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DETERMINATION OF GENTAMICIN IN SOME SYRIAN PHARMACEUTICAL PRODUCTS BY A NEW VISIBLE SPECTROPHOTOMETRIC METHOD

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ABSTRACT

A Simple and sensitive spectrophotometric method has been described for the assay of Gentamicin in pure form and pharmaceutical products. The proposed method was based on the oxidation of the studied drug by a known excess of cerium (IV) sulfate in acidic medium and estimating the unreacted oxidizing agent by adding a known amount of methyl orange in the same acid medium and measuring the absorbance of the resulted solution at λmax = 500 nm. Different variables affecting the reaction were studied and optimized. Beer's law is obeyed in the concentration range of 10-250 µg/mL (R^2 = 0.9999). The limit of detection (LOD) and limit of quantification (LOQ) were 2.52 µg/mL and 7.63 µg/mL respectively. The method was validated, with percentage recovery ranging from 99.26% to 101.34% and a relative standard deviation (RSD) of 3.07%. Precision was evaluated both intra-day and inter-day (over three days), yielding relative standard deviations (RSDs) of 2.11% and 2.16%, respectively. The robustness of the method was assessed, and percentage recovery ranged from 98.73% to 102.51%, with an RSD not exceeding 4.50%. Recovery of gentamicin from pharmaceutical formulations was investigated. The amount of added reference standard was determined using the developed method, and recovery was close to 100%, with a relative standard deviation (RSD) not exceeding 3.30%. The method was successfully validated and can be applied to the determination of Gentamicin in several Syrian pharmaceutical products.

KEYWORDS: Visible Spectrophotometry, Gentamicin determination, Cerium (IV), methyl orange, Method validation.

1- INTRODUCTION

Gentamicin is an antibiotic belonging to the aminoglycoside class, known for its efficacy against Gram-negative and certain Gram-positive bacteria. Gentamicin was first isolated in 1963 from the bacterium Micromonospora purpurea, it has since served as a key agent in combating severe bacterial infections.

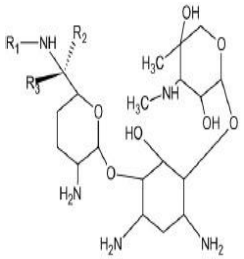
Gentamicin is a complex mixture of related compounds most notably gentamicin C1, C2, and C1a which differ in their methyl groups; this variation influences the drug's pharmacological efficacy and pharmacokinetics.

Gentamicin is used to treat respiratory tract infections, urinary tract infections, and peritonitis. It is administered via injection, though it is also available as ointments and creams for skin infections, and as eye drops for ocular infections.

The chemical name for Gentamicin C1 (one of the major components) is.

(2R,3R,4R,5R)-2-[(1S,2S,3R,4S,6R)-4,6-diamino-3-[(2R,3R,6S)-3-amino-6-[(1R)-1-(methylamino)ethyl]oxan-2-yl]oxy-2-hydroxycyclohexyl]oxy-5-methyl-4-(methylamino)oxane-3,5-diol

Its molecular formula is C21H43N5O7, and its molecular weight is 477.6 g·mol^{-1} and its structural formula is as follows.



Gentamicins	R_1	R_2	R_3
C_1	CH_3	H	CH_3
C_{1a}	H	H	H
C_2	H	H	CH_3
C_{2a}	H	CH_3	H
C_{2b}	CH_3	H	H

GENTAMICIN

Figure (1): Structural formula of gentamicin.

2- Research Significance and Objectives

a– Investigating the suitability of the new spectrophotometric method for the determination of gentamicin.

b– Applying the new method to determine gentamicin in certain Syrian pharmaceutical preparations.

3- Materials and Equipment Used

3-1 Materials Used

- Gentamicin sulfate (98.8% purity), manufactured by Sichuan Long March Pharmaceutical (China).
- Sulfuric acid (H2SO4): molecular weight 98 g/mol, 98% purity, density 1.835 g/mL; manufactured by The British Drug Houses (UK).
- Ammonium cerium sulfate ((NH4)4Ce(SO4)4·2H2O): molecular weight 632.56 g/mol, 98% purity; manufactured by The British Drug Houses (UK).
- Methyl orange: manufactured by Riedel-de Haën (Germany) (95% purity).

3-2 - Instruments and Tools

- Jasco-V630 UV-Vis spectrophotometer (Manufactured in japan).
- Sartorius (Manufactured in Germany) high-precision electronic analytical balance (sensitivity: ±0.01 mg).
- Ultrasonic bath (DIGITAL PRO).
- Variable-volume micropipettes, volumetric flasks, and other laboratory glassware.
- Whatman 3 filter paper (Manufactured in UK) and all necessary glassware for sample preparation and conducting the research.

3-3- Preparation of standard mother solutions

a- Gentamicin sulfate standard solution: A gentamicin sulfate standard solution with a concentration of 1000 µg/mL was prepared by weighing 101.21 mg of the gentamicin sulfate raw material (accounting for purity) and dissolving it in 50 mL of double-distilled water. The resulting solution was transferred to a 100 mL volumetric flask, and the volume was made up to the mark with double-distilled water to obtain a solution with a concentration of 1000 µg/mL.

b- Ammonium cerium sulfate standard solution: An ammonium cerium sulfate standard solution (molecular weight: 632.56 g/mol) with a concentration of 2×10^{-3} M was prepared by weighing 129.10 mg of ammonium cerium sulfate (accounting for purity) and dissolving it in 50 mL of double-distilled water. The resulting solution was transferred to a 100 mL volumetric flask, and the volume was made up to the mark with double-distilled water.

c- Methyl orange standard solution: A methyl orange standard solution with a concentration of 0.5 g/L was prepared by weighing 0.05 g of methyl orange and dissolving it in 50 mL of double-distilled water. The resulting solution was transferred to a 100 mL volumetric flask, and the volume was made up to the mark with double-distilled water. d- Standard sulfuric acid solution: A 5 M standard sulfuric acid solution was prepared by transferring 13.63 mL of concentrated sulfuric acid into a 50 mL volumetric flask containing double-distilled water, then diluting to the mark with double-distilled water.

3-4- Preparation of pharmaceutical sample solutions

a- Medico Gentamicin drops: No stock solution was prepared from the drops; instead, a fixed volume of 0.3 mL (at a concentration of 3 mg/mL) was taken directly from the drops and used in the subsequent stages of the proposed method.

b- Asia Gentamicin ampoule: Transfer 1 mL of the product (batch no. 77153), with a concentration of 80 mg/2 mL, into a 100 mL volumetric flask and make up the volume with double-distilled water to achieve a final concentration of 0.4 mg/mL.

c- Obari Gentamicin ampoule: Transfer 1 mL of the product (batch no. A3943), with a concentration of 80 mg/2 mL, into a 100 mL volumetric flask and make up the volume with double-distilled water to achieve a final concentration of 0.4 mg/mL.

d- Gentocil 80: Transfer 1 mL of the product (LOT: 246025), with a concentration of 80 mg/2 mL, into a 100 mL volumetric flask and make up the volume with double-distilled water to achieve a final concentration of 0.4 mg/mL.

e- Gentalina ointment: Weigh 10 g of the ointment (concentration 3 mg/g; batch no. 120) equivalent to 30 mg of gentamicin and dissolve it in a chloroform-water (1:40, v/v). Shake well until completely dissolved and allow the mixture to stand until the aqueous and organic layers separate. Collect the aqueous layer, filter it into a 100 mL volumetric flask, and make up the volume to the mark with distilled water to obtain a gentamicin solution with a concentration of 0.3 mg/mL.

4- Procedure

Transfer an appropriate volume (depending on the concentration to be studied) of a 1000 µg/mL gentamicin solution into a 10 mL volumetric flask. Add 4.5 mL of 5 M sulfuric acid, followed by 1 mL of 2×10^{-3} M ammonium cerium sulfate. Place the flask in a water bath at 80°C for 15 minutes. Afterward, cool the solution to room temperature, add 0.3 mL of 0.5 g/L methyl orange, and dilute to the mark with double-distilled water. Finally, measure the absorbance at a wavelength of 500 nm.

5- RESULTS AND DISCUSSION

5-1- Validation of Method

a- Linearity

By applying the optimal conditions established in previous research, we determined the linear range for gentamicin adherence to the Beer-Lambert law. Increasing volumes of a 1000 µg/mL gentamicin standard solution were added to 10 mL volumetric flasks, followed by the addition of 1 mL of 2×10^{-3} M cerium (IV) sulfate and 4.5 mL of 5 M sulfuric acid to each. After heating to 80°C, cooling to room temperature, adding 0.3 mL of 0.5 g/L methyl orange, and diluting to the mark with distilled water, the absorbance of the added methyl orange was measured at the wavelength of maximum absorption (500 nm). The method exhibited excellent linearity over the concentration rang of 10-250 µg/mL, with a relative standard deviation (%RSD) not exceeding 2.13%.

b- Limit of Detection (LOD) and Limit of Quantification (LOQ)

The Limit of Detection (LOD) and Limit of Quantitation (LOQ) for gentamicin were determined by calculating the standard deviation (SD) from replicate calibration curves based on five standard concentrations within the linear range, with each concentration measured five times. The LOD was found to be 2.52 µg/mL and the LOQ was 7.63 µg/mL, as shown in Figure (2).

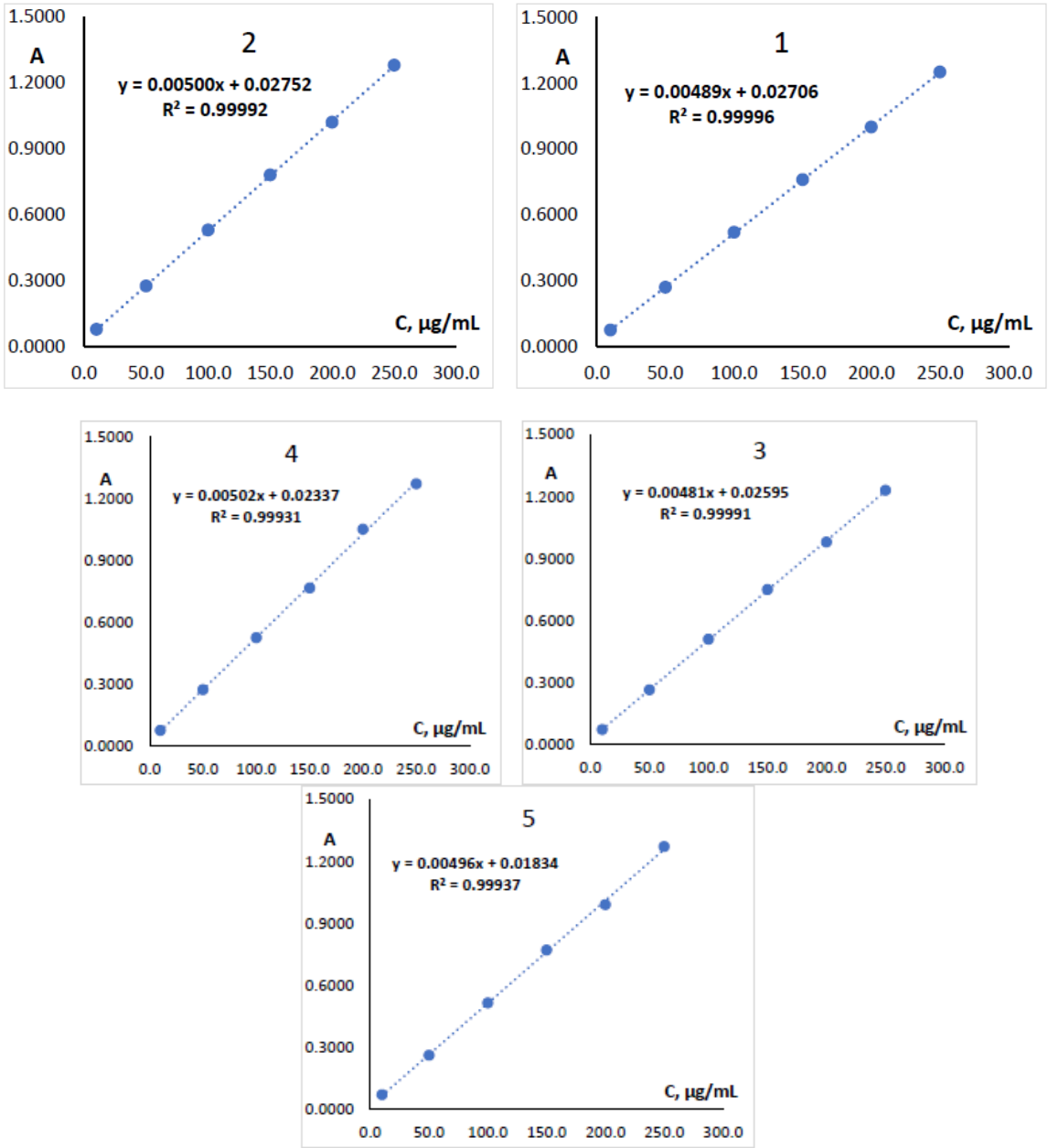


Figure (2): Calibration curves for added methyl orange at the wavelength of maximum absorption (λmax = 500 nm) for various gentamicin concentrations.
1) 10 µg/mL; 2) 50 µg/mL; 3) 100 µg/mL; 4) 150 µg/mL; 5) 200 µg/mL; 6) 250 µg/mL

c- Accuracy

Five standard solutions of gentamicin were prepared, and their relative percentage measurements were calculated. Table (1) shows the results of the relative percentage measurements, where the relative percentage deviation did not exceed 3.07%.

Table (1): Validation Results for the Proposed Method for Determining Gentamicin.

*C_{taken}, µg/mL	*C_{found} µg/mL	SD, µg/mL	RSD %	RM%
10.00	10.11	0.31	3.07	101.1
50.00	49.63	1.02	2.06	99.27
100.00	99.43	1.08	1.09	99.43
150.00	152.01	1.56	1.03	101.34
200.00	199.65	2.68	1.34	99.83
250.00	249.63	4.08	1.63	99.85

*n=5

d- Precision

The precision of the method is expressed by the relative standard deviation (RSD %) of the results obtained using the proposed method. Precision was evaluated through intra-day and inter-day studies. Intra-day by taking measurements immediately after preparation at three times on the same day, then at a two-hour interval (n=5), and intra-days on three consecutive days (n=5), with the sample re-prepared for each measurement. The results are presented in Tables 2 and 3.

Table 2: Intra-day precision results.

Prepared Concentration* µg/mL	Specific Concentration* µg/mL					
	Time Ⅰ*	Precision	Time Ⅱ*	Precision	TimeⅢ*	Precision
50	50.63	1.89	49.33	2.11	49.58	1.92
100	101.53	1.36	101.22	1.47	99.78	1.82
150	152.44	1.55	149.36	1.78	151.82	1.32

*n=5

Table 3: Inter-day precision results.

Prepared Concentration* µg/mL	Specific Concentration* µg/mL					
	Day Ⅰ*	Precision	Day Ⅱ*	Precision	Day Ⅲ*	Precision
50	50.45	1.65	49.36	1.98	49.71	2.16
100	99.69	1.72	100.77	2.03	101.24	2.09
150	150.85	1.45	149.15	1.66	151.29	1.52

*n=5

e- Robustness

We prepared three standard solutions with concentrations of (50.00, 100.00, 150.00) µg/mL, and adjusted the slit width by 1 nm on the spectrophotometer used. The absorption spectra were recorded at both slow and fast scan speeds, while also varying the wavelength at which absorbance was measured (500 ± 2 nm).

Table (4) shows the results of the robustness of the proposed method, where the relative percentage measurement fell within the range (98.73–102.51) %, and the relative standard deviation did not exceed 4.50 %.

Table 4: Assessment of the Robustness of the Proposed Method for Determining Gentamicin.

Parameter	Measured deviation	*Ctaken (µg/mL)	RSD %	per%	*Ctaken (µg/mL)	RSD %	per%	*Ctaken (µg/mL)	RSD %	per%
Slit rang 1 nm	**2 nm**	50	2.14	101.66	100	1.30	100.52	150	1.24	101.66
	1 nm	50	2.42	99.26	100	1.18	99.30	150	0.66	101.11
Scan speed fast	**Fast Slow**	50	4.01	101.28	100	2.24	102.51	150	1.21	102.14
		50	4.21	100.30	100	1.94	101.23	150	1.74	100.91
Wavelength 500 nm	**+2 nm**	50	4.50	100.46	100	1.86	101.46	150	1.50	100.86
	-2 nm	50	4.30	98.94	100	1.45	98.73	150	1.42	99.28

***n = 5**

f- Recovery of Gentamicin in pharmaceutical products

To validate the proposed method, and to ensure the suitability, reliability, and accuracy of the method and results, we studied the recovery of Gentamicin in Syrian pharmaceutical preparations by adding three standard concentrations—at levels of approximately 80%, 100%, and 120% to a fixed concentration of each preparation:

Gentamicin Medico 3 mg/mL (Medico), Gentamicin Asia 80 mg/2 mL (Asia), and Gentamicin Obari 80 mg/2 mL (Obari), Gentosil 80 80 mg/2 mL (Alpha), and Gentalina 3 mg/g (Delta).

The results validated the proposed method, as the drug substance recovery results in the studied pharmaceutical preparations were close to 100%, with a relative standard deviation not exceeding 3.30%, The recovery was performed using the standard addition method due to the effect of excipients.

Table 5: Results of Gentamicin Recovery in the Studied Pharmaceutical Preparations Using the Standard Addition Method.

Product	Dose	Sample Concentration µg/mL	Added Concentration* µg/mL	Determined Concentration* µg/mL	*Recovery %	**SD %	**RSD%	Average Recovery%
Gentamicin Medico Drops	3 mg/mL	96.00	76.80	174.47	102.17	1.15	1.13	101.65
			96.00	192.66	100.69	0.95	0.94	
			115.20	213.60	102.08	1.72	1.68	
Gentamicin Asia Ampoule	80 mg/2mL	40.00	32.00	72.35	101.09	1.62	1.60	100.70
			40.00	80.70	101.75	3.02	2.97	
			48.00	87.65	99.27	2.07	2.09	
Gentamicin obari Ampoule	80 mg/2mL	82.00	65.80	149.17	102.08	1.52	1.49	101.69
			82.00	165.56	101.90	2.43	2.38	
			98.40	181.46	101.08	2.11	2.09	
Gentocel 80 Liquid Vial	80 mg/2mL	80.00	64.00	145.31	102.05	1.92	1.88	101.80
			80.00	161.54	101.93	1.47	1.44	
			96.00	177.37	101.43	1.33	1.31	
Gentalina Ointment	3mg/g	60.00	48.00	106.97	97.85	1.89	1.93	98.80
			60.00	119.54	99.23	1.75	1.76	
			72.00	131.52	99.33	3.28	3.30	

*Average of five replicates, **Calculated for recovery.

5-2- Applications of the method

The proposed method was applied to the determination of gentamicin in the following Syrian pharmaceutical products: Gentamicin Medico 3 mg/mL (manufactured by Medico), Gentamicin Asia 80 mg/2mL (manufactured by Asia), Gentamicin Obari 80 mg/2mL (manufactured by Obari), Gentocil 80 80 mg/2mL (manufactured by Alpha), and Gentalina 3 mg/g (manufactured by Delta)

As an application of the new method, as illustrated in Table (6).

Table (6): Results of gentamicin determination in Syrian pharmaceutical preparations.

Product	Sample concentration	specific concentration	SD	RSD%	Percentage%
Gentamicin Medico	3.00 mg/mL	3.19 mg/mL	0.0921 mg/mL	2.89	106.33
Gentamicin Asia	80 mg/2mL	80.58 mg/2mL	0.2871 mg/2mL	0.36	100.73
Gentamicin Obari	80 mg/2mL	82.30 mg/2mL	0.5896 mg/2mL	0.72	102.88
Gentocil 80	80 mg/2mL	80.58 mg/2mL	0.3652 mg/2mL	0.45	100.73
Gentalina	3.00 mg/g	3.05 mg/g	0.09 mg/g	2.95	101.67

5-3- Comparison of Results

To verify the validity of the proposed analytical method, we compared its results with those of a reference method (fluorometric determination).[17] A statistical comparison was conducted by calculating the t-test value as a criterion for assessing the method's accuracy (trueness) and the F-test as a criterion for assessing the method's precision relative to the reference method. Table (7) presents the obtained results; the calculated t-test representing the statistical difference between the two methods was 0.08, well below the tabulated value of 2.776 (at a 95% confidence level), indicating the validity of the proposed method. Similarly, the calculated F-test reflecting the statistical agreement between the methods was 4.51, below the tabulated value of 6.39 (at the same confidence level), demonstrating the precision of the

proposed method. The mean recovery and standard deviation values were calculated from the results summarized in Table (6).

The results indicate no significant difference between the two methods regarding validity and precision at the same confidence level; this supports the adoption and use of the proposed method for determining gentamicin in both the raw material and its pharmaceutical formulations.

Table (7): Results of the t-test and F-test.

Preparation	Recovery* % ± SD*	
	Proposed Method	**Reference Method [17]**
Gentamicin Asia	100.70 ± 2.24 t = 0.08 F = 4.51	100.62 ± 1.05

*****Average of five trials with four degrees of freedom and a 95% confidence level, where the tabulated t-value is 2.776 and the tabulated F-value is 6.39.

6- CONCLUSIONS AND RECOMMENDATIONS

The proposed method is simple, rapid, sensitive, and cost-effective, yielding satisfactory results for the determination of gentamicin; its suitability and applicability for determining gentamicin in Syrian pharmaceutical preparations have been demonstrated.

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