

## IMPROVEMENT OF RELATED COMPOUND ANALYTICAL METHOD IN AMIKACIN BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Islam Sofiqul<sup>1, \*</sup>, Murugan V<sup>2</sup> and Prema Kumari KB<sup>2</sup>

<sup>1,2</sup> Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences,  
Dayananda Sagar University, Bengaluru-560078, Karnataka, India.

\* E-mail: [Sofi59964@gmail.com](mailto:Sofi59964@gmail.com)

### ABSTRACT

Amikacin is an antibiotic worn in the treatment of bacterial infectious diseases. An easy chromatographic technique was evaluated for the fortitude of the related compound. Newly improved method attained through isocratic elution on X bridge C<sub>18</sub> (250 x 4.6 mm, 5 $\mu$ m) column at 30 °C using a mobile phase consisting of phosphate buffer and methanol (70:30 v/v) with a flow rate of 1.0 mL/min. The UV detection was carried at 340 nm. The analytical execution of the new method was validated as per International Council for Harmonization (ICH) guideline

**Keywords:** Amikacin, chromatographic technique, related compounds, quantification

© RASAYAN. All rights reserved

### INTRODUCTION

This molecule belongs to a class of antibiotics (aminocyclitol glycoside) that show a wide spectrum towards severe infection caused by gram-negative bacteria. Amikacin is used mostly in the treatment of various skin disorders. This AG antibiotics molecule is almost found in white clear powder form and is generously soluble in water. It is synthesized by acylation of the amino group of Kanamycin<sup>2,3</sup>, hence Kanamycin can be considered as a possible related compound that might be produced during the manufacturing of the drug substances. An assessment of prose shows that various chromatographic techniques like Thin Layer Chromatography (TLC)<sup>4</sup>, Microbiological<sup>5</sup> were used for the detection. All the cited technique has some concern like the repeatability issue as well as the length of experimental time. Other detection techniques like Pulse Electrochemical Detection (PED) and Charged Electrochemical Detection (CAD) have also been used as detection techniques<sup>6</sup>. Several highly sensitive techniques like the Liquid chromatography-Mass spectroscopy (LC-MS) method enclosed for the study of Amikacin in animal tissue, human serum. The above-mentioned techniques primarily focused on the determination of the main antibiotic compounds other than its impurities<sup>7</sup>. Although these techniques can be used to detect these aminoglycosides, they are not widely available and required skilled operatives to use this detection technique. AG (aminoglycoside) are enormously hydrophilic compounds since they contain an elevated number of amino and hydroxyl moieties in their chain. Due to its polar nature, the use of an easy liquid chromatographic technique is not quite simple. The absence of a chromophore in the Amikacin structure made it difficult to detect it in the UV-visible region. Thus, an attempt was made to develop a chromatographic method to quantify the specified compound by High Performance Liquid Chromatographic technique using derivatization<sup>9</sup>. It is essential to compute and identify this related compound in drug substance to ensure drug safety and quality<sup>10</sup>. The major objective of this present work was to develop and validate a simple chromatographic process for quantification of the related compound in Amikacin by pre-column derivatization technique. Derivatization technique used as a straight detection mode for non-chromophoric molecules and can be deemed as an alternative for complicated detection methods<sup>11</sup>. The future method shows a distinct advantage regarding simplicity and easygoing pre column derivatization technique procedure. Pre column

derivatization is usually preferred over the post column technique because it doesn't require any exceptional tools<sup>12</sup>. Pre column derivatization technique reduces the consumption and exposure of larger amount solvent, any other extraction time.<sup>13,14</sup>

## EXPERIMENTAL

### Material and Methods

Amikacin, Kanamycin material procured from Merck. Methanol and outstanding chemicals like Potassium dihydrogen phosphate, 0.1M potassium hydroxide (KOH) solution, acetic acid, pyridine and Derivatizing agent Picryl sulfonic acid solution were taken from Merck.

The High-performance Liquid chromatography instrument consists of an isocratic pump (Water e2695 Alliance) equipped autosampler, column oven compartment and photodiode array detector (PDA) for detection. X bridge C<sub>18</sub> column (Make: Waters, Dimension: 250 x 4.6 mm, 5µm particle size) used as a suitable column in this study. The mobile phase comprises of mixture Buffer: Methanol (70:30) pump at 1.0 mL/min flow rate. Injection volume was set as 20 µL to run the sample set. The analysis was monitored at a wavelength of 340 nm in a photodiode array detector. Before the analysis of the system, the proposed column was pre-equilibrated with the mobile phase for 2 hr.

Various alternative columns like Spherisorb ODS C<sub>18</sub>, Dimension 250 X 4.6 mm; 5µm (Waters) and Agilent Zorbax C<sub>18</sub> Dimension 250 X 4.6 mm; 5µm (Agilent) were used during the initial method development stage.

Amikacin and its related compound Kanamycin (Amikacin Imp A) are lacking the presence of any active moiety in their molecular structure, hence peaks were not detected in the absence of any derivatizing reagents. Various Derivatizing reagents were used, but Picryl sulfonic acid solution was found as suitable reagent.

### Derivatizing Procedure

Volumetrically transferred 2.0 mL of derivatizing reagents, 3.0 mL of pyridine and closed the flask (containing sample solution). Shaken the flask vigorously for 30 sec. Added 1.0 mL acetic acid and vortex for 30 sec.

### Chromatographic Procedure

The mobile phase consisted of 2.7 g of phosphate buffer and methanol in the ratio 70:30 v/v. Prepared buffer was filtered through a 0.45-micron filter paper. The LC flow rate was 1 mL/min. An isocratic elution technique was performed to obtain a good separation with a short period. Conditioned the column with the mobile phase until the baseline was stable. LC column temperature was maintained at 30 °C for the duration. 20 µL of the solution was injected onto the column.

### Solution Preparation

#### Standard Stock

Hundred milligram of Amikacin and Ten milligram of Kanamycin (Amikacin Imp A) was accurately weighed and transferred into 100 mL flask separately, dissolved in diluent and then made up to the volume. Aliquots of standard stock diluted using diluent (mobile phase) to obtain the individual working standard solution.

#### SST Solution (System Suitability)

Pipetted out 0.1 mL of standard stock (Amikacin and Imp A) into 10 mL flask then adjusted the volume up to the mark.

#### Test Solution

Five Hundred milligram of Amikacin was accurately weighed and transferred into 100 mL flask. Dissolved and adjusted the volume up to the mark. Pipetted out 2.0 ml of stock and alienated in 10 mL flask. Made up to the mark with diluent.

### Validation Studies

The optimized chromatographic condition was progressed for validation study as per the ICH Q2 guideline.

Table-1: Solution Preparation for Precision, Linearity and Accuracy

Solution Name	Imp A Standard Stock (milliliter)	Amikacin Stock (milliliter)	Final Volume (milliliter)
TSR 0	-	2.0	10
TSR 50	0.5	2.0	10
TSR 100	1.0	2.0	10
TSR 150	1.5	2.0	10
Concentration of solution			
Solution Name	Imp A (mg/mL)	Amikacin (mg/mL)	Not Applicable
TSR 0	-	1.0	
TSR 50	0.005	1.0	
TSR 100	0.01	1.0	
TSR 150	0.150	1.0	

Where, TSR: Test solution of a related compound

### SST (System Suitability Test)

SST (System suitability test) was prepared, injected to confirm the accurate performance of the LC.

System execution was evaluated by six replicate of system suitability solution injection.

% RSD (Relative standard) of each peak area in six replicate injection, Tailing and theoretical plate count of Amikacin and Kanamycin peak was calculated. All the result is within the acceptable limit and met the criteria.

System suitability results are presented in Table-2. Chromatogram was presented in Fig.-1.

Table-2: Results for SST Solution

Parameter	Acceptance Criteria	Results	
		Amikacin	Kanamycin (Amikacin Imp A)
SST	Theoretical plates should be Not less than (NLT) 2000	5124	4563
	Tailing Factor should be Not more than (NMT) 1.5	1.2	1.2
	% RSD of six replicate injection should be NMT 5.0%	1.3	2.1

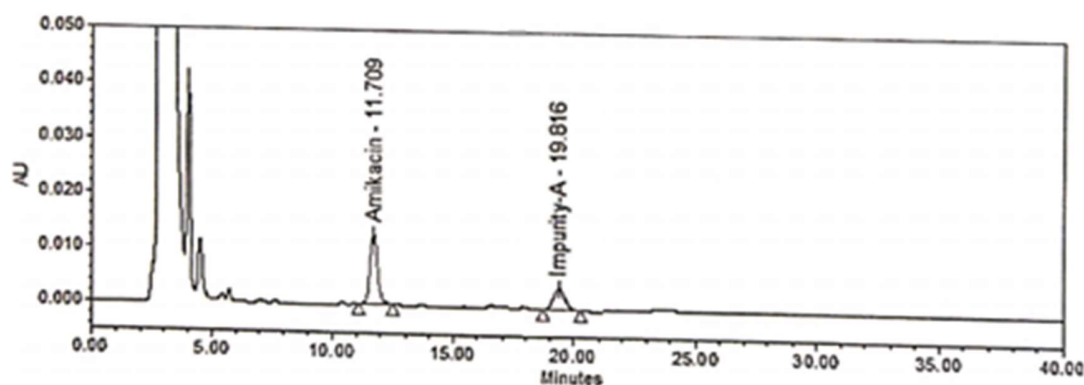


Fig.-1: Chromatogram for SST Injection

### Specificity

Method Selectivity was carried out to check any interference from blank to the main analyte. For ascertaining specificity, an injection of diluent, along with SST solution and Kanamycin (Amikacin Imp A) standard was infused.

No interfering peak was originated at the Rt (retention time) of Amikacin and its related compound in blank injection. The Rt (retention time) of the related compound was confirmed by injecting standard solution. Specificity results presented in Table-3.

Table-3: Results for Specificity Parameter

Injection	Results
Blank (diluent)	No interference was observed at the retention time (Rt) of Amikacin and its related compound
Acceptance criteria	No interference at the retention time (Rt) of Amikacin and its related compound. Even if it appears should be less than 50% of QL solution peak areas.

**Precision/ Repeatability**

Six replicate injection of 0.0100 mg/mL solution concentration was injected and premeditated the average Relative Standard Deviation (%RSD) of Experimental concentration values for the related compound (Kanamycin) in Amikacin. The results are presented in Table-4.

**Accuracy**

Accuracy was calculated using the test solution prepared at 50 % (LOQ concentration), 100% and 150 % level. Triplicate analysis of each test solution level was performed to determine the percentage recovery. The recovery of the kanamycin (Amikacin Imp A) was found between 95-105 %. A summary of % recovery results is presented in Table-4.

Table-4: Results for Precision, Linearity, Accuracy and LOQ Solution of Imp A

Parameter	Results		
	TSR 50	TSR 100	TSR 150
%RSD of Experimental Concentration (Precision)	3	1	3
Accuracy (% Recovery)	96	101	98
Signal to Noise at LOQ Solution	75	Not Applicable	
Linearity (Correlation Coefficient)	0.9919		

**Linearity and Limit of Quantification (LOQ)**

Under the proposed chromatographic condition, Experimental concentration Vs Theoretical concentration of the related compound. Method found to be linear over the assortment of LOQ level (50%), 100% and 150 % at the specified limit from LOQ concentration 0.005 mg/mL to 0.150 mg/mL (150%). The linearity graph was plotted using Experimental concentration against the Theoretical concentration. The value of the Correlation coefficient was premeditated using the linear regression analysis. Linearity results are presented in Table-4. Linearity Graph are plotted on Figure 2.

The limit of quantification (LOQ) is the lowest concentration of analyte that can be determined with acceptable accuracy and precision.

The concentration of Kanamycin (Amikacin Imp A) at the LOQ level was measured as 0.005 mg/mL. Signal-to-noise (S/N) ratio at the LOQ level was presented in Table-4.

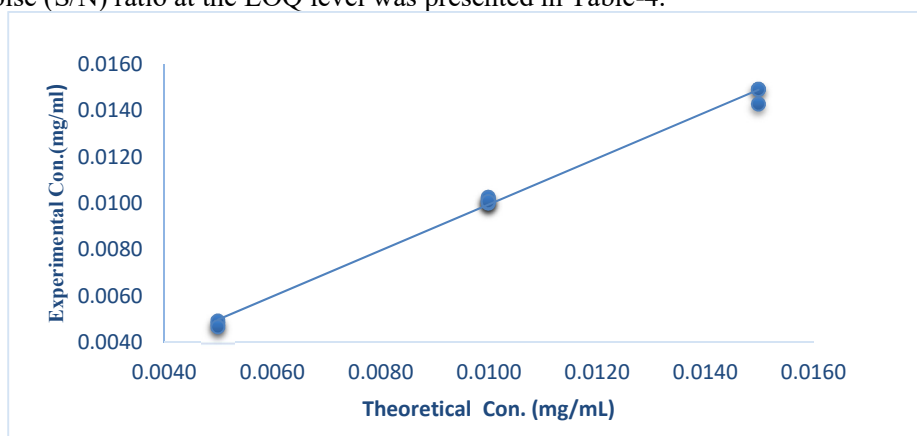


Fig.-2: Linearity Graph of Kanamycin

**Robustness**

Robustness was performed by a slight modification of chromatographic conditions (column temperature  $\pm 2$  °C). Method performance was evaluated by injecting the system suitability solution under modified chromatographic conditions. Robustness results are presented in Table-5 and Table-6.

Table-5: Results for SST Solution at Low Column Temperature (28 °C)

Parameter	Acceptance Criteria	Results	
		Amikacin	Kanamycin (Amikacin Imp A)
SST	Theoretical plates should be Not less than (NLT) 2000	6523	4856
	Tailing Factor should be Not more than (NMT) 1.5	1.1	1.2
	% RSD of six replicate injection should be NMT 5.0%	1.1	2.3

Table-6: Results for SST Solution at High Column Temperature (32 °C)

Parameter	Acceptance Criteria	Results	
		Amikacin	Kanamycin (Amikacin Imp A)
SST	Theoretical plates should be Not less than (NLT) 2000	5823	5746
	Tailing Factor should be Not more than (NMT) 1.5	1.2	1.3
	% RSD of six replicate injection should be NMT 5.0%	1.3	2.4

**RESULTS AND DISCUSSION****System Suitability**

The results of the SST parameter were studied for tailing factor, theoretical plate and peak area. All the system suitability results are satisfactory.

**Specificity**

There was no interference observed from the diluent.

**Precision, Linearity, Accuracy and Limit of Quantification**

Precision, linearity, accuracy and limit of quantification results are satisfactory. All the results met the acceptance criteria. Summary of results presented in Table 7.

Table-7

Parameter	Acceptance criteria	Results
Precision	% RSD of Experimental concentration at all the levels should not be more than 5 %	Table 4
Linearity	Plot a Linearity graph for Experimental concentration Vs. Theoretical concentration. The correlation coefficient should be NLT 0.99	Table 4 Figure 2
Accuracy	Recoveries of a related compound determined from QL to 150% of specification limit should be 80-120%	Table 4
LOQ	S/N ratio should be NLT 10	Table 4

**Robustness**

Robustness data generated in various conditions found satisfactory. Hence developed method found to be robust.

**CONCLUSION**

The estimated chromatographic method using the derivatization procedure gratifies all the parameters anticipated by ICH. A newly developed technique produces a good separation between the analyte peaks.

All the results obtained from the various parameters met the standard criteria. A newly improved simple procedure was found to be apposite for the estimation of the related compound in Amikacin aminoglycoside.

### REFERENCES

1. A. Soliven, I.A. Haidar, J. Tam N. Kadrichu, *Journal of Pharmaceutical and Biomedical Analysis*, **143**, 68 (2017), DOI: [10.1016/j.jpba.2017.05.013](https://doi.org/10.1016/j.jpba.2017.05.013)
2. S. Nicoli, P. Santi, *Journal of Pharmaceutical and Biomedical Analysis*, **41(3)**, 994(2006), DOI: [10.1016/j.jpba.2005.12.029](https://doi.org/10.1016/j.jpba.2005.12.029)
3. J.F. Ovalles, M.R. Brunetto, M. Gallignani, *Journal of Pharmaceutical and Biomedical Analysis*, **9**, 39(2005).
4. R. Hari, S. Taheruunisa, S.Y. Raut, S. Mutalik, K.B. Koteswara, *Journal of Applied Pharmaceutical Science*, **9(11)**, 19(2019), DOI: [10.7324/JAPS.2019.91118](https://doi.org/10.7324/JAPS.2019.91118)
5. S. Oguri, Y. Miki, *Journal of Chromatography*, 686(1996).
6. X. Zhang, J. Wang, Q. Wu, Y. Wang, *Molecules*, **1**, 24(2019), DOI: [10.3390/molecules24101902](https://doi.org/10.3390/molecules24101902)
7. Y. Bijleveld, T. de Haan, J. Tiersche, S. Jorjani, *Journal of Chromatography*, **8**, 110(2014), DOI: [10.1016/j.jchromb.2014.01.035](https://doi.org/10.1016/j.jchromb.2014.01.035)
8. B. Blanchaert, S. Huang, K. Wach. E. Adams, *Journal of Chromatographic Science*, **55**, 197(2017), DOI: [10.1093/chromsci/bmw169](https://doi.org/10.1093/chromsci/bmw169)
9. M.C. Caturla, E. Cusido, D. Westerlund, *Journal of Chromatographic Analysis*, **1**, 593(1992), DOI: [10.1016/0021-9673\(92\)80268-Y](https://doi.org/10.1016/0021-9673(92)80268-Y)
10. S.P. Vittal, S.V. Rao, K. Ramakrishna, *Rasayan Journal of Chemistry*, **12(3)**, 1601(2019), DOI: [10.31788/RJC.2019.1235183](https://doi.org/10.31788/RJC.2019.1235183).
11. E.D.A. Barbosa, F.R. Lourenco, *Brazilian Journal of Pharmaceutical Science*, **47(2)**, 261(2011), DOI: [10.1590/S1984-82502011000200007](https://doi.org/10.1590/S1984-82502011000200007)
12. K. Ruckmani, Z. Saleem, P. Khali, M. S. Muneera, *Pharmaceutical Method*, **2(2)**, 117(2011), DOI: [10.4103/2229-4708.84455](https://doi.org/10.4103/2229-4708.84455).
13. M. Usmani, S. Ahmed, M. Sheraz *et al.*, *Iranian Journal of Analytical Chemistry*, **5(2)**, 39(2018).
14. A.P. Topolyan, M.A. Belyaeva *et al.*, *Acta Naturae*, **8(3)**, 128(2016).

[RJC-5775/2020]