IRAQI Academic Scientific Journals

Samarra Journal of Pure and Applied Science

SJPAS

www.sjpas.com

p ISSN: 2663-7405
e ISSN: 2789-6838

Spectrophotometric Determination of Neomycin sulphate Via Association Ion Pair Complex with Erythrosine Dye

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https://doi.org/10.54153/sjpas.2025.v7i3.1084

Article Information

Received: 29/11/2024
Revised: 25/01/2025
Accepted: 30/01/2025
Published: 30/09/2025

Keywords:

Neomycin; sulphate; Ion association complex; Determination; Spectrophotometric; Erythrosine dye

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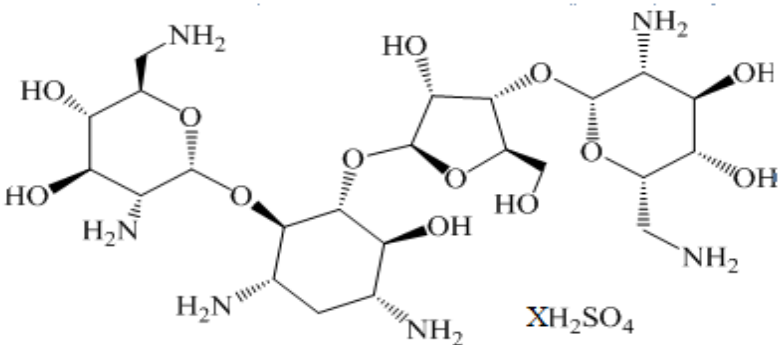
Abstract

A simple, rapid, sensitive and accurate analytical method for the quantitative determination of neomycin sulphate (NEO) in its pure form and in pharmaceutical dosage forms was proposed. The method is based on the formation of an ionic complex between erythrosine dye and neomycin sulphate in a buffer medium at an acidic pH of 4.5 to obtain a colored product associated with the amount of NEO, which gives a maximum absorption at 560 nm. A calibration curve was drawn which was linear over the concentration range of 1.0-12.0 µg/mL with a correlation coefficient of 0.9999 and the molar absorptivity value was 4.8×10^4 L/mol. cm and Sandall index was 0.0128 µg/cm^2. The LOD and LOQ values were 0.106 and 0.353 µg/mL, respectively. The method was successfully applied for the determination of NEO present in pharmaceutical preparations and the recovery was good.

Introduction

Neomycin sulphate (NEO) is an antibiotic found in many topical medications such as creams, ointments, and eye drops. It contains two or more amino sugars linked by glycosidic bonds. Neomycin sulphate was discovered in 1949 [1]. Neomycin sulphate is a white, odorless, tasteless, amorphous powder. Its chemical formula and structure are as in Fig. 1.



chemical composition of $C_{23}H_{46}N_6O_{13}.XH_2SO_4$
M.Wt. = 615.0 g/mol

Fig.1*: The chemical structure of Neomycin sulphate [2].*

NEO has excellent activity against Gram-negative bacteria and is partially effective against Gram-positive bacteria. The mechanism of action involves inhibition of protein synthesis by binding to the ribosome subunit of the bacterial ribosome, leading to misreading of the genetic code; it may also affect bacterial DNA [3]. The side effect was associated with reduced levels of methane in the breath. Reported side effects were gastrointestinal symptoms including nausea, bloating, abdominal pain, constipation, and diarrhea [4]

NEO has also been estimated in its pure form, in its various preparations, or even in animal products, by applying a number of spectrophotometric methods that depend on: remove the amine group [5], formation of ion-pair complex [6], direct reaction [7], oxidative degradation [8], oxidation reduction [9], Hantzsch condensation reagent [10], and ninhydrin reagent [11,12].

Ion-pair–based spectrophotometric methods had a dynamic evolution over the time [13]. Association ion pair reactions are considered delicate interactions, and the reagents used are few. The literature survey revealed that chronotropic acid azo dyes were used as reagents in the determination of NEO [14]. The present included using erythrosine dye to estimate NEO via the formation of the ion-pair complex, which was followed up spectroscopically, as we were able to estimate it in its preparation (eye drops).

Materials and Methods

Apparatus

All spectral measurements were performed using a Shimadzu UV-Vis. 1900i dual-beam spectrometer and 1.0 cm glass cells.

Chemical reagents

In this research, high purity chemical compounds were used, without pre-treatment.

Solution NEO working solution 100 µg/mL (1.63×10^{-4} M): Dissolved 0.0100 g of pure NEO in 10 ml of distilled water with shaking, then diluted to 100 ml with distilled water using a volumetric flask.

Erythrosine (ERY) solution 500 µg/mL: It is created by dissolving 0.0500 g of dye powder in distilled water and diluting it to 100 mL with the same solvent in a volumetric flask.

Preparing the buffer solution (pH=4.5): This buffer was prepared by adding 30.5 mL of 0.2 M acetic acid solution, then adding 19.5 mL of 0.2 M sodium acetate and completing the volume with distilled water to 100 ml using a volumetric flask after adjusting the pH value using 0.1 M sodium hydroxide solution [15].

Pharmaceutical preparation solution (100 µg/mL): The solutions of the pharmaceutical preparation drops Neodex(API, Jorden, 0.5%) and Metharan N (Brawn, India, 0.5%) were prepared by withdrawing 2 ml from each mixture of three containers of the two types of drops individually and completing the volumes to the mark with distilled water in two 100 ml volumetric flasks.

Principle of procedure and suggested chemical reaction

The proposed method consists of one step, which is the combination of neomycin sulphate with a known excess of erythrosine dye solution in an acidic medium to form a colored ion association complex product that gives the maximum absorption value at a wavelength of 560 nm and proportional to the concentration of NEO as in the equation**:**

$$\underset{\text{(excess)}}{\text{NEO} + \text{ERY}} \xrightarrow{H^+} \underset{\text{(colored product)}}{\text{NEO-ERY}}$$

Results and Discussion

Conditions for Optimal Reaction

The following experiments were performed in 10 ml volumetric flasks using 10 µg / mL of NEO, and the absorbance of the colored product was measured at 560 nm.

Effect of the acidity function and the type of buffer solution used

The acid function of the reaction was studied using a number of buffer solutions at different acidity levels with the aim of obtaining the best acid function that gives the highest absorption value for the colored product. (Table 1).

Table 1: The pH and the type of buffer solution used

Type of buffer solution (1 mL)	pH	Absorbance	Final pH
Acetate buffer	3.5	0.287	3.2
	4	0.507	3.7
	4.5	0.544	4.3
	5	0.335	4.6
Citrate buffer	3.5	0.240	3.34
	4	0.383	3.9
	4.5	0.378	4.22
KH-phthalate buffer	3.5	0.335	3.41
	4	0.394	3.8
	4.5	0.377	4.27

Effect of buffer solution amount

The amount of buffer solution was adjusted by adding increasing amounts of the solution and measuring the absorbance of the resulting solutions. A volume of 0.5 ml of buffer solution was chosen because it gave the highest absorbance. The results of this study were included in the Table 2.

Table 2: Effect of buffer solution amount

mL of buffer solution	**0.3**	**0.5**	**1**	**1.5**	**2.0**	**2.5**
Absorbance	0.545	**0.596**	0.578	0.553	0.518	0.516

Effect of ERY dye quantity

Different volumes of dye were used against a fixed volume of neomycin sulphate and the results shown in Table 3. The volume of 2 ml is the best amount of dye that can be adopted within the proposed method

.

Table 3: Effect of ERY dye quantity

mL of 500 µg/ml ERY sol.	**0.5**	**1**	**1.5**	**2.0**	**2.5**
Absorbance/sample vs blank at λ max 528	0.243	0.576	0.677	**0.758**	0.751
Absorbance/ blank vs water at λ max 528	0.055	0.104	0.105	**0.102**	0.216

Effect of adding surfactants

In this study, a number of available surface-active materials (neutral, negative and positive) were used at a concentration of 0.1% for each, and the results of this study were recorded in Table 4.

Table 4: Effect of adding surfactants

Surfactants	Absorbance/ml of surfactants			
(0.1 %)	Without	0.5	1	1.5
CPC	0.757	0.689	0.657	0.632
SDS		0.716	0.779	0.765
CTAB		No color contrast		
Triton-x100		No color contrast		

We note from the results in Table 4 that adding 1 ml of the surfactant SDS gave a good absorbance for the complex formed with stability in the absorbance over time, so it was fixed within the estimation method.

Temperature and time effects

The effect of temperature and time on the absorption value of the resulting solution was studied by applying the proposed method at different temperatures and waiting for different periods at each temperature. The results were recorded in Table 5.

Table 5: Effect of temperature and time

Temperature	Absorbance/Standing time, min.							
(^oC)	Immediately	5	10	20	30	40	50	60
10	0.381	0.353	0.564	0.456	0.442	0.433	0.420	0.411
RT(25± 2 ^oC)	0.770	0.776	0.778	0.777	0.775	0.773	0.774	0.772
40	Turbid	---	---	---	---	---	---	---

After studying the results of Table 5, the use of room temperature was confirmed within the proposed method because it gave the highest absorbance. The waiting time of 5 minutes was also chosen before measuring the absorption value as the reaction completion time, so it was adopted within the proposed method. The values were stable for approximately one hour, and this time allows for the required measurements to be carried out accurately during this period.

Effect of sequence of additions

This study was carried out by applying a number of different additive sequences and then measuring the absorbance of the resulting complex. The results are shown in Table 6.

Table 6: Effect of sequence of additions

Order of addition	Order of number	Absorbance
NEO+ Bu +ERY + S	I	0.775
NEO + S +Bu+ ERY	II	0.744
Bu + S + NEO + ERY	III	0.729

*__NEO__= Neomycin sulphate, **B**u= buffer solution, **ERY** = Erythrosine dye, **S**= Surfactant

From the results in Table 6, we find that the best sequence of addition of the reactants is (I) to give the highest absorbance, so sequence (I) was chosen and adopted in subsequent experiments.

Effect of solvents

A number of solvents available to us were used in order to choose the best solvent that gives the highest value for the molar absorbance coefficient. Most of the solvents gave cloudy solutions when used as a reaction medium, except for water, so its use was maintained in subsequent experiments. The results are recorded in Table 7.

Table 7: Effect of organic solvents

Solvent	Absorbance	ε (L.mol^{-1}.cm^{-1})
Water	0.776	4.76×10^4
Methanol	Turbid	---
Ethanol	Turbid	---
DMSO	No colors contrast	

Final absorption spectrum

The proposed method was applied at the previously established optimum conditions using two different concentrations of NEO in a final volume of 10 ml, and the resulting solutions were spectrophotometrically scanned and Fig. 2 shows the final spectra of these solutions.

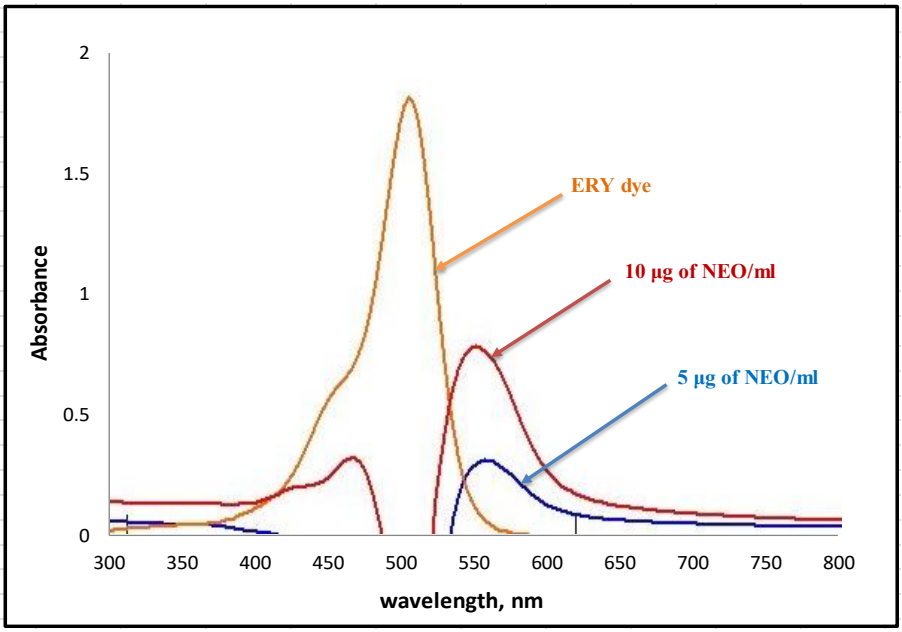


Fig. 2: *Final absorption spectra for proposed method*

From Fig. 2 ERY dye gave maximum absorption at 528 nm will the maximum absorption of the associated product at 560 nm, which is fixed in the next experiments.

The main procedure and calibration curve

Under the optimal conditions of the method and using a number of 10 mL volumetric flasks, different volumes of the working neomycin sulphate solution (0.1 - 1.5 mL) were added, giving solutions containing neomycin sulphate concentrations ranging from 1-15 µg/mL and 0.5 mL of acetate buffer solution was added, then 2 mL of ERY dye were added and waited for five minutes with shaking in order to complete the reaction, then 1 mL of 0.1 % of SDS solution was added with shaking then the volume was completed with distilled water to the mark, then the spectrophotometric measurement process was carried out for the resulting solutions and the standard curve of the method was drawn, which gave a linear relationship

following the Beer's law in a range of concentrations ranging from 1 - 12 µg/mL as shown in the Fig. 3, and the value of the determination coefficient (R^2) = 0.9999 and the value of the molar absorption coefficient was 4.8×10^4 L/mol.cm and the value of Sandall index 0.0128 µg/cm^2.

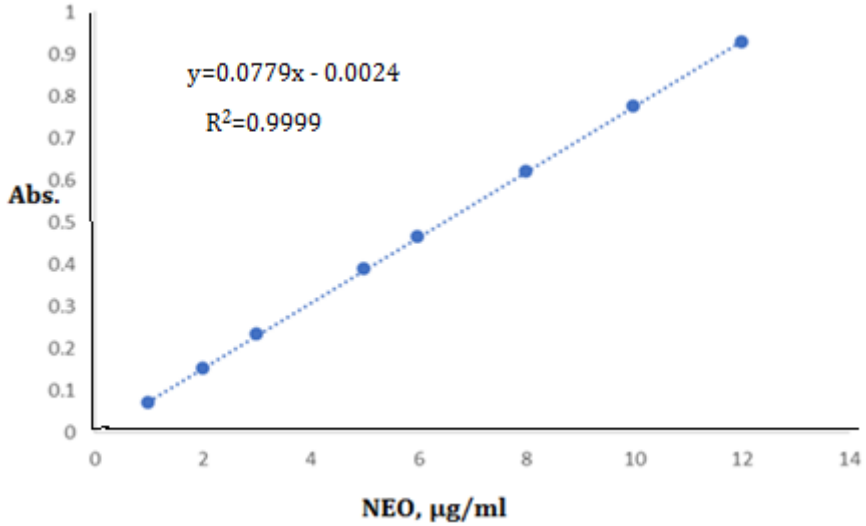


Fig. 3: *NEO Estimation Calibration Curve*

Nature of the product formed

In order to find the reaction ratio between NEO and the ERY reagent after coupling, the mole ratio method [16] was applied and Fig.4 shows that the coupling ratio is 1:1.

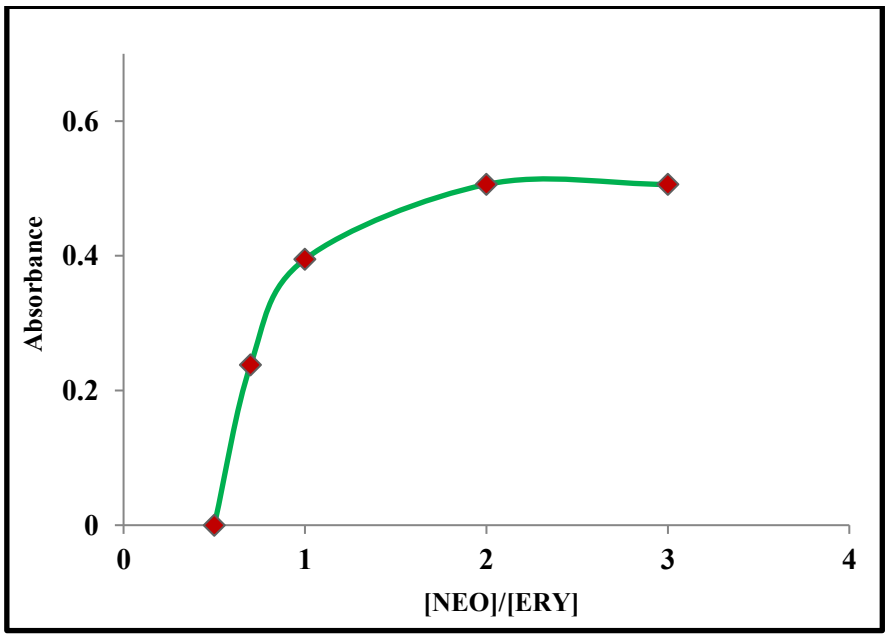
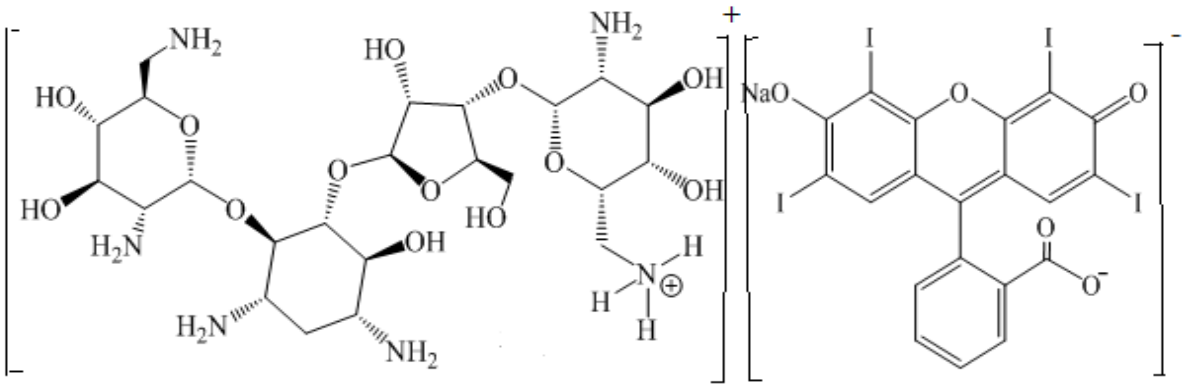


Fig. 4: *Plot of mole ratio method*

Based on the ratio determined in the previous experiment, the chemical structure of the ionic bonding complex was proposed as follows:



Accuracy and precision

The accuracy and precision of the proposed method were tested using three different concentrations (30, 50 and 100 µg) of pure neomycin sulphate solution, and the recovery percentage, relative error and relative standard deviation were calculated. The results in Table 8 show that the proposed method gave good accuracy and compatibility.

Table 8: Accuracy and precision of the method

Present amount of NEO, µg/ml	*Absorbance	Found amount of NEO, µg/ml	Recovery %*	RE, %*	RSD, %*
3	0.235	3.05	101.66	1.66	1.20
5	0.389	5.02	100.40	0.40	1.58
10	0.775	9.98	99.80	-0.20	0.46

*Average of three determinations

Application of the method

The proposed method was successfully applied to the determination of neomycin sulphate in pharmaceutical preparations (eye and ear drops), using three different concentrations (3, 5 and 10 µg/mL) of pure neomycin sulphate solution, and the results are shown in Table 9.

Table 9: Application of the method

pharmaceutical preparations	Present Amount of NEO µg/ml	Found Amount of NEO µg/ml	Recovery %*	RSD %*
Neodex drops, 0.5% of NEO (API, Jorden)	3	2.98	99.33	2.84
	5	4.79	95.80	2.29
	10	9.74	97.40	1.92
Metharan N drops, 0.5% of NEO (Brawn, India)	3	3.05	101.66	2.76
	5	5.01	100.20	3.55
	10	10.09	100.90	1.34

* The mean of three determinations

Referring to the results in Table 9, we find that the proposed method has good accuracy by calculating the relative standard deviation and finding the percentage of recovery.

Evaluation of the proposed method

In order to test the selectivity of the proposed method, the standard addition method [17] was applied to estimate neomycin sulphate in its pharmaceutical preparations (eye and ear drops), and the results are shown in Fig. 5 which are represented by the standard addition curves for the estimation of this pharmaceutical compound in its pharmaceutical preparations for concentrations (3 and 5 µg/mL).

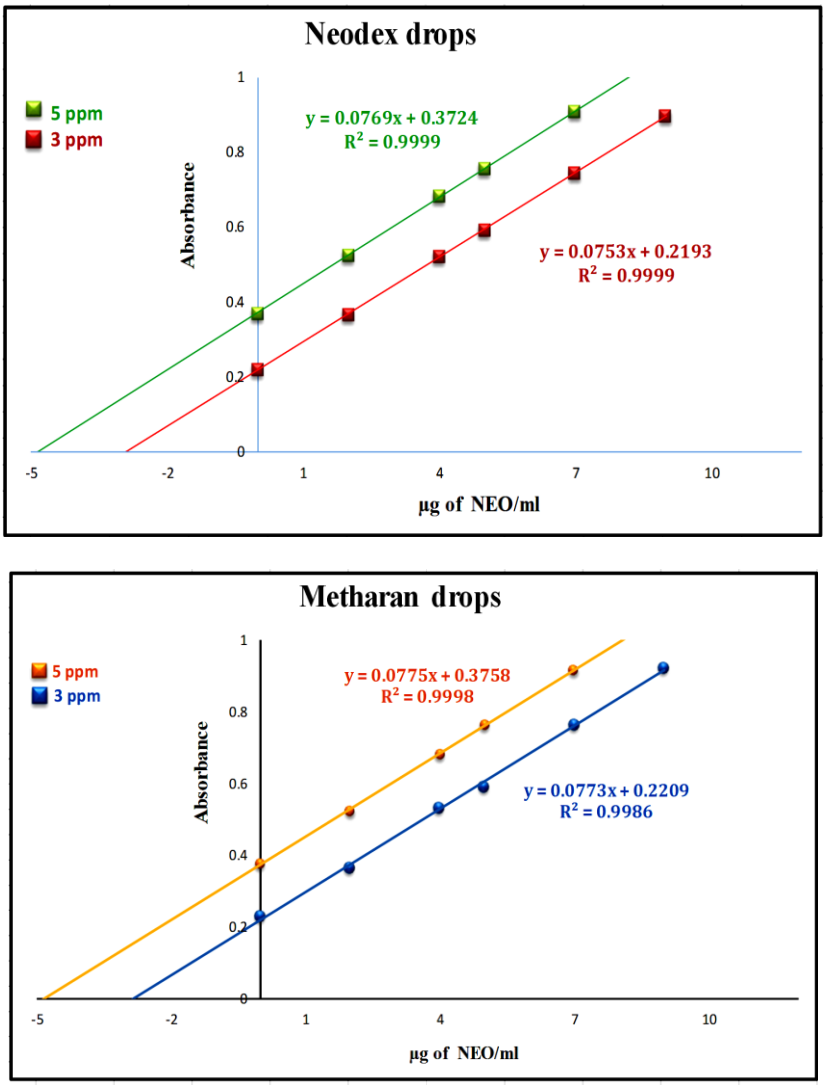


Fig.5: *Standard addition curves for estimation of NEO in pharmaceutical drug*

The concentrations and recovery ratio were calculated from the linear equations in Fig 5, and the results are shown in Table 10.

Table 10: Results of standard addition method

pharmaceutical preparation	Amount of NEO taken µg/mL	Amount of NEO measured µg/mL	Recovery %
Neodex drops, 0.5% of NEO (API, Jorden)	3	2.91	97.00
	5	4.84	96.80
Metharan N drops, 0.5% of NEO (Brawn, India)	3	2.86	95.3
	5	4.85	97.00

The results in Table 10 show that the standard addition method is in high agreement with the proposed method for the determination of neomycin sulphate in its pharmaceutical preparations (eye drops).

Comparison with other methods

The more important analytical parameters of proposed method were compared with two spectrophotometric methods (Table11)

Table 11: The comparison of the methods.

Variable	Present method	Literature method [12]	Literature method [10]
Type of reaction	Association ion pair complex	Condensation	Condensation
Reagent used	Erythrosine Dye	Ninhydrin	Ac-Ac-Formaldehyde
Maximum wavelength, nm	560	574	488
Linearity range	1.0-12.0 µg/ml	0.0002-0.0011mol/L (0.123-0.6765 µg/ml)	5-125 µg/ml
ε, l/mol.cmε	$4.8x10^4$	$9.35 x10^4$	$1.23 x10^3$
Sandell sensitivity index	0.0128 µg/cm^2	0.006582 mol/cm^2	0.5 µg/cm^2
LOD	0.106 µg/ml	$5.423x10^{-6}$ mol/L (0.000113 µg/ml)	1.1 µg/ml
LOQ	0.353 µg/ml	$1.643x10^{-5}$ mol/L (0.00374 µg/ml)	3.66 µg/ml
Stability, minute	60	6	60

The results fixed in Table 11 indicated that the second method [12], which includes a condensation reaction, gives better sensitivity than an ion pair complex (present work) and condensation reaction [10]. The proposed method is characterized by simplicity and does not require heating to high temperatures (65^0C in ref. 12), but rather at room temperature and the application of two eye drop preparations of different origins.

Conclusions

In this research, an easy and rapid spectroscopic method was developed for the estimation of NEO in its pure form and in its pharmaceutical preparations. This method depends on the formation of an ionic complex between ERY dye and NEO in a buffer medium at an acidic pH = 4.5 The method was successfully applied to the estimation of NEO in its pharmaceutical preparations (eye and ear drops) and gave good results in terms of accuracy and recovery.

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IRAQI Academic Scientific Journals
Samarra Journal of Pure and Applied Science
SJPAS
www.sjpas.com
p ISSN: 2663-7405
e ISSN: 2789-6838

التقدير الطيفي لكبريتات النيومايسين عن طريق معقد الترابط الايوني مع صبغة الإريثروزين

مينا عادل عبد الرحمن، نبيل صبيح عثمان، الاء محمد طيب الليلة
قسم الكيمياء، كلية العلوم، جامعة الموصل

معلومات البحث:
تاريخ الاستلام: 2024/11/29
تاريخ التعديل: 2025/01/25
تاريخ القبــول: 2025/01/30
تاريخ الـنشر: 2025/09/30
الكلمات المفتاحية:
كبريتات النيومايسين، معقد ايوني، التقدير، مطيافية ضوئية، صبغة إريثروزين

الخلاصة:
تم اقتراح طريقة تحليلية بسيطة وسريعة وحساسة ودقيقة للتقدير الكمي لكبريتات النيومايسين (NEO) في شكلها النقي وفي أشكال الجرعات الصيدلانية .تعتمد الطريقة على تكوين معقد أيوني بين صبغة الإريثروزين وكبريتات النيومايسين في وسط منظم عند دالة حامضية تبلغ 4.5 للحصول على ناتج ملون، يعطي أقصى امتصاص عند 560 نانومتر .تم رسم منحنى معايرة وكان خطيا على مدى نطاق تركيز 12.0-1.0 ميكروغرام / مل مع معامل ارتباط 0.9959 وكانت قيمة الامتصاص المولي 4.8 × 10^4 لتر / مول. سم وكانت دلالة ساندال 0.0128 ميكروغرام / سم ^{2}. كانت قيمتي حد الكشف وحد التقدير الكمي 0.106 و0.353 ميكروغرام / مل على التوالي .تم تطبيق الطريقة بنجاح لتقدير NEO الموجودة في المستحضرات الصيدلانية وكانت الاستعادة جيدة.