman No.42 filter paper. The first portion of the filtrate was rejected, and the solution was then completed to volume with bidistilled water to prepare a stock solution of 100 µg/ml. Aliquots covering the working concentration ranges for each method were transferred into a series of 10 ml volumetric flasks and the proposed methods were applied. The nominal content of the tablets was determined using the corresponding regression equations or the calibration graphs.

RESULTS AND DISCUSSION

Absorption spectra

The proposed spectrophotometric methods for the determination of SOF or DAC is indirect and involves two steps namely:

1. Oxidation of SOF or DAC with a known excess of cerium(IV) in acidic medium.
2. Determination of the residual cerium(IV) by reacting it with a fixed amount of IC or ARS dyes and measuring the increase in absorbance at λmax 610 and 360 nm, respectively (Scheme 1) (fig. 2a and 2b).

Fig. 2: Absorption spectra for the unreacted cerium(IV) oxidant that determined by reacting with a fixed amount of (a) IC and (b) ARS dyes and measuring the absorbance at 610 and 360 nm for IC and ARS methods, respectively

![Absorption spectra](image.png)
Optimization of the reaction conditions

The optimum conditions for the assay procedures and color development for each method have been established by varying the parameters one at a time, keeping the others fixed and observing the effect produced on the absorbance of the colored species.

Effect of cerium(IV) sulphate concentration

The influence of the concentration of cerium(IV) ammonium nitrate on the absorbance of the colored products was investigated using different volumes of $1.0 \times 10^{-3}$ mol/l cerium(IV) solution from (0.25 - 4.0 ml). The results indicate that the maximum and constant absorbance was obtained using 2.0 ml of $1.0 \times 10^{-3}$ mol/l cerium(IV) solution for both drugs (SOF and DAC) and the color intensity constant or decreased (fig. 3a and 3b).

Effect of dye concentration

The effect of IC or ARS dye concentration on the intensity of the color developed was carried out to obtain the optimum concentration of dyes that produces the maximum and reproducible color intensity by reducing the residual of cerium (IV). The effect dye concentration was studied using different volumes (0.25 – 2.0 ml) of the studied dyes ($1.0 \times 10^{-3}$ mol/l) (fig. 4a and 4b). It was observed that maximum color intensity of the oxidation products was achieved with 0.5 and 1.0 ml of IC and ARS dye solution, respectively. The color was found to be stable up to 10 h.

Fig. 3: Effect of volume of cerium (IV) ($1.0 \times 10^{-3}$ mol/l) on the reaction product of (a) SOF and (b) DAC and dyes in H$_2$SO$_4$ medium

Fig. 4: Effect of volume of dye ($1.0 \times 10^{-3}$ mol/l) on the reaction product of (a) SOF and (b) DAC with Ce(IV) in H$_2$SO$_4$ medium
Effect of acid type and concentration

Different types of acids were examined (HCl, H2SO4, H3PO4, HNO3, and CH3COOH) to achieve maximum yield of redox reactions. The results indicated that the sulphuric acid (H2SO4) (2.0 mol/l) was the most suitable acid with cerium (IV) as oxidant. Moreover, different volumes (0.25 – 3.0 ml) of 2.0 mol/l H2SO4 were tested, keeping the concentrations of oxidant and drug fixed. The results indicated that, at (1.5 -2.5 ml) of H2SO4 (2.0 mol/l), there were almost same absorbance values were obtained in the presence of the studied drugs. At the acid volumes less than 1.5 ml, reaction led to go slower and incomplete. Therefore, 2.0 ml of H2SO4 (2.0 mol/l) was the optimum volume for subsequent studies (fig. 5).

Effect of temperature and mixing time

The effect of temperature was studied by heating a series of sample and blank solutions at different temperatures ranging from 25 to 80 °C in water bath. It was found that raising the temperature does not accelerate the oxidation process and does not give reproducible results, so maximum color intensity was obtained at temperature (50 °C). The effect of mixing time required completing oxidation of SOF or DAC and for reducing the excess oxidant was studied by measuring the absorbance of sample solution against blank solution prepared similarly at various time intervals 2.0–30 min. It was found that the contact times gave constant and reproducible absorbance values at 15.0 min. After oxidation process, 5.0 min standing time was found necessary for the complete bleaching of the dye color by the residual cerium (IV) and the absorbance of the unreacted dye was stable for at least 10 h, thereafter (fig. 6).

Effect of sequence of addition

After optimizing all other experimental variables, further experiments were performed to ascertain the influence of sequence of addition of reactants on the color development by measuring the absorbance. The optimum sequence of addition for both drugs was (drug–cerium (IV) ammonium nitrate–H2SO4–dye). Other sequences gave lower absorbance values under the same experimental conditions.

Validation of the proposed methods

The validity of the methods was tested regarding linearity, specificity, accuracy, repeatability and precision according to International Conference on Harmonization (ICH) [37] guidelines.

Linearity, detection, and quantification limits

Following the proposed experimental conditions, linear regression equations were obtained. The regression plots showed that there was a linear dependence of the absorbance to the concentration in the range of 0.2–3.0, 0.2–4.0 for SOF and 0.5–5.0, 0.5–5.0 μg/ml for DAC using IC and ARS methods, respectively. Linear regression analysis of the data gave the following equations:

\[
A = 0.0342 + 0.3063C, \quad r^2 = 0.9991 \quad \text{for SOF using IC.}
\]

\[
A = 0.0799 + 0.0.2134C, \quad r^2 = 0.9996 \quad \text{for SOF using ARS.}
\]

\[
A = -0.0198 + 0.1413C, \quad r^2 = 0.9991 \quad \text{for DAC using IC.}
\]

\[
A = -0.0224 + 0.1694C, \quad r^2 = 0.9991 \quad \text{for DAC using ARS.}
\]

Where \( A \) is the absorbance, \( C \) is the concentration of drug, and \( r^2 \) is the correlation coefficient.

The limits of detection (LOD) were determined by establishing the minimum level at which the analyte can be reliably detected, and the limit of quantification (LOQ) was determined by establishing the lowest concentration that can be measured with acceptable accuracy and precision according to ICH [37, 38]. The results are also summarized in table 1. LOQ and LOD were calculated according to the following equations (Eqn. 1. and Eqn. 2):

\[
LOD = 3s/k \quad \text{Eqn. 1.}
\]

\[
LOQ = 10s/k \quad \text{Eqn. 2.}
\]

Where \( s \) is the standard deviation of ten replicate determinations values of the reagent blank and \( k \) is the sensitivity, namely the slope of the calibration graph. In accordance with the formula, for SOF and DAC the LOD were found to be 0.06 and 0.15 μg/ml and LOQ were found to be 0.20 and 0.50 μg/ml using IC and ARS, respectively.
variables did not significantly affect the results. The RSD% values of other parameters were kept unchanged, and the recovery experiments, one experimental parameter was changed while the others were in the ranges 0.60–2.10% and 0.40–1.50% for SOF and DAC, respectively (table 3). This indicated the reliability of the proposed method during its routine application for the analysis of SOF or DAC. The ruggedness and precision of the proposed method were assessed by applying the procedures using two different instruments in three different laboratories (instruments) at different times and three different analysts. The inter -instruments RSD% were in the ranges 0.56-1.90% and 0.58-1.90% for SOF and DAC, respectively, whereas the inter -instruments RSD% ranged from 0.56-1.80% and 0.60-2.20% for SOF and DAC, respectively; these results were found to be reproducible because the RSD did not exceed 3.0% (table 3).

### Table 1: Analytical and regression parameters of proposed oxidation spectrophotometric methods for determination of SOF and DAC

| Parameters | SOF | ARS | DAC |
|------------|-----|-----|-----|
| Wavelength (nm) | 610 | 360 | 610 |
| Beer’s law limits, µg/ml | 0.2-3.0 | 0.2-4.0 | 0.5-4.5 |
| Molar absorptivity, x10^3 l/mol. cm | 2.354 | 1.933 | 1.786 |
| Sandell sensitivity, ng cm^-2 | 22.47 | 27.37 | 45.45 |
| Regression equation | Intercept (a) | 0.0342 | 0.0799 | -0.0198 |
| | Slope (b) | 0.084 | 0.075 | 0.091 |
| | Standard deviation of intercept (S_a) | 0.3063 | 0.2134 | 0.1413 |
| | Standard deviation of slope (S_b) | 0.09 | 0.12 | 0.089 |
| | Correlation coefficient, (r) | 0.9991 | 0.9996 | 0.9991 |
| | Mean±SD | 99.20±0.60 | 99.40±0.80 | 98.80±0.90 |
| | RSD% | 0.60 | 0.80 | 0.90 |
| | RE% | 0.63 | 0.84 | 0.94 |
| | Limit of detection, µg/ml | 0.06 | 0.06 | 0.15 |
| | Limit of quantification, µg/ml | 0.20 | 0.20 | 0.50 |
| | Calculated t-value | 1.64 | 0.85 | 0.43 |
| | Calculated F-value | 1.33 | 0.91 | 3.0 |

The accuracy and precision expressed as percent relative error (RE%) and relative standard deviation (RSD%) values, respectively and found to be within 1.50-0.50% and 1.01-2.51%, respectively for SOF and found to be within 1.10-0.40% and 1.0-2.52%, respectively for DAC. The accuracy and precision study obtained by the proposed methods.

### Table 2: Results of intra-day and inter-day accuracy and precision study obtained by the proposed methods

| Method | Taken (µg/ml) | Recovery % | Precision RSD % | Accuracy RE % | Confidence limit | Recovery % | Precision RSD % | Accuracy RE % | Confidence limit |
|--------|--------------|------------|-----------------|---------------|----------------|------------|-----------------|---------------|-----------------|
| Intra-day | | SOF | DAC |
| IC | 1.0 | 99.00 | 2.0 | -1.0 | 0.99±0.02 | 99.40 | 1.01 | -0.60 | 0.994±0.01 |
| | 2.0 | 99.60 | 2.51 | -0.40 | 1.992±0.05 | 99.00 | 2.02 | -1.0 | 1.980±0.04 |
| | 3.0 | 100.50 | 1.99 | 0.50 | 3.015±0.06 | 99.70 | 1.0 | -0.30 | 2.99±0.03 |
| ARS | 1.0 | 99.20 | 1.01 | -0.80 | 0.992±0.01 | 98.90 | 2.02 | -1.10 | 0.989±0.02 |
| | 2.0 | 99.70 | 1.50 | -0.30 | 1.994±0.03 | 99.10 | 2.52 | -0.90 | 1.982±0.05 |
| | 3.0 | 98.50 | 1.69 | -1.50 | 2.955±0.05 | 100.40 | 2.32 | 0.40 | 3.012±0.07 |
| Inter-day | | SOF | DAC |
| IC | 1.0 | 100.20 | 2.94 | 0.20 | 1.02±0.03 | 99.50 | 1.05 | -0.50 | 0.995±0.01 |
| | 2.0 | 100.60 | 1.99 | 0.60 | 2.012±0.04 | 99.10 | 1.51 | -0.90 | 1.982±0.03 |
| | 3.0 | 99.30 | 1.68 | -0.70 | 2.979±0.05 | 99.00 | 1.35 | -1.0 | 2.978±0.04 |
| ARS | 1.0 | 99.80 | 2.95 | -0.20 | 0.996±0.02 | 100.30 | 1.0 | 0.30 | 1.003±0.01 |
| | 2.0 | 100.20 | 2.50 | 0.20 | 2.004±0.05 | 99.40 | 2.01 | -0.60 | 1.988±0.04 |
| | 3.0 | 99.00 | 1.35 | -1.0 | 2.970±0.04 | 98.70 | 2.03 | -1.30 | 2.961±0.06 |

RSD%, percentage relative standard deviation; RE%, percentage relative error. *Mean±standard error.

### Ruggedness and robustness

Robustness of the proposed method was assessed by evaluating the influence of small variation of experimental variables, including concentration of analytical reagents and reaction time, on the analytical performance of the proposed method. In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time. The analysis was performed with altered conditions by taking three different concentrations of SOF or DAC and it was found that the small variations in any of the variables did not significantly affect the results. The RSD% values were in the ranges 0.60–2.10% and 0.40-1.50 for SOF and DAC, respectively (table 3). This indicated the reliability of the proposed method during its routine application for the analysis of SOF or DAC.
fold excess with $1 \times 10^{-2}$ concentration of each drug was added and the absorbance values showed that there is no interference with Hopforhep tablets and DAC in Daclahepex tablets. The results of the proposed method were applied to the determination of SOF in Analysis of the pharmaceutical preparations

The proposed methods were applied to the determination of SOF in (Hopforhep tablets) and DAC in Daclahepex tablets. The results of recovery±SD values of the proposed methods agree well with the label claim and also were in agreement with the results obtained by the reported methods for SOF [4] and DAC [10] and were statistically compared with those obtained using the reference

| Methods | Nominal amount concentration (μg/ml) | RSD% | Robustness | Ruggedness |
|---------|-----------------------------------|------|------------|-----------|
|         | Acid volume (n=3) | Reaction time (n=3) | Different analysts (n=3) | Different instruments (n=3) |
| IC      | SOF     | 1.0 | 0.65 | 0.75 | 0.56 | 0.80 |
|         |         | 2.0 | 1.0 | 1.20 | 0.90 | 1.40 |
|         |         | 3.0 | 1.0 | 1.70 | 2.10 | 1.90 | 1.80 |
| ARS     | SOF     | 1.0 | 0.80 | 0.60 | 0.73 | 0.56 |
|         |         | 2.0 | 0.920 | 1.30 | 1.0 | 0.87 |
|         |         | 3.0 | 1.0 | 1.20 | 0.90 | 1.10 | 1.40 |
| IC      | DAC     | 1.0 | 0.40 | 0.70 | 0.58 | 1.18 |
|         |         | 2.0 | 0.90 | 0.87 | 0.80 | 0.60 |
|         |         | 3.0 | 1.0 | 1.30 | 1.50 | 1.20 | 2.20 |
| ARS     | DAC     | 1.0 | 0.76 | 0.48 | 1.18 | 0.85 |
|         |         | 2.0 | 0.95 | 0.86 | 1.45 | 1.16 |
|         |         | 3.0 | 1.32 | 1.58 | 1.90 | 2.10 |

*Volume of (2.0 mol/l) H$_2$SO$_4$ is (±0.2 ml) and reaction time is (±2.0 min) (after adding cerium (IV)) were used.

Effect of interferences and recovery studies

The specificity of the proposed method was investigated by observing any interference encountered from the common tablets excipients such as starch, talc, chloride, MgSO$_4$, and cellulose by 20-fold excess with $1 \times 10^{-2}$ concentration of each drug was added and the absorbance values showed that there is no interference with each one. Also, the standard addition method was applied by adding known amounts of pure SOF or DAC to a previously analyzed tablet solution. This study was performed by spiking three different levels of pure SOF or DAC (50, 100 and 150% of the level present in the tablet) to a fixed amount of drugs in tablet powder (pre-analysed) and the total concentration was found by the proposed methods. The determination with each level was repeated three times and the percent recovery of the added standard was calculated from Eqn. 3:

$$\%\ Recovery = \left[ \frac{C_F - C_R}{C_F} \right] \times 100 \quad \ldots \text{Eqn. 5}$$

Where $C_F$ is the total concentration of the analyte found, $C_R$ is a concentration of the analyte present in the tablet preparation; $C_R$ is a concentration of analyte (pure drug) added to tablets preparations. The results were recorded in table 4. The high recovery values of the proposed methods indicated that the excipients did not interfere with the proposed methods indicating the high selectivity of the proposed methods.

| Method | Taken drug in Tablet (μg/ml) | Pure drug added (μg/ml) | Total found (μg/ml) | Recovery% (±SD) | Reported method |
|--------|-------------------------------|-------------------------|---------------------|----------------|----------------|
| Hopforhep tablets | SOF  | 1.0 | 0.5 | 1.49 | 99.30±0.25 |
|         |     | 1.0 | 1.0 | 1.99 | 99.50±0.30 |
|         |     | 1.0 | 1.50 | 2.52 | 100.80±0.60 |
| IC     | DAC  | 1.0 | 0.5 | 1.485 | 99.00±0.40 |
|         |     | 1.0 | 1.0 | 2.008 | 100.40±0.50 |
|         |     | 1.0 | 1.50 | 2.485 | 99.30±0.70 |
| ARS    | DAC  | 1.0 | 0.5 | 1.487 | 99.10±0.20 |
|         |     | 1.0 | 1.0 | 2.01 | 100.50±0.30 |
|         |     | 1.0 | 1.50 | 2.475 | 99.00±0.50 |
| IC     | DAC  | 1.0 | 0.5 | 1.502 | 100.10±0.40 |
|         |     | 1.0 | 1.0 | 2.012 | 100.60±0.60 |
|         |     | 1.0 | 1.50 | 2.483 | 99.30±0.80 |
| ARS    | DAC  | 1.0 | 0.5 | 1.502 | 100.00±0.66 |
|         |     | 1.0 | 1.0 | 2.012 | 100.00±0.66 |
|         |     | 1.0 | 1.50 | 2.483 | 99.30±0.80 |

*Average of six determinations. *Theoretical values of t and F at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).
methods. Statistical analysis of the results, using Student’s t-test and the variance ratio F-test at 95% confidence level revealed no significant difference between the performance of the proposed and reference methods regarding the accuracy and precision, respectively (table 4)[38]. It is evident from these results that the proposed methods are applicable to the analysis of SOF and DAC in its dosage forms with comparable analytical performance.

CONCLUSION

A new, simple, rapid, useful and cost-effective spectrophotometric methods have been developed for determination of SOF and DAC in pure form and tablets using cerium (IV) as oxidizing agent and validated as per the current ICH guidelines. The present spectrophotometric methods are characterized by simplicity of operation, high selectivity, comparable sensitivity, low-cost instrument, they do not involve any critical experimental variable and are free from tedious and time-consuming extraction steps and use of organic solvents unlike many of the previous methods reported for SOF and DAC. The proposed methods have some additional advantages involve less stringent control of experimental parameters such as the stability of the colored system, accuracy, reproducibility, time of analysis, temperature independence and cheaper chemicals. These advantages encourage the application of the proposed methods in routine quality control analysis of SOF and DAC in pure and dosage forms.

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AUTHORS CONTRIBUTIONS

Prof. Dr. Muneer Zaky has generated the research idea and interpreted the data and helped to draft the manuscript. Prof. Dr. Atef Amer has suggested the research idea and participated in the design of the study. Mr. Basem El Gendy was prepared the solutions, carried out the experiments, interpreted the data and helped to draft the manuscript. Dr. Khaled El Gendy helped in check spelling, reducing the plagiarism, interpreting the data, reviewed the manuscript and submit the manuscript for publication.

CONFLICTS OF INTERESTS

The authors confirm that this article content has no conflict of interest.

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