International Journal of Current Pharmaceutical Research

ISSN- 0975-7066 Vol 10, Issue 1, 2018

Original Article

SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF AMIKACIN IN PURE AND PHARMACEUTICAL DOSAGE FORM

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Received: 21 Oct 2017, Revised and Accepted: 12 Dec 2017

ABSTRACT

Objective: 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 using the spectrophotometric method.

Methods: Due to lack of chromophoric group in the amikacin, it was derivatized with 0.1 mmol chloranillic acid reagent. For the estimation of amikacin, Shimadzu UV-1700 model spectrophotometer with UV probe software was used. The method was based on simple charge transfer complexation of the drug with a p-chloranillic acid reagent to give a purple coloured product which was measured at 524nm against blank solution.

Results: The derivatised product of amikacin was detected at a wavelength of 524 nm. Linearity was observed with the concentration range of 20-100 µg/ml with a regression coefficient of 0.9803. Results of all the parameters were within the acceptance criteria with % RSD less than 2.

Conclusion: The spectroscopic method was validated as per ICH guidelines and was found to be applicable for routine quantitative analysis of amikacin in marketed formulations also. The results of linearity, precision, accuracy LOD and LOQ were within the specified limits. The method is highly sensitive, robust, reproducible and specific.

Keywords: Amikacin, Analytical method development, ICH guidelines, Pharmaceutical dosage, Spectrophotometric.

DOI: http://dx.doi.org/10.22159/ijcpr.2018v10i1.24703

INTRODUCTION

Amikacin is an aminoglycoside antibiotic used for many gram-negative bacterial infections like infections in the urinary tract, infections in brain, lungs and abdomen which are resistant to gentamicin, kanamycin or tobramycin. When compared to other aminoglycosides amikacin has very narrow safety margin i.e., its therapeutic plasma concentration is 8-16 µg/ml. When it is given to renal impaired patients for over a period of time it shows ototoxicity and nephrotoxicity [1-3].

A detailed literature review indicated that there are few analytical and bioanalytical methods were reported like calorimetry [4], HPLC [5-8], LCMS [9] and immunoassay [9]. But till date, there were no reported methods for UV visible Spectroscopy by using choloranilic acid as a derivatizing agent. This method is simple, sensitive, rapid and can be possible to extend to HPLC method using similar reagent.

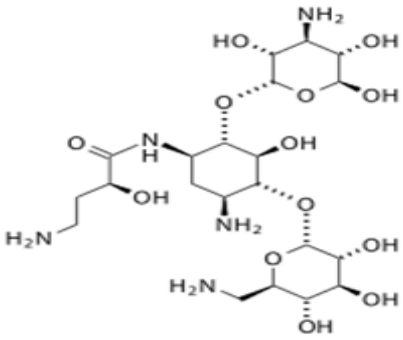

Fig. 1: Amikacin chemical structure

The 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 [10]. Direct UV methods are not available in literature because the drug is not absorbing in the UV region. Hence, it is essential to derivatize with 0.1% chloranilic acid using electron transfer reaction. The detailed chemical reaction between amikacin and chloranillic acid was shown in fig. 2.

METHODS AND MATERIALS

Equipment

Absorbance and spectral were measured by using Shimadzu UV-visible spectrophotometer model 1800 with 1 cm pair quartz cells. Shimadzu electronic weighing balance was used for weighing samples.

Chemicals and reagents

Amikacin Sulfate was procured from Shri Chem, Mumbai and Chloranillic acid was procured from Loba chem and acetonitrile from Merck.

Amikacin standard stock solution

100 mg pure drug of amikacin was taken into 100 ml volumetric flask and dissolved with distilled water and made up to mark with distilled water. Further 10 ml was taken from the above solution and diluted to 100 ml with distilled water to get 100µg/ml solution. From these serial dilutions were made to get 20,40,60,80 and 100 µg/ml solutions.

0.1% mmol chloranilic acid reagent [11, 12]

Solution A (1% chloranilic acid): 0.208 gms of cholranilic acid was weighed into 100 ml volumetric flask and dissolved in few ml of acetonitrile. Volume was made up to mark with acetonitrile.

Working solution B (0.1% choloranilic acid): From solution A pipette out 10 ml and dilute to 100 ml using acetonitrile.

Assay procedure

1 ml of the intramuscular injection containing 250 mg was transferred into 10 ml volumetric flask. It was dissolved using distilled water. Finally, volume was made up to 10 ml using distilled water. The solution was further diluted for analysis to get a concentration of 25µg/ml. The assay results are tabulated in table 2.

RESULTS AND DISCUSSION

Validation of the method [13, 14]

According to ICH guidelines validation of the method was carried out. Linearity, accuracy, precision, selectivity, robustness and ruggedness parameters were done.

Linearity

A series of amikacin sulfate solutions were prepared in the range of 20-100µg/ml from the stock solution of 1000µg/ml. The resultant solution was measured at 524 nm against the reagent blank. The overlay graphs of absorption of the standard drug and calibration graph were shown in the fig. 2 and 3 respectively.

Accuracy

Accuracy is the nearness of the measured value to the obtained value of the sample.

To determine this three different standard concentrations of 50%, 100% and 150% are added to the sample which is procured from the market. The results obtained for the spiked drug are given in % recovery (94.44–106.4%) shown in the table 3.

Precision

The precision of the analytical method was determined by measuring the fixed concentration of the drug solution for 6 times within the Beer's range and absorbance was found. The results of amikacin were given in the table 4-7.

Limit of detection (LOD) and limit of quantitation (LOQ)

The LOD and LOQ for amikacin sulfate were determined using calibration standards. The LOD and LOQ were calculated as 3.3* standard deviation/slope and 10* standard deviation/slope respectively.

Robustness and ruggedness

To determine the robustness of the method, reaction time and reagent concentrations were slightly altered with optimum values in spectrophotometry. To check the ruggedness, the analysis was done by four different analysts and on three different spectrophotometers using the same analyst. The robust data are expressed in % RSD. The results of amikacin were in table 8 and 9.

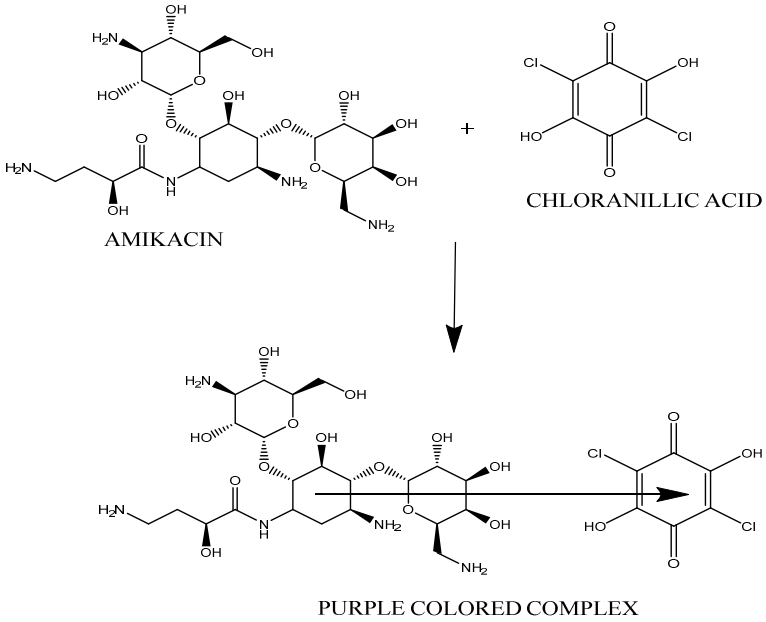

Fig. 2: Chemical reaction between amikacin and reagent

Sandell's sensitivity

The serial dilutions of 20-100µg/ml solutions absorbance were taken and the sensitivity is calculated using the formula: Sandell's Sensitivity (*л*) = Conc. (µg/100 ml) x 0.001/D1 value. The results were given in the table 10.

Selection of chloranillic acid reagent was based on the higher reactivity due to its stronger chromophore group in its structure when compared to other reagents. It enables its use for colourimetric determination of several amino groups.

The drug shows maximum absorption at 524 nm with a linearity range of 20-100 µg/ml. The method is also validated for precision, accuracy, LOD, LOQ. The precision of the method was found to be 1.78µg/ml,1.76 µg/ml and1.38 µg/ml.

The percentage recovery was ranging from 94.44% to 106.94%. And the LOD and LOQ were found to be 6.49 µg/ml and 19.68 µg/ml respectively. These data's confirms the method is very sensitive and effectively used for quantification of Amikacin sulfate.

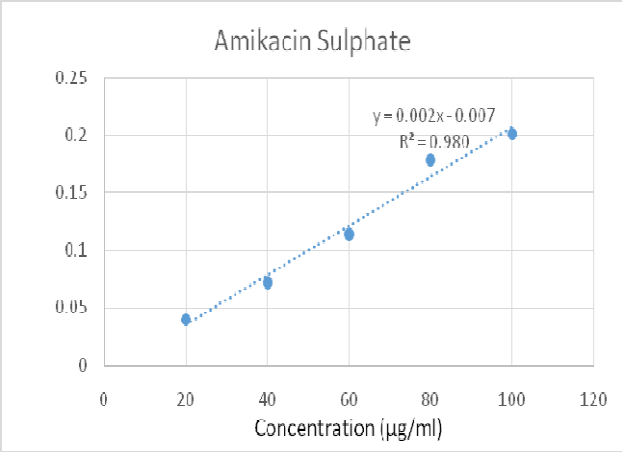

Fig. 3: Linearity plot for amikacin sulfate

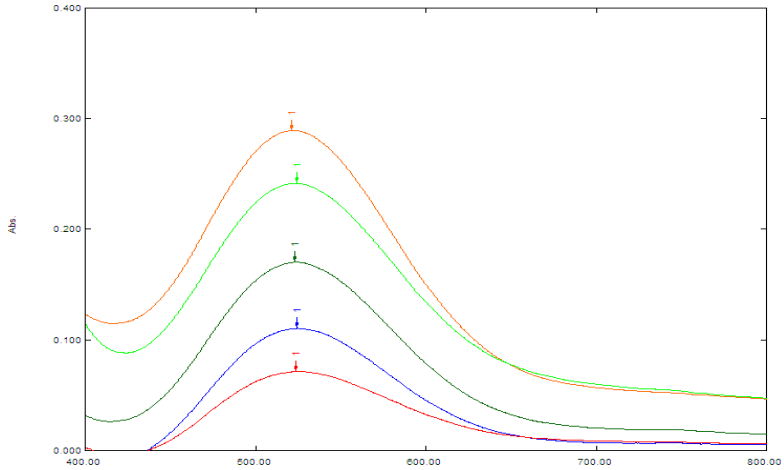

Fig. 4: Overlay absorption spectra of amikacin sulphate

Table 1: Optical parameters of the method

Parameters	Method
λmax	524 nm
Beers law limits µg/ml	20-100
Regression equation y=mx+c	Y=0.0021x-0.0075
Slope,m	0.0021
Intercept,c	-0.0075
LOD, µg/ml	6.49
LOQ, µg/ml	19.68
Correlation coefficient(r^2)	0.9803
Sandell's Sensitivity, µg/cm^2/0.001 A. U.	0.23
Molar absorpitivity, $cm^{-1}M^{-1}$	$0.122*10^4$

Table 2: Assay procedures

Brand name	Available form	Label claim	Amount found	Assay
MIKACIN	IM injection	250 mg/ml	248.97 mg/ml	99.58

Table: 3 Accuracy/% recovery

Level of recovery	Amount of formulation (µg/ml)	Amount of pure drug (µg/ml)	Total amount of drug (µg/ml)	Absorbance	Difference	% recovery	Mean
	40	20	60	0.113	0.073	101.39	100.46
50	40	20	60	0.111	0.071	98.61	
	40	20	60	0.113	0.073	101.39	
	40	40	80	0.143	0.071	98.61	100.92
100	40	40	80	0.149	0.077	106.94	
	40	40	80	0.142	0.07	97.22	
	40	60	100	0.189	0.075	104.17	99.07
150	40	60	100	0.182	0.068	94.44	
	40	60	100	0.185	0.071	98.61	

Table 4: Method precision (intraday)

Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance
	0.0401		0.11		0.203
20	0.0409	60	0.109	100	0.201
	0.042		0.112		0.21
	0.0405		0.107		0.209
	0.0409		0.11		0.201
	0.04		0.106		0.203
Avg	0.0407	Avg	0.109	Avg	0.2045
SD	0.0006	SD	0.002	SD	0.003
% RSD	1.63	%RSD	1.83	%RSD	1.77

Avg: Average SD: Standard Deviation RSD: Relative Standard Deviation

Table 5: Method precision (interday)

Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance
	0.041		0.109		0.209
20	0.04	60	0.11	100	0.21
	0.042		0.113		0.205
	0.04		0.112		0.203
	0.041		0.11		0.207
	0.04		0.115		0.213
Avg	0.0406	Avg	0.115	Avg	0.2078
SD	0.0007	SD	0.002	SD	0.003
%RSD	1.83	%RSD	1.84	%RSD	1.58

Avg: Average SD: Standard Deviation RSD: Relative Standard Deviation

Table 6: System precision (intraday)

Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance
20	0.04	60	0.109	100	0.199
	0.04		0.11		0.203
	0.042		0.114		0.201
	0.04		0.112		0.2
	0.04		0.109		0.197
	0.0401		0.113		0.203
Avg	0.0405	Avg	0.111	Avg	0.2005
SD	0.0007	SD	0.001	SD	0.002
%RSD	1.86	%RSD	1.75	%RSD	1.06

Avg: Average SD: Standard Deviation RSD: Relative Standard Deviation

Table 7: System precision (interday)

Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance	Concentration µg/ml	Absorbance
	0.0401		0.112		0.201
20	0.04	60	0.114	100	0.199
	0.0403		0.109		0.203
	0.042		0.11		0.2
	0.0399		0.109		0.201
	0.0401		0.11		0.206
Avg	0.0405	Avg	0.11	Avg	0.201
SD	0.0007	SD	0.001	SD	0.002
%RSD	1.82	%RSD	1.62	%RSD	1.13

Avg: Average SD: Standard Deviation RSD: Relative Standard Deviation

Table 8: Robustness data of the developed method

Wavelength (nm)	Concentration µg/ml	Absorbance	Wavelength (nm)	Concentration µg/ml	Absorbance
523	60	0.114	525	60	0.114
	60	0.112		60	0.111
	60	0.113		60	0.113
	Avg	0.113		Avg	0.112
	St Dev	0.001		St Dev	0.001
	%RSD	0.88		%RSD	1.35

Table 9: Ruggedness of the developed method

Concentration µg/ml	Linearity absorbance	Change in instrument absorbance	Mean	SD	%RSD
0	0	0	0	0	0
20	0.04	0.041	0.04	0.0007	1.74
40	0.072	0.074	0.073	0.001	1.94
60	0.114	0.112	0.113	0.001	1.25
80	0.179	0.176	0.177	0.002	1.98
100	0.201	0.205	0.203	0.003	1.39

SD: Standard Deviation RSD: Relative Standard Deviation

Table 10: Sandell's sensitivity

S. No.	Concentration(µg/ml)	Absorbance	Sensitivity	Mean sensitivity
1	20	0.04	0.5	
2	40	0.072	0.278	0.23
3	60	0.114	0.175	
4	80	0.179	0.112	
5	100	0.201	0.010	

CONCLUSION

The developed spectrophotometric method was easy, responsive and authentic with good precision and accuracy. The procedure did not involve any critical steps; hence it can be used routinely for determination of amikacin in pure and in the marketed formulation.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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