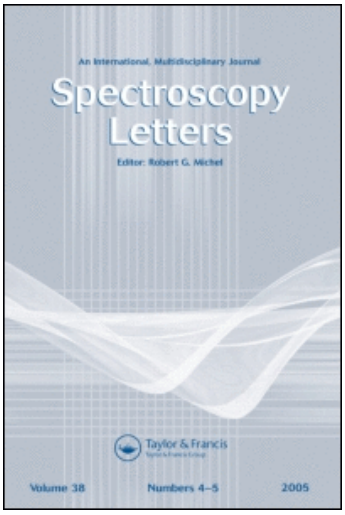

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FLUORIMETRIC DETERMINATION OF AMINOGLYCOSIDE ANTIBIOTICS IN PHARMACEUTICAL PREPARATIONS AND BIOLOGICAL FLUIDS

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FLUORIMETRIC DETERMINATION OF AMINOGLYCOSIDE ANTIBIOTICS IN PHARMACEUTICAL PREPARATIONS AND BIOLOGICAL FLUIDS

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ABSTRACT

2-Hydroxyl-1-naphthaldehyde has been utilized as a fluorigenic reagent for the determination of four aminoglycoside antibiotics namely: neomycin, tobramycin, amikacin and kanamycin, in pure form, in some pharmaceutical preparations and in biological fluids. The method is based on the reaction of the reagent with aminoglycosides through their amino groups. The method is thus selective for those aminoglycosides which contain primary amino groups(s). The reaction products exhibit a maximum fluorescence intensity at 434 nm after excitation at 366 nm. A linear relationship was found between the fluorescence intensity and concentration over the range, 0.5 to 5 ug/mL for neomycin and tobramycin, and 0.25 to 4 ug/mL for amikacin and kanamycin. Percentage recoveries range from 99.67% to 100.26% with standard deviations ranging from ± 1.32 to ± 1.69, and a limit of detection ($S/N = 2$) of 10 ng/mL for all drugs. The optimum reaction conditions were studied and the results obtained

compared favorably with a published fluorimetric method using fluorescamine reagent.

Key Words: Fluorimetry; Aminoglycoside antibiotics; 2-Hydroxyl-1-naphthaldehyde; Pharmaceutical analysis; Biological fluids

INTRODUCTION

Aminoglycosides are one of the most important broad spectrum antibiotics[1]. They are active against Gram positive and Gram negative bacteria. Due to the wide application of aminoglycosides for human uses and in poultries, their determination in pharmaceuticals and in biological fluids is an important goal for pharmacists and chemists. Numerous proecedure are available for their determination such as spectrophotometric [2,3], densitometric[4], fluorimetric[5,6], polarographic[7], gas chromatographic[8], radioimmunoassay[9], HPLC[10,11], MS[12], capillary electrophoresis[13] and NMR[14]. Many of these method have been reviewed[15–17]. The official methods usually involve microbiological procedures[18,19].

2-Hydroxyl-1-naphthaldehyde (HNA) has been successively used as a sensitive chromogenic and fluorogenic reagent for the determination of hydrazine derivatives [20–24].

The aim of this work is to study the reaction of HNA with aminoglycosides is an attempt to develop a sensitive method for the determination of this important class of antibiotics in pharmaceutical preparations and biological fluids.

EXPERIMENTAL

Apparatus

An amino-Browman model J_4-8969 spectrogluorimeter was used with the excitation and emission slit controls set at 5 nm. The measurements were performed using a 1-cm quartz cell.

Materials and Reagents

Neomycin sulphate, tobramycin sulphate, amikacin sulphate and kanamycin sulphate were obtained from Sigma Chemical Company. The 2-Hydroxyl-1-naphthaldehyde was obtained from Aldrich Chemical Company.

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All chemicals were of Analytical Reagent grade. The following solutions were prepared:

1) Stock solutions of aminoglycosides were freshly prepared as 1 mg.mL in deionized water, then serially diluted as appropriate.
2) 2-Hydroxyl-1-naphthaldehyde solution (5 mg HNA in 100 mL) was prepared in acetone; the solution was kept in a refrigerator.
3) Borate buffer was prepared by adjusting 0.2 M boric acid solution with 0.4 M sodium hydroxide solution to the required pH.
4) Trichloroacetic acid (10% w/v) aqueous solution.

PROCEDURE

Calibration

An aliquot volume of standard aminoglycosides antibiotic solution equivalent to 5–50 ug neomycin sulphate and tobramycin sulphate; 2.5–40 ug amikacin sulphate and kanamycin sulphate was transferred into a 10 mL calibrated flask. 1 mL of HNA solution and and 5 mL of borate buffer solution of pH 6.3 ± 0.2 were added. The solution was heated in a water bath at 80°C for 30 minutes, cooled and brought to volume with the same buffer solution. The relative fluorescence intensity was measured at 434 nm (excitation at 366 nm). Calibration graphs were constructed. Alternatively, the regression equations were calculated.

Applications of the Proposed Method

For Injections

An accurate measured volume of the mixed contents of 5 vials, or ampoules, equivalent to 10 mg of drug was transferred into a 100 calibrated flask then brought to volume with deionized water. 10 mL of this solution was transferred to another 100 mL calibrated flask and brought to volume with deionized water. 2 mL of this solution was assayed as described under calibrated lines.

For Tablets

Weigh and pulverize twenty tablets. To a quantity of powder equivalent to 10 mg add 10 mL of deionized water then sonicate for 20 minutes

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filter into a 100 mL calibrated flask, wash the powder three times each with 5 mL of water. The combined filtrates were mixed well and brought to volume with deionized water. Complete as described under calibration lines.

Assay of Drugs in Spiked Human Serum

To 5 mL of human serum add a solution of the studied compounds within the concentration range cited in Table 1. Add 5 mL of 10% (w/v) trichloroacetic acid for deproteination. Blend on a vortex mixer and centrifuge at 3000 rpm at 15 minutes transfer 1 mL of the protein free supernatant into a 10 mL calibrated flask and bring to volume with deionized water. Complete the procedure as described under calibration line.

Assay of Drugs in Spiked Human Urine

To 5 mL of human urine add a solution of one of the studied compounds within the concentration range cited in Table 1. Add 5 mL of methanol then blend on a vortex and centrifuge at 1500 rpm for 3 minutes. Transfer 1 mL of the supernatant into a 10 mL calibrated flask and bring to the volume with deionized water. Complete the procedure as described under calibration line.

RESULTS AND DISCUSSION

The aminoglycosides possessing primary amino group were found to react with HNA to produce highly fluorescent products (Fig. 1). The

Table 1. Regression Data, Correlation Coefficients, and Concentration Ranges of Aminoglycosides Antibiotics by the Proposed Method

Aminoglycoside Sulphate Salt	Regression Data*			Conc Range Ug/mL
	a	b	r	
Neomycin	−0.2838	19.9806	0.9994	0.50–5
Tobramycin	−0.8898	18.8370	0.9999	0.25–4
Amikacin	0.0679	21.8617	0.9998	0.25–4
Kanamycin	0.8285	20.5676	0.9997	0.25–4

Where a is the intercept, b is the slope, r is the correlation coefficient.
*Average of at least seven triplicate determinations within the concentration range.

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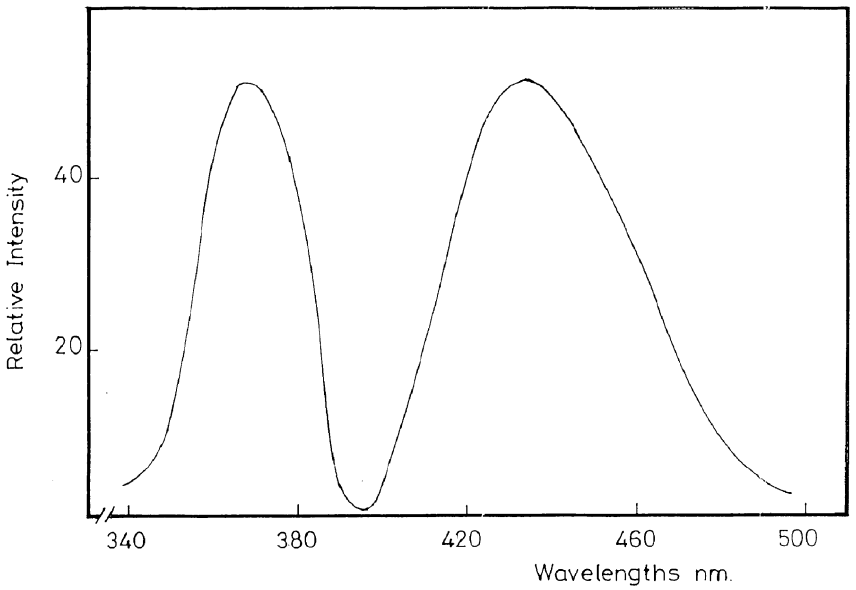

Figure 1. Fluorescence spectrum of neomycin-2-hydroxy-1-napthaldehyde reaction products (2.5 $\mu g \cdot ml^{-1}$).

products are proposed to be Schiff's base. Studying the experimental factors affecting the reaction revealed that 1 mL of the reagent solution in a total volume of 10 mL gave the maximum fluorescence intensity (Fig. 2). Increasing the reagent concentration decreased the fluorescence intensity probably due to the quenching effect of the reagent.

Effect of Buffer and pH

Figure 3 shows that the reaction of aminoglycosides with HNA is pH dependent and must be controlled between pH 6 and 6.5 using borate buffer. Acidic buffers like acetate, phosphate, MOPS, MES and THAM buffer systems at the same pH value produced a little fluorescence intensity, so, they were not suitable in such study. At pH values below 4.5 a sudden decrease in the fluorescence intensity was observed due to competition between the proton and aldehyde group of the reagent to the amino group of the aminoglycoside and consequent decrease of the formation of Schiff's bases. At pH values above 8 a similar decrease in the intensity was also observed, the same observation was also reported with hydrazines [19–24].

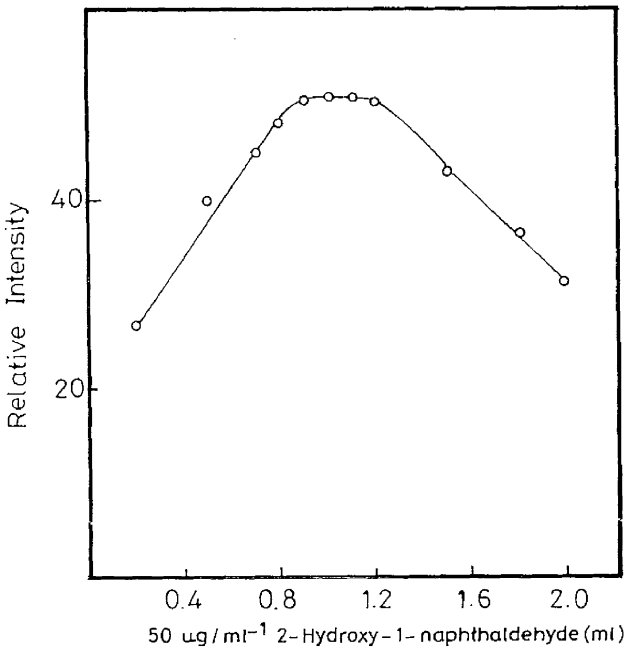

Figure 2. Effect of 2-hydorxy-1-napthaldehyde on the reaction product of 2.5 $\mu g \cdot ml^{-1}$ neomycin sulphate.

Effect of Time, Temperature, and Light

Stable fluorescence intensity readings were observed after heating in a water bath at 80°C for 20 minutes. The reaction products formed between HNA and aminoglycoside were stable for at least 24 hrs. Light has no effect of fluorescence intensity and stability of the reaction products.

Effect of Ionic Strength

Ionic strength is a factor that sometimes cannot be neglected. The effect of ionic strength on the fluorescence intensity was studied by varying the concentration of the background electrolyte, sodium chloride. The choice of sodium chloride was based on the fact that Na^+ ion is very weakly bound, if at all, to the aminoglycoside binding sites, and Cl^{-1} ion acts as a counter ion. Three different concentrations corresponding to 0.1, 0.1 and 0.1 M sodium chloride were added as background electrolyte. The addition of

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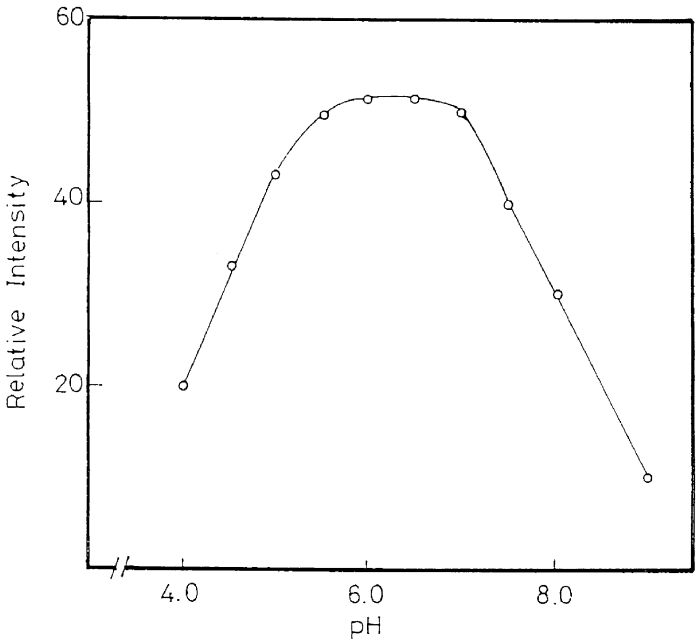

Figure 3. Effect of pH on the fluorescence intensity of the reaction products of 2.5 $\mu g \cdot ml^{-1}$ neomycin sulphate.

background electrolyte was found to have no effect on the fluorescence intensity of reaction products.

PERFORMANCE CHARACTERISTICS

Table 1 shows regression data, correlation coefficients, and concentration ranges of aminoglycosides using the proposed method. As compared with conventional fluorescent probes using fluorescamine, the proposed method offers several advantages. It is highly selective for the determination of aminoglycosides that have a free NH_2 group. In addition, the reaction products have an exceptionally wide Stok's shift, over 70 nm while with fluorescamine it is about 50 nm.

Table 2 shows the assay results of aminoglycosides by the proposed method and fluorescamine fluorimetric methods[5] as a reference one. The proposed method, when compared to the official microbial assays, offers advantages with regards to rapidity, simplicity and precision, so, these

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Table 2. Assay Results of Aminoglycoside Antibiotics in Bulk Using the Proposed Method and a Reference Method

Aminoglycoside Sulphate Salt	% Recovery*, ± SD Proposed Method	Reference Method**
Neomycin	100.26 ± 1.54	100.04 ± 1.48
	$t = 0.731\ (2.179)^{+}$	$F = 1.079\ (4.28)^{+}$
Tobramycin	99.67 ± 1.69	99.68 ± 2.28
	$t = 0.005\ (2.179)^{+}$	$F = 1.820\ (4.28)^{+}$
Amikacin	100.09 ± 1.45	97.82 ± 2.27
	$t = 1.060\ (2.179)^{+}$	$F = 3.366\ (4.28)^{+}$
Kanamycin	100.10 ± 1.32	99.14 ± 2.49
	$t = 0.482\ (2.179)^{+}$	$F = 3.558\ (4.28)^{+}$

* Average of at least seven triplicate determinations.
** Fluorometric determination of aminoglycosides using fluorescamine[5].
$^{+}$ Values in parenthesis are theoretical values of t and F at $P = 0.05$.

results were compared with the fluorimetric method using fluorescamine. The calculated student's t test and Variance ratio F test were less than the respective tabulated ones at $p = 0.05$, indicating that the results obtained by the proposed method is in good agreement with those obtained by the reference fluorimetric method. Regarding the sensitivity the proposed procedure is as sensitive as the fluorescamine fluorimetric method. However the proposed method is simpler, and the reaction products are more stable than the conventional fluorimetric methods.

Results of the determination of aminoglycosides in different dosage forms by both the proposed method and the fluorescamine fluorimetric method are presented in Table 3. Ingredients other than aminoglycosides such as additives, stablilizers and antioxidants of tablets and ampoules did not interfere with the recommended method.

Aminoglycosides are poorly absorbed from gastrointestinal tract and are excreted through the kidney. These drugs are mainly administered by intravenous and intramuscular routes. Oral administration of 3 gm of neomycin produces peak plasma concentration of 1–4 ug/mL. The peak plasma concentrations of other aminoglycosides ranged from 5–20 ug/mL after administration of the recommended therapeutic doses[26]. Such plasma concentrations of aminoglycosides are within the determinable concentration ranges. The proposed procedure was applied for the analysis of human serum and urine samples spiked with the neomycin as a model example. It is found that percentage recoveries were 94.34 and 96.72 with standard

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Table 3. Assay Results of Aminoglycoside Antibiotics in Some Pharmaceutical Preparations by the Proposed Method and a Reference Method[5]

Drug	% Recovery*, ±SD Proposed Method	Reference Method**
Neomycin Tablets 500 mg	100.37 ± 0.56	99.49 ± 1.18
(Memphis Co., Egypt)	$t = 0.953$ $(3.306)^+$	$F = 4.433$ $(6.39)^+$
Nebcin Amp. 20 mg Tobramycin	101.87 ± 1.07	100.03 ± 1.93
(Lily France S.A. France)	$t = 1.348$ $(3.306)^+$	$F = 3.253$ $(6.39)^+$
Amikin Amp. 1 g Amikacin	99.79 ± 1.21	99.75 ± 0.91
(Bristol Co., USA)	$t = 0.037$ $(3.306)^+$	$F = 1.768$ $(6.39)^+$
Kanamycin vial, 1 g per vial	100.92 ± 0.86	98.92 ± 1.53
(Misr Co., Egypt)	$t = 1.612$ $(3.306)^+$	$F = 3.165$ $(6.39)^+$

* Average of at least three triplicate determinations, calculated relative to nominal content.
** Fluorometric determination of aminoglycosides using fluorescamine[5].
$^+$ Values in parenthesis are theoretical values of t and F at $P = 0.05$.

deviations of ± 1.89 to ± 2.63, respectively for the human serum and urine sample respectively. Moreover, no interferences arose from endogenous compounds. Thus, the proposed procedure can be used for routine analysis and quality control of aminoglycosides in pharmaceutical preparations and in spiked biological fluids.

CONCLUSION

From the foregoing discussion fluorimetric technique using 2-Hydroxy-naphthaldehyde succeeded for the determination of four aminoglycosides in pure form, in pharmaceutical preparations and in biological fluids. Urine, blood contents and other additives like DTA, sodium citrate, phenol and other injection and tablet additives did not interfere with the assay. The method is selective, highly sensitive with reasonable accuracy and precision.

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