Quantitation of Amikacin, Kanamycin, Neomycin, and Tobramycin in Pharmaceutical Dosage Forms Using the Hantzsch Reaction

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Abstract □ A spectrophotometric assay method for the quantitative determination of amikacin, kanamycin, neomycin, and tobramycin in pharmaceutical dosage forms has been developed. The method is based on the Hantzsch reaction, forming dihydrolutidine derivatives which can be measured spectrophotometrically. The excipients EDTA, phenol, sodium bisulfite, and sodium citrate do not interfere, while salts of ammonia do interfere. The relative standard deviations based on seven readings were 1.64, 1.88, 2.10, and 1.93% for amikacin, kanamycin, neomycin, and tobramycin, respectively. Assay results have been compared with microbiological assay results provided by the manufacturers. The assay method appears to be stability indicating.

Keyphrases □ Amikacin—quantitation in pharmaceutical dosage forms using the Hantzsch reaction, kanamycin, neomycin, tobramycin □ Kanamycin—quantitation in pharmaceutical dosage forms using the Hantzsch reaction, amikacin, neomycin, tobramycin □ Neomycin—quantitation in pharmaceutical dosage forms using the Hantzsch reaction, amikacin, kanamycin, tobramycin □ Tobramycin—quantitation in pharmaceutical dosage forms using the Hantzsch reaction, amikacin, kanamycin, neomycin

The aminoglycoside antibiotics, amikacin sulfate (I), kanamycin sulfate (II), neomycin sulfate (III), and tobramycin sulfate (IV) are usually quantified by the microbiological procedures established by the Food and Drug Administration (1). The high experimental errors inherent in microbiological assays have been reported previously (2). A spectrophotometric assay technique for I using purified kanamycin acetyltransferase has been reported (3). In addition, a radioimmunoassay procedure for I has been developed (4) and compared with the microbiological assay (5). A previously described spectrofluorometric method (6) involves the purification of I–IV in urine using ion-exchange chromatography followed by formation of the dehydrolutidine derivative. Recently, this methodology, involving the formation of the dehydrolutidine derivative has been extended to provide a colorimetric assay for neomycin sulfate in urine (7). This study reports a spectrophotometric assay technique for the quantitative of amikacin, kanamycin, neomycin, and tobramycin in pharmaceutical dosage forms, based on the formation of the dehydrolutidine derivative (Hantzsch reaction) (7).

EXPERIMENTAL

Chemicals and Reagents—All chemicals and reagents were USP, NF, or ACS quality and were used without further purification. Amikacin sulfate[1], kanamycin sulfate[1], neomycin sulfate[2], and tobramycin sulfate[3] powders were used as received.

Preparation of Stock and Standard Solutions—Stock solutions of I–IV containing 50.0, 40.0, 60.0, 40.0 mg/100 ml (as the free base) in water, respectively, were prepared daily. These stock solutions were diluted further with water to obtain standard solutions as needed. The usual concentrations of I–IV standard solutions were 50.0, 40.0, 60.0, 40.0 μg/ml, respectively.

Preparation of Assay Solutions—All commercial injectable formulations were diluted stepwise with water to obtain antibiotic concentrations similar to the standard solutions. Ten tablets/capsules were weighed, and the tablets or capsule contents were ground to a fine powder. An appropriate quantity of the powder (containing 40–60 mg of the antibiotic) was weighed, mixed with 40 ml of water, brought to volume (100 ml) with water, and mixed thoroughly. The mixture was filtered, rejecting the first 20 ml of the filtrate, and a portion of clear filtrate was diluted further to an appropriate concentration (similar to standard solutions).

Preparation of a Solution of Acetylacetone and Formaldehyde—A buffer solution containing 0.2*M* each of acetic acid, boric acid, and phosphoric acid in water was prepared. The pH of this solution was adjusted to 2.5 (±0.05) with ~1 *N* NaOH, measured by a pH-meter[4]. To a 10-ml portion of the buffer, 0.8 ml of acetylacetone and 1.5 ml of formaldehyde solution (40% in water) were added; the mixture was brought to 30 ml with water and thoroughly mixed. The buffer solution was stable for at least 30 days at room temperature. The reagent was prepared daily.

Assay Procedure—A 2-ml quantity of standard/assay solution was mixed with a 2-ml portion of the acetylacetone–formaldehyde solution in a glass tube. The tube was sealed, placed in a boiling water bath for 20 min, cooled to room temperature, and the contents mixed with 5 ml of water. The absorbance of the solution was measured at 356 nm using a spectrophotometer[5]. A reagent blank was prepared by substituting water for the antibiotic solution.

Calculations—Concentrations were calculated by comparing the absorbance value of the assay solution with that of a standard solution of identical concentration. Since Beer's law (7) was followed:

$$\frac{(A_a)}{(A_s)} \times \text{Actual concentration of the standard solution} = \text{concentration of assay solution found}$$

where (A_a) is the absorbance of the assay solution and (A_s) the absorbance of the standard solution.

Determination of Excipient Interference—To determine the possibility of interference from inactive ingredients, the above assay procedure was followed substituting the inactive ingredient(s) for the antibiotic.

Selectivity of the Method—To determine if the method was stability indicating, solutions of amikacin (50 μg/ml) and tobramycin (40 μg/ml) were mixed with a 250-μg/ml concentration of carbenicillin disodium. Aminoglycoside antibiotics are known to be unstable in the presence of carbenicillin and other penicillins (8). The mixtures of aminoglycoside antibiotics with carbenicillin were stored at 50° and reassayed after 48 hr. Solutions of amikacin, tobramycin, and carbenicillin disodium alone were also stored at 50° for 48 hr and reassayed as controls. The results were calculated as described above, except that the absorbance values were corrected for a slight interference from carbenicillin disodium, measured using a carbenicillin blank.

[1] Supplied by Bristol-Myers Laboratories, Rochester, N.Y.
[2] The Upjohn Co., Kalamazoo, Mich.
[3] Supplied by Eli Lilly and Co., Indianapolis, Ind.
[4] Beckman Zeromatic, SS-3.
[5] Bausch and Lomb Spectronic 20.

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Table I—Assay Results of Various Dosage Forms

Dosage Form	Label Claim, mg/ml	Antibiotic	Potency Found, % (Based on the Label Claim) Developed Method[a]	Microbiological Method[b]
Injectable				
Lot 1	250	Amikacin	103.7	101.2
Lot 2	250	Amikacin	108.1	104.8
Lot 3	250	Amikacin	104.1	103.6
Lot 4	250	Amikacin	100.6	104.0
Injectable	50	Amikacin	101.7	101.6
Injectable	333.3	Kanamycin	103.6	105.3
Injectable	37.5	Kanamycin	104.2	104.0
Capsule	500	Kanamycin	103.4	101.0
Tablet	350	Neomycin	99.6	100.9
Injectable				
Lot 1	40	Tobramycin	106.3	108.8
Lot 2	40	Tobramycin	104.2	107.5

[a] The relative standard deviations based on seven readings were 1.64, 1.88, 2.1, and 1.93% for I–IV, respectively. [b] As provided by the manufacturers.

RESULTS AND DISCUSSION

The results indicate (Table I) that the method can be used for the quantitation of amikacin, kanamycin, neomycin, and tobramycin in pharmaceutical dosage forms. The relative standard deviations based on seven readings were 1.64, 1.88, 2.10, and 1.93% for amikacin, kanamycin, neomycin, and tobramycin, respectively. It is well known that standard deviations are usually very high when microbiological assay techniques are used.

A 20-min reaction time was determined to be optimal for the completion of the reaction. The reaction usually starts 5–6 min, after the mixture reaches the water bath temperature. The results are highly reproducible and precise (see Table I for standard deviations) when the standard solution is assayed simultaneously with each sample determination. The excipients present in the injectable formulations [EDTA (ethylenediaminetetraacetic acid), phenol, sodium bisulfite, and sodium citrate] did not interfere with the assay procedure (Table II). Also, methylparaben and propylparaben, which are present in injectable formulations of some other aminoglycoside antibiotics, showed no interference (Table II), while salts of ammonia did interfere with the assay procedure (Table II).

Using the recommended wavelength of 356 nm (7) provided a low blank reading and high absorbance value for the dihydrolutidine derivatives. The mechanism of the reaction has been postulated (6). It has been determined that it is not necessary to add all the buffering agents (acetic acid, boric acid, and phosphoric acid) to the solution as recommended (7). Either boric acid or phosphoric acid (0.6 *M*) can be used at pH 2.5. The color that developed was stable for at least 20 min.

Aminoglycoside antibiotics contain amino groups, which react with acetylacetone to form dihydrolutidine derivatives. The method developed appears to be stability indicating (Table III) when applied to the interaction of aminoglycoside antibiotics with penicillins. Tobramycin assayed for only 47.2% of the original after 48 hr of storage at 50° in the presence of carbenicillin disodium, and amikacin assayed for 73.5% in a similar solution. These results are in agreement with an earlier report (8) in which tobramycin was reported to be less stable in the presence of carbenicillin disodium than other aminoglycoside antibiotics, *i.e.*, gentamicin and netilmicin. Tobramycin is also less stable than amikacin in the presence of carbenicillin.

Table II—Effect of Excipients on Absorbance

Inactive Ingredient	Concentration in Solution[a], μg/ml	Absorbance Value Against Blank
EDTA	2.5	0.00
Methyparaben	3.6	0.00
Propylparaben	0.4	0.00
Phenol	10	0.00
Sodium bisulfite	14	0.00
Sodium citrate	5	0.00
Ammonium chloride	14	0.14

[a] These are at least 2 times the concentrations expected in the assay solutions except ammonium chloride, which is not usually added to the dosage forms of these antibiotics.

Table III—Assay Results of Solutions Stored at 50°

Solution Composition	Assay Results (Percent of Label Claim) 0 hr	48 hr
Amikacin (50 μg/ml)	100.5	100.1
Tobramycin (40 μg/ml)	99.8	100.4
Carbenicillin disodium (250 μg/ml)	19.8[a]	14.3[a]
Amikacin (50 μg/ml) and carbenicillin disodium (250 μg/ml)	97.4[b]	73.5[b]
Tobramycin (50 μg/ml) and carbenicillin disodium (250 μg/ml)	97.1[b]	47.2[b]

[a] When assayed as tobramycin, *i.e.*, the assay absorbance value was compared with the absorbance value from a standard solution of tobramycin (40 μg/ml). [b] After correcting for interference from carbenicillin disodium.

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