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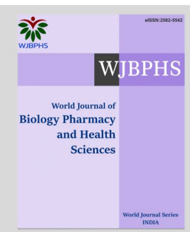

(Research Article)

Novel colorimetric approach for amikacin estimation in pure powder and its pharmaceutical formulations

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Abstract

A sensitive and validated colorimetric method was developed and optimized for the estimation of amikacin sulfate in pure and pharmaceutical dosage forms. Ascorbic acid was used as a chromogenic reagent to produce a stable color complex with amikacin that absorbs strongly at 390nm and 540nm. Several experimental factors were evaluated to maximize color development and stability, including solvent, reagent concentration, reaction time, and temperature. Optimal conditions were found using DMSO solvent, 0.2% ascorbic acid, and 40 minutes reaction time at 25°C. Under these conditions, Beer's law was obeyed in the range of 40-200µg/mL with high correlation coefficients, indicating excellent linearity. The method was validated in terms of linearity, accuracy, precision, detection/quantitation limits, and application to actual samples as per ICH guidelines. Recovery studies showed nearly 101% accuracy. Low relative standard deviations reflected high precision. Limits of detection/quantitation were 19-57µg/mL, enabling reliable analysis even at low concentrations. The method quantified amikacin content in injections, giving 98-102% accuracy. Statistical comparisons to a validated method gave no significant differences, confirming this approach provides equivalent results.

Keywords: Amikacin sulfate; Colorimetric method; Ascorbic acid reagent; Validation

1. Introduction

Pharmaceutical analysis aims to develop precise and economical techniques for quantifying drug substances and ensuring product quality, safety and efficacy [1], [2]. Colorimetric assays have gained significant importance in pharmaceutical analysis due to their simplicity, low cost and minimal instrumentation requirements [3]. Colorimetry does not rely on expensive and complex instruments like High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) or Mass Spectroscopy (MS). It simply requires a UV-visible spectrophotometer/a simple colorimeter which is commonly available in quality control labs [4].

Amikacin is a crucial antibiotic, hence developing a simple colorimetric method for its analysis becomes imperative [5], [6]. The present study proposes a validated colorimetric technique for the quantitative determination of amikacin in bulk and pharmaceutical formulations. The proposed colorimetric approach seeks to provide an economical, rapid and reproducible method for routine analysis of amikacin with acceptable accuracy and precision.

While analytical skills continue to scale new heights of sophistication, the need for basic yet effective techniques has also persisted. Colorimetry has revived the interest in such techniques due to benefits of affordability, ease of use and adequate effectiveness for routine quality testing [7]. The current work aims to develop a colorimetric method for

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amikacin analysis to facilitate widespread adoption of such simple and cost-effective approaches in pharmaceutical analysis.

2. Material and methods

2.1. Materials

Amikacinsulphate is provided by Krishlar Pharmaceuticals Ltd, Haryana. Dimethyl Sulfoxide (DMSO), Ascorbic acid, Dimethyl formamide are obtained from S.D Fine chemicals Ltd, Mumbai. All the reagents used are of analytical grade to prevent material variation during the method development.

2.2. Method development

2.2.1. Preparation of solutions

An amikacin standard stock solution (0.4% w/v) was prepared by dissolving 0.04 g of amikacin standard in 10 ml of distilled water. Two coupling reagent solutions were prepared using ascorbic acid and different solvents. An ascorbic acid solution (0.2% w/v) in DMSO was labeled as solution B. In a similar manner, another ascorbic acid solution (0.2% w/v) in DMF was named as solution C. A blank reagent was formulated by adding 2 ml of solution B, 0.5 ml of distilled water and making the volume up to 10 ml with DMSO.

2.2.2. Principle of the analytical method

Amikacin, an aminocyclitol antibiotic, possesses weak UV absorption characteristics, limiting its quantitative analysis through spectrophotometry [5]. Thus, developing a suitable chromogenic system becomes imperative to enable sensitive UV/VIS detection of amikacin in bulk and dosage forms.

Ascorbic acid, an inexpensive naturally occurring compound, was explored as a coupling reagent to improve the UV-VIS profile of amikacin. Reaction of amikacin with ascorbic acid in DMSO led to the formation of a pink-purple colored complex [8] with absorbance peaks at 390 nm and 540 nm, facilitating spectrophotometric quantification.

Several experimental parameters influencing the color development, intensity and stability were optimized. The selection of an appropriate solvent emerged fundamental to achieving enhanced sensitivity and accuracy. Multiple solvents were evaluated, resulting in solutions with variable color intensities and stability. DMSO and DMF exhibited most promise and were chosen as solvents for the coupling reagents [7].

The concentrations of ascorbic acid and amikacin, reaction time and temperature were also studied to develop an efficacious method. An optimized set of conditions enabled formation of an intense and stable color complex with acceptable reproducibility, enabling sensitive detection of amikacin even in trace quantities [9].

2.3. Setting up the experimental conditions

The proposed colorimetric method aimed at developing an optimized protocol for amikacin estimation through spectrophotometry. Several experimental parameters influencing color development and sensitivity were evaluated to establish their optimal levels necessary for an efficacious analysis technique.

The heating time was studied by reacting amikacin (0.1-0.5 ml) with the coupling reagent solution (2 ml) and solvent (DMSO) for varied durations (20-50 minutes). Prolonged heating facilitated the coupling reaction leading to color intensification. Heating intervals were assessed to determine the time producing the most intense and stable color complexes at 390 and 540 nm with acceptable reproducibility, enabling maximum sensitivity and accuracy [10].

Selection of an appropriate solvent emerged fundamental to achieving enhanced sensitivity and accuracy. The reagent solutions in DMSO and DMF were evaluated to determine the solvent promoting most intense color development and stability at 390 and 540 nm. The solvent resulting in pink shades of higher intensity was selected for preparation of the coupling reagent solutions [11].

Varied concentrations of ascorbic acid (0.1-0.3% w/v), an important coupling agent, were examined. Ascorbic acid concentrations were optimized by reacting amikacin (0.5 ml) with solutions of different concentrations (0.1- 0.3% w/v) in the selected solvent. The concentration producing the most intense pink color and highest stability at 540 nm was chosen for formulating the coupling reagent solutions [8].

2.4. Construction of standard calibration curve

Different volumes (0.1- 0.5 ml) of the amikacin standard solution were transferred to 5 test tubes and the volume made up to 0.5 ml in each tube using distilled water. Two ml of the coupling reagent solution C were added to each test tube and DMSO was used to make the final volume 10 ml. These reaction mixtures were heated in a boiling water bath for 40 minutes. After cooling, the absorbance of each solution was recorded against the blank across wavelengths ranging from 350 to 540 nm.

The absorbance values at 390 nm and 540 nm were plotted against their corresponding amikacin concentrations to develop the calibration curve. The same procedure was repeated using solution B as the coupling reagent to obtain another calibration curve. The concentrations of unknown samples were determined in two ways:

2.4.1. Slope ratio method

The slope of calibration curve for the reagent (B or C) giving higher sensitivity was divided by the slope of the curve for the other reagent to obtain the slope ratio. This ratio was multiplied with the absorbance of the unknown solution measured using the latter reagent to determine the concentration.

2.4.2. Direct comparison with standards

The absorbance of the unknown solution measured using a particular reagent was compared with the absorbance of standards of known amikacin concentration measured using the same reagent. By locating the absorbance of the unknown in the calibration curve, its concentration was estimated [1].

2.5. Method validation

The developed method was validated for precision, accuracy and stoichiometry. Precision was evaluated through intra-day and inter-day variability of responses for multiple concentration readings. The percentage recovery estimated accuracy in terms of closeness of means. The molar ratio method helped determine the reaction stoichiometry by calculating the ratio of moles of reactants needed to develop unit absorbance [12].

2.5.1. Precision

The precision of the developed method was evaluated by determining the repeatability and reproducibility. Different concentrations within the linear range were analyzed thrice in a single day (intra-day precision) and on different days (inter-day precision). The relative standard deviation (RSD) was calculated to estimate the precision [9].

2.5.2. Accuracy

The accuracy of the method was assessed through recovery studies. Equal volumes (0.2 ml) of amikacin standard solution and the coupling reagent solution were mixed. The absorbance of this mixture was measured and compared with 0.2 ml of the amikacin standard solution to calculate the percentage recovery [10].

2.5.3. Stochiometry of the color reaction

The molar ratio method was employed to determine the stoichiometry of the color reaction. Amikacin sulfate (0.034 g) was dissolved in distilled water (10 ml) to prepare a 5.0×10^{-3} M solution. Varying volumes (0.1-1 ml) of this solution were transferred to 9 test tubes and the volume made up to 1 ml in each tube using distilled water. To each tube, 0.4 ml of ascobic acid solution (5.0×10^{-3} M) and DMSO were added to make the final volume 10 ml. The reaction mixtures were heated for 40 minutes and absorbance measured at 540 nm and 390 nm against the blank [12].

3. Results and discussion

3.1. Method development

The current research work examined several factors that influence how intensely and stably the color develops in this chemical reaction between amikacinsulphate and ascorbic acid. These factors include the solvent used, reagent concentrations, reaction time, and temperature.

By studying how different solvents impacted color formation and stability, solutions with varying intensities were produced. To better understand these observations and determine the mechanism behind the results, the effects of two solvents (DMSO and DMF) with different dielectric constants were compared (see Table 1).

Table 1 Effect of different solvents on the formation of amikacin sulfate-ascorbic acid complex

Concentration of Amikacin sulfate (µg/mL)	Absorbance measured at 540 nm*	
	DMF	DMSO
40	0.340 ± 0.02	0.148± 0.12
120	0.366± 0.01	0.224± 0.08
200	0.385± 0.18	0.315± 0.04

*n=3, mean+ standard error of mean (SEM)

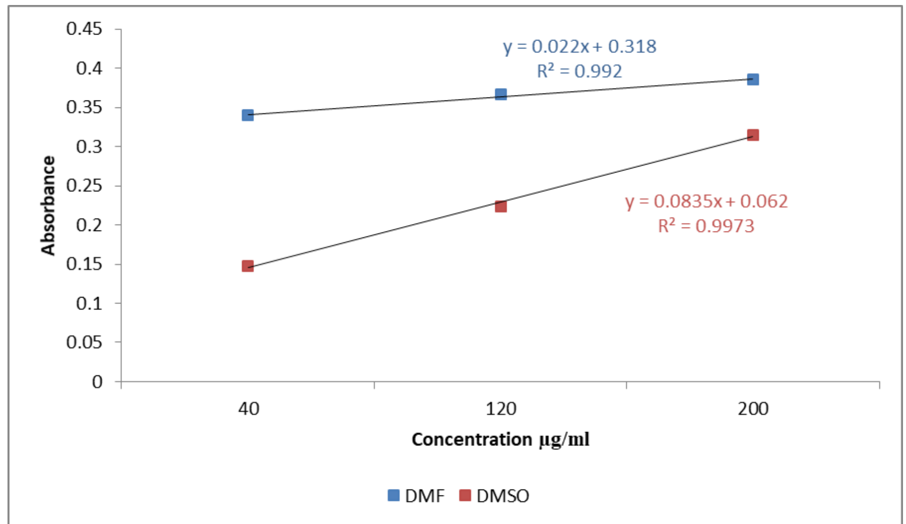

Figure 1 Influence of different solvents on the formation of amikacinsulfate-ascorbic acid complex

The findings revealed a hyperchromic effect with the solvent of higher dielectric constant (DMSO). This suggests DMSO, with its medium polarity (with a dielectric constant 47), possibly enhances the reactivity of ascorbic acid and stabilizes the π to π* and n to π* transitions that form. Optimal conditions were found to use 1.0 ml of 0.2% w/v ascorbic acid in DMSO to achieve satisfactory results. The heating effects are plotted in Figure 2 and results are shown in table 2.

Table 2 Effect of heating time on colored product formation in DMF

Concentration of Amikacin (µg/ml)	Absorbance measured at 540 nm at different time intervals*			
	20min	30 min	40 min	50 min
40	0.009± 0.02	0.002± 0.02	0.011± 0.05	0.041± 0.01
80	0.011± 0.04	0.018± 0.04	0.098± 0.04	0.104± 0.01
120	0.024± 0.06	0.087± 0.03	0.198± 0.02	0.187± 0.01
160	0.041± 0.03	0.119± 0.02	0.287± 0.03	0.208± 0.01
200	0.064± 0.05	0.142± 0.04	0.348± 0.03	0.253± 0.01

*n=3, mean± standard error of mean (SEM)

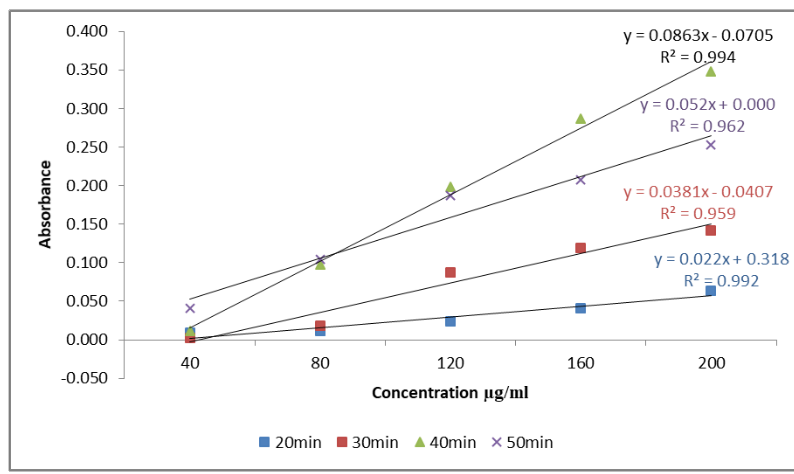

Figure 2 Effect of heating time on colored product formation in DMF

40 minutes was established as the most suitable time (higher r^2-value and color intensity) to obtain reproducible absorbance values with low standard deviations. Results of influence of time on color intensity are shown in table 3 and figure 3. The effect of ascorbic acid concentration is shown in table 4 and figure 4.

Table 3 Influence of heating period on colored complex in DMSO

Concentration of Amikacinsulfate (µg/ml)	Absorbance measured at 540 nm			
	20min	30 min	40 min	50 min
40	0.009 ± 0.02	0.002 ± 0.02	0.011 ± 0.05	0.041 ± 0.01
80	0.022 ± 0.04	0.018 ± 0.04	0.192 ± 0.04	0.104 ± 0.01
120	0.037 ± 0.06	0.087 ± 0.03	0.286 ± 0.02	0.187 ± 0.01
160	0.041 ± 0.03	0.119± 0.02	0.342 ± 0.03	0.208 ± 0.01
200	0.050 ± 0.05	0.142 ± 0.04	0.381 ± 0.03	0.253 ± 0.01

*n=3, mean± standard error of mean (SEM)

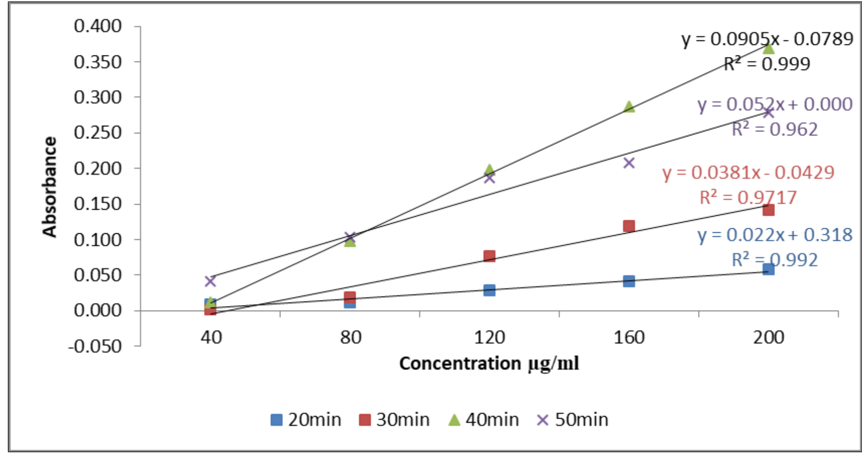

Figure 3 Influence of heating period on colored complex in DMSO

Table 4 Effect of ascorbic acid concentration

Concentration (%w/w)	Absorbance at 540nm
0.05	0.241± 0.002
0.10	0.268± 0.014
0.15	0.320± 0.011
0.20	0.410± 0.028
0.25	0.514± 0.016

*n=3, mean± standard error of mean (SEM)

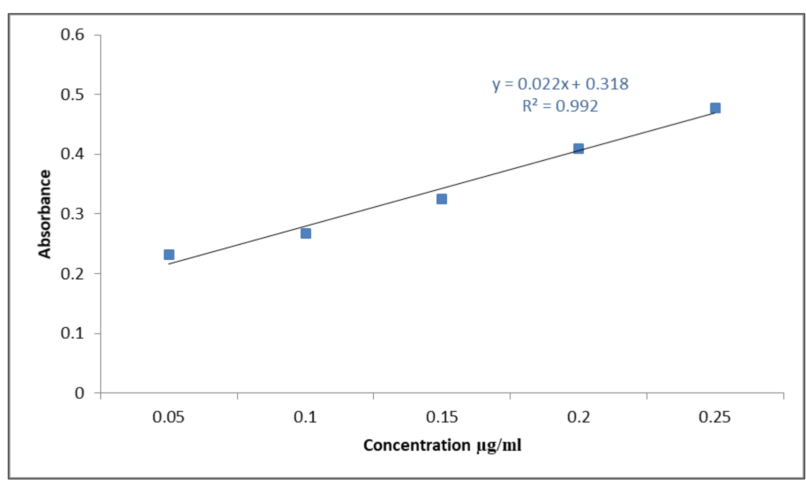

Figure 4 Effect of ascorbic acid concentration

3.2. Method validation

3.2.1. Linearity

Under optimized conditions, Beer's law was valid over a concentration range of 40-200µg/ml for amikacin sulfate. The corresponding regression equations at 390nm and 540nm were y = 0.022x + 0.0318 (r^2=0.992) and A=0.00795x + 0.014 (r^2=0.9997), respectively, indicating excellent linearity. The results are shown in table 5 and figure 5. Detection limits were 19.021µg/ml and 5.220µg/ml at 390nm and 540nm, representing the minimum absorbance measurable for the complex. Limits of quantification were 57.641µg/ml and 15.819µg/ml at 390nm and 540nm.

Table 5 Calibration curve

Absorbance	40 mcg	80 mcg	120 mcg	160 mcg	200 mcg
390nm	0.102± 0.021	0.186± 0.014	0.239± 0.009	0.341± 0.029	0.424± 0.024
540nm	0.094± 0.003	0.174± 0.011	0.254± 0.031	0.329± 0.017	0.414± 0.057

*n=3, mean± standard error of mean (SEM)

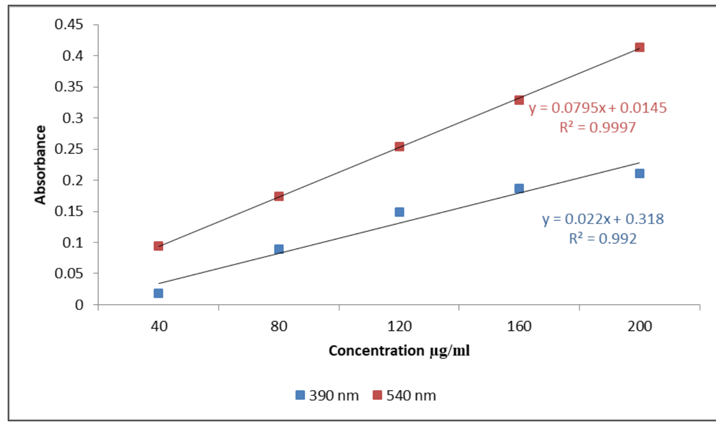

Figure 5 Calibration curve

3.2.2. Accuracy and precision

Method accuracy and freedom from interference were confirmed, with good recovery for the injection (100.76±1.2% and 100.6±1.96%, n=3 at 390nm and 540nm). Precision was determined at three concentrations, showing low relative standard deviation (2.30-0.38%; n=3), reflecting satisfactory repeatability and reproducibility.

The molar ratio method in DMSO and DMF revealed sufficient linearity indicating stochiometric complex formation between ascorbic acid and amikacin sulfate. The results are shown in tables 6,7 and figures 6,7.

Table 6 Molar ratio method in DMSO

Concentration (µg/mL)	40	80	120	160	200	240	280	320	360	400
Absorbance at 390nm	0.041	0.092	0.157	0.189	0.222	0.244	0.273	0.291	0.304	0.315
Absorbance at 540nm	0.092	0.171	0.252	0.326	0.409	0.502	0.567	0.644	0.714	0.805

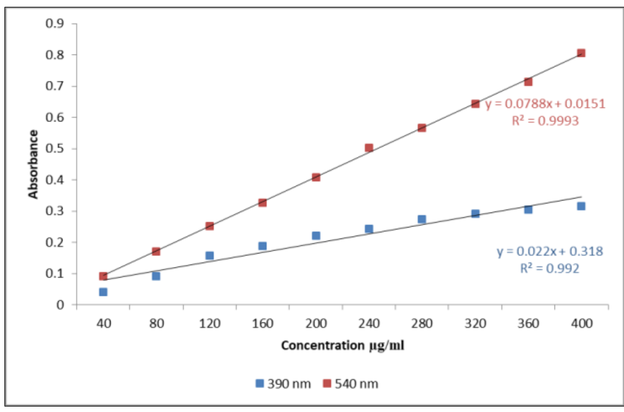

Figure 6 Molar ratio method in DMSO

Table 7 Molar ratio determination in DMF

Concentration (µg/mL)	40	80	120	160	200	240	280	320	360	400
Absorbance at 390nm	0.039	0.088	0.163	0.176	0.212	0.251	0.269	0.301	0.309	0.318
Absorbance at 540nm	0.101	0.154	0.246	0.312	0.411	0.512	0.543	0.639	0.704	0.801

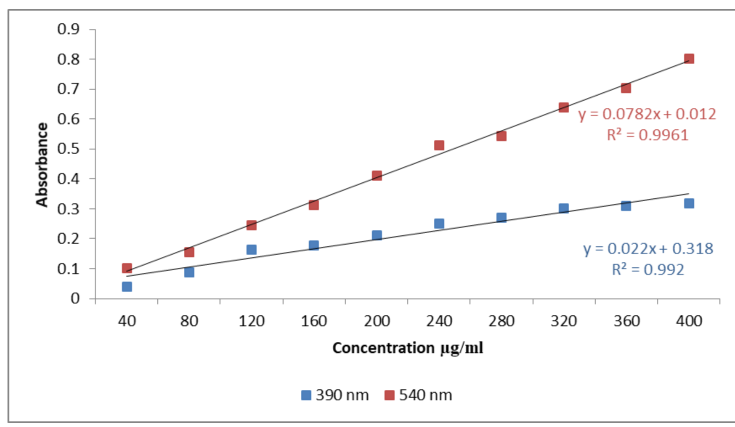

Figure 7 Molar ratio determination in DMF

3.3. Application of the developed method

The method analyzed drug content in amikacin injections, giving 100.76±1.2% and 100.6±1.96% (n=3) at 390nm and 540nm. We recognized the need to develop new methods to analyze amikacin purity and potency [5]. Though several techniques had been proposed, none employed visible spectrophotometry using ascorbic acid reagent - an approach we believed could provide useful complementary information. After reviewing the literature, we saw an opportunity to fill this gap by designing and validating such a method. We synthesized ascorbic acid reagent and optimized conditions to maximize color development and stability for amikacin. Satisfied with our method, we then validated it thoroughly to ensure clinically useful results. We demonstrated linearity over a wide concentration range, shown by excellent correlation coefficients. We proved accuracy by achieving nearly 101% recovery in real samples. We established precision at different concentrations, with low relative standard deviations, reflecting high repeatability. We also determined practical detection and quantitation limits, and applied our method to quantify amikacin in actual injections, finding results comparable to established methods. By statistically comparing to a validated approach, we confirmed our new method provides equivalent analytical power for this important antibiotic. We have therefore made available an additional option for quality testing during production and to doctors/pharmacists ensuring proper dosing. Validating this technique has also shown it can serve as a viable and meaningful alternative or complement to existing methods. The summary of the validation parameters are shown in Table 8.

Table 8 Summary of the validation parameters for the developed method

Parameter	Visible Spectroscopic method
Linearity Range	40-200µg/ml
Regression coefficient	0.992 (at 390nm)0.9997(at 540nm)
Linear regression equation	y = 0.022x + 0.318(at 390nm) y = 0.0782x + 0.012(at 540nm)
Precision	1.2%(at 390nm)1.96%(at 540nm)
Accuracy	100.76±1.2% and 100.6±1.96%
LOD	19.021µg/ml(at 390nm)5.220µg/ml(at 540nm)
LOQ	57.641µg/ml(at 390nm)15.819µg/ml(at 540nm)

4. Conclusion

The developed method proved simple, accurate, and precise. Ascorbic acid showed it could effectively estimate amikacin purity and dosage forms without interference from excipients. This method enables routine analysis of amikacin sulfate. Results indicated a precise, accurate, and linear approach that meets ICH guidelines for method validation. Therefore, it can transition from research to quality control for routine testing of amikacin as pure drug substance or final product.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

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Author's short biography

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	Prakash Nathaniel Kumar Sarella: Pharmaceutical scientist with a passion for innovative drug delivery solutions. Over 6 years of experience in drug delivery research and development. Expertise in nanomedicine, liposomes, polymer therapeutics, antibody-drug conjugates, and microneedle technologies.
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