Spectrophotometric Determination of Sodium Sulfacetamide Using Pyrocatechol as an Oxidative Coupling Agent

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Abstract: *Oxidative coupling reaction is one of the simplest and fastest spectrophotometric methods for measuring sodium sulfacetamide (SCS) in pure formulations and pharmaceutical formulations (eye ointment) when compared to other methods that require difficult and costly operating conditions. Here, a new approach for quantifying SCS drug spectrophotometrically by oxidative coupling was described. The research is based on the oxidation of the drug with potassium periodate and then conjugation with pyrocatechol reagent in the neutral medium, which results in a water-soluble and stable reddish-brown product that had an absorption band at 500.5 nm. In this work, oxidant agent quantity, coupling reagent quantity, oxidation time, temperature effect, stoichiometry between SCS and pyrocatechol, interference effect, and calibration curve were all studied. Beer's law linearity ranged from 6.25–112.5 μg mL^{-1}. The molar absorptivity, Sandell's index, detection limit, determination coefficient, and average recovery percentage were 5185.7 L mol^{-1} cm^{-1}, 0.049 μg cm^{-2}, 0.0889 μg mL^{-1}, 0.9988, and 100.34% respectively. The determination of sulfacetamide in pharmaceutical preparation eye, ointment was successful using this method.*

Keywords: *oxidative coupling; potassium periodate; pyrocatechol; sulfacetamide sodium*

■ INTRODUCTION

Sulfa drugs (or sulfonamides) are among the first bacteriostatic inhibitors, effective against Gram-positive and Gram-negative bacteria. Sulfacetamide sodium (SCS) is the most common antibacterial agent used to treat eye infections [1]. SCS is one of the most important and frequently used drugs in the medical field. It was a white or yellowish-white crystalline powder [2]. It has biological activity and is used as an antibacterial. The SCS is derived from (*N*-4-aminophenyl)acetamide. It was a white or yellowish crystalline powder, soluble in water, dissolved in an amount of ethanol, insoluble in ether, and has a melting point of 257 °C [3]. SCS had the molecular formula $C_8H_9N_2O_3NaS{\cdot}H_2O$, and the molecular weight was 254.2 g mol^{-1} [4]. The chemical structure of the sulfacetamide sodium is shown in Fig. 1 [5].

SCS has several trade names: Albucid (Ankerpharm), Ak-Sulf (Akorn), Sulten-10 (Bausch and Lomb), Beocid-Puroptal (Metochem), Anteacutebor (Biologiques), Bleph-10 (Allergan), Cetamide (Alcon), and Prontamid (SIT) [6-7]. SCS is one of the sulfa drugs that inhibit the action of bacteria and is used in the form of eye drops with a ratio of 10–20% and in acute conjunctivitis. Its solutions of 30% are used in the treatment of infections of the ear, eye, and throat [8].

Due to the importance of the SCS compound, it is under medical study [9]. It was evaluated using a variety of analytical methods, such as spectrophotometric [10-14], electrochemical [15-19], and high-performance liquid chromatography techniques [20-24]. Although the drug has many beneficial aspects, it may cause some side effects. The use of SCS on the skin or eyes causes the emergence of some side effects, the most important of

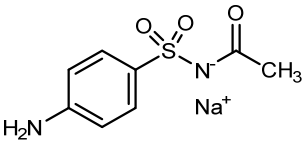


Fig 1. The chemical structure of sulfacetamide sodium

of which is skin irritation, redness, or peeling when used. The oxidative coupling reaction method is very important in pharmaceutical preparations, and it has been used for the determination of several drugs, such as metoclopramide hydrochloride [25].

By combining SCS with pyrocatechol reagent in a neutral medium and utilizing potassium periodate as an oxidizing agent to produce a reddish-brown product dissolved in water. The aim of this work is to implement a photometric approach for the determination of SCS by drug evaluation in a simple and sensitive spectrophotometric method based on the oxidation of the drug with its reactant.

■ EXPERIMENTAL SECTION

Materials

In this study, high-purity analytical chemicals and reagents, i.e., SCS (99.8%, Samarra Pharmaceutical Factory), potassium periodate (99%, BDH), pyrocatecol (99%, BDH), sodium hydroxide (98.8%, BDH), and hydrochloric acid (37%, Fluka) were used.

Instrumentation

Shimadzu Uv-vis 160 spectrophotometer (Japan), pH meter (WTW 720, Germany), sensitive balance Precisa (XR-205gm, Sweden), water bath, and magnetic stirrer (BIOSAN MSH 300, Germany) were used for all spectral measurements.

Procedure

Prepare stock solutions

The standard SCS solution 3.9339×10^{-3} M (1,000 ppm) was prepared by dissolving 0.1000 g of pure SCS powder with distilled water in a volumetric flask of 100 mL capacity, and the lowest concentration solutions were prepared by dilution with distilled water. A 0.1 M pyrocatechol reagent solution was prepared by adding 1.10 g of the reagent with distilled water in a 100 mL volumetric flask. The 0.01 M potassium periodate oxidizing agent solution was prepared by dissolving 0.23 g of the oxidizing agent in a quantity of distilled water and completing the volume to 100 mL with distilled water. The 1 M NaOH solution was prepared by dissolving 4.00 g of NaOH in water and completing the volume with distilled water to the mark of 100 mL. The HCl solution with an approximate concentration of 1.00 M was prepared by adding 8.50 mL of the concentrated acid (11.80 M) to the distilled water in a 100 mL volumetric flask then, the volume was completed to the mark.

Predmacin eye ointment solution (Domna Pharmaceutical Industries – Damascus, Syria) was bought from the local market and prepared for a 250 μg mL^{-1} concentration. The SCS was weighed to an equivalent of 0.10 g by taking one tube of ointment, weighing 50 g, and dissolving in 50 mL of diethyl ether in a suitable beaker, and then transferred to a suitable separation funnel and extracted (three times) by distilled water (25 mL each one). Following the collection of the aqueous layer that included SCS, it was transferred to a 100 mL volumetric flask and the volume was completed to the mark with distilled water to obtain a solution that has a concentration of 1,000 μg mL^{-1}. After that, 25 mL of the solution was transferred into a 100 mL volumetric flask, and the volume was completed to the mark with distilled water to obtain a solution with a concentration of 250 μg mL^{-1}.

Optimization of the experimental conditions

The experiments were carried out using 1.00 mL of 0.01 M oxidizing agent solution, 1.00 mL of 0.10 M pyrocatechol solution, and 2 mL of 250 μg mL^{-1} SCS.

■ RESULTS AND DISCUSSION

The general principle of the color formation involves two steps. The first step was the reaction of the pyrocatechol reagent with potassium periodate to produce cyclohexa-2,4-diene-1,2-dione. The second step was the reaction of cyclohexa-2,4-diene-1,2-dione with SCS in the medium to form a reddish-brown product.

Preliminary Test

At the preliminary test, it was observed that when the SCS solution was mixed with the pyrocatechol solution in the presence of potassium periodate in a neutral medium with a little shaking, a reddish-brown soluble complex was formed. It showed maximum absorption while the blank solution did not exhibit any

absorption at 500.5 nm. In addition, an effort was made to achieve the optimum condition in order to create a straightforward and accurate spectrophotometric method for the measurement of SCS.

The Effect of Oxidizing Agent Type

The experiments were conducted to find the best oxidizing agent for the formation of the colored product, as solutions of several oxidizing agents were used, including potassium periodate (KIO_4) and potassium chromate (K_2CrO_4), and potassium ferricyanide [$K_4Fe(CN)_6$] at a concentration of 0.01 M for each of them. The study showed that the oxidant agent that gives the best results is potassium periodate, used in subsequent experiments.

The Effect of Oxidizing Agent Amount

Fig. 2 shows the effect of the volume of the KIO_4 solution on the absorption of the colored product. As in Fig. 2, the results helped to choose the best volume of the oxidizing agent solution of KIO_4 (0.01 M), as different volumes were used 0.3–3.5 mL.

The Effect of Reagent Type

Fig. 3 shows the effect of pyrocatechol reagent volume on the absorbance of the colored product. Many coupling reagents solutions were used, including pyrocatechol ($C_6H_6O_2$), *m*-aminophenol (C_6H_7NO), and 4-chloroaniline (C_6H_6CIN) at a concentration of 0.01 M, for each of them. The study showed that the reagent that gives the best results is pyrocatechol ($C_6H_6O_2$), which was used in subsequent experiments.

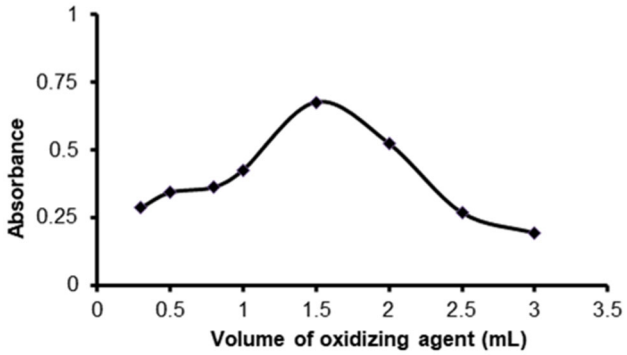


Fig 2. The effect of the volume of KIO_4 solution on the absorption of the colored product

Coupling Reagent Effect

The optimal amount of the pyrocatechol reagent solution was studied, which gives the highest absorption of the colored product and increases the volumes of the reagent solution from 0.30–3.50 mL of the reagent solution at a concentration of 0.1 M and 1 mL of a potassium periodate solution at a concentration of 0.01 M and 2 mL of the drug were added. The results indicate that 2 mL of the reagent used is the optimal volume because it provided the greatest absorbance of the product formed.

The Effect of Acid Amount

Table 1 shows the acid effect on absorbance by monitoring. The outcomes indicate the impact of the acidic medium at a wavelength of 500.5 nm by adding different volumes of 0.5–2.5 mL of a 1 M hydrochloric acid solution to volumetric flasks of 10 mL containing 2 mL of a sulfacetamide sodium solution was added at a concentration of 250 μg mL^{-1}, along with 1.5 mL of the oxidizing agent solution and 2 mL of pyrocatechol reagent solution. The solutions were then standing for 5 min. After diluting with distilled water, the absorbance

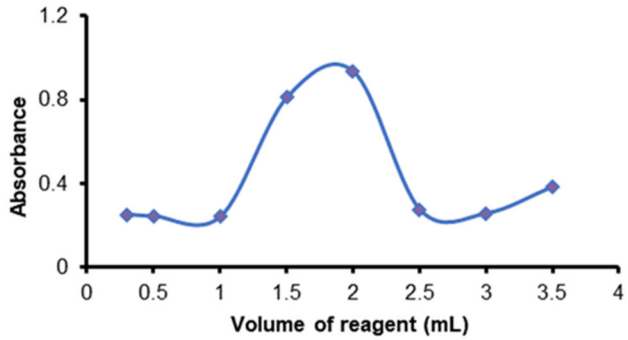


Fig 3. The effect of pyrocatechol reagent volume on the absorption of the colored product

Table 1. The effect of acid amount on absorbance

Volume of HCl (mL)	Absorbance
0.0	0.934
0.5	0.510
1.0	0.491
1.5	0.378
2.0	0.321
2.5	0.295

was measured at 500.5 nm. Since the inclusion of the acid reduced absorbance, it was found that it should not be used in the following trials.

The Effect of Oxidation Time

The time needed for potassium periodate to oxidize SCS was determined by using a series of volumetric flasks with a 10 mL capacity containing 2 mL of SCS solution at a concentration of 250 μg mL^{-1}, and 1.5 mL of potassium periodate solution at a concentration, of 0.01 M. The solutions were left for varying amounts of time, ranging from 2.5 to 30 min, as shown in Fig. 4. After that, 2 mL of the 0.1 M pyrocatechol reagent solution was added. It was then diluted to a final volume of 10 mL with distilled water and the absorbance of the combined solutions was compared to the blank solutions at 500.5 nm. Then, 10 min was chosen for the standing solution before dilution.

The Effect of Temperature on the Absorbance of the Color

Table 2 shows the effect of the temperature on the absorbance of the color. The effect of temperatures 15–50 °C on the absorbance of the colored product was studied using the ideal conditions obtained from previous experiments. As results in Table 2, the optimal temperature is 20–30 °C, and absorbance decreases as the temperature increases, so 25 °C was used in subsequent experiments.

The Stability of the Complex Dye

Table 3 shows the effect of settling time (stability of the resulting product). The effect of time was done to find the time needed for the colored product to reach a stable state and complete its formation at the optimal reaction conditions (1.5 mL of the oxidizing agent solution, 2.0 mL of the drug solution, and 2.0 mL of the used reagent solution). Under these conditions, it was observed that the absorbance of the colored product stabilizes after 5 min at least and remains stable for 1 h.

The Effect of Solvent Type

Table 4 shows the effect of the solvent type used in dilution on absorbance. Following the addition of all reaction components at the optimal values determined in

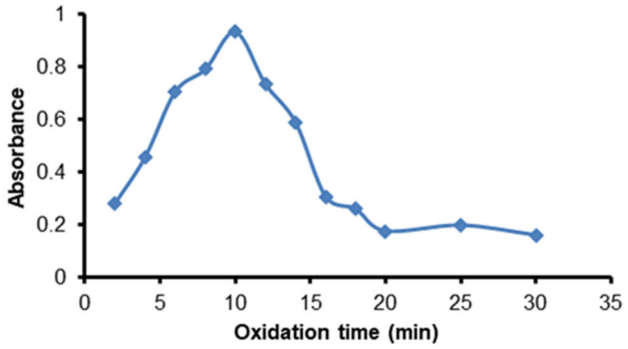


Fig 4. The effect of oxidation time on the absorbance of the colored product

Table 2. The effect of temperature on the absorbance of the colored

Temperature (°C)	Absorbance
15	0.4327
20	0.7861
25	0.9325
30	0.9043
35	0.6472
40	0.4761
45	0.1809
50	0.1337

Table 3. The stability of product

Time (min)	Absorbance
After addition	0.7456
5	0.9343
10	0.9341
15	0.9344
20	0.9343
25	0.9342
30	0.9345
35	0.9343
40	0.9341
50	0.9341
60	0.9339
70	0.8754

Table 4. The effect of solvent type

Solvents	λ_{max} (nm)	Absorbance
Water	500.5	0.9343
Ethanol	412.5	0.2321
Acetone	450.0	0.3093
Methanol	498.5	0.3135

previous tests, a variety of solvents were employed to complete the volumes to the mark in a volumetric flask (10 mL). Water as a solvent gives the highest absorbance to the solution formed in contrast to the solvents used because it was a good mediator for the reaction in addition to its availability, cheapness, and non-toxicity, so it was chosen in the subsequent experiments.

Final Adsorption Spectrum

Fig. 5(a) shows the absorption spectrum of the product formed versus the blank. Before preparing the calibration curve, under ideal working conditions, the wavelength of the greatest absorption for the colored product produced by the oxidative coupling process between SCS and pyrocatechol was validated. The product generated against the blank solution was tested for its absorbance in the 400–800 nm region. As shown in Fig. 5(b), the blank solution did not exhibit any absorption at the wavelength of the maximum absorption, which was 500.5 nm.

Absorption Spectrum and Calibration Curve

The standard curve was created as follows once the ideal circumstances for the measurement of SCS. A volume of 1.5 mL of potassium periodate solution and 0.25–4.50 mL of SCS with 250 μg mL^{-1} and a final concentration of 6.25–112.50 μg mL^{-1} was added to a series of volumetric flasks with a capacity of 10 mL. The samples were left for 10 min to complete the oxidation, and then 2 mL of the 0.1 M pyrocatechol reagent solution was added. Then the volume was completed to the mark with distilled water and leave the samples for 5 min and measure the absorbance against the blank solution at the wavelength 500.5 nm. Fig. 6 represents the calibration curve that follows Beer's law for a range of concentrations between 6.25–112.50 μg mL^{-1} of SCS. The molar absorbance of the method was 5.1857×10^3 L mol^{-1} cm^{-1}, Sandell's index was 0.049 μg cm^{-2}, and the determination coefficient value was 0.9988, which indicates the good linear specifications of the standard curve. The molar absorptivity was calculated from Eq. (1).

$$\varepsilon = \text{slope} \times 1000 \times \text{M.wt} \quad (1)$$

and Sandell's index (S) inference from Eq. (2).

$$S = \frac{\text{M.wt}}{\varepsilon} \quad (2)$$

whereas ε is molar absorptivity (L mol^{-1} cm^{-1}) and M.wt is the gram molecular weight of the substance to be measured (g mol^{-1}).

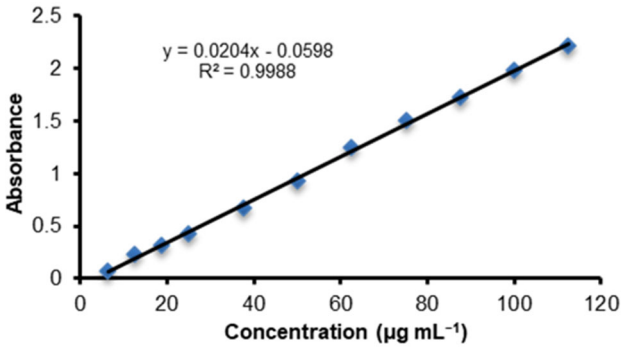


Fig 6. The calibration curve for SCS determination via suggested method

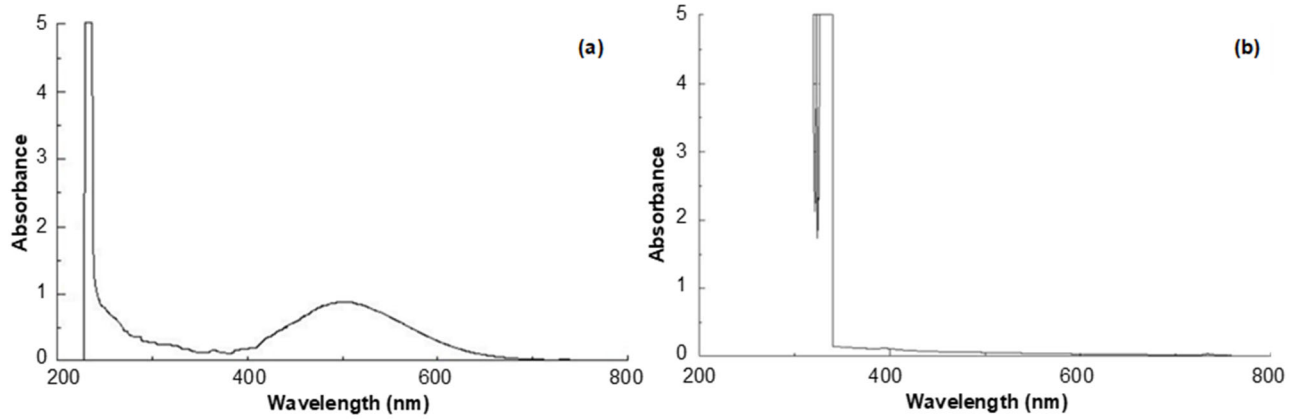


Fig 5. UV-vis spectrum of (a) the product formed versus the blank and (b) a blank solution versus distilled water

Accuracy and Precision of the Proposed Method

The ideal conditions were used in the working method to test the accuracy and compatibility of the method. An average of six readings were taken for two different concentrations of SCS solution within the limits of Beer's law. The relative error, recovery, and relative standard deviation were calculated, and the results are listed in the Table. The results obtained are summarized in Table 5, and they clearly show that the proposed method has high compatibility and good accuracy, as shown in Eq. (3) [26]:

$$\%RE = \frac{O - T}{T} \times 100 \quad (3)$$

whereas RE is a relative error, O is practical value, and T is true value. The recovery value is calculated from Eq. (4):

$$Rec = \frac{X_i}{u} \times 100 \quad (4)$$

whereas X_i = analytical value and u = true value

As for calculating the percentage value of the relative standard deviation, Eq. (5) is applied:

$$RSD = \frac{S}{\bar{x}} \times 100 \quad (5)$$

whereas S = standard deviation and, $\bar{x}$ = rate of reads

Calculating the LOD and LOQ Limits

The absorbance of the lowest concentration taken from the calibration curve was measured 10 times under the same circumstances at 500.5 nm to determine the detection limit value (LOD) and quantity limit value (LOQ) of the method. The results are shown in Table 6 and the LOD and LOQ can be expressed by the following two relationships (Eq. (6) and (7)) [27].

$$LOD = \frac{3.3S}{b} \quad (6)$$

$$LOQ = \frac{10S}{b} \quad (7)$$

whereas S is standard deviation and b is the slope of the standard curve.

The Nature of the Colored Product

The continuous variations method (Job's method) was used to find out the ratio of the interaction of SCS with the reagent pyrocatechol. A number of solutions were prepared to contain different volumes of sulfacetamide sodium 1–9 mL and reagent 9–1 mL at a concentration of 250 μg mL^{-1} (9.8348×10^{-4} M) for each one in a final volume of 10 mL, and then 1.5 of the oxidizing, agent 0.01 M potassium periodate at a concentration was added and diluted with distilled water to the mark. The absorption of the resulting product was measured against the blank solution at a wavelength of 500.5 nm. Fig. 7 shows the reaction ratio of sulfacetamide sodium with the reagent ratio of 1:1 [28].

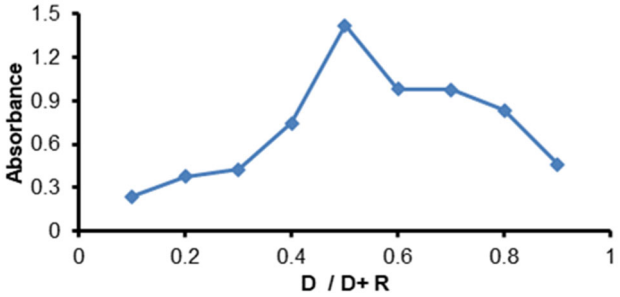


Fig 7. The continuous variation curve for the colored SCS-pyrocatechol product

Table 5. The accuracy and compatibility

SCS conc. taken (μg mL^{-1})	RE* (%)	Recovery* (%)	Average recovery* (%)	RSD* (%)
12.50	- 0.10	99.90		1.85
18.75	0.77	100.77	100.34	0.68

*Average of six determinations

Table 6. Values for detection and quantitative limits

Concentration (μg mL^{-1})	b	S	LOD (μg mL^{-1})	LOQ (μg mL^{-1})
6.25	0.0204	0.00055	0.0889	0.2696

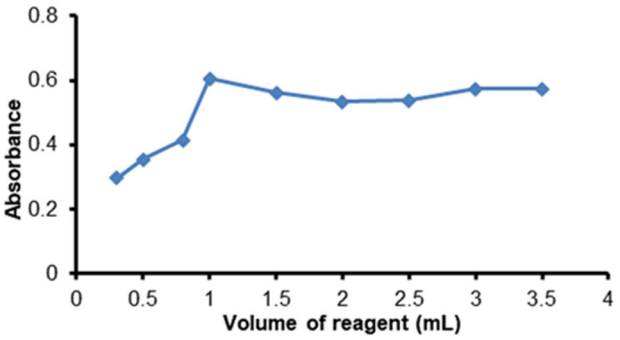
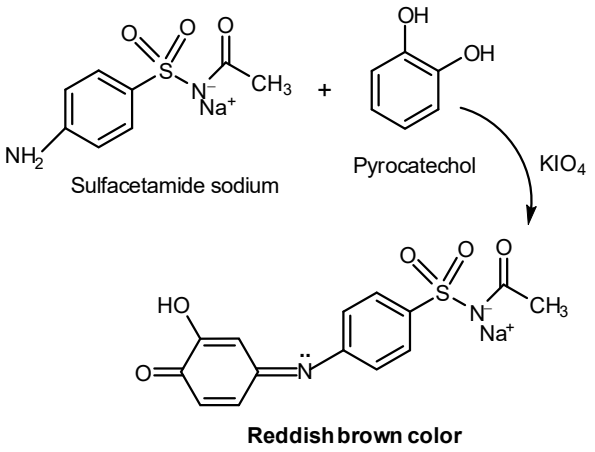


Fig 8. The molar ratio curve of the SCS-pyrocatechol product



Scheme 1. The suggested equation for a reddish-brown product

The mole-ratio method to prove that the 1:1 ratio is correct. A series of volumetric flasks with a capacity of 10 mL were constructed, and 1.5 mL of the oxidizing agent and 1 mL of SCS solution at a concentration of 250 μg mL^{-1} (9.8348×10^{-4} M) each were added. Following the addition of various amounts of 0.3–3.5 mL of pyrocatechol reagent with the same concentration of SCS, the volume was brought up to the desired level with distilled water, the absorbance of each sample was measured in comparison to its fake solution. Fig. 8 confirms that the reaction ratio is 1:1 [28]. Scheme 1 shows the proposed chemical equation for the oxidative conjugation reaction of SCS.

The Additives Effect Study

In order to ensure the selectivity of the method in order to benefit from its applicability to pharmaceutical preparations, the effect of the interaction of some additives used in the manufacture of pharmaceutical preparations on the absorbance of the resulting product was studied by adding different volumes of each of these additives 1, 2, and 3 mL at a concentration of 1000 μg mL^{-1} to a series of the volumetric flasks of 10 mL capacity containing 2 mL of SCS solution and 1.5 mL of 0.001 M potassium periodate. The samples were left for 10 min to complete the oxidation, then 2 mL of the 0.1 M pyrocatechol reagent solution was added. The volume was completed to the mark with distilled water. The samples were left for 5 min to complete the color. The absorbance was measured against its blank solutions at 500.5 nm. Then, by calculating the recovery for each addition, it was found that there was no effect of the additives used on the absorption, which makes the possibility of applying the method to pharmaceutical preparations. The results are shown in Table 7.

Comparison of the Proposed Method with Other Modalities

The proposed method for the determination of SCS has been compared with other spectrophotometric methods as shown in Table 8, that the proposed method is no less good than similar spectroscopic methods used

Table 7. The effect of additives on absorbance

Foreign compound	Recovery (%) of 50 μg mL^{-1} of sulfacetamide sodium per μg mL^{-1} another compound added		
	100	200	300
Mannose	99.44	98.65	100.54
Glucose	100.60	101.74	99.50
Lactose	100.59	99.73	101.77
Sucrose	101.11	99.19	100.10
Maltose	99.43	100.47	101.52

Table 8. Comparison with other methods

Analytical parameter	Present method	Literature methods
Reagent	Pyrocatechol	2,4-dinitrophenylhydrazine
Beers law range (μg mL^{-1})	6.25–112.50	2.5–25.0
Solvent	Water	Water
Molar absorptivity (L mol^{-1} cm^{-1})	5.1857×10^3	2.6500×10^4
λ_{max} (nm)	500.5	485.0
Average Recovery (%)	100.34	98.57
RSD (%)	0.68–1.85	1.595–2.817
Temperature (°C)	20–30	RT
LOD (μg mL^{-1})	0.0889	0.2000
LOQ (μg.mL^{-1})	0.2696	-
Color of the dye	Reddish-brown	Pink-reddish
Sandell's index (μg cm^{-2})	0.0490	0.0095
Pharmaceutical preparation	Eye ointment Predmacin	Eye drops

Table 9. The results of the determination of sulfacetamide sodium in an eye ointment solution by the direct method

Pharmaceutical preparation	SCS present (μg mL^{-1})	SCS found (μg mL^{-1})	RSD (%)	RE (%)	Recovery (%)	Average recovery
Eye ointment (Predmacin)	37.50	37.64	0.13	0.38	100.38	
Domna Pharmaceutical	50.00	50.84	2.02	1.68	101.68	100.42
Industries – Damascus, Syria	62.50	62.00	0.76	-0.80	99.20	

in the determination of SCS, but this method gives good sensitivity and stability for the product formed and does not need the use of organic solvents that may be expensive and unavailable or need an extraction process. Thus, it was applicable in the actual determination of SCS in its pharmaceutical preparations (eye ointment solution), the method is considered reliable and highly accurate.

Application of the Method

The three readings were averaged for each concentration and the recall was calculated as shown in Table 9. The method was applied to the pharmaceutical preparation, which is an ointment of SCS 250 mg. In that method, three different concentrations were prepared at 37.5, 50.0, and 62.5 μg mL^{-1} from a pharmaceutical solution with a concentration of 250 μg mL^{-1}. The solutions were treated with the same steps used in preparing the calibration curves, and their absorbances were measured at 500.5 nm against the blank solution.

■ CONCLUSION

In this study, the oxidative coupling method using pyrocatechol reagent via spectrophotometric determination of sulfacetamide sodium (SCS) was detailed. A simple, rapid, and inexpensive spectrophotometric method was evaluated to determine SCS. The process was based on the oxidative coupling reaction, where SCS is coupled with the reagent pyrocatechol in the presence of potassium periodate as an oxidizing agent in a neutral medium. The output is stable, and the method does not require organic solvents or an extraction process. This method follows the Beer's Law within the range of 6.25–112.50 μg mL^{-1}. The molar absorbance, Sandall's index, determination coefficient value, recovery percentage value, detection limit, and detection amount were 5.1857×10^3 L mol^{-1} cm^{-1}, 0.0490 μg cm^{-2}, 0.9988, 100.3400, 0.0889, and 0.2696 μg mL^{-1}, respectively. The method was successfully applied to pharmaceutical preparation (eye ointment).

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■ AUTHOR CONTRIBUTIONS

Israa Talib Humeidy and Marib Ismail Ali contributed to the conception and design of the study. Material preparation, data collection and analysis were performed by Marib Ismail Ali. The first draft of the manuscript was written by Israa Talib Humeidy. All authors read and approved the final manuscript.

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