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Spectrofluorimetric determination of streptomycin in dosage forms and in spiked plasma using 9,10-phenanthraquinone

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Abstract

A simple and highly sensitive method is proposed for the fluorimetric determination of streptomycin in dosage forms and in biological fluids. The method involves the reaction of streptomycin with 9,10-phenanthraquinone in alkaline medium to give a highly fluorescent derivative. The experimental parameters were carefully studied and incorporated into the procedures. The results obtained compared favourably with those obtained by the official methods. The concentration–fluorescence plots were rectilinear over the range 0.025–0.4 μg/ml, with minimum detectability ($S/N = 2$) 0.006 μg/ml (4.19×10^{-9} M). The proposed method was applied for the determination of streptomycin in dosage forms. The results obtained were in good agreement with those obtained by the official method. The proposed method was further applied to the determination of streptomycin in human plasma. The percentage recovery was 101.82. A proposal of the reaction pathway was presented.

Keywords: Fluorimetry; Streptomycin; 9,10-Phenanthraquinone; Plasma

1. Introduction

Streptomycin classified as broad spectrum aminoglycoside antibiotic, it is active against numerous gram-negative and gram-positive bacteria. One of the greatest virtues of 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 [1]. Many analytical techniques have been employed for the determination of streptomycin, these include spectrophotometry after reaction with ninhydrin in alkaline medium [2], nitrosation followed by reaction with 2-cyanoacetamide in ammonia solution [3] and through reaction with iodine/wool fast blue BL [4]. Streptomycin was also determined in presence of ceftriaxone by 'ratio spectra' second derivative and 'zero crossing' third derivative spectrophoto-

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metry [5]. Continuous flow chemiluminometric methods for the determination of streptomycin with *N*-bromosuccinimide [6] and by the interaction of streptomycin and glucose was also adopted [7]. A review for the high-performance liquid chromatography (HPLC) bioanalysis of aminoglycosides, was reported by Soltas [8]. Streptomycin residues were determined in food by solid phase extraction liquid chromatogrpahy with post column derivatisation and fluorimetric detection using 1,2 naphthoquinone [9]. Rapid detection of streptomycin in milk could be achieved by enzyme linked immuno-filtration assay [10]. Other reported methods for the determination of streptomycin include capillary electrophoresis [11], gas chromatography–mass spectrometry (GC–MS) [12] and fluorimetrically using lanthanide probe ion spectroscopy [13].

9,10-Phenanthraquinone used as a reagent for the determination of some metals as palladium by column chromatography [14], iron [15] and ruthenium [16] by spectrophotometry. Also used for the determination of arginine in proteins [17] and for spectrofluorimetric determination of guanthidine in pharmaceutical preparations and in biological fluids [18]. The present method involves the reaction between streptomycin as a compound containing guanidine moiety with 9,10-phenanthraquinone to give a highly fluorescent product. The proposed method was successfully applied to the determination of streptomycin in dosage forms and in plasma. The results obtained were satisfactorily accurate and precise.

2. Experimental

2.1. Apparatus

An Aminco-Bowman model J_4-9860-spectrofluorimeter with the excitation and emission slit control set at 5nm, and 1 cm quartz spectrofluorimetric cells, were used all over the measurements.

2.2. Materials and reagents

All reagents were of analytical grade. Distilled water was used.

1. Streptomycin was obtained from (El-Nile Co., Cairo, Egypt).
2. 9,10-Phenanthraquinone (Aldrich, USA), 2 g/ml in dimethyl formamide Gold Label, free from anthraquinone.
3. Sodium hydroxide 1 M aqueous solution.
4. Hydrochloric acid (Prolabo, France) 10 M.
5. Plasma was obtained from Mansoura University Hospital, Mansoura, Egypt.

2.3. Standard solution

Stock solution (1.0 mg/ml) of streptomycin was freshly prepared in distilled water and was further diluted with the same solvent to obtain the nominal concentration of the working range. The prepared solutions were stable for 24 h.

2.4. Formulations

The following commercial formulations were subjected to the analytical procedures: Streptomycin vial (El-Nil Co.) containing 500 mg streptomycin sulphate, streptophenicol capsules and suspensions (Misr Co., Cairo, Egypt) containing 125 mg streptomycin sulphate and 125 mg chloramphenicol.

2.5. General procedures

2.5.1. Procedures for calibration curve

Stock solution of streptomycin in distilled water was prepared in distilled water, and was further diluted with the same solvent to obtain a final concentration of 5.0 μg/ml of the analyte. Transfer aliquots (0.25–0.8 ml) of this solution covering the concentration range stated in Table 1 into 10 ml standard measuring flasks. Add 2 ml of 9,10-phenanthraquinone reagent, and 0.25 ml of sodium hydroxide (1 M). Leave for 30 min at room temperature then add 0.1 ml of concentrated HCl and complete to volume with distilled water. Measure the fluorescence intensity at 310/395 nm.

2.5.2. Analysis of dosage forms

2.5.2.1. Capsules. Empty the contents of ten capsules. Transfer an accurately weighed amount of

the powder equivalent to 50.0 mg of streptomycin into 50 ml measuring flask then complete to volume with distilled water. Sonicate to dissolve the powder. Filter into a small flask, reject the first few millilitres of the filtrate. Transfer 0.5 ml of filtrate into a 100 ml measuring flask and complete to volume with water to give an analysed concentration of 5.0 μg/ml. Transfer aliquot volumes into 10 ml measuring flasks and proceed as described for pure sample. Calculate the nominal content from the corresponding regression equation.

2.5.2.2. Suspension (streptophenicol suspension). Mix the contents of five bottles, transfer an aliquot volume (0.5 ml) equivalent to 15.625 mg into a 100 ml measuring flask, complete to volume with water, transfer 5 ml into a small separating funnel, extract using 3×3 ml of $CHCl_3$ and reject organic layer. Transfer aqueous layer into 25 ml measuring flask, complete to volume with distilled water. Transfer 5 ml of the final solution into 25 ml measuring flask to obtain final concentration of 6.25 μg/ml and continue as described for pure sample Calculate the nominal content from the corresponding regression equation.

Table 1
Analysis of pure sample of streptomycin sulphate by the proposed and official methods

Proposed method			Official method [20]
Taken (μg)	Found (μg)	Found (%)	
0.025	0.0251	100.4	
0.05	0.0501	100.2	
0.10	0.099	99.0	
0.15	0.1501	100.0	
0.20	0.202	101.0	
0.25	0.2501	100.4	
0.35	0.3501	100.03	
0.40	0.4	100.0	
$\bar{X} \pm$ S.D.		100.09 ± 0.55	100.37 ± 0.907
R.S.D.		0.549	
S.A.E.		0.371	
Confidence limit		0.460	

2.5.2.3. Vials. Mix the contents of five vials, transfer an accurately weighed amount of the powder, equivalent to 10 mg of streptomycin into a 50 ml measuring flask. Complete to the volume with water. Transfer 2.5 ml into 100 ml measuring flask to obtain final concentration of 5 μg/ml and continue as described before.

2.5.3. Determination of streptomycin in spiked human plasma

To 1 ml of a plasma sample contained in a centrifuge tube add 0.2 ml of aqueous streptomycin solution (0.25 mg/ml), mix well using a vortex mixer, then add 2 ml of acetonitrile. Centrifuge at 2400 rpm for 5 min. Transfer the supernatant as completely as possible into 10 ml measuring flask complete to volume. Proceed as described above.

3. Results and discussion

3.1. Optimisation of the parameters

The reaction of streptomycin as a guanidine containing compound with 9,10-phenanthraquinone was found to yield strongly fluorescent product. Fig. 1 shows the excitation and emission spectrum of the obtained fluorophore of standard streptomycin. The different experimental parameters affecting the intensity of the fluorophore were studied by changing one parameter while keeping the others constant. Increasing the volume of 9,10 phenanthraquinone (0.0002% w/v) solution was found to increase the fluorescence intensity up to 2.5 ± 0.1 ml, after which the fluorescence intensity began to decrease (Fig. 2). The fluorescence intensity increased with increase volume of NaOH (1 M) up to $0.25 \pm$ ml then began to decrease (Fig. 3). The fluorescence intensity increased with increase the reaction time up to 30 ± 2 min then become stable as shown in (Fig. 4). The produced fluorophore was found to be stable for 1 h.

3.2. Sensitivity, limit of detection

The calibration graph was linear over the concentration range 0.025–0.4 g/ml. Regression anal-

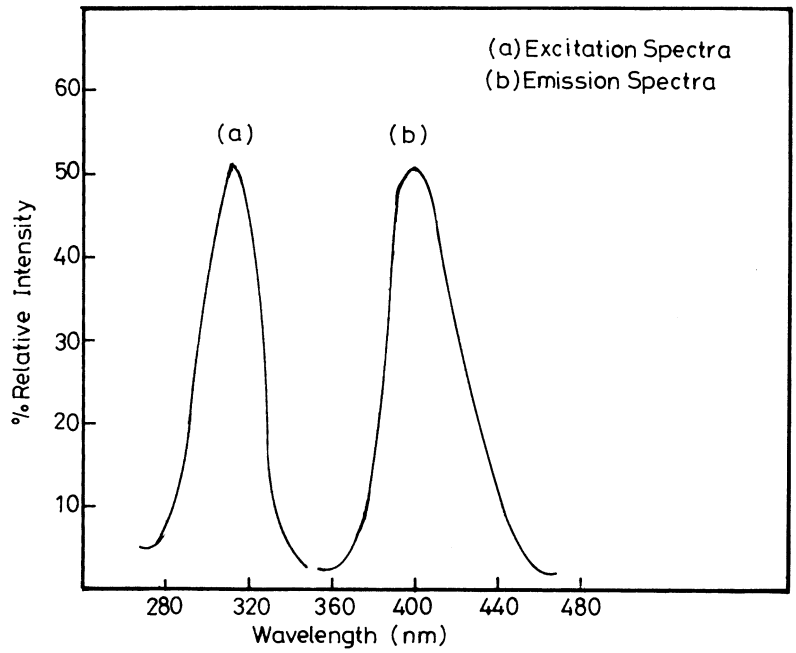

Fig. 1. Fluorescence spectra of the reaction product of streptomycin (0.25 μg/ml) and 9,10-phenanthraquinone.

ysis indicated a linear relationship between fluorescence intensity and concentration

$$C = -0.000087 + 0.005RI \qquad r = 0.9999.$$

The minimum detection limit ($S/N = 2$) was calculated and was found to be 0.006 g/ml (4.19×10^{-9} M). Statistical analysis [19] of the regression line gave values of 0.142, 0.586 and 0.39 for $S_{y/x}$, S_a, and S_b, respectively. These small values indicate the high precision of the proposed method.

3.3. Precision, and accuracy of the procedure

In order to determine the precision and accuracy of the method solutions containing known amount of the drug were prepared and analysed in five replicates. The analytical results obtained from these investigations are summarised in Table 1. The mean relative standard (R.S.D.) and the mean standard analytical error (S.A.E.) are considered to be very satisfactory.

The method was further applied to some dosage forms containing streptomycin. Table 2 shows that the results of analysis of some dosage forms containing streptomycin are in

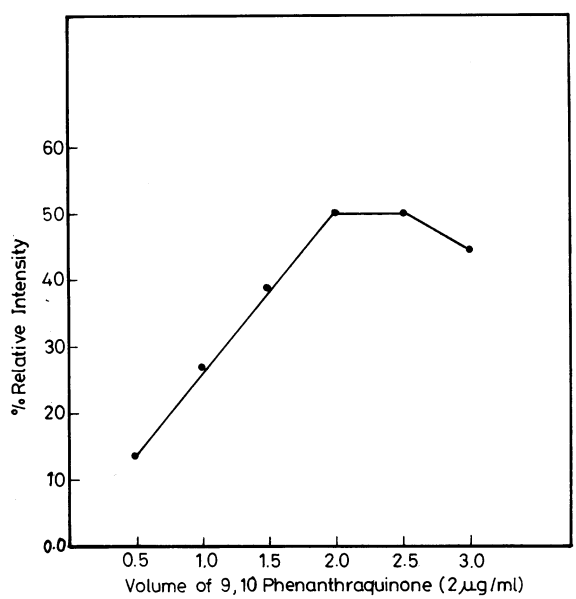

Fig. 2. Effect of volume of 9,10-phenanthraquinone (2 μg/ml) on the development of the fluorophore.

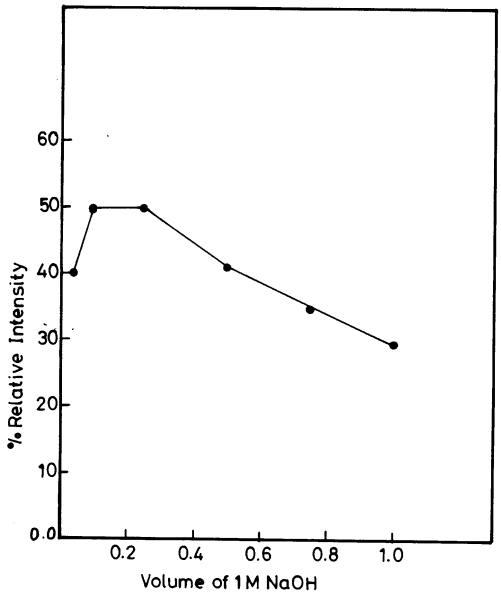

Fig. 3. Effect of volume of NaOH (1 M) on the development of the fluorophore streptomycin (0.25 μg/ml).

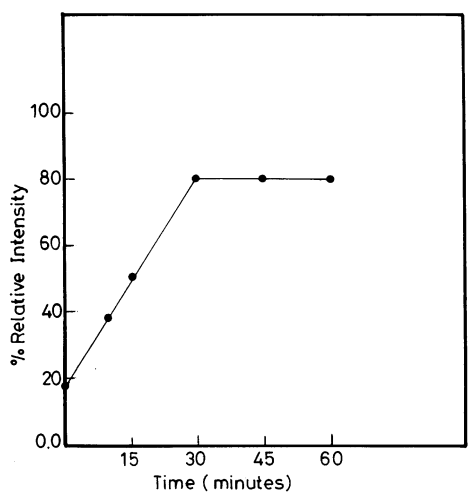

Fig. 4. Effect of the reaction time on the formation of the reaction product streptomycin (0.4 μg/ml).

good agreement with those obtained with the official method [20]. Statistical analysis of the results obtained by both methods using Student's t-test and the variance ratio F-test. The results obtained show no significant difference between the performance of the two methods regarding accuracy and precision.

Chloramphenicol, which is frequently co-formulated with streptomycin did not interfere with the assay. The proposed method could be successfully applied for the determination of streptomycin in spiked plasma. Streptomycin is administered intramuscularly in a dose of 500 mg, leading to final plasma concentration of about 10 μg/ml. This concentration is high above the working range of the proposed method. Therefore, the proposed method could be successfully applied to the analysis of streptomycin in human plasma. The results obtained in (Table 3) are satisfactorily accurate and precise.

Table 2
Analysis of some dosage forms containing streptomycin by the proposed and official methods[a]

Preparation	Proposed method	Official method [20]
Streptomycin[b]	100.20	
Vials	100.80	
Streptomycin sulphate/vial (500 mg)	99.6	
$\bar{X} \pm$ S.D.	100.2 ± 0.6	100.4 ± 0.74
t	0.36 (2.776)	
F	4.2 (19.00)	
Streptophenicol[c]	99.5	
Capsules	100.5	
Chloramphenicol 125 mg and streptomycin sulphate 125 mg	100.9	
$\bar{X} \pm$ S.D.	100.3 ± 0.72	100.47 ± 0.95
t	0.25 (2.776)	
F	1.74 (19.00)	
Streptophenicol[c]	100.2	
Suspension	99.8	
Chloramphenicol 125 mg and streptomycin sulphate 125 mg per 5 ml	101.2	
$\bar{X} \pm$ S.D.	100.4 ± 0.71	100.3 ± 0.75
t	0.2 (2.776)	
F	1.125 (19.00)	

[a] Each result is the average of three separate determinations and values between brackets are the tabulated t and F values at $P = 0.05$.

[b] Product of El-Nile Co.

[c] Product of Misr Co.

Table 3
Fluorimetric determination of streptomycin in spiked human plasma by the proposed method

Taken (μg)	Found (μg)	Found (%)
0.3	0.3127	104.23
	0.3074	102.46
	0.3127	104.23
0.5	0.5140	102.8
	0.5030	100.6
	0.4830	96.60
$\bar{X}$		101.82
±S.D.		±2.9

The method of extraction reported by Diaz-Marot et al. [21] was adopted in our study.

The most striking advantage of the proposed method is its high specificity for streptomycin, as it is based on the presence of guanidine group. Other aminoglycosides in particular, and antibiotics — in general, do not interfere with the assay.

A proposed mechanism for the reaction pathway is presented in Fig. 5. The reaction proceeds in two steps, the first one involves condensation of the reagents through its carbonyl groups with the guanidine group in alkaline medium with the elimination of two molecules of water. The second step involves hydrolysis of the reaction product in acid medium with the formation of the amino derivative, which is highly fluorescent.

4. Conclusion

In conclusion streptomycin can be determined in pharmaceutical preparations and in plasma by a simple accurate and precise spectrofluorimetric method. The proposed method is specific for streptomycin as a guanidine containing compound alone or in presence of other antibiotics do not have guanidine moiety in their structure.

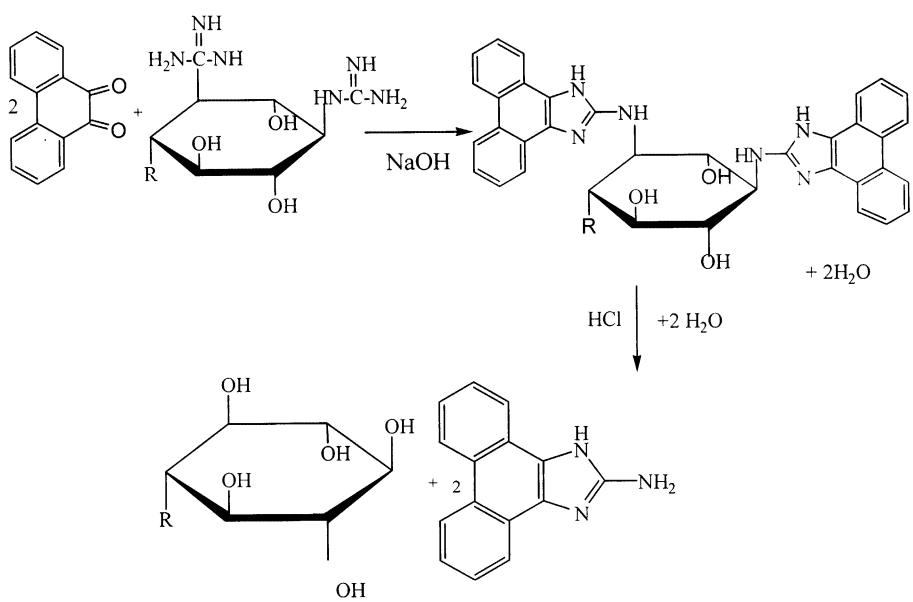

Fig. 5. The proposed mechanism for the reaction between streptomycin and 9,10-phenanthraquinone.

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