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RESEARCH ARTICLE……!!!
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NEW, SIMPLE AND VALIDATED SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF AMIKACIN IN BIOLOGICAL SAMPLES AND ITS PHARMACEUTICAL FORMULATIONS

Hany A. Omara[a*] and Alaa S. Amin[b]

[a] Chemistry Department, Faculty of Science, Sirt University, Sirt, Libya.

[b] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt.

KEYWORDS:

Amikacin sulphate, Ninhydrin, Spectrophotometric, Spiked human plasma, Urine, Pharmaceutical analysis.

For Correspondence:

Hany A. Omara*

Address:

Chemistry Department, Faculty of Science, Sirt University, Sirt, Libya.

ABSTRACT

A simple and fast spectrophotometric procedure has been developed for the determination of amikacin sulphate (AMK). The method is based on the interaction of ninhydrin in *N*,*N*′-dimethylformamide (DMF) medium, with primary amines present in amikacin. This reaction produced a blue-purple product which absorbed maximally at 590 nm. The effects of variables such as reagent concentration and reaction time were investigated to optimize the experimental conditions. Beer's law is obeyed in the concentration range of 1.0 - 21 µg/ml with RSD of 0.463% and molar absorptivity of 3.33 x 10^4 l/mol.cm. The proposed method has been applied successfully to the analysis of the bulk drug and its dosage forms and spiked human plasma. No interference was observed from common pharmaceutical adjuvant. Statistical comparison of the results with the reference method shows excellent agreement and indicates no significant difference in accuracy and precision.

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INTRODUCTION:

Amikacin sulphate ($C_{22}H_{43}N_5O_{13}$. $2H_2SO_4$) is an amino glycoside antibiotic used to treat different types of bacterial infections. Amikacin works by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth.

Amikacin is most often used for treating severe, hospital-acquired infections with multi-drug resistant gram negative bacteria such as Pseudomonas Aeruginosa, and Enterobacter. Amikacin is used in the systemic treatment of serious infections, but also locally for the treatment of skin infections [1]. Amikacin may be combined with a beta-lactam antibiotic for empiric therapy for people with Neutropenia and fever.

The analytical methods reported for amikacin sulphate include HPLC [2-5], liquid chromatography [6], volumetric method [7], high-performance capillary electrophoresis with fluorescence detection [8], Chemiluminescence's [9], long-wavelength fluorimetry [10] and capillary zone electrophoresis [11].

Flow-injection stopped-flow kinetic spectrophotometric methods [12], fluorimetric [13] and spectrophotometric methods [14], have been reported for its determination. These methods were sophisticated to perform and/or time consuming.

Spectrophotometry is considered as the most convenient analytical technique in pharmaceutical analysis because of its inherent simplicity and availability in most quality control and clinical laboratories [15-18].

Ninhydrin (2, 2-Dihydroxyindane-1,3-dione) is a chemical used to detect ammonia or primary and secondary amines. When reacting with these free amines, a deep blue or purple color known as Ruhemann's purple is evolved [19].

Ninhydrin is known to yield a complex, which are applied in the determination of many pharmaceutical compounds [20-25].

The present work aims to develop a simple, rapid and sensitive method for the determination of AMK in pure form and in their pharmaceutical preparations and can be used for the quality control and assurance of these drugs in industry.

EXPERIMENTAL:

Apparatus:

All the spectral measurements were made using either Perkin Elmer Lambda 12 and Perkin Elmer 73B spectrophotometers, with scanning speed 400 nm/min and band width 2.0 nm, equipped with 10 mm matched quartz cells. A thermostat water bath, JOUAN, J18 Bain Universal (France) was used to carry out the temperature studies. A centrifuge Model 90-1 with speed 50000 rpm (USA) was used to carry out for the spiked plasma samples.

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Reagents and materials

All chemicals used were of analytical or pharmacopoeia grade purity and doubly distilled water were used. Standard amikacin sulphate (AMK) was obtained from Egyptian Organization for Control and Pharmaceutical Research – Cairo, Egypt. Standard solution 100 µg/ml of (AMK) was prepared by dissolving pure drug in the least amount of bidistilled water and made up to 100 ml in measuring flask with bidistilled water. The solution was remained stable for two months when kept refrigerated.

The ninhydrin was obtained from E. Merck Darmstdt F. R. Germany. Stock solution of ninhydrin (5.0×10^{-3} M), was prepared in N, N' - dimethylformamide (DMF) and further diluted according to the need with DMF.

Recommended procedures

A volume of the drug 0.5 – 1.5 ml (100 µg/ml dissolved in bidistilled water) was pipetted into a series of boiling test tubes. To each test tube 0.5 ml (5.0×10^{-3} M) of ninhydrin solution (which prepared in DMF) was added, mixed well and heated on a water bath at 100 ± 1 ^{o}C for 5 min. After heating the solution, tubes were cooled to room temperature. The content of the tube was transferred to a 10-ml volumetric flask and diluting to volume with DMF. The absorbance of the complex product was measured at the recommended λ_{max} 590 nm, against a reagent blank prepared in the same manner without addition of the drug.

Determination of amikacin in injections

Three vials injections were carefully evacuated; their contents were measured. An accurately volume quantity of the vials contents equivalent to 125 and 500 mg of AMK was transferred into a 100 ml calibrated flask, and dissolved in about 40 ml of distilled water, and then completed to volume with water. The prepared solution was diluted quantitatively with distilled water to obtain a suitable concentration for the analysis.

Procedure for spiked plasma and urine samples

Aliquots of 1.0 ml of plasma and/or urine were spiked with different concentration levels of AMK. The spiked samples were treated with 0.1 ml of 70 % perchloric acid and vortexed for 1.0 min. The samples were centrifuged for 20 min at 13000 rpm. The supernatants were transferred carefully into test tubes and neutralized with 0.2 ml of 1.0 M NaOH solution, then treated as described above under the recommended procedure. A blank value was determined by treating drug-free plasma and/or urine in the same way. The absolute recovery was determined by comparing the representative absorbance of samples with the absorbance of the standard drug at the same concentration.

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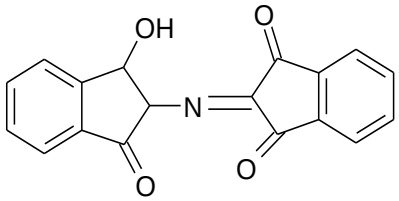
RESULTS AND DISCUSSION

The use of ninhydrin for the detection and quantitative estimation of amino acids and imino acids depends on the formation of Ruhemann's purple [26]. It was reported that in alkaline medium ninhydrin is converted to o-carboxyphenylglyoxal which would reduce ninhydrin to 2-hydroxyindan-1,3-dione. The primary amino group of AMK reacted with 2- hydroxyindan-1,3-dione in alkaline medium to form the amino compound which condensed with ninhydrin to give diketohydrindylidene-diketohydrindamine (Scheme 1), which interacts with amino group of the drug resulting in the formation of a blue colored product (Ruhemann's purple) which absorbed maximally at 590 nm.

Several parameters such as heating time and reagent concentration were optimized to achieve high sensitivity, stability, low blank reading and reproducible results.



Scheme 1. diketohydrindylidene-diketohydrindamine

Effect of heating time

The optimum reaction time was determined by heating the reaction mixture on water bath at 100 ± 1 ^{o}C. It is apparent from Figure 1, that complete color development was attained after 5 min of heating and remained constant up to 10 min. Therefore, the optimum heating time was fixed at about 6 min throughout the experiment.

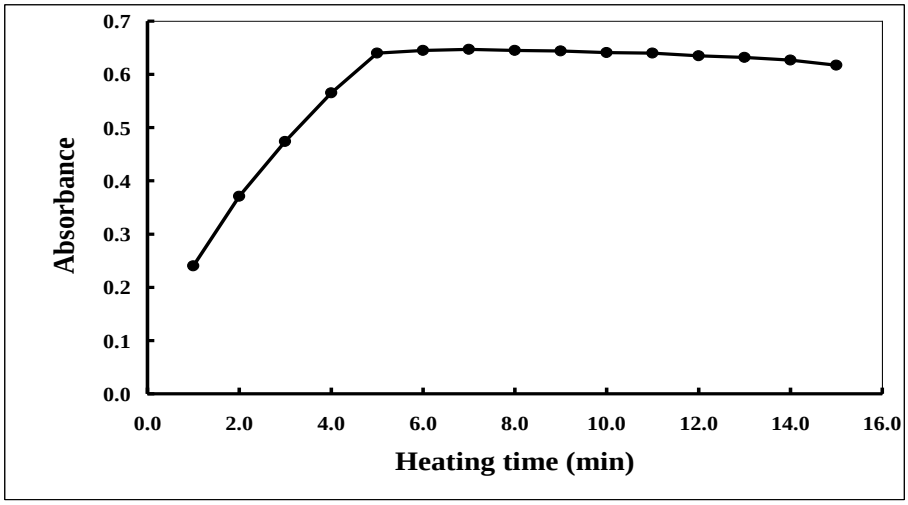


Figure. 1 Effect of heating time on the formation of colored product (AMK = 15 µg/ml).

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Effect of the Reagent Concentration

The effect of ninhydrin concentration on the color development was investigated using different volumes (0.1 - 1.6 ml) of 5.0×10^{-3} M ninhydrin were added to a fixed amount of AMK (15 µg/ml). The results are presented in Figure 2, showing that the highest and most stable absorbance was obtained after addition of 0.5 ml of 5.0×10^{-3} M ninhydrin. A 0.5 ml of the reagent was used as an optimum value for color development.

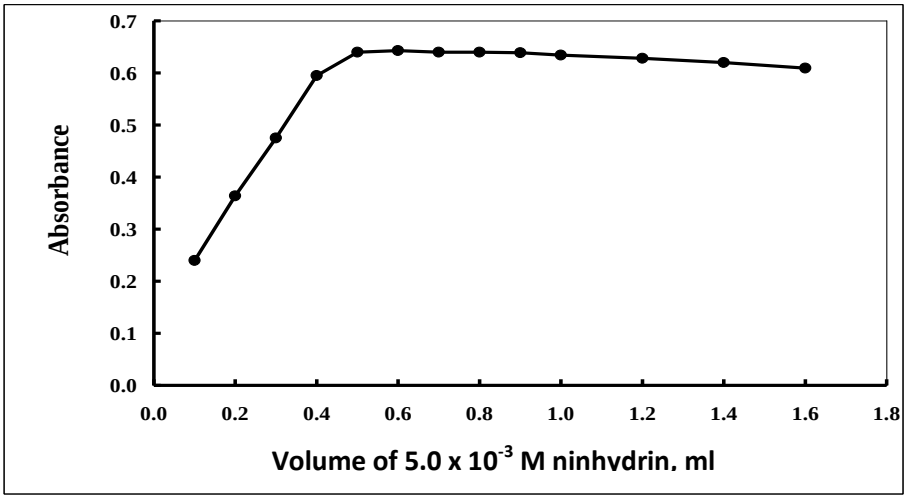

Figure. 2 Effect of ninhydrin (5.0×10^{-3} M) volume on the absorbance of the colored product (AMK = 15 µg/ml).

Interference

The effects of common excipients that often accompany the studied drug (AMK) in various pharmaceutical dosage forms (commercial injections) were tested for possible interference in the assay. An attractive feature of the procedure is its relative freedom from interference by the usual injections diluents and excipients such as glucose, lactose, fructose and magnesium stearate. Amounts far in excess of their normal occurrences (up to 100 fold excess) in dosage forms were added, and no effect due to these excipients was noted in the experimental procedure, meaning the common excipients present in injection formulations did not interfere in the determination of AMK.

Analytical data

Beer's law was verified up to (1.0-21.0) µg/ml of AMK with ninhydrin. The molar absorptivity (ε) calculated and found to be 3.33 x 10^4 l/mol.cm, indicating high sensitivity of the reagents under investigations for the determination of AMK. The regression equations (A = a + b C) where A = absorbance, a = intercept, b = slope and C = concentration in µg/ml), calculated from the calibration graph, were evaluated and recorded in Table 1.

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Table. 1 Optical and regression characteristics of AMK with ninhydrin

Parameters	Ninhydrin
λ_{max} (nm)	590
Stability (h)	16
Heating time (min.)	6
Beer's Law Limits (µg/ml)	1.0 – 21.0
Ringbom Limits (µg/ml)	1.4 – 20.2
Molar Absorptivity (l/ mol cm)	3.33×10^4
Sandell Sensitivity (ng/cm^2)	23.47
Detection Limits (µg/ml)	0.123
Quantitation Limits (µg/ml)	0.411
Regression Equation [a] Slope (b)	0.0426
RSD% of Slope	0.0026
Intercept (a)	0.0034
RSD% * of Intercept	0.0017
Correlation Coefficient (r)	0.9999
Range of Error	± 1.01
RSD%	0.463
Calculated t–values (2.57) [b]	1.13
Calculated F- test (5.05) [b]	2.07

[a] With respect to A = a + bC where C is concentration of drug in µg/ml and A is absorbance.
[b] Values in parentheses are the theoretical values for t- and F- values at 95% confidence limits and five degrees of freedom.
***** Relative standard deviation for six determinations.

The intercept of the lines were very small indicating that there is no systematic difference between determined and expected concentration within the investigated rang using the present method. For more accurate results, Ringbom concentration range was determined by plotting log [drug] in µg/ml against % transmittance from which the linear portion of the curve gave accurate range for the determination of the drug under investigation Table 1.

Statistical analysis of the results obtained, indicated that the proposed methods were accurate and precise. The limits of detection (LOD) and limits of quantitation (LOQ) were determined [27] using the formula:

$$\text{LOD or LOQ} = \kappa SD_a / b$$

Where $\kappa = 3$ for LOD and 10 for LOQ, SD_a is the standard deviation of the intercept, and b is the slope. Based on the basis of six replicate measurements, the limit of detection was 0.123 µg/ml and the limit of quantification was 0.411 µg/ml. Both LOD and LOQ values confirmed the sensitivity of the proposed methods.

In order to determine the accuracy and precision of the present method, solutions containing five different concentrations of drug were prepared and six replicate determinations, converting the usable concentration

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range, were carried out for the pure form and the pharmaceutical of the drugs under investigation. The recovery values almost reach 100% recovery, revealing a high accuracy of the results Table 2.

Table. 2 Evaluation of the accuracy and precision of the proposed procedure.

Reagent	Taken µg/ml	Recovery %	RSD %[a]	RE %[b]	Confidence limits [c]
Ninhydrin	5.0	101.0	0.26	0.27	05.05 ± 0.0136
	10.0	100.1	0.11	0.22	10.01 ± 0.0220
	15.0	99.80	0.19	0.20	14.97 ± 0.0304
	20.0	100.1	0.18	0.19	20.02 ± 0.0378

[a] Relative standard deviation for six determinations.

[b] Relative error.

[c] 95% confidence limits and five degrees of freedom.

The calculated standard deviations are compared with those obtained by the pharmacopoeia method of amikacin sulphate [28] (Examine by liquid chromatography which may be carried out using: a stainless steel column 0.25 m long and 4.6 mm in internal diameter packed with octadecylsilyl silica gel for chromatography R (5 µm), as mobile phase at a flow rate of 1 ml/min a mixture of 30 volumes of a 2.7 g/l solution of potassium dihydrogen phosphate R adjusted to pH 6.5 with a 22 g/l solution of potassium hydroxide R and 70 volumes of methanol R, as detector a spectrophotometer set at 340 nm, maintaining the temperature of the column at 30 °C and that of the solutions to be examined at 10 °C. Inject 20 µl of reference solution (b). Adjust the sensitivity of the system so that the height of the principal peak is at least 50 % of the full scale of the recorder. Inject reference solution (b) six times).

Analytical data: The proposed method was successfully applied to determine AMK in its dosage forms in spiked serum plasma and urine samples. The accuracy of the proposed methods is evaluated by applying standard addition technique, in which variable amounts of the drug were added to the previously analyzed portion of pharmaceutical preparations and in spiked serum plasma and urine samples. The validity of the present method was tested by standard addition method. For this purpose, solutions containing three different concentrations of AMK were prepared by adding a known amount of pure drug to the pre-analyzed commercial dosage forms and determined in six replicates. The results are summarized in Table 3, which can be considered to be very satisfactory.

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Table. 3 Determination of AMK in pharmaceutical formulations using standard addition technique.

Samples	Taken µg/ml	Added µg/ml	AMK	
			Found µg/ml	Recovery %
Amikacin 125 mg [(1)] / 2 ml injection	5.0	0.0	5.01	100.20
		5.0	10.02	100.20
		10.0	14.94	99.60
		15.0	19.99	99.95
Amikacin 500 mg [(1)] / 2 ml injection	4.0	0.0	3.97	99.25
		3.0	6.98	99.71
		8.0	11.97	99.75
		14.0	18.02	100.11

* Average of six determinations.

(1) Laboratories Normon, S. A., Nieremberg, 10-28002 Madrid (Spain).

The results were compared statistically with the official method (BP, 2007) by Student's t-test (for accuracy), and variance ratio F-test (for precision) [29], at 95 % confidence level as recorded in Table 4.

Table. 4 Determination of AMK in injection and spiked plasma and urine samples by the proposed and official methods [PB, 2007]

Parameter	Ninhydrin	Official method
Amikacin 125 mg [(1)] / 2 ml injection		
Recovery% [a]	99.4 ± 0.46	100.1 ± 0.7
± Standard deviation	1.28	0.86
Number of experiments	6	6
Variance	1.21	0.39
Student t-value [b]	0.77	
Variance ratio F-test [b]	3.23	
Amikacin 500 mg [(1)] / 2 ml injection		
Recovery% [a]	99.9 ± 1.29	99.7 ± 0.80
± Standard deviation	0.97	0.73
Number of experiments	6	6
Variance	0.69	0.57
Student t-value [b]	1.42	
Variance ratio F-test [b]	2.44	
spiked plasma sample		
Recovery% [a]	99.6 ± 0.39	100.1 ± 1.1
± Standard deviation	0.95	0.99
Number of experiments	6	6
Variance	0.58	0.46
Student t-value [b]	0.78	
Variance ratio F-test [b]	2.45	
spiked urine sample		
Recovery% [a]	99.2 ± 0.51	99.1 ± 1.1
± Standard deviation	1.06	1.37
Number of experiments	6	6
Variance	0.67	0.94
Student t-value [b]	1.24	

Variance ratio F-test [b]	2.69	

[a] Average values of six determinations were used for the official and the proposed methods, respectively.
[b] Theoretical values for *t* and *F* at 95% confidence limit are 2.57 and 5.05, respectively.

(1) Laboratories Normon, S. A., Nieremberg, 10-28002 Madrid (Spain).

Such comparison showed that there is no significant difference, at 95 % confidence level, between the values obtained by the proposed and the pharmacopoeia method. This indicates the high accuracy and precision of the present method.

CONCLUSIONS: The proposed method for the estimation of AMK using ninhydrin is advantageous over many of the reported methods, due to its sensitivity, rapidity and good agreement with the pharmacopoeia methods. The high recovery percentage and lows relative standard deviation reflect the high accuracy and precision of the proposed method. Moreover, the method is easy, applicable to wide ranges of concentration, beside less time consuming and depend on simple reagents which are available. This offering economic and acceptable method for the routine determination of the cited drug, Beer's law up to 1.0 µg/ml. So it is recommended for the routine determination in pure samples and in their pharmaceutical formulations.

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