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Review Article

Highly Sensitive Spectrofluorimetric Method for Determination of Certain Aminoglycosides in Pharmaceutical Formulations and Human Plasma

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Abstract. A simple, reliable, highly sensitive and selective spectrofluorimetric method has been developed for determination of certain aminoglycosides namely amikacin sulfate, tobramycin, neomycin sulfate, gentamicin sulfate, kanamycin sulfate and streptomycin sulfate. The method is based on the formation of a charge transfer complexes between these drugs and safranin in buffer solution of pH 8. The formed complexes were quantitatively extracted with chloroform under the optimized experimental conditions. These complexes showed an excitation maxima at 519–524 nm and emission maxima at 545–570 nm. The calibration plots were constructed over the range of 4–60 pg mL^{-1} for amikacin, 4–50 pg mL^{-1} for gentamicin, neomycin and kanamycin, 4–40 pg mL^{-1} for streptomycin and 5–50 pg mL^{-1} for tobramycin. The proposed method was successfully applied to the analysis of the cited drugs in dosage forms. The proposed method was validated according to ICH and USP guidelines with respect to specificity, linearity, accuracy, precision and robustness. The high sensitivity of the proposed method allowed determination of amikacin and gentamicin in spiked and real human plasma.

KEY WORDS: aminoglycosides; dosage forms; human plasma; safranin; spectrofluorimetry.

INTRODUCTION

Aminoglycosides are important class of antibiotics that are active against both gram-positive and gram-negative bacterial infections. They have found widespread use in both human and veterinary medicine. However, the use of aminoglycosides may cause side effects of ototoxicity and nephrotoxicity (1,2). Careful monitoring of the aminoglycosides in plasma is routinely used as a guide to dosing, to prevent toxicity and to ensure efficacy. The chemical structures of the studied drugs are shown in Table I.

A wide variety of analytical methods was reported for determination of these drugs in pure forms, pharmaceutical formulations and biological fluids. These methods include spectrophotometric (3–11), spectrofluorimetric (12–18), chemiluminescence (19–23), thin-layer chromatographic (24–26), capillary electrophoresis (27–29), high-performance liquid chromatographic (30–34), polarographic (35,36), near infrared (37) immunoassay (38,39) and microbiological methods (40–42). In comparison with the previously published spectrofluorimetric methods that determined these drugs in micrograms per milliliter or nanograms per milliliter levels, the proposed method determines the concerned drugs in picograms per milliliter level. Therefore, the proposed method increases the sensitivity and allowed determination of amikacin and gentamicin in real human plasma.

The wide use of these drugs necessitates the development of a simple, rapid, accurate, sensitive, applicable and cheap method for their determination in pure forms, pharmaceutical formulations and spiked and real human plasma. So this study describes a simple and very sensitive spectrofluorimetric method for determination of these drugs depending on charge transfer complexation of these drugs with safranin.

GENERAL EXPERIMENTAL

Apparatus

A Perkin Elmer LS 45 Luminescence spectrometer (UK) connected to an IBM PC computer loaded with the FL WINLABTM software, Milwakee SM 101 pH meter (Portugal) and Laboratory centrifuge 4,000c/s (Bremsen ECCO, Germany) were used in this work.

Materials and Reagents

All materials were of analytical grade, and the solutions were prepared with double distilled water. Samples of aminoglycosides were generously supplied by their respective manufacturers and were used without further purification; amikacin sulfate and tobramycin (Egyptian International Pharmaceutical Industries Co.; E.I.P.I.CO., El Asher Ramadan City, Cairo, Egypt), neomycin sulfate and gentamicin sulfate (Memphis Co. For Pharmaceutical & Chemical Industries, Cairo, Egypt), kanamycin sulfate (Miser Co. for Pharm. Ind. S.A.E., Cairo, Egypt) and streptomycin sulfate (The Nile Co. for Pharmaceutical & Chemical Industries, Cairo, Egypt.). Safranin (Sigma–

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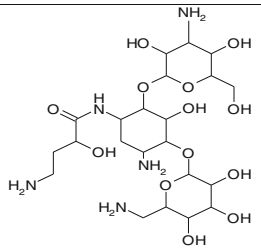
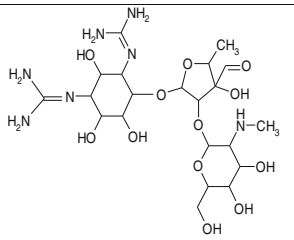
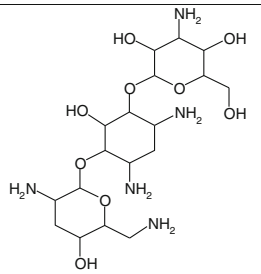
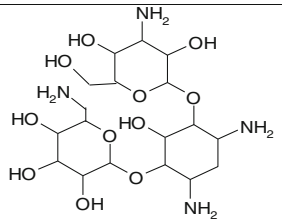
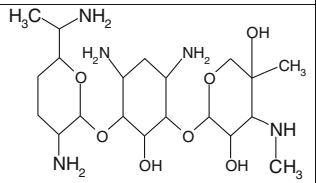
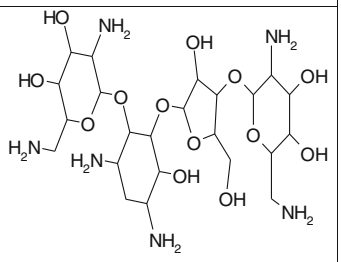
Table I. Chemical Structures of Studied Drugs

Drug	Structure	Drug	Structure
Amikacin		Streptomycin	
Tobramycin		Kanamycin	
Gentamicin		Neomycin	

Aldrich Chemie GmbH, Steinheim, Germany) was 3% (*w*/*v*) in water. All solvents used were of analytical grade and obtained from El Nasr chemical Co., Egypt.

Pharmaceutical Formulations

The following available commercial preparations were analysed; Amikin® vials (Smith Kline Beecham; an affiliated co. to GlaxoSmithKline, Egypt) labeled to contain 100 mg amikacin sulfate in 2 ml aqueous solution, Neomycin® tablets (Memphis Co. For Pharmaceutical & Chemical Industries, Cairo, Egypt.), labeled to contain 500 mg Neomycin sulfate per tablet, Tobrin® eye drops and Tobrin® eye ointments (Egyptian International Pharmaceutical Industries Co.; E.I.P.I.CO., El Asher Ramadan City, Cairo, Egypt), labeled to contain 0.3% *w*/*v* and *w*/*w* tobramycin, respectively. Tobradex® eye drops (Alcon-Couvreur), labeled to contain 0.3% tobramycin and 0.1% dexamethasone. Streptomycin® vial (Manufactured by Amriya Pharm. Ind. Co. for the Nile Co. For Pharmaceutical & Chemical Industries, Cairo, Egypt.), labeled to contain 1 g streptomycin sulfate equivalent to 1 g streptomycin. Diakan-M® syrup (Misr Co. for Pharm. Ind. S.A.E., Cairo, Egypt), labeled to contain 100 mg kanamycin as sulfate. Garamycin® ampoules (manufactured by Memphis Co. For Pharmaceutical & Chemical Industries, Cairo–A.R.E. under authority of Schering-Plough Corporation/USA), labeled to contain 40 mg/ml gentamicin sulfate and Garamycin® ointment (Manufactured by Memphis Co. For Pharmaceutical & Chemical Industries, Cairo–A.R.E. under authority of Schering-Plough Corporation/U.S.A.), each gram ointment labeled to contain 1 mg gentamicin sulfate.

Preparation of Solutions

Preparation of Stock Standard Solutions

Stock solution containing 1 mg mL^{-1} of each drug was prepared in distilled water, and working standard solutions containing 100–1,500 pg mL^{-1} were prepared by suitable dilution of the stock solutions with ethanol.

Buffer Solutions

Teorell and Stenhagen buffer solution (43) of the pH range 5.0–8.5 was prepared in distilled water.

Safranin Reagent

Safranin was prepared by diluting a certain volume of stock solution with distilled water to obtain concentration of 0.004% *w*/*v*.

General Analytical Procedure

One half milliliter of Torell and Stenhagen buffer solution (pH 8.0) was transferred into 60-ml separatory

funnels. Accurate volumes of the standard solution of each drug, within the concentration range and 0.8 ml safranin (0.004% *w*/*v*), were added. The contents of each separator were mixed and then extracted with 10-, 5- and 5-ml portions of chloroform. The chloroform extracts were dried with anhydrous sodium sulphate, filtered through dry filter paper into 25-ml volumetric flasks and completed to the volume with chloroform. The fluorescence intensities were measured at 545–570 nm emission wavelengths with excitation at 519–524 nm against reagent blanks treated similarly. The fluorescence intensities were plotted against the corresponding concentrations to obtain the calibration graphs.

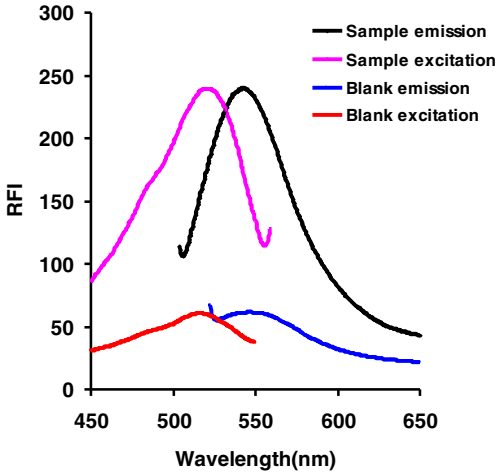


Fig. 1. Excitation and emission spectra of reaction product of gentamicin (16 pg ml^{-1}) with safranin (0.004% *w*/*v*)

Determination of the Studied Drugs in Pharmaceutical Formulations

Procedures for Tablets

Twenty tablets of neomycin were weighted accurately, finely powdered and mixed thoroughly. An accurate weighted amount from the powdered tablets equivalent to 25 mg was transferred into a 50-ml calibrated flask, dissolved in amount of distilled water, swirled and sonicated for 5 min, completed to volume with the same solvent and filtered to obtain a solution of 0.5 mg mL^{-1}. Further dilutions with ethanol were made to obtain sample solutions within the concentration range for calibration (100–1,250 pg mL^{-1}), and then the general analytical procedure was followed.

Procedures for Ophthalmic Ointment (44)

An equivalent amount of 3 mg of tobramycin in Tobrin® ophthalmic ointment was vigorously shaken with 2 ml chloroform. The investigated drug was extracted with 1 ml of distilled water with vigorous shaking, and centrifuged for 15 min. The clear upper aqueous layer containing drug diluted with ethanol to obtain concentration within the concentration ranges for calibration (125–1,250 pg mL^{-1}), and then the general procedure was followed.

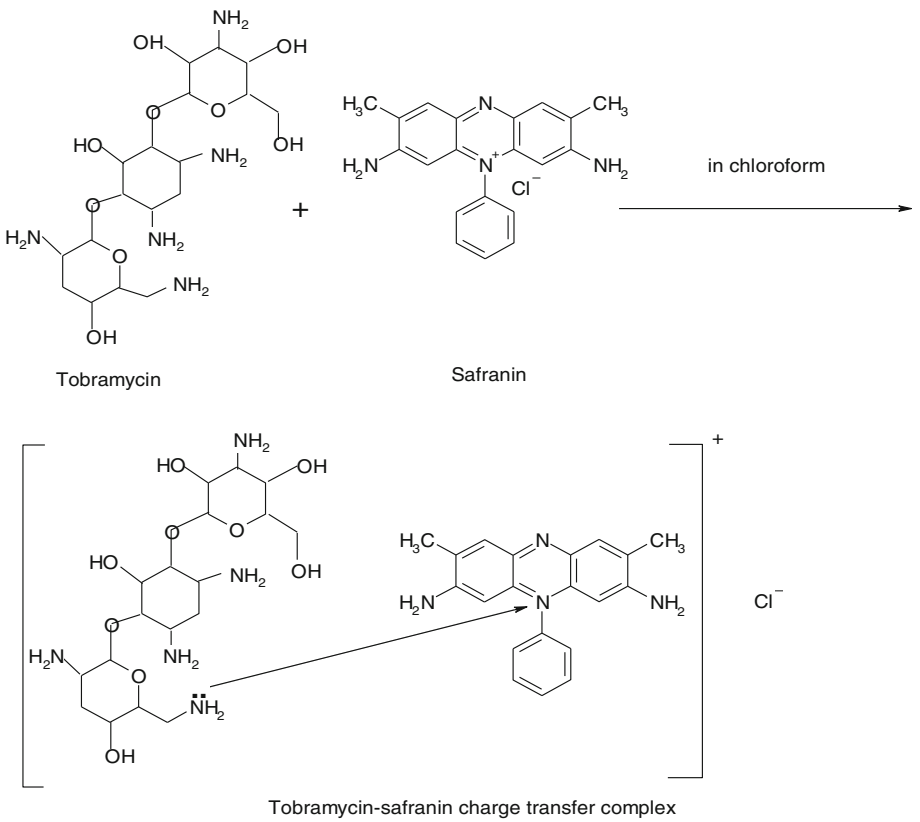


Fig. 2. Suggested reaction mechanism between tobramycin as a representative example of the studied drugs and safranin

Procedures for Ophthalmic Drops (44)

The solution of Tobrin® and Tobradex® drops was diluted with distilled water to obtain a solution of 0.5 mg mL^{-1}, and then further diluted with ethanol to obtain concentration within the concentration ranges for calibration (125–1,250 pg mL^{-1}), and then the general procedure was followed.

Procedures for Amikin® Vial and Garamycin ® Ampoule (44)

The solution of Amikin ® vials and Garamycin ® ampoules was diluted with distilled water to obtain a solution of 0.5 mg mL^{-1}, and then was further diluted with ethanol to obtain concentration within the concentration ranges for calibration (100–1,500 pg mL^{-1}), and then the general procedure was followed.

Procedures for Streptomycin® Vial (44)

An amount of Streptomycin® vial content equivalent to 25 mg of streptomycin was dissolved in 50 ml distilled water to obtain a solution of 0.5 mg mL^{-1}. Then, the resultant solution was further diluted with ethanol to obtain concentration within the concentration ranges for calibration (100–1,000 pg mL^{-1}), and then the general procedure was followed.

Procedures for Syrup

A certain volume of suspension containing 25 mg kanamycin was transferred into a 50-ml calibrated flask, completed to the volume with distilled water and then filtered to obtain a solution of 0.5 mg mL^{-1}. Further dilutions with ethanol were made to obtain sample solutions within the concentration ranges for calibration (100–1,250 pg mL^{-1}), and then the general analytical procedure was followed.

Procedure for Spiked Human Plasma

A sample of 5.0 ml of drug-free human blood sample was taken from healthy volunteers into a heparinized tube, centrifuged at 4,000 rpm for 30 min. Then, into a 10-ml stoppered calibrated tube, 1.0 ml of the drug-free plasma (supernatant) was spiked with either 1.0 ml of amikacin or gentamycin. Two milliliters of acetonitrile was added as precipitating agent for protein, and the resultant mixture was diluted to 10.0 ml with distilled water, and then centrifuged at 4,000 rpm for about 20 min. A certain volume of the resultant supernatant was diluted with ethanol to obtain concentration within the concentration range of both studied drugs. Then, the general procedure was followed. A blank value was determined by treating the antibiotic-free blood sample in the same manner.

Procedure for Real Human Plasma

Either amikacin (Amikin 500 mg vial) or gentamycin (Garamycin 80 mg® ampoule) was intramuscularly injected into healthy human volunteers, then a 5.0-ml sample of human blood was taken by using calibrated heparinized syringe after 1.5 h for amikacin and 1 h for gentamicin (45), centrifuged at 4,000 rpm for 30 min. Then, into a 10-ml stoppered calibrated tube, 1.0 ml of obtained plasma was treated with 2.0 ml of acetonitrile, and the resultant solution was diluted to 10.0 ml with distilled water. The sample was centrifuged at 4,000 rpm for 20 min. A certain volume of this supernatant was diluted with ethanol to obtain concentration within the concentration ranges for both studied drugs. Then, the general procedure was followed. This process was repeated three times to accomplish intraday and interday assay.

RESULTS AND DISCUSSION

The aim of this work is to establish a simple, sensitive, reliable, selective and cheap spectrofluorimetric method for the analysis of investigated drugs in pure forms, pharmaceutical formulations, spiked and real human plasma. The developed method is based on reaction of investigated drugs with safranin to form a highly fluorescent product which can be measured fluorometrically. Aminoglycosides possess amino group (electron donor) that can form charge transfer complex with safranin (electron acceptor) which is easily extracted with chloroform. In polar solvent, complete electron transfer from the donor to the acceptor moiety takes place with the formation of highly fluorescent complex. The complex exhibits maximum fluorescence intensity at 545–570 nm with excitation

Table II. Analytical Parameters of the Proposed Method for Determination of Investigated Drugs with Safranin

Parameters	Amikacin	Neomycin	Tobramycin	Gentamicin	Kanamycin	Streptomycin
λ_{ex} (nm)	524	523	519	521	523	524
λ_{em} (nm)	570	555	557	545	555	556
Linear range (pg ml^{-1})	4–60	4–50	5–50	4–50	4–50	4–40
Intercept (a)	16.7	14.2	−52.8	−29.8	12.83	−44.5
SE of the intercept (Sa)	5.1	4.8	6.7	4.9	4.6	4.9
Slope (b)	14.1	13.2	14.8	13.6	13.1	13.8
SE of slope (Sb)	0.16	0.18	0.25	0.18	0.17	0.22
SD of residual (Sy.x)	8.7	7.5	10.4	7.8	7.3	6.9
Correlation coeff. (r)	0.9996	0.9995	0.9992	0.9995	0.9995	0.9993
Determination coeff.(r^2)	0.9991	0.9989	0.9984	0.9989	0.9990	0.9987
LOD (pg ml^{-1})	1.2	1.2	1.5	1.2	1.2	1.2
LOQ (pg ml^{-1})	3.6	3.6	4.5	3.6	3.5	3.6

LOD limit of detection (picograms per milliliter), *LOQ* limit of quantitation (picograms per milliliter)

Table III. Evaluation of Accuracy of the Investigated Analytical Procedure at Five Concentration Levels Within the Specified Range

Sample number	Taken pg ml^{-1}	Found[a] pg ml^{-1}	% Recovery	Taken pg ml^{-1}	Found[a] pg ml^{-1}	% Recovery	Taken pg ml^{-1}	Found[a] pg ml^{-1}	% Recovery
	Amikacin			Neomycin			Tobramycin		
1	8	7.9	99.1	8	8.1	100.8	8	8.0	99.8
2	12	12.1	100.7	16	16.1	100.6	20	19.8	99.1
3	16	16.0	99.9	20	19.9	99.6	30	30.1	100.3
4	20	20.0	100.2	30	30.0	100.0	40	40.1	100.3
5	30	29.9	99.7	40	39.91	99.7	50	50.0	100
Mean			99.9			100.1			99.9
SD			0.59			0.52			0.50
RSD			0.59			0.52			0.51
	Gentamicin			Kanamycin			Streptomycin		
1	12	12.1	100.8	8	7.9	99.00	8	8.1	100.6
2	16	16.1	100.4	16	15.8	98.8	12	12.1	100.7
3	20	19.9	99.5	20	20.1	100.5	16	15.9	99.5
4	30	30.0	100.1	30	30.1	100.3	20	20	100
5	40	40.0	100.0	40	39.9	99.8	30	30.1	100.4
Mean			100.2			99.7			100.2
SD			0.46			0.75			0.50
RSD			0.46			0.75			0.50

SD standard deviation, *RSD* relative standard deviation
[a] Mean of three replicate measurements

wavelength at 519–524 nm. Figure 1 shows excitation and emission spectra of reaction product of gentamicin, as representative example for the studied drugs with safranin.

Optimization of Variables

The spectrofluorimetric properties of the fluorescent product as well as the different experimental parameters affecting the development of the charge transfer complex and its stability were carefully studied and optimized. These factors were changed individually, while the others were kept constant. The studied factors include pH, type of buffers, volume of buffer, safranin volume, solvent used for extraction, reaction time and stability of complex.

Effect of pH

In order to select the suitable pH for complex formation, the reaction of safranin with studied drugs was

Table IV. Comparison Between the Proposed Spectrofluorimetric and the Reported Methods for the Determination of the Studied Aminoglycoside Drugs in their Pharmaceutical Dosage Forms

Pharmaceutical dosage forms	Percent recovery±SD[a]		*t* value[c]	*F* value[c]
	Proposed method	Reported method[b] (49–51)		
Amikacin sulfate				
Amikin 100 mg® vials	99.7±1.8	99.1±1.0	0.50	3.2
Neomycin sulfate				
Neomycin 500 mg ® tablets	99.2±0.80	98.7±1.6	0.40	3.8
Tobramycin				
Tobradex ® eye drops	100.2±1.5	102.2±0.8	2.2	3.7
Tobrin ® eye drops	100.8±1.3	100.3±1.5	0.4	1.5
Tobrin ® eye ointment	100.8±0.6	101.4±1.1	0.9	3.6
Gentamicin				
Garamycin ® 80 ampoule	100.3±0.6	99.9±1.1	0.5	3.1
Garamycin ® ointment	101.0±1.2	100.2±1.2	0.8	1.0
Kanamycin				
Diakan M ® syrup	99.8±1.3	99.1±0.9	0.8	2.2
Streptomycin				
Streptomycin ® 1 g vial	99.4±0.3	99.3±0.6	0.189	4.89

[a] Average of five determinations
[b] (49) For amikacin, tobramycin and neomycin, (50) For gentamicin and kanamycin, (51) For streptomycin
[c] Tabulated values at 95% confidence limit are t=2.306, F=6.338

Table V. Evaluation of the Precision of the Proposed Spectrofluorimetric Method

		20 pg ml^{-1}	30 pg ml^{-1}	40 pg ml^{-1}	20 pg ml^{-1}	30 pg ml^{-1}	40 pg ml^{-1}	20 pg ml^{-1}	30 pg ml^{-1}	40 pg ml^{-1}
		Amikacin (% found)[a]			Neomycin (% found) [a]			Tobramycin (% found) [a]		
Intraday	1	101.0	99.2	99.5	100.0	98.7	100.6	99.7	100.2	98.4
	2	99.9	99.6	100.1	99.9	99.3	100.0	99.0	99.9	99.2
	3	99.0	98.8	100.1	98.9	100.2	99.2	98.4	99.1	98.1
	Mean	100.0	99.2	99.9	99.6	99.4	99.9	99.0	99.7	98.6
	SD	1.02	0.39	0.34	0.60	0.73	0.71	0.68	0.56	0.55
	RSD	1.02	0.39	0.34	0.60	0.74	0.71	0.69	0.56	0.55
Interday	1	99.9	100.0	100.1	99.6	100.2	99.3	98.6	99.1	101.3
	2	100.0	99.6	99.9	100.1	100.2	99.2	99.0	99.7	99.8
	3	99.3	99.0	99.8	99.9	100.0	98.3	99.1	99.0	99.0
	Mean	99.7	99.54	99.9	99.8	100.1	98.9	98.9	99.3	100.0
	SD	0.39	0.53	0.19	0.25	0.12	0.51	0.27	0.40	1.17
	RSD	0.39	0.53	0.19	0.25	0.12	0.52	0.27	0.40	1.17
		Gentamicin (% found) [a]			Kanamycin (% found) [a]			Streptomycin (% found) [a]		
Intraday	1	100.6	98.6	101.4	99.25	99.6	99.31	99.7	100.3	101.6
	2	100.7	98.1	100.6	100.75	100.8	97.27	101.4	100.7	100.8
	3	100.1	99.1	99.4	99.40	101.4	97.04	101.0	102.1	99.8
	Mean	100.5	98.6	100.5	99.80	100.6	97.87	100.7	101.0	100.8
	SD	0.31	0.50	1.01	0.826	0.92	1.249	0.90	0.93	0.88
	RSD	0.31	0.50	1.01	0.828	0.91	1.276	0.90	0.92	0.87
Interday	1	100.1	99.9	98.6	101.2	100.8	99.09	100.1	100.4	101.0
	2	100.0	99.1	99.0	101.65	101.0	101.6	99.7	99.2	100.2
	3	99.8	100.9	100.4	100.61	100.5	101.1	98.0	101.8	100.8
	Mean	100.0	100.0	99.3	101.15	100.8	100.6	99.2	100.5	100.6
	SD	0.18	0.89	0.95	0.52	0.26	1.33	1.12	1.30	0.42
	RSD	0.18	0.89	0.96	0.52	0.25	1.32	1.13	1.30	0.42

SD standard deviation, *RSD* relative standard deviation
[a] Mean of three replicate measurements

carried out in different pH (6.5–10). It was found that pH 8±0.1 was considered to be the best pH for the fluorescence development proved by the highest RFI observed relative to other pH values.

Volume of Buffer

The results obtained from optimizing this factor indicated that the maximum fluorescence intensity was attained when

Table VI. Evaluation of the Robustness of the Proposed Spectrofluorimetric Method

	% Recovery[a]±SD					
Variation	Amikacin (20 pg ml^{-1})	Neomycin (20 pg ml^{-1})	Tobramycin (12 pg ml^{-1})	Gentamicin (16 pg ml^{-1})	Kanamycin (12 pg ml^{-1})	Streptomycin (20 pg ml^{-1})
Optimum conditions	99.8±0.8	100.2±1.1	99.0±0.9	99.2±1.1	100.1±1.2	100.0±0.5
	1- PH of buffer solution			pH 8		
pH 7.9	99.3±1.0	100.2±0.8	99.2±0.6	100.1±1.0	99.0±1.3	99.1±0.8
pH 8.1	100.8±1.1	99.9±1.5	98.8±1.0	98.9±1.4	99.7±0.5	100.1±0.8
	2- Buffer volume			0.5 ml		
0.25 ml	99.0±1.4	100.7±1.0	99.0±1.5	99.1±1.1	99.7±1.2	99.5±1.1
0.75 ml	99.3±1.3	99.5±0.8	99.6±0.8	100.0±0.7	99.2±1.4	100.2±0.9
	3- Safranin volume			0.8 ml		
0.6 ml	99.9±1.4	100.3±1.6	99.7±0.9	100.1±1.3	99.9±1.0	100.3±1.1
1.0 ml	98.4±1.7	100.6±1.3	98.8±0.9	99.4±0.9	100.1±1.1	98.9±1.4
	4- Reaction time			2 min		
0 min	98.8±1.0	99.1±0.8	98.4±0.8	99.2±1.5	99.4±0.7	100.1±0.9
5 min	99.4±0.9	99.6±0.8	98.2±1.2	100.2±1.0	100.2±1.4	99.0±0.6

[a] Mean of three replicate measurements

Table VII. Application of the Proposed Method in Determination of Amikacin and Gentamicin in Spiked Human Plasma

Percent recovery[a]			
Concentration (pg ml^{-1})	20 pg ml^{-1}	30 pg ml^{-1}	40 pg ml^{-1}
Amikacin	96.3±0.8	95.9±1.3	95.0±1.5
Concentration (pg ml^{-1})	23.2 pg ml^{-1}	34.8 pg ml^{-1}	46.4 pg ml^{-1}
Gentamicin	98.6±1.3	97.6±1.0	99.0±1.2

[a] Mean of three replicate measurements

using 0.25–1.0 ml of Teorell and Stenhagen buffer. Therefore, further experiments were carried out using 0.5 ml buffer.

Volume of Safranin

The fluorescence intensity was dependent on safranin volume. The higher fluorescence intensity was attained when using 0.6–1 mL safranin (0.004% *w*/*v*). Therefore, further experiments were carried out using 0.8 ml safranin.

Reaction Time

Different time intervals were tested before extraction of complex. It was found that complete fluorescence development was attained after 0–5 min for neomycin, kanamycin and streptomycin; after 0–10 min for amikacin, tobramycin and gentamicin. Therefore, in further experiments, extraction was carried out immediately.

Stability of Complex

The fluorescence of the formed complex was measured at different time intervals. It was found that the fluorescence intensity remained stable for at least 1 h.

Extraction Solvent

In order to select the suitable solvent for complex extraction, the reaction was carried out using different solvents. The studied solvents were chloroform, acetonitrile, ethanol, methanol, methylene chloride and 1,2-dichloroethane. It was found that chloroform was considered to be the best solvent for complex extraction proved by the highest RFI observed compared with other solvents.

Stoichiometry and Mechanism of the Reaction

The stoichiometry of the reaction mechanism was studied through the Job's method of continuous variation (46). The molar ratio of safranin to each of investigated drugs was 1:1. The reaction pathway was proposed in Fig. 2.

Validation of the Proposed Method

Concentration range (47) was established by confirming that the analytical procedure provides a suitable degree of precision, accuracy and linearity when applied to the sample containing amount of analyte within or at the extreme of the specified range of the analytical procedure (44,48). In this work, concentration ranging from 4 to 60 pg ml^{-1} (for amikacin), 4 to 50 pg ml^{-1} (for gentamicin, neomycin and kanamycin), 4 to 40 pg ml^{-1} (for streptomycin) and 5 to 50 pg ml^{-1} (for tobramycin) were studied for the investigated drugs. The whole set of experiments were carried out within this range to ensure the validation of the proposed procedure. Linear calibration graphs were obtained for all the studied drugs by plotting the RFI of the studied drugs *versus* the drug concentration (picograms per milliliter) within the specified range.

Linearity was indicated by high correlation coefficient obtained. The correlation coefficients (r) of the formed products were in the range from 0.9992 to 0.9996 indicating good linearity, as shown in Table II.

Table VIII. Application of the Proposed Method in Real Human Plasma

Drug	Delivery$_{in\ vitro}$ (μg ml^{-1})	Percent recovery$_{in\ vitro}$[a]	Delivery$_{in\ vivo}$ (μg ml^{-1})	Percent recovery$_{in\ vivo}$[a]
(A) Intraday assay				
Amikacin sulfate	19.3	96.3	18.5	92.3
			19.0	95.0
			18.8	94.0
Mean±S.D.				93.8±1.4
Gentamicin sulfate	5.7	98.6	5.1	87.9
			5.2	90.2
			4.9	84.5
Mean±S.D.				87.5±2.9
(B) Interday assay				
Amikacin sulfate	19.5	97.6	18.30	91.5
			18.80	94.0
			18.62	93.1
Mean±S.D.				92.9±1.3
Gentamicin sulfate	5.7	97.8	4.8	82.6
			4.7	80.9
			4.9	84.5
Mean±S.D.				82.7±1.8

[a] Mean of three replicate measurements

Accuracy (44)was checked at five concentration levels within the specified range. Three replicate measurements were recorded at each concentration level. The results were recorded as percent recovery±standard deviation as shown in Table III. The results obtained show the close agreement between the measured and true values. Meanwhile, comparison of the obtained results from the analysis of the drug products by the proposed procedure with those obtained from the reported methods (49–51) revealed that there is no significant difference between them with respect to accuracy as indicated by *t* and *F* tests as shown in Table IV.

Precision (44) was checked at three concentration levels; three replicate measurements were recorded at each concentration level; the results were summarized in Table V. The calculated relative standard deviations were below 2.2% indicating excellent precision of the proposed procedure at both level of repeatability and intermediate precision

Limit of detection (LOD) and limit of quantitation (LOQ) (47) were calculated based on standard deviation of response and the slope of calibration curve (48). The limit of detection and limit of quantitation (44) were expressed as**:** LOD=$3\sigma/S$, LOQ=$10\sigma/S$, respectively.

Where σ is the standard deviation of intercept and S is the slope between measured and predicted concentration.

The results were summarized in Table II. The calculated detection limits for all the studied drugs were less than 1.486 pg ml^{-1}, while the calculated quantitation limits for all the studied drugs were less than 4.5 pg ml^{-1} indicating high sensitivity of the proposed method.

ROBUSTNESS

Robustness of the procedure was assessed by evaluating the influence of small variation in experimental variables on the analytical performance of the method (pH of buffer solution, volume of buffer, safranin volume and reaction time). The small variations in any of the variables did not significantly affect the results (Table VI). This gave an indication for the reliability of the proposed method during routine work.

Application to Pharmaceutical Dosage Forms

The proposed method was applied for determination of investigated drugs in commercial pharmaceutical dosage forms. The results were statistically compared with those of reported methods (49–51), in respect to accuracy and precision. The obtained mean recovery values were 98.73–102.23±0.265–1.83%, as shown in Table IV. According to *t* and *F* tests, no significant difference was found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicates good level of precision and accuracy.

Application to Spiked Human Plasma

The high sensitivity attained by the proposed method allowed the possible determination of amikacin and gentamicin in spiked human plasma. The concentration of both studied drugs was computed from their corresponding regression equations. The obtained mean recovery values of the obtained amount were 95.02–99.02±0.819–1.45%, as shown in Table VII, indicating the possibility of the analysis of investigated drugs in real human plasma using the proposed method.

Analysis of Cited Drugs in Real Human Plasma

Aminoglycosides are excreted as intact form in the urine. They are neither metabolized nor eliminated in the bile (52).

Amikacin is absorbed rapidly after intramuscular injection of 500 mg producing a mean peak plasma concentration of 20 mg/L in 1.5 h (45).

A single IM dose of 1 mg/kg of gentamicin produced a mean peak plasma concentration of 5.8 mg/L in 0.5 to 1 h (45).

So, percent recovery of the investigated drugs in plasma was calculated by using the following equation (53).

$$\text{Percent recovery } \textit{in vivo} = \text{delivery } \textit{in vivo} \times \text{percent recovery } \textit{in vitro}/\text{delivery } \textit{in vitro}$$

Where:

Percent recovery *in vivo* is the percentage of recovery for drug in real human sample.

Delivery *in vivo* is concentration of the drug in real human sample.

Percent recovery *in vitro* is percentage of recovery for drug in spiked human sample.

Delivery *in vitro* is the concentration of the drug in spiked human sample.

Percent recoveries after application of the proposed method in real human plasma sample by intra- and interday assay were shown in Table VIII.

CONCLUSION

In conclusion, the present study described a simple, rapid, accurate, reliable and validated spectrofluorimetric method for the analysis of certain aminoglycosides in their dosage forms. Moreover, it could be applied in quality control analysis of these drugs owing to their improved simplicity, sensitivity, low cost and their independence on expensive instruments, or critical analytical reagents. In addition, the proposed method is a highly sensitive method where it determines these drugs in picograms per milliliter. Therefore, it determines amikacin and gentamicin in real human plasma.

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