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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION OF AMIKACIN IN PURE AND MARKETED FORMULATION USING HPLC

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Keywords:

Amikacin, ICH guidelines, Pre column derivatization, RP-HPLC, Validation

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ABSTRACT: The aim of the study was to develop an easy, sensible and rapid method for the estimation of amikacin in both pure and marketed formulation by pre derivatisation technique using reverse phase C_{18} column in HPLC. In this RP-HPLC method Shimadzu LC 20 AD of PDA detector was used with LC solutions software. In this method gradient elution with a mobile phase of acetonitrile and acetate buffer in the ratio of 25:75 v/v at the flow rate of 2 mL/min and run time of 10 min were used. Validation parameters of HPLC were found at detection wavelength of 272 nm. Linearity was observed with a concentration range of 10 - 50 µg/mL with R^2 = 0.99. The method was robust with wavelength 271 and 273 nm with a flow rate of 1.8 and 2.2 mL/min showed good results. The retention time of drug was found at 8.9 min and assay showed 98.1%. RP-HPLC method was validated as per ICH guidelines and can be used for routine quantitative analysis of amikacin by RP-HPLC using PDA detector in both pure and marketed formulation. All the results of linearity, accuracy, precision were within the limits. The proposed method was highly sensitive, reproducible and reliable.

INTRODUCTION: Amikacin (AMK) is an antibiotic belongs to amino glycosides. It is used for bacterial infections like sepsis, meningitis, pneumonia, joint infections urinary tract infections and intra-abdominal infections. It is also used in multidrug resistant tuberculosis. It is given in the form of injections to veins or muscles. Usage of amikacin causes deafness, renal damage, and paralysis. If it is given to pregnant women there may be chances of permanent loss of hearing in newborn baby [1, 2, 3, 4]. A detailed literature review indicated that there are few analytical and bioanalytical methods were reported like calorimetry [5], HPLC [6, 7, 8, 9, 10], LCMS [11] and immunoassay.

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But till date there were no reported methods for amikacin using HPLC with PDA detector. The chemical structure of amikacin was shown in **Fig. 1**, which has four primary amine groups, one secondary amine group, one primary OH group and seven secondary OH groups [12]. Direct HPLC-PDA methods are not available because drug is non UV absorbent. Hence, it is derivatized with mixture of HPLC grade acetyl acetone, formaldehyde and sodium acetate buffer. It undergoes Hantzsch condensation where the amine group of amikacin reacts with the two molecules of acetyl acetone and one molecule of formaldehyde gives a dihydro lutidine derivative which is yellow in color. The reactions were shown in **Fig. 2**.

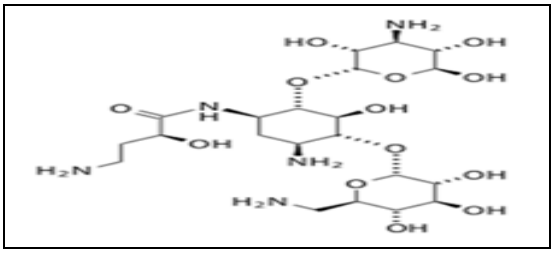

FIG. 1: CHEMICAL STRUCTURE OF AMIKACIN

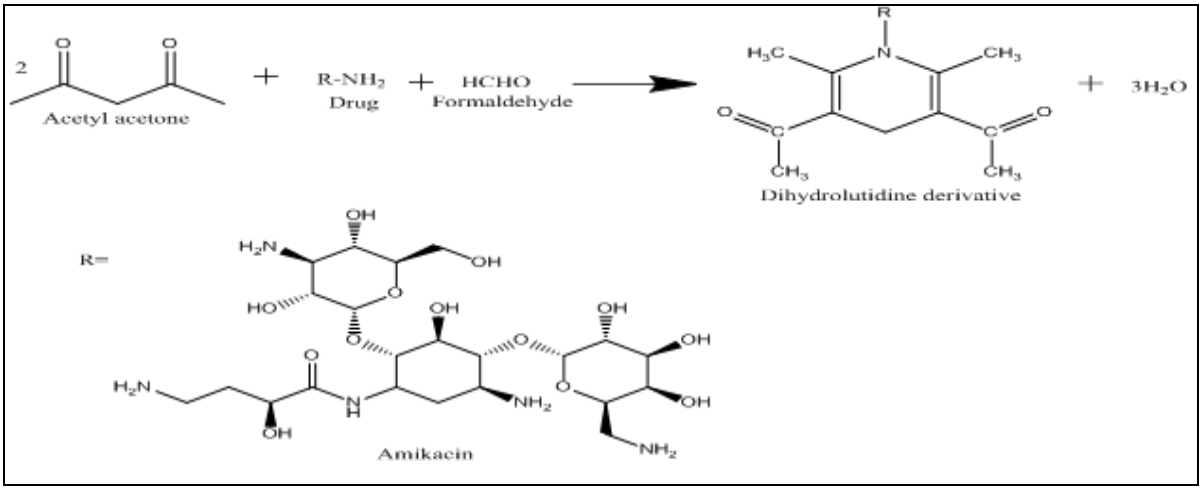

FIG. 2: DERIVATIZATION REACTION OF AMIKACIN WITH REAGENT

METHODS AND MATERIALS:

Equipment: Shimadzu LC-20AD with PDA detector were used for chromatographic separations. LC solution software was used for data analysis. Shimadzu electronic weighing balance was used for weighing samples and mark ultra sonicator was used for sonication of mobile phases and samples.

Chromatographic Conditions: Mobile phase of acetonitrile and acetate buffer in the ratio of 25:75 v/v with a flow rate of 2 mL/min was used in binary gradient elution. 50 µL volume of sample was injected and amikacin was detected at 272 nm. The separations were achieved with phenomenex C_{18} column (5µ, 230 × 4.6 mm) with ambient temperature.

Chemicals and Reagents: Amikacin sulfate was procured from Shri Chem, Mumbai. All chemicals used of analytical grade and was procured from loba chemicals. HPLC grade solvents are purchased from Merck India Ltd., Mumbai.

Amikacin Standard Stock Solution: The standard stock solution of amikacin was prepared by taking 100 mg of pure drug into 100 mL volumetric flask and dissolved with HPLC grade water and made upto mark with HPLC grade water. Further serial dilutions were made to get 10,20,30,40 and 50 µg/mL solutions.

Preparation of 0.1 M Sodium Acetate buffer Solution: Buffer solution was prepared by taking 13.6 gm of sodium acetate in 800 mL of HPLC grade water, to that 6 mL of glacial acetic acid was added and pH was adjusted to 5 with 25% NaOH. Volume was made upto 1000 mL with HPLC grade water.

Preparation of Derivatizing Agent: Derivatizing agent was prepared by mixing 1 mL of 0.1M sodium acetate buffer, 1 mL of formaldehyde, 2mL of acetyl acetone and 6 mL of water. It should be freshly prepared. The above solution was sonicated for 20 min and should be stable for 1 h.

Preparation of Mobile Phase for HPLC Analysis: The mobile consists of acetonitrile and 0.1M acetate buffer, filtered through 0.45 µ membrane filter and used for analysis.

Procedure for Derivatization Reaction:

A. Preparation of Blank Solution: The blank solution was prepared by mixing 1 mL of HPLC grade water with 2 mL of derivatizing agent into a 10 mL volumetric flask. The mixture was heated in a water bath at 55 ºC for 15 min. The solution was cooled and transferred into 50 mL volumetric flask, the volume was made upto the mark with the mobile phase consisting of acetonitrile and buffer in the ratio of 25:75. Blank chromatogram is shown in **Fig. 3**.

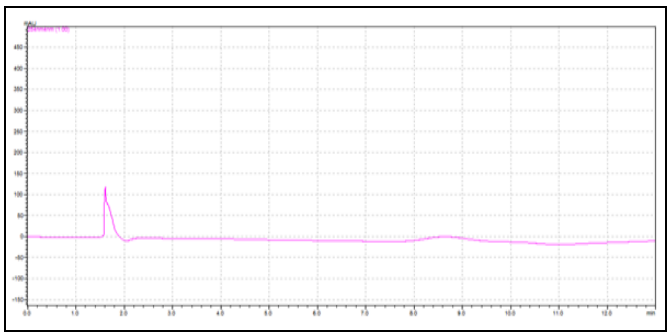

FIG. 3: BLANK CHROMATOGRAM

B. Preparation of Amikacin Working Standard Solutions: Working standard amikacin solution was prepared by mixing different volumes of each stock solution (1000 µg/mL) *i.e.*, 0.5, 1.0, 1.5, 2.0 and 2.5 mL with 2 ml of derivatizing agent in 10mL volumetric flask. The mixture was heated in a water bath at 55 ºC for 15 min. The solution was cooled and transferred into 50 mL volumetric flask, the volume was made upto the mark with the mobile phase consisting of acetonitrile and buffer in the ratio of 25:75 v/v. Standard and sample chromatogram of amikacin was shown in **Fig. 4** and **5**.

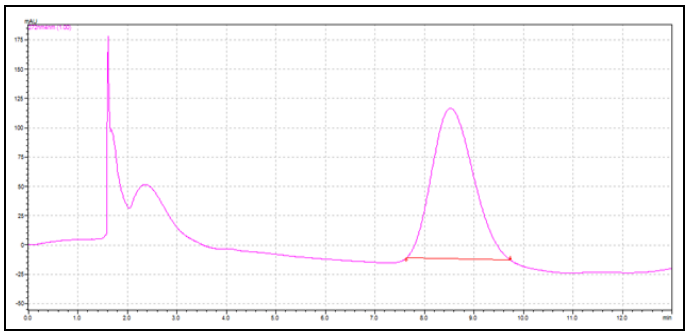

FIG. 4: STANDARD CHROMATOGRAM OF AMIKACIN SULFATE

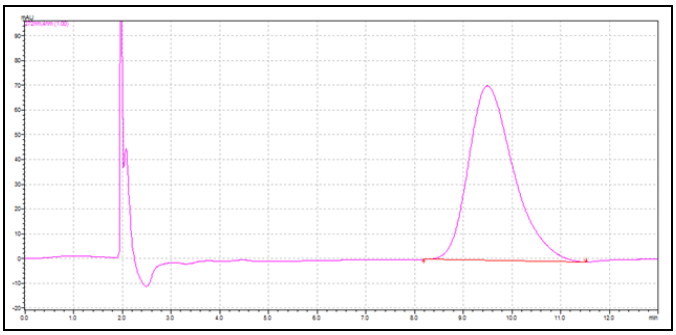

FIG. 5: SAMPLE CHROMATOGRAM OF AMIKACIN SULFATE

RESULTS AND DISCUSSION:

Validation of HPLC Method: Method was validated as per ICH guidelines [13, 14].

Linearity: A direct relationship ought to be assessed over the scope of logical methodology with a base five fixations. A series of 10-50 µg/mL standard stock solutions were injected to HPLC system and peak areas were noted down. A plot of peak area and analyte concentration was assessed by proper statistical strategies where by slant, intercept and regression (R^2) were ascertained and the information was given in the **Fig. 6**. All the optical parameters are given in the **Table 1**.

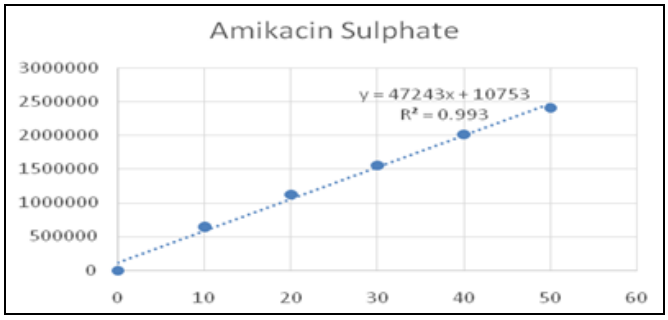

FIG. 6: LINEARITY PLOT FOR AMIKACIN SULFATE

TABLE 1: CHROMATOGRAPHIC PARAMETERS OF THE DEVELOPED METHOD

Parameters	Method
λ_{max}	272 nm
Column	Phenomenex C_{18}
Mobile phase	Acetonitrile : Acetate Buffer (75:25)
Flow rate (mL/min)	2
Injection volume	50 µL
Beers law limits (µg/mL)	10-50
Regression equation y = mx+c	Y= 47243x + 107532
Slope, m	47243
Intercept, c	107532
LOD, µg/mL	8.08
LOQ, µg/mL	24.50
Correlation coefficient (r^2)	0.9933

Accuracy: The accuracy of the tactic could be outlined because the closeness of the check outcomes got by the technique to the real esteem. It is the measure of the precision of the diagnostic technique created. Exactness may frequently express according to percent recovery by the measure of a known measure of analyte included. Exactness might be controlled by applying the technique to sample where in which known measure of analyte have been included both above and beneath the typical levels expected in the samples. Exactness is then ascertained from the test score as percent of analyte regained by the assay. Accuracy of the method was tested by spiking 50, 100 and 150% of standard amikacin solution. The % recovery and % RSD were calculated and results were given in the **Table 2**.

Precision: The exactness of the method is the level of agreement among singular test outcomes when the strategy is repeated over and over to various samplings of homogenous examples. This is normally communicated as the standard deviation or the relative standard deviation (RSD).

TABLE 2: ACCURACY / % RECOVERY OF THE DEVELOPED METHOD

Level of recovery	Amount of formulation (µg/mL)	Amount of pure drug (µg/mL)	Total amount of drug (µg/mL)	Peak area	Difference	% Recovery	Mean
50	20	10	30	1672525	1028300	91.55	99.49
	20	10	30	1899289	1255064	111.74	
	20	10	30	1713486	1069261	95.20	
100	20	20	40	2269988	1146787	102.10	101.41
	20	20	40	2296785	1173584	104.48	
	20	20	40	2219853	1096652	97.64	
150	20	30	50	2611697	1059130	94.29	100.15
	20	30	50	2500291	947724	84.38	
	20	30	50	2920395	1367828	121.78	

Repeatability incorporates examination of imitates by the specialist using a comparable instrument and method and coordinating the precision over a short period while reproducible. The RSD esteems are vital for demonstrating level of assortment anticipated when the methodology is reiterated a couple of time in a standard situation. The % RSD ought to be fewer than 2 as indicated by ICH rules. The consequences of accuracy contemplates were given in the **Table 3** and **4**.

TABLE 3: METHOD PRECISION (INTRADAY)

Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
10	648976	30	1582987	50	2426105
10	645125	30	1552731	50	2401388
10	643739	30	1572355	50	2407605
10	646691	30	1552589	50	2408667
10	643822	30	1552173	50	2401499
10	644925	30	1562561	50	2411241
AVG	645546.3	AVG	1562566	AVG	2409418
SD	1820.08	SD	11669.93	SD	8294.79
% RSD	0.28	% RSD	0.75	% RSD	0.34

SD: Standard Deviation RSD: Relative Standard Deviation

TABLE 4: METHOD PRECISION (INTERDAY)

Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area	Concentration (µg/mL)	Peak area
10	653278	30	1598754	50	2436105
10	645753	30	1557313	50	2418858
10	649852	30	1576355	50	2427695
10	646852	30	1559570	50	2489267
10	648229	30	1554138	50	2425926
10	645842	30	1565643	50	2421241
AVG	648301	AVG	1568629	AVG	2436515
SD	2640.07	SD	15254.67	SD	24214.06
% RSD	0.407	% RSD	0.97	% RSD	0.99

SD: Standard Deviation RSD: Relative Standard Deviation

Limit of Detection (LOD) and Limit of Quantification (LOQ): The limit of detection is the most reduced level of analytes that can be distinguished, but cannot be quantified under the standard conditions. The limit of quantification is the grouping of analyte in an example that might be resolved with adequate exactness and precision when required technique is connected. It is estimated by dissecting tests containing known amounts of the analyte and deciding the most reduced level at which adequate level of precision and accuracy are feasible. By and large the LOQ is roughly double the LOD. The LOD and LOQ were calculated as 3.3^{*} standard deviation/slope and 10^{*} standard deviation/slope respectively. Serial dilutions of 10 - 50 µg/ml solutions are injected thrice and calculated the average, standard deviation and slope of three trials. The results of this method were given in the **Table 5**.

TABLE 5: LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION

Average of SD	Average of SD (3 trails)	LOQ = 10*(SD/slope)
36663 48451 56113	47075.67	3.29
Average of slope	**Average of Slope (3 trails)**	**LOQ = 10*(SD/slope)**
47238 46810 47251	47099.66	9.99

Robustness: The hardiness of a technique is assessment of its ability to check unaffected by little, yet ponder varieties in proficiency parameters. The robustness was checked by varying different parameters like detection wavelength by ± 2 nm, flow rate by ± 0.2 mL and temperature by ± 5 ºC. The strategy was observed to be hearty and results were given in the **Table 6**.

TABLE 6: ROBUSTNESS

Condition		Tailing	% RSD
As such condition (optimized method)		1.473	
Flow rate	Decreased (-0.2 mL/min)	1.436	1.79
	Increased (+0.2 mL/min)	1.504	1.47
Column temperature	Decreased (-5°C)	1.486	0.62
	Increased (+5°C)	1.469	0.19
Wave length	Decreased (1nm)	1.489	0.76
	Decreased (2nm)	1.451	1.06
	Increased (1nm)	1.464	0.46
	Increased (2nm)	1.448	1.21

CONCLUSION: The planned pre column derivatised HPLC method intended for the determination of amikacin in pure and pharma-ceutical formulation has showed results within the specified limits and it can be applied for routine analysis. This method is validated according to ICH guidelines. The results of linearity, exactness, repeatability, LOD, LOQ and robustness were within the limits. Hence this RP-HPLC method is simple, sensitive and real with exactness and precision.

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CONFLICT OF INTEREST: Nil

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