

과망간산포타슘에 의한 산화에 바탕을 둔 아미노글리코사이드 항생제의 분광광도법적 정량

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Spectrophotometric Determination of Aminoglycoside Antibiotics Based on their Oxidation by Potassium Permanganate

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요 약. 순수 형태 또는 약제 중 네오마이신과 스트렙토마이신을 신속하게 분석하는 인증된 분광광도법적 방법을 기술하였다. 이 방법은 산성 용액 속에서 기지 과량의 과망간산포타슘으로 약품을 산화시킨 다음 미반응의 과망간산을 아마란스(방법 A), 산성 오렌지 II(방법 B), 인디고카민(방법 C) 및 메틸렌블루(방법 D) 색소로 각각 λ_{\max} =521, 485, 610 및 664 nm에서 흡광도를 측정하여 정량하는 방법이다. 네오마이신과 스트렙토마이신의 농도 범위 5-10 및 2-7 $\mu\text{g mL}^{-1}$ 에서 Beer의 법칙이 성립되었으며 겉보기 몰흡수계수와 sendell 감도 값은 네오마이신과 스트렙토마이신에 대하여 각각 $5.47\text{-}6.20 \times 10^4$, $2.35\text{-}2.91 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ 및 $7.57\text{-}8.59$, $5.01\text{-}6.2 \text{ ng cm}^{-2}$ 였다. 반응에 영향을 미치는 여러 변수를 조사하여 최적화하였으며 이 방법을 순수 형태 및 약제 형태의 약품에 적용하여 좋은 정확도와 정밀도로 정량하였다. 첨가제의 방해는 관찰되지 않았으며 얻어진 결과는 공인 분석법을 사용하여 얻은 결과와 잘 일치하였다.

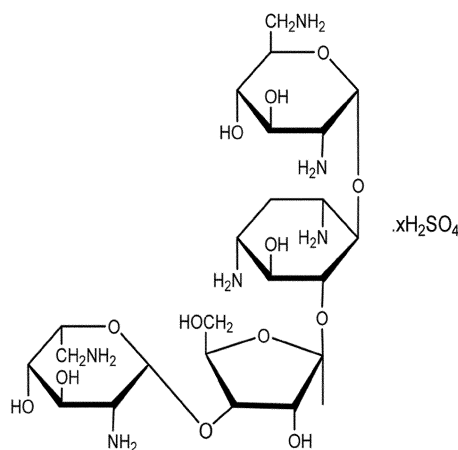
주제어: 분광광도법, 황산 네오마이신, 황산 스트렙토마이신, 과망간산포타슘, 산화반응, 약제

ABSTRACT. A rapid, simple and sensitive validated spectrophotometric methods have been described for the assay of neomycin and streptomycin either in pure form or in pharmaceutical formulations. The proposed methods were based on the oxidation of the studied drugs by a known excess of potassium permanganate in acidic medium and estimating the unreacted permanganate with amaranth dye (method A), acid orange II (method B), indigocarmine (method C), and methylene blue (method D), in the same acid medium at a suitable λ_{\max} =521, 485, 610 and 664 nm, respectively. Beer's law is obeyed in the concentration range of 5-10 and 2-7 mg mL^{-1} for neomycin and streptomycin, respectively. The apparent molar absorptivity and sandell sensitivity values are in the range $5.47\text{-}6.20 \times 10^4$, $2.35\text{-}2.91 \times 10^5 \text{ L mol}^{-1} \text{ cm}^{-1}$ and $7.57\text{-}8.59$, $5.01\text{-}6.2 \text{ ng cm}^{-2}$ for neomycin and streptomycin, respectively. Different variables affecting the reaction were studied and optimized. The proposed methods were applied successfully to the determination of the examined drugs either in a pure or pharmaceutical dosage forms with good accuracy and precision. No interferences were observed from excipients and the results obtained were in good agreement with those obtained using the official methods.

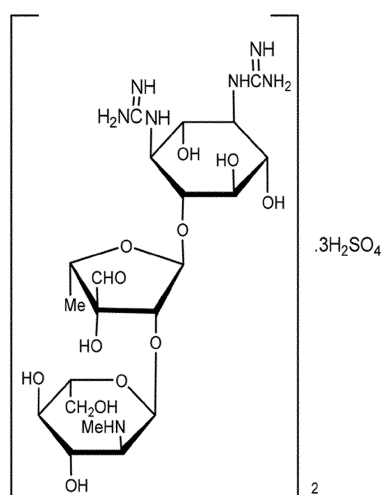
Keywords: Spectrophotometry, Neomycin sulphate, Streptomycin sulphate, Potassium permanganate, Oxidation reactions, Pharmaceutical formulations

INTRODUCTION

Aminoglycoside antibiotics have similar chemical and biological properties as well as mechanism of action. Neomycin (Scheme 1), belongs to a group of broad spectrum aminoglycoside antibiotics which are widely used in clinical therapy of serious infections. It inhibits the growth of both gram-positive and gram-negative bacteria.¹ Streptomycin (Scheme 2), is a human antibiotic drug which also is used as a pesticide, to control fungi and algae, it is active against numerous gram-negative and gram-positive bacteria. One of the greatest virtues of



Scheme 1.



Scheme 2.

streptomycin is its effectiveness against tubercle bacillus. In itself it is not a cure, but it is valuable adjunct to the standard treatment of tuberculosis. Streptomycin remains one of the agents of choice for the treatment of certain 'occupational' bacterial infections, such as brucellosis, tularemia, bubonic plague, it is used rather widely in the treatment of infections of the intestinal tract.²

Many analytical techniques have been employed for the detection and determination of aminoglycoside antibiotics include potentiometric,³ voltammetric,⁴ amperometric,⁵ fluorometric,⁶⁻¹⁰ immunoassay,¹¹ spectropolarimetric,¹² electrophoresis,¹³ colorimetric,¹⁴ spectrophotometric,¹⁵⁻²⁰ gas chromatographic,²¹ thin layer chromatographic,²²⁻²⁴ liquid chromatographic,²⁵⁻³¹ and high performance liquid chromatographic.³²⁻³⁴ To the best of our knowledge, there is no work in the literature reported about the application of potassium permanganate for the determination of neomycin and streptomycin.

The present communication describes four visual spectrophotometric methods (A-D) for the assaying of the cited drugs in bulk form and in commercial pharmaceutical formulations. Methods A-D are indirect procedures, involving the addition of an excess KMnO_4 and the determination of unreacted oxidant by the decrease in absorbance of the different dyes.

EXPERIMENTAL

Apparatus. Absorbance measurements were carried out using Biotech (UV-VIS) spectrophotometer (Cyprus), with scanning speed 400 nm/min and band width 2.0 nm, equipped with quartz cells of 10 mm path length.

Reagents and materials. All chemical and reagents used were of analytical or pharmaceutical grade and doubly distilled water were used throughout.

Pure neomycin and streptomycin were obtained from the Egyptian International Pharmaceutical Industries Company (EIPICO). Stock solutions of the studied drugs were freshly prepared daily by dissolving 20 mg of the drug in distilled water and

then, completed to the mark in a 100 mL calibrated flask with distilled water. Working standard solutions were prepared by suitable dilution of the stock.

An aqueous solution of amaranth (AM) (Merck; 5.0×10^{-4} M), acid orange II (AO) (Merck; 5.0×10^{-4} M), indigocarmine (Aldrich; 5.0×10^{-4} M), and methylene blue (MB) (Merck; 1.0×10^{-4} M) were prepared by dissolving an accurate weight of dye in least amount of water and completed to the mark in a 100 mL calibrated flask. The stock solutions of dyes were allowed to stand at room temperature for a few weeks without any significant decay.

A stock solution of 5.0×10^{-3} M KMnO_4 (Aldrich) was prepared by dissolving an accurate weight in 10 mL of warm distilled water, then completed to the mark in a 100 mL calibrated flask. Standardized using sodium oxalate and kept in a dark bottle. A 5.0×10^{-4} M solution of KMnO_4 was prepared by diluting the previously stock solution with water and 2.0 M H_2SO_4 was prepared.

General procedure and calibration

Pipette a 1.0 mL aliquot of the examined drugs solution in a series of 10 mL calibrated flasks, followed by acidification by adding 0.5 mL of 2.0 M H_2SO_4 . A 2.5 mL and 1.2 mL of 5.0×10^{-4} M KMnO_4 were added and heated in a boiling water bath for 25 min and 20 min with neomycin and streptomycin, respectively. The mixture was cooled to laboratory temperature, then 1.3 mL of 5.0×10^{-4} M of AM was used for method A, 1.8 mL of 5.0×10^{-4} M of AO for method B, 1.7 mL of 5.0×10^{-4} M Indigo for method C, and 2.5 mL of 5.0×10^{-4} M of MB for method D, with neomycin and streptomycin, respectively. The volume was completed to 10 mL with water. The decrease in color intensities in A, B, C and D, were measured spectrophotometrically at their corresponding maximum wavelengths. The concentration of each drug was found from a calibration graph constructed under the same conditions.

Procedure for pharmaceutical formulations:

Procedure for tablet

The contents of twenty tablets of neomycin sulphate, were crushed powdered, weighed out and the

average weight of one tablet was determined. An accurate weight equivalent to 20 mg of pure drug was dissolved in 20 mL distilled water and then filtered. The filtrate was diluted to 100 mL with distilled water in a 100 mL calibrated flask. This solution was further diluted stepwise to the request concentration with water and then analyzed by the recommended procedure.

Procedure for vials

Mix the content of ten streptomycin vials and weigh an accurate amount of the powder equivalent to 20 mg in a 100 mL calibrated flask. The above stated procedures described were applied to determine drug concentrations.

RESULTS AND DISCUSSION

Absorption spectra

The absorption spectra of the reaction products from the reduced drugs with amaranth dye (method A), acid orange II (method B), indigocarmine (method C) and methylene blue (method D) show characteristics λ_{max} values at 521, 485, 610 and 664 nm, respectively, (Fig. 1). The calibration graph are linear over a concentration range of 5 - 11 $\mu\text{g mL}^{-1}$ with neomycin sulphate and 2-7 $\mu\text{g mL}^{-1}$ with streptomycin sulphate, (Table 1).

Effect of reaction temperature

In order to obtain the highest and most stable absorbance, the effect of heating time on the oxida-

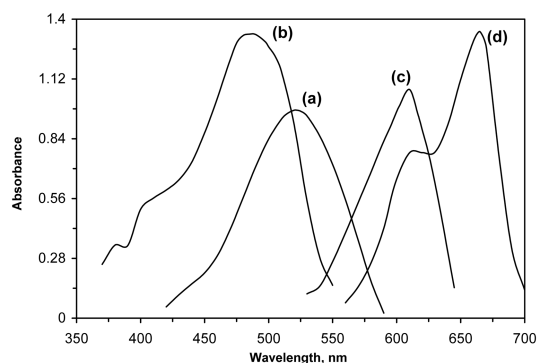
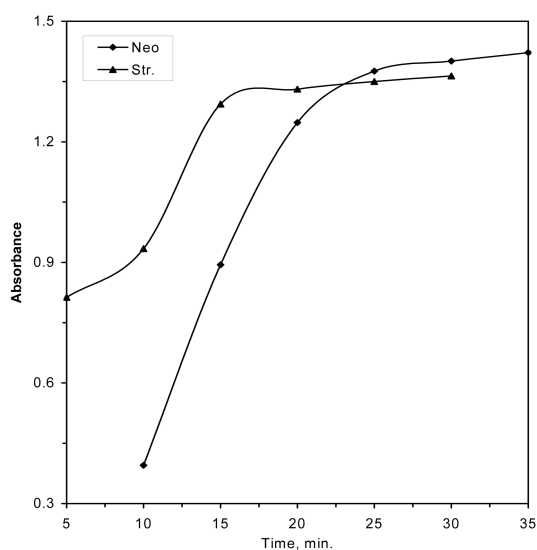
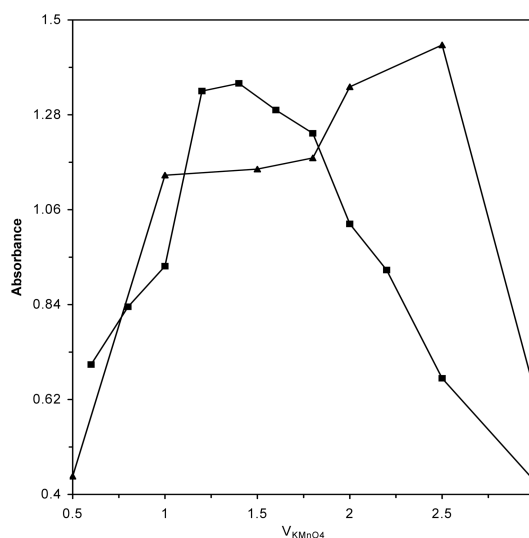


Fig. 1. Absorption spectra of the oxidation product between KMnO_4 and, a- AM, b- AO, c- Indigo and d- MB

Table 1. Analytical parameters and optical characteristics of the proposed methods with neomycin sulphate

Parameter	A	B	C	D
λ_{max}	521	485	610	664
Beer's law limits ($\mu\text{g mL}^{-1}$)	5 - 10	6 - 10	6 - 11	6 - 10
Molar absorptivity ($\text{L mol}^{-1}\text{cm}^{-1}$)	6.2×10^4	5.84×10^4	5.64×10^4	5.47×10^4
Sandell sensitivity ($\mu\text{g mL}^{-1}$)	7.57	8.04	8.33	8.59
Detection limits ($\mu\text{g mL}^{-1}$)	9×10^{-3}	5.19×10^{-3}	4.74×10^{-3}	7.64×10^{-3}
Quantification limits ($\mu\text{g mL}^{-1}$)	0.029	0.017	0.015	0.025
Regression equation ^a				
Slope (b)	0.1812	0.2080	0.1685	0.1980
Intercept (a)	- 0.446	- 0.751	- 0.521	- 0.734
$S_{y/x}$	0.3311	0.3803	0.3077	0.3615
SD of slope (S_b)	0.1047	0.1202	0.0973	0.1143
SD of intercept (S_a)	1.638	2.151	1.957	2.044
Correlation coefficient (r)	0.9989	0.9986	0.9994	0.9995
Stoichiometric ratio				
[Drug] : [Oxid.]	1 : 4	1 : 4	1 : 4	1 : 4
[Drug] : [Dye]	1 : 4	1 : 4	1 : 4	1 : 4
[Oxid.] : [Dye]	1 : 1	1 : 1	1 : 1	1 : 1

^a $A = a + bC$, where C is the concentration in $\mu\text{g mL}^{-1}$.Fig. 2. Effect of heating time on the oxidation of: (■) $8.0 \mu\text{g mL}^{-1}$ neomycin-MB and (▲) $6.0 \mu\text{g mL}^{-1}$ streptomycin-AO.Fig. 3. Effect of volume of $5.0 \times 10^{-4} \text{ M KMnO}_4$ on the development of the reaction product: (▲) $8.0 \mu\text{g mL}^{-1}$ neomycin with MB and (■) $6.0 \mu\text{g mL}^{-1}$ streptomycin with AO.

tion reaction was studied. The reactions were performed on a boiling water bath at $100 \pm 2^\circ\text{C}$ for the periods ranging from 5.0-35 min. Maximum and constant absorbance was obtained after 25 min for neomycin, where as 20 min for streptomycin. The results are shown in Fig. 2.

Effect of the concentration of KMnO_4

The influence of the volume of $5.0 \times 10^{-4} \text{ M KMnO}_4$ on the reaction has been studied. It is apparent from Fig. 3, that the absorbance increased with increasing volume of $5.0 \times 10^{-4} \text{ M KMnO}_4$ solution and reached maximum when 2.5 mL and 1.2 mL of

KMnO₄ solution were added to the total volume of 10 mL for neomycin and streptomycin, respectively. The color intensity decreased above the upper limits. Therefore, 2.5 mL and 1.2 mL of KMnO₄ were recommended for all measurements (Fig. 3).

Effect of acid concentration

To study the effect of sulphuric acid concentration the reaction was performed in a series of 10 mL volumetric flask containing 6.0 µg mL⁻¹ of the cited drugs, different volumes (0.1 - 3.0 mL) of 2.0 M H₂SO₄ and 2.5, 1.2 mL of KMnO₄ with neomycin and streptomycin, respectively. It was found that the maximum absorbance was obtained at 0.5 mL of 2.0 M H₂SO₄, beyond which the absorbance decreases. Thus 0.5 mL of 2.0 M H₂SO₄ was used through out the experiment.

Effect of dye concentration

In order to ascertain the linear relationship between the volume of added KMnO₄ and the decrease in absorbance of AM, AO, Indigo and MB, experiments were performed in 0.5 mL of 2.0 M H₂SO₄ with varying volumes of KMnO₄. The decrease in absorbance was found to be linear up to 2.5 mL and 1.2 mL of 5.0×10⁻⁴ M KMnO₄ with 1.3 mL of AM,

1.8 mL of AO, 1.7 mL of indigo and 2.5 mL of MB with neomycin and streptomycin, respectively. The color was found to be stable up to 24 h.

Stoichiometry

Job's method of continuous variation,³⁵ was employed to determine the stoichiometry of neomycin, oxidant and dyes. Keeping the sum of the molar concentration of both fixed, the ratio of the concentrations of each two in the mixture was varied and the absorbances of the mixture were recorded at the suitable wavelength against reagent blank. The maximum absorbance, as well as known, corresponds to the stoichiometric ratio. Stoichiometric was found to be 1 : 4 for neomycin to oxidant; neomycin to dyes and 1 : 1 for oxidant to dyes (Table 1).

The stoichiometry between streptomycin, oxidant and dyes were determined by continuous variation of potassium permanganate concentration and the concentration of streptomycin being constant. The plot obtained by the molar ratio method,³⁶ indicated that the reaction proceed by molar ratio of 1 : 20. Additionally, the stoichiometric ratio between drugs, dyes and between dyes and oxidant were examined as shown in Table 1, 2.

Table 2. Analytical parameters and optical characteristics of the proposed methods with streptomycin sulphate

Parameter	A	B	C	D
λ_{\max}	521	485	610	664
Beer's law limits (µg mL ⁻¹)	3 - 7	3 - 7	2 - 7	3 - 7
Molar absorptivity (L mol ⁻¹ cm ⁻¹)	2.91×10 ⁵	2.87×10 ⁵	2.84×10 ⁵	2.35×10 ⁵
Sandell sensitivity (µg mL ⁻¹)	5.01	5.08	5.13	6.2
Detection limits (µg mL ⁻¹)	9.13×10 ⁻³	4.77×10 ⁻³	5.58×10 ⁻³	6.74×10 ⁻³
Quantification limits (µg mL ⁻¹)	0.027	0.014	0.017	0.020
Regression equation*				
Slope (b)	0.1625	0.1810	0.2113	0.1677
Intercept (a)	0.165	0.05	- 0.0852	- 0.033
S _{y/x}	0.2972	0.3342	0.3860	0.3065
SD of slope (S _b)	0.0939	0.1057	0.1220	0.0969
SD of intercept (S _a)	1.050	1.1814	1.0916	1.0836
Correlation coefficient (r)	0.9988	0.9891	0.9995	0.9994
Stoichiometric ratio				
[Drug] : [Oxid.]	1 : 20	1 : 20	1 : 20	1 : 20
[Drug] : [Dye]	1 : 20	1 : 20	1 : 20	1 : 20
[Oxid.] : [Dye]	1 : 1	1 : 1	1 : 1	1 : 1

*A = a + b C, where C is the concentration in µg mL⁻¹.

Quantification

Beer's law limits, molar absorptivity, Sandell's sensitivity, regression equations and correlation coefficients obtained by linear square treatment of the results are given in Table 1, 2. The standard deviation of the absorbance measurements was obtained from a series of 13 blank solutions. The detection ($k = 3$) and quantification ($K = 10$) limits of the methods were established according to the IUPAC definitions ($C_1 = K S_0 / s$) where C_1 is the detection limit, S_0 is the standard error of blank

determination, s is the slope of the standard curve, and K is the constant related to the confidence interval.³⁷ In order to study the accuracy and precision of the proposed methods, three concentration levels of the studied drugs within the linearity range were selected and analyzed in five replicates. The measured standard deviation (SD), relative standard deviation (RSD) and confidence limit were, summarized in (Table 3, 4) and can be considered satisfactory, at least for the levels of concentrations examined.

Table 3. Evaluation of accuracy and precision of the proposed methods with neomycin sulphate

Method	Taken $\mu\text{g mL}^{-1}$	Recovery %	RSD ^a %	RE ^b %	Confidence limits ^c
A	5.0	99.96	1.724	1.979	$0.395 \pm 7.82 \times 10^{-3}$
	7.0	100.04	1.089	1.252	0.868 ± 0.0108
	9.0	100.04	1.039	1.192	1.214 ± 0.0144
B	6.0	100.17	1.240	1.423	$0.463 \pm 6.59 \times 10^{-3}$
	7.0	99.96	0.8837	1.0150	$0.731 \pm 7.42 \times 10^{-3}$
	8.0	100.03	0.6411	0.7367	$0.942 \pm 6.94 \times 10^{-3}$
C	8.0	99.98	0.9859	1.132	0.998 ± 0.0113
	9.0	100.12	0.7666	0.8803	$1.103 \pm 9.71 \times 10^{-3}$
	10	99.97	0.8819	1.0135	1.270 ± 0.0128
D	6.0	99.94	2.132	2.449	$0.343 \pm 8.4 \times 10^{-3}$
	7.0	99.92	1.375	1.581	0.657 ± 0.0103
	8.0	100.05	0.5172	0.5949	$1.042 \pm 6.19 \times 10^{-3}$

^aRelative standard deviation for five determinations.

^bRelative Error.

^c95% confidence limits and five degree of freedom.

Table 4. Evaluation of accuracy and precision of the proposed methods with streptomycin sulphate

Method	Taken $\mu\text{g mL}^{-1}$	Recovery %	RSD ^a %	RE ^b %	Confidence limits ^c
A	5.0	100.03	0.6827	0.7840	$0.977 \pm 7.66 \times 10^{-3}$
	7.0	99.90	0.6061	0.6965	$1.183 \pm 8.24 \times 10^{-3}$
	9.0	100.29	0.7306	0.8397	1.344 ± 0.0112
B	3.0	99.98	1.936	2.224	$0.448 \pm 9.96 \times 10^{-3}$
	5.0	100.04	0.6201	0.7122	$0.994 \pm 7.07 \times 10^{-3}$
	7.0	100.06	0.6027	0.6921	$1.241 \pm 8.59 \times 10^{-3}$
C	5.0	99.96	0.5974	0.6865	$0.954 \pm 6.65 \times 10^{-3}$
	6.0	100.06	0.4890	0.5617	$1.182 \pm 6.64 \times 10^{-3}$
	7.0	99.78	0.5747	0.6605	$1.204 \pm 7.95 \times 10^{-3}$
D	4.0	99.98	0.9746	1.119	$0.670 \pm 7.5 \times 10^{-3}$
	5.0	100.06	0.6259	0.7186	$0.885 \pm 6.36 \times 10^{-3}$
	6.0	100.07	0.5290	0.6076	$1.017 \pm 6.18 \times 10^{-3}$

^aRelative standard deviation for five determinations.

^bRelative Error.

^c95% confidence limits and five degree of freedom.

Table 5. Assay results of neomycin sulphate in some pharmaceutical preparation by the proposed and official methods

Preparation and Supplier	Taken $\mu\text{g mL}^{-1}$	Proposed methods Recovery \pm SD (%) ^a				Official method
		A	B	C	D	
Neomycin sulphate tablet, 500 mg/tablet Memphis Co.	5.0	99.90 \pm 0.57 F ^b = 0.79 t = 0.20				99.84 \pm 0.64
	6.0	100.15 \pm 0.64 F = 2.30 t = 0.29	100.07 \pm 0.54 F = 1.26 t = 0.15	100.06 \pm 0.48 F = 1.12 t = 0.28	100.09 \pm 0.56 F = 1.31 t = 0.34	99.96 \pm 0.42
	7.0	100.07 \pm 0.58 F = 0.65 t = 0.23	100.10 \pm 0.80 F = 1.21 t = 0.23	100.03 \pm 0.63 F = 0.76 t = 0.37	99.94 \pm 0.68 F = 0.89 t = 0.38	98.95 \pm 0.72
	8.0	100.08 \pm 0.87 F = 1.02 t = 0.38	100.05 \pm 0.62 F = 0.51 t = 0.17	99.94 \pm 0.57 F = 0.43 t = 0.32	99.98 \pm 0.72 F = 0.71 t = 0.12	99.94 \pm 0.86
	9.0	99.98 \pm 0.60 F = 0.31 t = 0.12	99.96 \pm 0.75 F = 0.49 t = 0.26	99.96 \pm 0.84 F = 0.62 t = 0.43	100.07 \pm 0.69 F = 0.42 t = 0.25	98.39 \pm 1.06
	10		99.96 \pm 0.52 F = 0.37 T = 0.27	99.96 \pm 0.82 t = 0.26 F = 0.90	99.98 \pm 0.84 F = 0.95 t = 0.19	98.92 \pm 0.86

^aThe average of six determinations.^bTheoretical values for t- and F-values for five degree of freedom and 95% confidence limits are 2.57 and 5.05, respectively.

Interferences

The effects of various excipients associated with the drugs were investigated on the determination of neomycin and streptomycin in dosage forms. The results indicated that the talc, starch, glucose, alginate, gelatin and magnesium stearate.

The results indicated that the talc, starch, glucose, alginate, gelatin and magnesium stearate do not interfere with the assay of the drugs mentioned above even though they exist in excess amount. This is clear from the results obtained for the pharmaceutical preparations (Table 5, 6).

Analytical Application

The proposed methods were, successfully applied to determination of the studied drugs in their pharmaceutical dosage forms (Table 5, 6). The performance of the proposed methods were assessed by calculation of t- and F- values compared with the official method.^{38,39} At 95% confidence level, the calculated t- and F- test,⁴⁰ were less than the critical value, indicating that the proposed and official

methods are equally accurate and precise. The results demonstrate the suitability of the proposed method for routine analysis of pharmaceutical preparations containing the studied drugs.

Chemistry of Colored Species

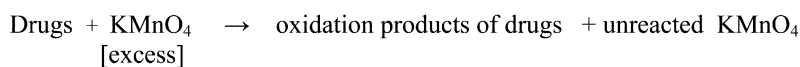
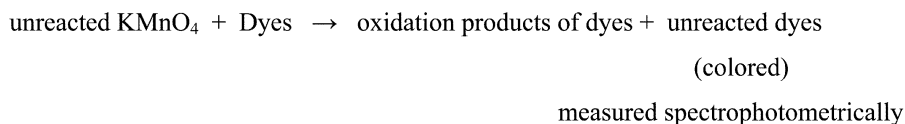
The proposed methods are based on the oxidation of the cited drugs by excess of KMnO_4 to form oxidation products besides unreacted KMnO_4 (step 1), and followed by the estimation of unreacted KMnO_4 using AM (method A), AO (method B), Indigo (method C) and MB (method D), (step 2). The possible sequence of reactions are presented in Scheme 3.

CONCLUSION

The order of λ_{max} values among the proposed methods in the determination of the cited drugs is $D > C > A > B$. The higher λ_{max} of the visible spectrophotometric methods over reported UV and visible spectrophotometric methods is a decisive and advan-

Table 6. Assay results of streptomycin sulphate in some pharmaceutical preparation by the proposed and official methods

Preparation and Supplier	Taken $\mu\text{g mL}^{-1}$	Proposed methods Recovery \pm SD (%) ^a				Official method
		A	B	C	D	
Streptomycin sulphate vial 1.0 gm/vial Nile Co.	3.0	100.20 \pm 1.23	99.94 \pm 0.84	100.11 \pm 0.96	99.96 \pm 0.95	99.92 \pm 1.11
		F = 1.22	F = 0.58	F = 0.75	F = 0.73	
		t = 0.18	t = 0.20	t = 0.28	t = 0.24	
	4.0	100.06 \pm 0.66	100.07 \pm 0.84	100.10 \pm 0.91	99.98 \pm 0.89	98.94 \pm 1.04
		F = 0.40	F = 0.65	F = 0.76	F = 0.73	
		t = 0.20	t = 0.33	t = 0.17	t = 0.20	
	5.0	99.96 \pm 0.79	99.94 \pm 0.87	100.08 \pm 0.72	99.94 \pm 0.97	99.58 \pm 1.10
		F = 0.52	F = 0.63	F = 0.42	F = 0.79	
		t = 0.53	t = 0.31	t = 0.30	t = 0.15	
	6.0	99.94 \pm 0.71	99.98 \pm 0.99	99.96 \pm 1.08	100.05 \pm 1.08	99.72 \pm 0.98
		F = 0.52	F = 1.02	F = 1.21	F = 1.22	
		t = 0.26	t = 0.16	t = 0.22	t = 0.23	
	7.0	100.01 \pm 0.62	100.03 \pm 0.70	100.06 \pm 0.65	100.05 \pm 0.77	99.96 \pm 0.64
		F = 0.92	F = 1.16	F = 1.03	F = 1.43	
		t = 0.49	t = 0.25	t = 0.33	t = 0.38	

^aThe average of six determinations.^bTheoretical values for t- and F-values for five degree of freedom and 95% confidence limits are 2.57 and 5.05, respectively.**Step1:****Step 2:**

Scheme 3.

tage since the interference from the excipients should far less at higher wavelengths. The proposed methods are simple sensitive and can be used for routine analysis and in quality control laboratories for quantitative determination of the cited drugs in the pure or in pharmaceutical formulations.

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