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Development and validation of colorimetric method for the quantitative analysis of kanamycin in bulk and pharmaceutical formulation

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ABSTRACT

The aim of this study was to develop a simple spectrophotometric method for the determination of Kanamycin (KM) in pure bulk form and in its pharmaceutical formulations. Being an amino group containing molecule, KM 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 analyzing KM under the optimum experimental conditions. Beer's law was found valid over the concentration range (40-200µg /ml) with an excellent correlation coefficient (less than 0.999). The repeatability and reproducibility results showed a low relative standard deviation values (RSD % < 2), which reflected the precision of the developed method. The good percentages added recovery (100.09 ± 0.28 % and 99.98 ± 0.88 %, n = 3) at 390nm and 530nm, respectively, reflected the method freedom from interferences.

INTRODUCTION

Kanamycin (Fig.1) is an aminoglycoside antibiotic obtained from the soil bacterium *Streptomyces kanamyceticus*, used parenterally in the treatment of various infections, especially those caused by gram-negative bacteria (Pestka, 1975). The coupling reagent (ascorbic acid) was reported to be used for determination of many primary amines containing drugs (Krishna and Sanker, 2007; Adam *et al.*, 2015), penicillins and cephalosporins having *α*-aminoacyl functionality (Gadkariem *et al.*, 2009; EL-Obeid *et al.*, 1999). Although several methods were reported for the analysis of KM in bulk form, pharmaceutical dosage form, and also in biological fluids (Mahmoud *et al.*, 2013; Mirela *et al.*, 2007, Ahmed *et al.*, 2007; Sekkat *et al.*, 1989; Kim *et al.*, 2001), these methods are either expensive, require many chemical reagents or sophisticated instruments.

Therefore, the aim of the present work was to develop simple and accurate colorimetric method for the determination of KM in bulk and pharmaceutical forms using ascorbic acid.

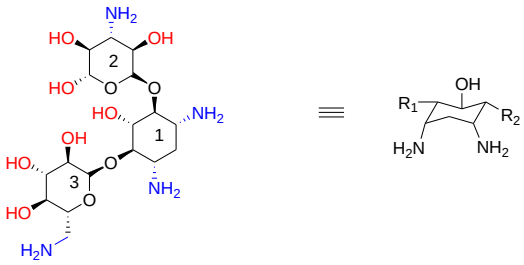

Fig 1: Chemical structure of KM.

MATERIALS AND METHODS

Reference and sample

KM sulphate RS was obtained from Aladdin Industrial Corporation, Shanghai, China. Kanamycin injection contains kanamycin acid sulphate B.P. equivalent to 1g kanamycin base, Shanghai Medicines & Healthproducts, China.

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Chemicals

L-ascorbic acid, Labtech chemicals, India. Dimethyl sulphoxide (DMSO; 99.5%), 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 HPLC grade, CHEM-LAB, Belgium.

Instruments

The spectrophotometric studies were carried on UV Spectrophotometer-1800, Eng240v, Shimadzu, Japan.

METHODOLOGY

Preparation of Stock Solutions

KM standard stock solution

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

KM sample stock solution

A quantity of KM powder for injection equivalent to 0.04g of KM was accurately weighed and transferred into 10 ml volumetric flask. The volume was then completed to mark with distilled water (solution B; 4000 µg/ml or 0.4% w/v).

Coupling reagent solutions (Ascorbic acid)

Ascorbic acid solution (0.2%w/v) was prepared using different solvents (DMSO and DMF; solution C and D, respectively).

Blank reagent

2 ml of freshly prepared solution C was added to 0.5 ml distilled water in stoppered glass tube. The volume was then completed to 10 ml with DMSO.

Reaction Conditions Optimization

Effect of heating time

Serial volumes from solution A (0.1- 0.5ml) were transferred into five stoppered glass tubes. The volumes were completed to 0.5ml with distilled water. 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 reagent.

Effect of different solvents

Serial dilutions were made from solution A by transferring 0.1 ml, 0.3 ml and 0.5 ml into three stoppered glass tubes. The volume was then completed to 0.5ml with distilled water. 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. After cooling, the absorbance values were measured against blank at 390 and 530nm. The above procedure was repeated using solution D instead of solution C and the volumes were completed to 10 ml using DMF.

Effect of ascorbic acid concentration

Two ml of 0.1%, 0.2% or 0.3% w/v ascorbic acid solution in DMSO were added separately 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 reagent.

Construction of Calibration Curve:

Serial aliquots of solution A (0.1- 0.5ml) were transferred into five stoperred glass tubes. The volumes were completed to 0.5ml with distilled water. 2 ml of freshly prepared solution C were added to each flask. The volumes were then completed to 10 ml with DMSO. The mixture solutions were heated for 40 minutes in a boiling water bath. After cooling, the solutions were scanned at 350-600nm against the blank. The measured absorbance values at 390nm and 530nm were plotted against the corresponding concentrations to obtain the calibration curve.

Solution B was also treated as under calibration curve. The injection content was determined by the slope ratio method and direct sample/ standard comparison.

Method precision

The precision of the developed method was evaluated by the repeatability and reproducibility results. Different concentrations within the linearity range were analysed three times in the same day and between-days. The relative standard deviation (RSD) was then calculated.

Percentage Added recovery

0.2 ml of each solution A and B was transferred into separate stoppered glass tubes. 0.2ml of solution A was mixed with 0.2ml of solution B in a third tube. These solutions were treated as under calibration curve and the percentage recovery was calculated (Adam *et al.*, 2016).

Molar ratio method for determination of the stoichiometry

In a volumetric flask (10 ml), 0.034g of KM standard was dissolved in distilled water (concentration 5.0×10^{-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. Distilled water was added to adjust the volumes to 1.0 ml. 0.4 ml of freshly prepared ascorbic acid solution (5.0×10^{-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 at $100\ ^0$C. After cooling, the absorbance values were measured at 530 nm and 390 nm against the blank.

RESULTS AND DISCUSSION

KM is composed of 2-deoxystreptamine (aminocyclitol moiety) glycosidically linked to amino sugars. It exhibits weak UV- absorption, thus a suitable chromogen is needed to obtain a more UV/VIS light absorbing chromophore that can be useful as a sensitive spectrophotometric method for its determination in bulk and dosage forms.

Ascorbic acid, naturally occurring cheap organic compound (sugar acid), was found to react with KM 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 the solvent, the reagent concentration, the reaction time and temperature. During the study of the effect of different solvents on the color formation and stability, solutions of variable color intensities were obtained. In an attempt to reach an explanation for this observation on the reaction process, the effect of two solvents of different dielectric constants (D.E.) were studied (Table 1). The results obtained reflected that a hyperchromic effect was observed with the solvent with higher D.E. (DMSO). An assumption was drawn that DMSO with its medium polarity (DE 47) possibly enhances the reactivity of the ascorbic acid and stabilizes the formed π to π* and n to π* transitions.

The optimal volume and concentration for ascorbic acid to give satisfactory results were found to be 1.0 ml of 0.2% w/v in DMSO. The results obtained for heating effect are shown in figure 2. A fixed time of 40 minutes was established as the most suitable time (best r-value and color intensity) to give reproducible absorbance values with low standard deviations.

Table 1: Effect of different solvents on the formation of kanamycin-ascorbic acid complex.

KM conc. (µg/ml)	λ_{max} (nm)	Absorbance in DMF	Absorbance in DMSO
40	390	0.125	0.226
	530	0.043	0.076
120	390	0.420	0.714
	530	0.142	0.238
200	390	0.652	0.851
	530	0.219	0.382

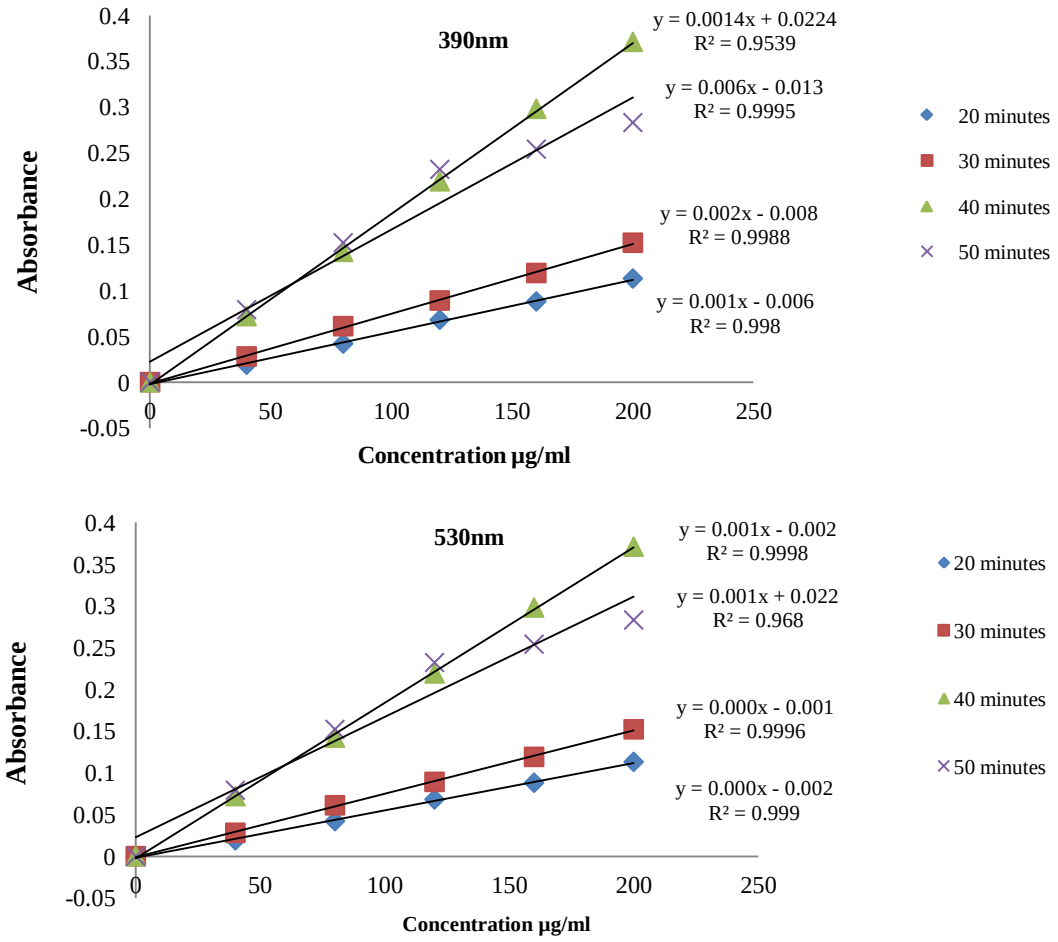
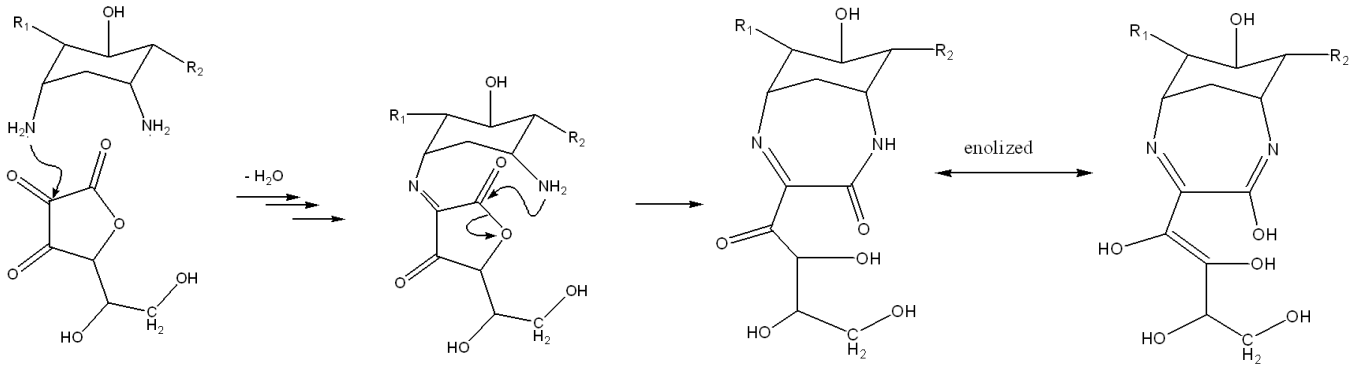


Fig. 2: Effect of heating time on colored product formation.



Scheme 1: Proposed pathway for KM-ascorbic acid complex formation.

Method validation

Linearity

Under the optimum experimental conditions, Beer's law was valid over the concentration range (40-200µg /ml) of KM. The corresponding regression equations at 390nm and 530nm were A= 0.007 + 0.0058 C (r= 0.9992) and A = 0.002 + 0.0019 C (r= 0.999), respectively, which indicate an excellent linearity. The detection limits were 8.58µg/ml and 9.6µg/ml at 390nm and 530nm, respectively which represent the minimum absorbance value that can be measured for the color produced by complex. The limits of quantification were 32.15µg/ml and 28.6µg/ml at 390nm and 530 nm, respectively.

Accuracy and precision

The accuracy of the developed method and freedom from interference by the injection excipients was confirmed by the obtained results for recovery testing of added amount of authentic KM to the injection solution in ratio of 1:1.The results showed good recovery for the injection (100.76± 1.2% and 100.6 $\pm$ 1.96%, n=3 at 390nm and 530nm, respectively).

The precision of the developed method was determined on three different concentrations of KM. The results obtained showed a low relative standard deviation values (RSD) varying from 2.30 to 0.38%; n=3, which reflect that the developed method is satisfactory repeatable and reproducible.

Application of the developed method

The developed method was applied for drug content testing in KM injection. The results were found to be 100.09 $\pm$ 0.28% and 99.98 $\pm$ 0.88%, n=3 at 390nm and 530 nm, respectively.

The accuracy of the proposed method was tested against the official biological assay method for KM (Eu.P., 2012). The biological assay method results gave a100% potency; this result was taken as the true mean (µ). The formula for t-value calculation when true mean is known was used (Shantier *et al.*, 2011).

The calculated t-value (0.29 and 0.037 at 390 nm and 530nm, respectively for 2 degrees of freedom) was less than the tabulated t-value, which indicates no significant difference between the chemical and biological methods.

Reaction stoichiometry

The reaction stoichiometry was found to be a 1:1 ratio reaction using the molar ration method. Accordingly, the proposed reaction pathway between the drug and the reagent is expected to proceed through a nucleophilic addition and acyl substitution between the two cis and equatorial amino group of the drug and the carbonyl group and lactone functionality of the reagent as illustrated in Scheme 1.

CONCLUSION

Ascorbic acid was found to be a suitable reagent for the determination of KMin pure form and in its dosage forms without interference from the excipients. Thus, the developed method is simple, accurate and precise method and can be used for the routine quality control analysis of KM.

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Conflict of Interests: There are no conflicts of interest.

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