ejpmr, 2017,4(2), 235-239

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF AMIKACIN SULPHATE IN PURE AND PHARMACEUTICAL FORMULATIONS USING ASCORBIC ACID

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Article Received on 14/12/2016 **Article Revised on 05/01/2016** **Article Accepted on 26/01/2017**

ABSTRACT

In this study, a simple spectrophotometric method was developed for the determination of amikacin sulphate (AK) in pure bulk form and in its pharmaceutical formulations. Amikacin reacted with ascorbic acid to form a water soluble, purple pink, 1:1 complex that showed two wavelengths maxima (λ_{max}) at 390 nm and 530 nm. The color was developed after heating for 40 minutes at 100 ˚C and remained stable for at least 48 hours. The validity of Developed method was tested by analysing amikacin working standard under the optimum experimental conditions. Beer`s lambert law was obeyed over the concentration range 40-200µg /ml with an excellent correlation coefficient (not less than 0.999). The repeatability and reproducibility results showed a low relative standard deviation values (RSD % < 2) at λ_{max} 390nm and 530nm, which reflected the precision of the developed method. The assay results for commercial amikacin injection (500mg/2ml) were (99.89 ± 0.75 %, n=4) and (99.9 ± 0.5 %, n=4) at λ_{max} 530 nm and 390 nm, respectively. The percentage added recovery was found to be about (100.76 ± 1.2 %, n=3) at both wavelength, which reflected the freedom from interferences.

KEYWORDS: Amikacin, Spectrophotometric, Ascorbic acid.

INTRODUCTION

AK is a semisynthetic aminoglycoside antibiotic derived from kanamycin A (1-N-L-(-)-amino-ά-hydroxybutyric acid) (Figure1). It is used parenterally in the treatment of bacterial infection, particularly those caused by gram-negative organisms.[1]

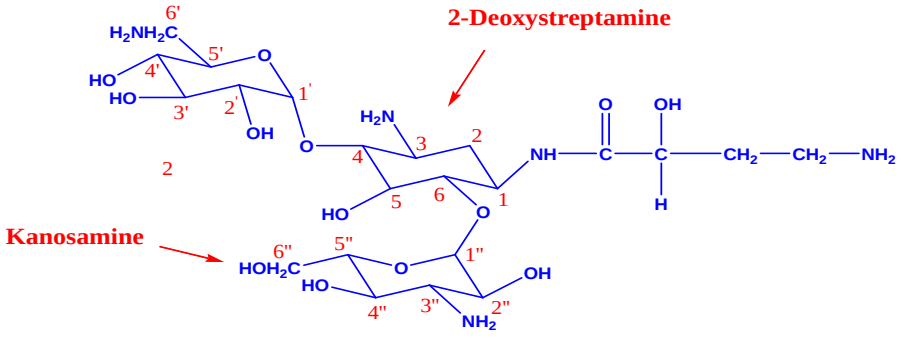


Figure 1: Chemical structure of AK

Several methods were reported for the analysis of AK in bulk form, pharmaceutical dosage form and also in biological fluids. These methods include spectrophotometric methods(2-4) and HPLC methods[5,6]. The reported method are either expensive and time consuming or require many experimental conditions.

Ascorbic acid[7], the chromogen used in this study, is reported to react with number of primary amine containing drugs such as lisinopril[8] and tobramycin.[9] Therefore the aim of the present work was to develop simple and accurate method for the analysis of AK using ascorbic acid.

MATERIALS AND METHODS

Chemicals

Amikacin sulphate RS was obtained from Aladdin Industrial Corporation, China.

Amikacin sulphate injection (MIKAJECT 500 ®, 500mg/2ml) was obtained from Troikaa Pharmaceutical Ltd. India, Batch NO.: M14131, Reg. NO.:AMV/051/21036, Man. Date: 2/2013, Exp. Date: 1/2015.

L-ascorbic acid Labtech chemicals, India.

Dimethyl sulphoxide AR (DMSO). 99.5%. Exp. Date: 5/2015 S.d.Fine-chem limited, India.

Dimethylformamide (DMF), S.d. Fine-Chem limited, India.

Potassium dihydrogen orthophosphate CDH (Central Drug House Ltd) Newdelhi- India

Potassium hydroxide CDH (Central Drug House Ltd), Newdelhi- India

Methanol Chem-Lab, Belgium

Instruments

UV Spectrophotometer-1800, ENG240V, Shimadzu, Japan

HPLC chromatograph, DGU20A3, Shimadzu, Japan. C_{18} stainless steel column (250 x 4.6 mm), UV detector.

METHODOLOGY

PREPARATION OF STOCK SOLUTIONS

Amikacin Standard Stock Solution

0.04g of AK standard was accurately weighed and transferred into 10 ml volumetric flask. The volume was then completed to mark with distilled water (solution A; 4000 µg /ml ; 0.4% w/v).

Amikacin Injection Stock Solution

0.4 ml of AK injection was pipetted and transferred into 25 ml volumetric flask. About 20 ml of distilled water was added and the solution was shaken well. The volume was then completed to 25 ml with distilled water (solution B; 4000 µg/ml or 0.4%w/v).

Coupling Reagent Solutions

Different solvents, DMSO and DMF, were used to prepare 0.2%w/v solutions of ascorbic acid (solution C & D, respectively).

Blank reagent

0.5 ml of distilled water was transferred into stoppered glass tube, 2 ml of solution C was added. The volume was then completed to 10 ml with DMSO.

Reaction Conditions Optimization

Effect of Heating Time

Serial dilutions were made from solution A (0.4%w/v) by transferring 0.1ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml into five stoppered glass tubes. Then 0.4 ml, 0.3 ml, 0.2 ml, 0.1 ml and 0.0 ml of distilled water were added, respectively. 2 ml of freshly prepared solution C and 7.5 ml of DMSO were added to each tube. The above dilutions were repeated four times and heated in a boiling water bath for a time ranged between 20–50 minutes. After cooling to room temperature, the absorbance values were measured against the blank.

Effect of Different Solvents on Absorption Spectra

Serial dilutions were made from solution A by transferring 0.1 ml, 0.3 ml and 0.5 ml into three stoppered glass tubes. Then 0.4 ml, 0.2 ml and 0.0 ml of distilled water were added, respectively. 2 ml of freshly prepared solution C and 7.5 ml of DMSO were added to each tube. The solutions were heated for 40 minutes in a boiling water bath then allowed to cool at room temperature.

The above solutions were scanned against blank using UV/ visible spectrophotometer at range of 350-600 nm.

The same procedure was repeated using solution D instead of solution C and the volumes were completed to 10 ml using DMF instead of DMSO.

Effect of Ascorbic Acid Concentration

Two ml of 0.1%, 0.2% and 0.3% w/v ascorbic acid solution in DMSO were added to three stoppered glass tubes containing 0.5 ml of solution A. 7.5 ml of DMSO was added to each tube and the solutions were heated in a boiling water bath for 40 minutes. After cooling at room temperature, the absorbance values were measured against the blank.

Estimation of Color Stability

0.3 ml of freshly prepared solution A was transferred into stoppered glass tube. 0.2 ml of distilled water and 2 ml of solution C were added and the volume was completed to 10 ml using DMSO. The solution was then heated in a boiling water bath for 40 minutes.

After cooling, The absorbance values were measured at different time intervals for 48 hours to estimate the stability of the formed colored product.

METHOD VALIDATION

Construction of Calibration Curve

Different volumes of distilled water (0.4, 0.3, 0.2, 0.1 & 0.0 ml) were added to five stoppered glass tubes containing 0.1, 0.2, 0.3, 0.4 and 0.5 ml of solution A, respectively. 2 ml of freshly prepared solution C was added to each tube. The volumes were then completed to 10 ml with DMSO and heated in a boiling water bath for 40 minutes. After cooling, the solutions were scanned against the blank with UV/ visible spectrophotometer at scanning mode range 350-600 nm.

Aliquots of solution B were treated as under calibration curve. The content of AK injection was then calculated by the direct sample/standard comparison.

Repeatability and Reproducibility of the proposed method

Three different concentrations within the linearity range were treated as under calibration curve. The solutions were analyzed three times within the same day and between days to evaluate the repeatability and reproducibility, respectively.

Added Recovery (%) Measurement

0.2ml of each solution A and B were transferred to separate stoppered glass tubes. Another 0.2ml of solution B was mixed with 0.2 of solution A in a third tube. The above solutions were then treated as under calibration curve. The percentage recovery was calculated using the following equation

$$\frac{Amix - Asam}{Astd} \; x\; 100$$

Where A_{mix} = absorbance of the mixture solution, A_{sam} = absorbance of the sample solution, A_{std} = absorbance of the standard solution

Molar ratio method for determination of the stoichiometry

In a volumetric flask (10 ml), 0.034g of AK standard was dissolved in distilled water (5.0 x 10^{-3} M). Then 0.1ml, 0.2ml, 0.3ml, 0.4ml, 0.5ml, 0.6ml, 0.7ml, 0.8ml & 1 ml of this solution were transferred into nine stoppered glass tubes. 0.9ml, 0.8ml, 0.7ml, 0.6ml, 0.5ml, 0.4ml, 0.3ml, 0.2ml & 0.0 ml of distilled water were then added, respectively. 0.4 ml of freshly prepared ascorbic acid solution (5 x 10^{-3}M) was added to each tube, and the volumes were then completed to 10 ml with DMSO.

The above solutions were heated for 40 minutes in a boiling water bath. After cooling the absorbance values of the resulting solutions were measured at At 390nm and 530nm against the blank.

RESULTS AND DISCUSSION

AK is composed of 2-deoxystreptamine (aminocyclitol moiety) glycosidically linked to amino sugars. It lacks a conjugation in its structure so it has no UV/ Visible absorption. Therefore, a suitable chromogen is needed to form UV absorbing chromophore that can be used for determination of amikacin in bulk and in different pharmaceutical formulations.

Ascorbic acid is reported to have a selective reaction with ammonia, and primary aliphatic amines. This coupling reagent has been used for colorimetric determination of a number of drugs containing primary amine such as Alfuzocin[10], aminocaproic acid[11], tobramycin[9], penicillins and cephalosporines.[12]

Based on these reports, a simple, sensitive and selective assay procedure was proposed for the determination of the aminoglycoside (amikacin) in its bulk and pharmaceutical formulation. It was found that ascorbic acid react with AK in presence of DMSO to produce pink-purple colored complex absorbing at 390nm and 530nm .The different experimental factors affecting the color development, intensity and stability were studied. These factors include solvent, the reagent concentration, the reaction time and temperature. The optimal volume and concentration of ascorbic acid which give satisfactory results were found to be 1.0 ml of 0.2% w/v in DMSO. The obtained results for the effect of the different solvents are illustrated in Table 1.

Table 1: Effect of different solvents on the absorption spectra at λ_{max} 390nm and 530 nm

Concentration µg/ml	Absorbance in DMF		Absorbance in DMSO	
	390nm	530nm	390nm	530nm
40	0.275	0.093	0.385	0.132
120	0.790	0.265	1.185	0.395
200	1.374	0.458	1.972	0.674
R	0.9996	0.9994	0.9999	0.9998

The effect of heating time on product formation, color intensity and stability was also studied. The formation of colored product increased with heating time till 40 minutes after which the color intensity started to decrease. This indicated that the optimal heating time for maximum product formation and color stability is 40 minutes (Figure 2).

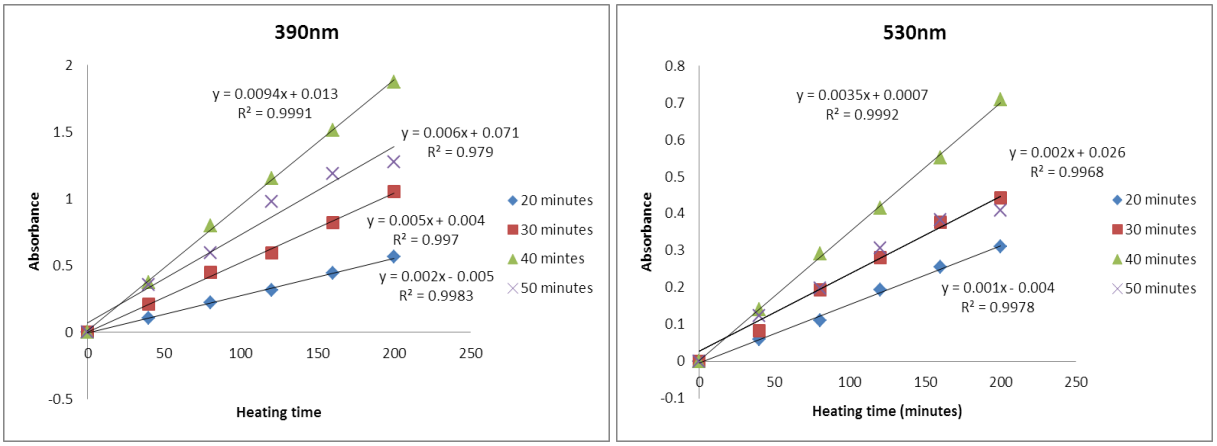


Figure 2: Effect of heating time on colored product formation

Method Validation

The optimized experimental conditions were utilized to construct the calibration curve. The coloured product remained stable for at least 24 hours. Beer's law was found to be valid over the concentration range (40-200µg /ml) of AK at λ_{max} 390nm & 530nm.The corresponding regression equations are:

A = 0.0239 + 0.0092 C (390nm)

And A = -0.0033 + 0.0034 C (530nm)

The linearity of the method were detected by the excellent values of correlation coefficient(not less than 0.9995). The detection limits were 6.796µg/ml at 390nm and 4.53µg/ml at 530nm, which represents the minimum amount that can be detected by the developed method. The limits of quantification were 22.65µg/ml at 390 nm and 15.08µg/ml at 530 nm.

Precision

The method precision was evaluated by determining the repeatability and reproducibility. The calculated RSD% values were found to be within the accepted limits(less than 2%) Table 2.

Table 2. Repeatability and Reproducibility results

Concentration µg/ml	λ (nm)	Within-day precision RSD%, n =3	Between-days precision RSD%, n = 3
40	390	0.98	1.20
	530	2.00	1.97
120	390	1.74	1.18
	530	1.28	1.23
200	390	0.19	0.44
	530	1.12	0.56

Accuracy

The accuracy of the procedure and freedom from interference by the injection excipients was confirmed by the obtained results for recovery testing of added amount of authentic AK to the injection solution in ratio of 1:1.The results showed good recovery for the injection (100.6 ± 1.96%, n=3).

The developed method was applied for drug uniformity testing in amikacin sulphate injection USP(500mg/2ml), where good results were obtained (Table 3).

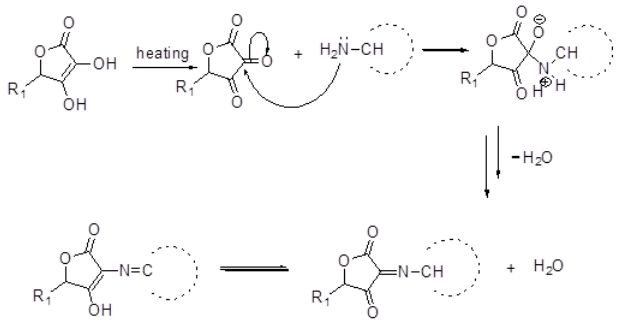
Table 3. Validation results of the developed method

Method		% ± SD, n=3	t cal., (t tab.)	F cal., (F tab)
Developed method	390nm	99.90 ± 0.50%	0.62 (2.78)	1.9 (19)
	530nm	99.89 ± 0.75 %	0.44 (2.78)	4.3 (19)
Official method		99.68 ± 0.36%	-	-

The validity of the method for the determination of AK in bulk or pharmaceutical form was assessed by comparison of the obtained statistical results with those of the official BP liquid chromatography method (13). Data of Table 3 showed the obtained assay results and the calculated *t*- and *F*-values as compared to the corresponding tabulated values at 95% confidence level. As the calculated *t*- and *F*-values were less than tabulated ones, The developed method can be considered as accurate as the official method.

Reaction stoichiometry and pathway

The molar ratio method revealed a 1:1 ratio reaction. Accordingly, the proposed reaction pathway between the drug and the reagent is expected to proceed as illustrated in Scheme 1.



Tautomer II Tautomer I

Scheme 1. Proposed reaction pathway between ascorbic acid and AK

CONCLUSION

The developed method was proved to be simple, accurate and precise. Ascorbic acid was found to be a suitable reagent for the determination of AK in pure form and its dosage forms without interference from excipients. The developed method can be used for the routine analysis of AK.

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