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Spectrophotometric Determination of Sulfanilamide in Pure and in Synthetic Sample based on Condensation Reaction Method

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Abstract

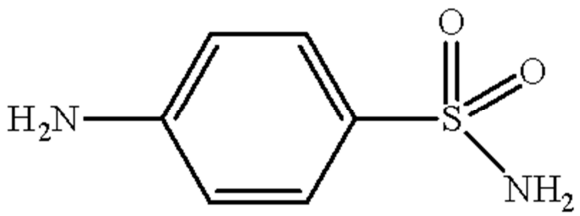
A new, Simple, sensitive and accurate spectrophotometric methods have been developed for the determination of sulfanilamide (SNA) drug in pure and in synthetic sample. This method based on the reaction of sulfanilamide (SNA) with 1,2-napthoquinone-4-sulphonic acid (NQS) to form N-alkylamono naphthoquinone by replacement of the sulphonate group of the naphthoquinone sulphonic acid by an amino group. The colored chromogen shows absorption maximum at 455 nm. The optimum conditions of condensation reaction forms were investigated by: (1) univariable method, by optimizing the effect of experimental variables; (different bases, reagent concentration, borax concentration and reaction time), (2) central composite design (CCD) including the effect of three experimental factors (reagent concentration, borax concentration, and reaction time). The linearity ranges of sulfanilamide are (5-30 µg.mL^{-1}) at 455 nm with molar absorptivity (6.9568×10^4 - 7.0774×10^4 L.mol^{-1}.cm^{-1}), Sandell's sensitivity index (2.4753 - 2.4330 µg.cm^{-2}) and detection limit of (0.546 – 0.536 µg.mL^{-1}) for each procedure respectively. The results showed there are no interferences of excipients on the determination of the drug. The proposed method has been successfully applied for the determination of sulfanilamide in pure and in synthetic sample.

Key words: Spectrophotometric determination, Sulfanilamide, Central composite design, 1, 2-napthoquinone-4-sulphonic acid (NQS).

Introduction

Sulfanilamide (SNA) is a sulfonamide antibacterial and chemically name is 4-aminobenzenesulfonamide with molecular formula $C_6H_8N_2O_2S$ and molecular weight of 172.205 g.mol^{-1}; the basic structure of the drug, is shown in Scheme (1). White or yellowish-white crystals or fine powder is a medicinal compound used to guard against certain bacterial infections [1-3]. It is frequently used in the form of a topical cream or powder to treat surface infections, as well as a pill for internal infections. It falls into the category of sulfonamide antibacterial drugs; Common infections treated by sulfanilamide include urinary tract infections, vaginal infections, strep throat, and some staph infections, depending on the type of infection, either a cream or a pill will be prescribed [4].

Some analytical methods which include HPLC [5-7], flow injection analysis [8] and spectrophotometric method [9], have been reported in the Literature for the determination of (SNA) in pharmaceutical preparations. Chemometrics is a field of science that studied the application of statistical and mathematical methods in chemistry one of the chemometrics methods is multivariate central composite design (CCD). The aim of the present work is to provide an optimized spectrophotometric method using the univariate and multivariate central composite design (CCD). In the central composite design method [10-12], three-interest factors concentration of (reagent concentration, borax concentration and reaction time) were designated as independent variables and absorbance as response.



Scheme (1): The chemical structure of sulfanilamide.

Experimental

Instruments

Cecil 7200 CE double beam UV-visible spectrophotometer possessing a fixed slit width (1.8 nm) with quartz cells of 1.0 cm path length connected to a P IV computer loaded.

Materials and reagents

All reagents were of analytical grade. Sulfanilamide was obtained from State Company for Drug Industries and Medical Appliance (SDI) Samarra-Iraq.

Preparation of standard stock solutions

Solution of 1000 $\mu g.ml^{-1}$ Sulfanilamide was prepared by dissolving accurate weighted 0.100 g of pure drug in 10 mL of 0.4 M HCl and further diluted to the mark in volumetric flask 100 mL with distilled water and stored in a cool (< 25 ^{0}C) and dark place, working solution were prepared fresh daily by subsequent dilutions. Sodium 1,2-Naphthoquinone-4-sulphonate (NQS) solution 0.5% (m/v) in distilled water was prepared fresh daily. Sodium hydroxide 0.01 M prepared by dissolving 0.20 gm of pure substance in 100 ml distilled water, Sodium tetraborate decahydrate (Borax) 0.03 M prepared by dissolving 0.57207 g in 25.0 mL double distilled water and diluting to the mark in a 50 mL volumetric flask.

Preparation of synthetic sulfanilamide drug sample

1- To 0.025 g of the bulk drug, 0.005 g of interfering substance mixture (consisting of equal weights of each substance: glucose, sucrose, lactose, starch soluble, and vanillin) was added.
2- 0.0125 g of the resulted mixture was dissolved in 10 mL of 0.4 M HCl and diluted to the mark with distilled water in volumetric flask 100 mL in the same manner as used for the preparation standard drug to obtain 100 $\mu g.mL^{-1}$.

General recommended procedure

Under univariate conditions

Aliquots of the standard solution (1000 $\mu g.mL^{-1}$) containing (50, 100, 150, 200, 250, and 300 µg) of sulfanilamide were transferred into a series of 10 mL volumetric flasks. A volume of 1.0 mL of 0.03 M borax solution was added to each flask, followed by 1.0 mL of 1.0 % (m/v) NQS solutions were added, and then the mixture was shaken gently until the appearance of orange color. Left to stand for 3.0 min., and the contents were diluted up to the mark with distilled water. The absorbance of each solution was measured at 455.0 nm against the reagent blank.

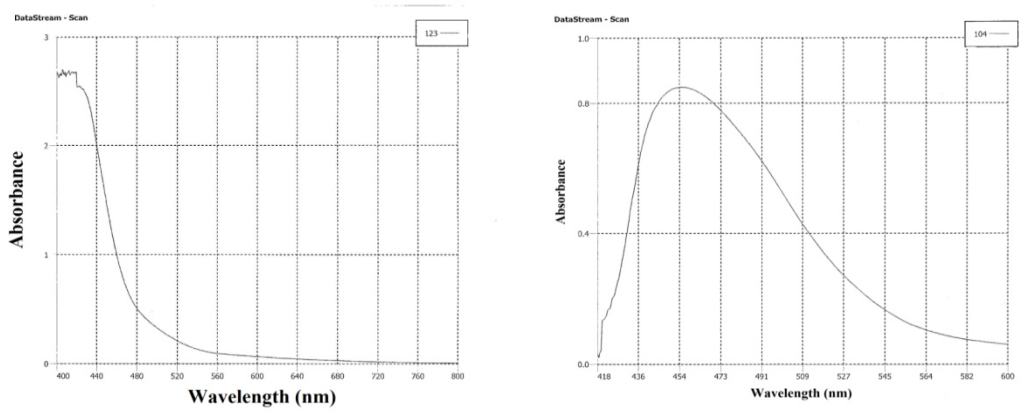
Under multivariate conditions

Aliquots of the standard solution (1000 $\mu g.mL^{-1}$) containing (50, 100, 150, 200, 250 and 300 µg) of sulfanilamide were transferred into a series of 10 mL volumetric flasks. A volume of 1.0 mL of 0.036 M borax solution was added to each flask, followed by 1.0 mL of 0.975 % (m/v) NQS solutions were added, and then the mixture was shaken gently until the appearance of orange color, then left to stand for 22.0 sec., and the contents were diluted up to the mark with distilled water. The absorbance of each solution was measured at 455.0 nm against the reagent blank.

Results and discussion

Absorption spectra

When the solution of sulfanilamide was mixed with NQS in alkaline medium at room temperature, intense coloration was developed, showing a broad band in the region of 420-600 nm). It was found that the product is orange colored exhibiting (λ_{max}) at 455 nm, against reagent blank (Figure 1-B), and the λ_{max} of derivative chromogenic reagent (sodium 1,2-naphthoquinone-4-sulfonic) is at 430 nm. (Figure 1-A), which indicates the formation as sulfanilamide possesses amino groups, it involves in yielding colored produced by nucleophillic displacement of the sulfonic acid group of 1,2-naphthoquinone-4-sulfonic acid in alkaline conditions. The intensity of this band increased with increased concentration of sulfanilamide.



(A) **(B)**

Figure (1): Absorption spectra of (A) Reagent blank against distilled water and (B) (20 µg.mL^{-1}) SNA against the reagent blank under the primary test and optimization condition.

Optimization of reaction variables

In order to optimize the conditions, a number of parameters namely reagent concentration, borax concentration and reaction time. The optimum conditions were established univariatly by changing one variable and observing its effect on the absorbance of the colored product, shows the results in Table (1) and (Figure (2).

Table (1): Effect of different bases on condensation reaction and effect of coupling reaction time.

Alkaline solution (0.01M)	Absorbance	Time (min.)	Absorbance
NaOH	0.541	1	0.768
$Na_2B_4O_7.10H_2O$	0.583	2	0.801
NH_4OH	0.512	3	0.821
KOH	0.438	4	0.825
Na_2CO_3	0.350	5	0.830
-	-	6	0.825
-	-	8	0.828
-	-	10	0.830
-	-	60	0.826

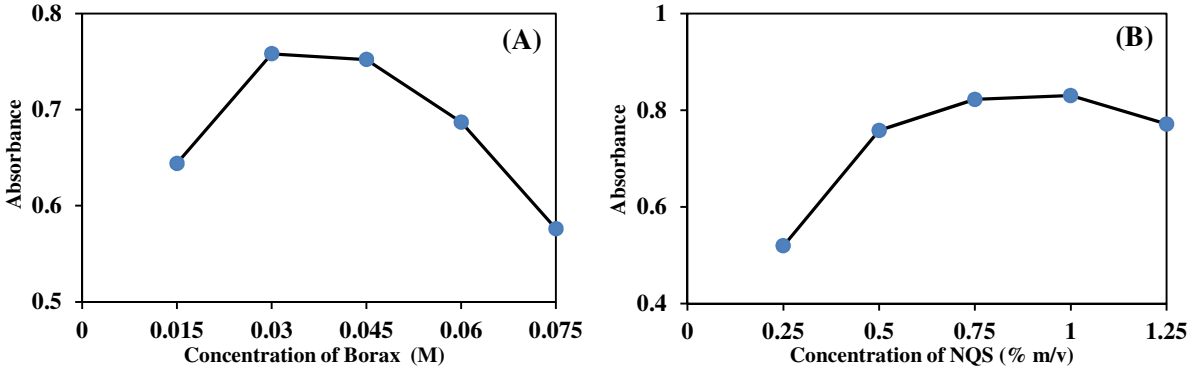


Figure (2): Effect of 1.0 mL of each (A) Borax concentration and (B) NQS concentration on the color development of dye on the estimation of (20 µg.mL^{-1}) SNA.

On the other hand, experimental design methodology, central composite design model, was used to multivariate optimization of three of factors that could have an important effect on the reaction. The factors of interest were reagent concentration, borax concentration and reaction time. To study these factors, 1 ml of (0.25-1.25 % m/v) NQS and 1 ml of (0.015-0.075 M) borax concentration and coupling of reaction time (1-10 min.). Table (2) shows the equivalent matrix of the central composite design as well as the absorbance data. The experiment corresponding to the central point executed in four replicates. All experiments were carried out with solution of sulfanilamide set at (20 µg.mL^{-1}).

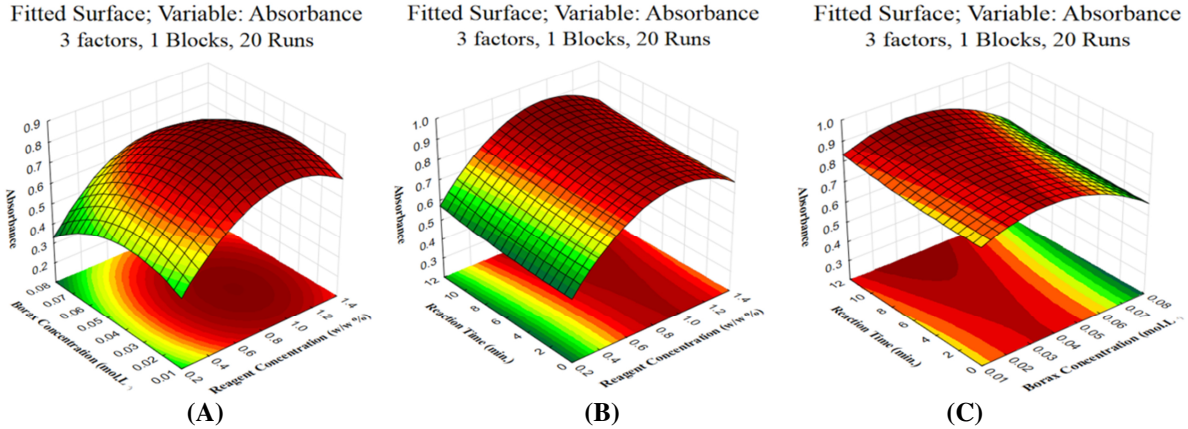
Table (2): Matrix and absorbance data results from of the central composite design of SNA drug. **(20 µg.mL^{-1})**

Exp. No.	Reagent conc. (% m/v)	Borax conc. (M)	Reaction time (min.)	Abs.
1	0.750	0.045	10.0	0.825
2	1.250	0.045	5.5	0.823
3	0.750	0.045	5.5	0.832
4	0.750	0.045	5.5	0.832
5	1.250	0.075	10.0	0.848
6	0.250	0.045	5.5	0.538
7	0.750	0.075	5.5	0.713
8	0.750	0.045	1.0	0.826
9	0.750	0.045	5.5	0.832
10	0.750	0.045	5.5	0.832
11	0.250	0.075	1.0	0.232
12	1.250	0.075	1.0	0.812
13	0.250	0.015	10.0	0.766
14	0.750	0.045	5.5	0.832
15	0.250	0.015	1.0	0.667
16	1.250	0.015	10.0	0.612
17	0.750	0.045	5.5	0.832
18	0.250	0.075	10.0	0.288
19	0.750	0.015	5.5	0.741
20	1.250	0.015	1.0	0.633

The experimental data show an excellent fitting using a linear-quadratic second-order polynomial main effects model (r = 0.9999) according to the given equation:

$$\textbf{Abs.} = 0.1957 + 1.0729\,X1 + 7.3558\,X2 - 0.0003\,X3 - 0.5504\,(X1)^2 - 101.2121\,(X2)^2 + 0.0004\,(X3)^2$$

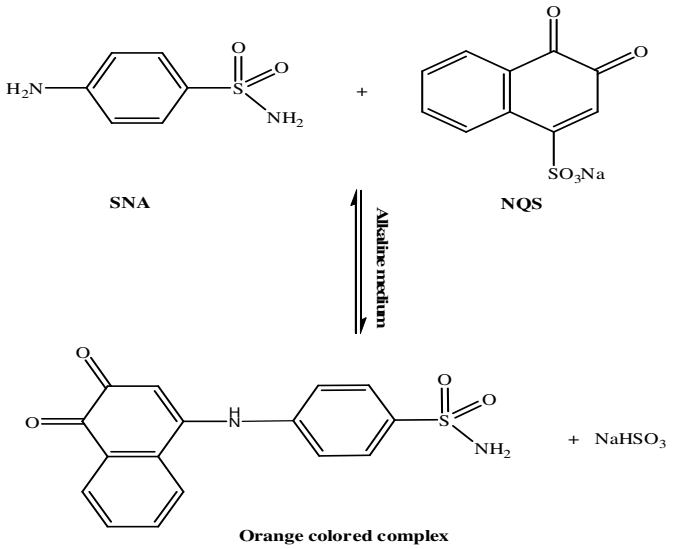
The results show that the optimum values of the studied parameters are: reagent concentration solution is 1 ml of (0.975 % m/v), borax concentration is 1 ml of (0.036 M), and reaction time is (22.0 seconds). A three-dimensional response surface graph which represent the absorbance values obtained experimentally from central composite design is given in Figure(3).



(A) **(B)** **(C)**

Figure (3): The response surface for the absorbance of SNA-NQS complex as a function of Response surface of quadratic model for absorbance values as a function of (A) reagent concentration and borax concentration (At constant optimum value of reaction time, 22.0 sec.). (B) reagent concentration and reaction time (At constant optimum value of borax concentration, 0.036 M). (C) borax concentration and reaction time (At constant optimum value of reagent concentration, 0.975 % m/v).

Accordingly, the reaction mechanism between sulfanilamide and NQS is suggested on the basis of results mentioned Scheme (2).



Scheme (2): The proposed mechanism of the reaction between sulfanilamide and NQS.

Procedure for synthetic sulfanilamide drug sample

Aliquots of the synthetic sample (1000 µg.mL^{-1}) containing (50, 100, 200 µg) of sulfanilamide were transferred into a series of 10 mL volumetric flasks. A volume of 1.0 mL of 0.036 M borax solution was added to each flask, followed by 1.0 mL of 0.975 % (m/v) NQS solutions were added, and then the mixture was shaken gently until the appearance of orange color. Left to stand for 22.0 sec., and the contents were diluted up to the mark with distilled water. The absorbance of each solution was measured at 455.0 nm against the reagent blank.

Calibration curves and analytical data

I. Univariate method

The effect of concentration on the absorbance behavior at optimum conditions of univariable method was investigated using authentic standard.

Beer's law was obeyed in the range of 5.0-30.0 µg.mL^{-1} of SNA the results are shown in Figure (4). The regression equation, correlation coefficient, molar absorptivity, Sandell's sensitivity and detection limit (DOL) and quantification of limit (QOL) are calculated and listed in Table (3).

II. Multivariate method (CCD)

Optical characteristics and statistical data for the regression equation of the central composite desigen method are given in Table (3) and Figure (4).

Quantification

With univariate and multivariate experimental design conditions, the intensity of absorption at 455 nm was found to be a function of the concentration of the sulfanilamide. In both cases studied, Beer's law plots were linear in two concentration ranges of the drug. The molar absorptivity values were 6.9568×10^4 L. mol^{-1}.cm^{-1} when calibration curve was constructed under those experimental conditions obtained univariatly, while two linear regression equations were attained for the proposed procedures under multivariate experimental conditions with values of molar absorptivity of 7.0774×10^4 L. mol^{-1}.cm^{-1}. Table (3) shows other quantitative and statistical parameters for the determination of sulfanilamide by univariate and multivariate conditions.

Table (3): Quantitative parameters for the reaction of the studied sulfanilamide with NQS.

Parameter	Univariate conditions	Multivariate conditions
λmax (nm)	455.0	
Color	Orange	
Linearity range (µg.mL^{-1})	5.0-30.0	
Regression equation	Y=0.0404[SNA. µg.mL^{-1}]+0.0301	Y=0.0411[SNA. µg.mL^{-1}]+0.0347
Calibration sensitivity (mL.µg^{-1})	0.0404	0.0411
Correlation coefficient (r) %	0.9998	0.9999
Correlation of linearity (r^2) %	0.9997	0.9999
Molar absorptivity(L. mol^{-1}.cm^{-1})	ε =6.9568×104	ε=7.0774×104
Sandell's sensitivity (µg.cm^{-2})	2.4753	2.4330
Detection limit (µg.mL^{-1})	0.546	0.536
Quantification limit (µg.mL^{-1})	1.654	1.626

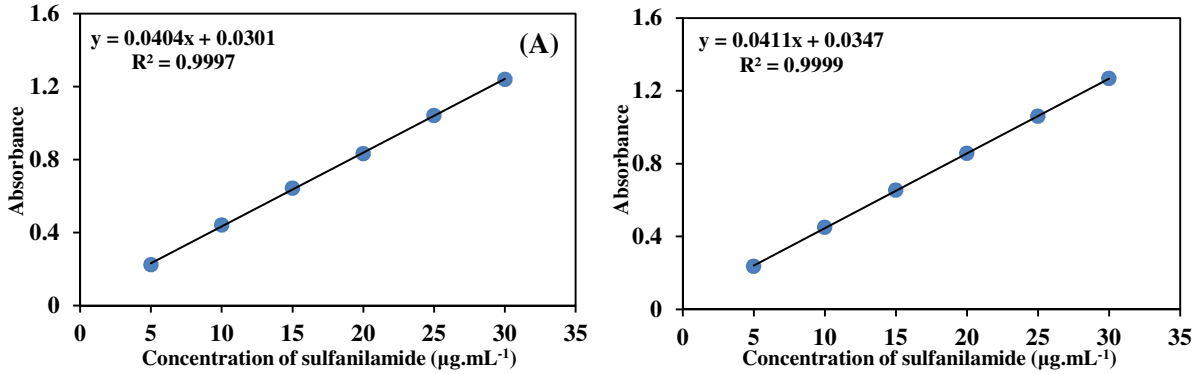


Figure (4): Calibration curve for the determination of SNA by (A) Univariate optimal condition and (B) DOE optimal condition (central composite design).

Precession and accuracy of method

The precision and the accuracy of the proposed method was checked under univariate and multivariate central composite conditions by calculating the relative standard deviation percent , relative error percent and coefficient of variation for five replicates at four different concentration levels of the drug. Table (4) shows the method precise and accurate results.

Table (4): Evaluation of accuracy and precision for the determination of SNA by the proposed method.

	Concentration of SNA (µg.mL^{-1})		Relative Error %	C.V %
	Taken	Found*		
For univariate	5.0	4.968	-0.640	1.381
	10.0	9.993	-0.070	0.991
	20.0	19.790	-1.050	0.542
For DOE	5.0	5.043	0.860	2.127
	10.0	10.157	1.570	0.429
	20.0	19.958	-0.210	0.367

*Average of five determinations.

Interference Study

In pharmaceutical analysis, it is important to test the selectivity towards the excipients added to the pharmaceutical preparations. Commonly encountered excipients such as (vanillin, glucose, lactose, starch, sucrose) did not interfere in the determination of SNA and did not effect on the reaction between the SNA and NQS. (20.0 µg.mL^{-1}) of SNA was analyzed and design of experiment method was used for analyzing Table (5).

Table (5): Percent recovery for (20.0 µg.mL^{-1}) of sulfanilamide in the presence of different concentration of Excipients.

Excipients	Concentration (µg.mL^{-1})	Sulfanilamide Concentration Taken (20 µg.mL^{-1})	
		Conc. Found µg.mL^{-1}	Recovery %
Vanillin	1000	19.971	99.85
Glucose		20.046	100.23
Lactose		19.912	99.56
Starch		19.731	98.65
Sucrose		20.086	100.43

*Average of three determinations

Application in synthetic sample

Synthetic sample of sulfanilamide was successfully analyzed by the proposed method. The results obtained for the determination of SNA in its synthetic sample by the proposed method (CCD), are presented in Table (6).

Table (6): Application of the proposed method to the SNA concentration measurements in synthetic sample.

Sample	Conc. taken (µg.mL^{-1})	Conc. found* (µg.mL^{-1})	Recovery %	C.V* %
SNA	5.0	5.042	100.832	0.4827
	10.0	10.126	101.266	0.2573
	20.0	19.980	99.900	0.0512

*Average of three determinations.

Application in synthetic sample by standard additions method (SAM)

The proposed method (CCD) also could be useful and suitable for the determination of SNA in synthetic sample by standard addition method. The data presented in Table (7) reveals the good recovery and coefficient of variation. Figure (5) shows the plot of standard addition method for the determination of SNA.

Table (7): Application of the proposed method to the SNA concentration measurements in synthetic sample (SNA) by (SAM).

Sample	Conc. taken (µg.mL^{-1})	Conc. found* (µg.mL^{-1})	Recovery %	C.V* %
SNA	500.00	505.266	101.0532	0.2180

*Average of three determinations.

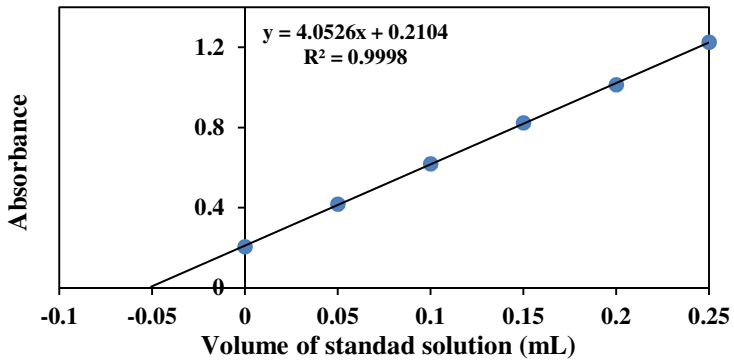


Figure (5): Determination of SNA in synthetic sample by standard additions method (SAM).

Conclusions

A simple, rapid, and inexpensive spectrophotometric method for determination of sulfanilamide is proposed, which provide gain of sensitivity without the need of additional step as extraction or heating. The method involve mild reaction conditions and gives precise and accurate results. Its usefulness for the cited drug determination in pharmaceutical formulations was demonstrated, suggesting its use as an attractive alternative to many other previously reported methods for analysis of sulfanilamide.

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