J Young Pharm, 2016; 8(1): 28-32
A multifaceted peer reviewed journal in the field of Pharmacy
www.jyoungpharm.org

Original Article

Quantitative Determination of Amikacin Sulfate using Vanillin from pure and commercial brands available in Pakistan

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ABSTRACT

Objective: To determine the amikacin sulfate by developing a new spectrophotometric method by derivatization using double beam spectrophotometer from pure and commercial brands. **Method:** A quantitative analytical method was developed by amikacin sulfate using spectrophotometer after derivatization with vanillin under optimized parameters like pH, heating time and temperature, volume of reagent, Impact of mixing order, Effect of addition of solvents and Effect of excipients. The method was successfully applied on bulk and different brands containing amikacin sulfate. **Results:** The bulk and pharmaceutical analysis was carried out of different brands with formation of slight yellow colored imine base. The product showed absorbance at 400 nm with molar absorptivity of 5.27×10^3 L/mole/cm. In a concentration of 10-50 µg ml^{-1} a linear relationship was established with absorbance which follow the beer's law with coefficient of determination r^2 0.9991-0.9998. The procedure was valid because it did not show change in absorbance of the derivative up to 3 days. The sandells sensitivity was calculated as 0.004 at 0.45 µg mL^{-1} of amikacin sulfate with vanillin. The percentage of recovery was 103-106.4% with RSD of 0.004-0.005.

Key words: Amikacin Sulfate, Vanillin, Spectrometric, Pakistan.

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DOI: 10.5530/jyp.2016.1.7

INTRODUCTION

Amikacin sulfate belongs to group of aminoglycosides and chemically it is {O-3-amino-3-desoxy-á-d-glucopyranosyl-(1-6)-O-[6-amino-6-desoxy-á-d-glucopyranosyl-(1-4)]-N1-(4-amino-2-hydroxy-1-oxobutyl)-2-desoxy-d-streptamine,($C_{22}H_{43}N_5O_{13}$)} sulphate.[1] There are different types of aminoglycosides which shows broad activity against pathogens specially to treat infections which are resistant to gentamicin and tobramycin. It is effective against both gram (+ve) and gram (-ve) microorganism.[2] Amikacin like other aminoglycosides causes nephrotoxicity and ototoxicity therefore drug monitoring is essential.[3]

Amikacin is useful in both humans and animals because it is effective against both types of bacterial species i:e gram (+ve and –ve). Amikacin is useful in the treatment of fatal nosocomial gram-negative bacillary infectious diseases and also used against aerobic gram-ve bacilli, strains of *P.aeruginosa*, proteus and serratia. It is also useful against strains of *E. coli*, *Enterobacter* and *Klebsiella*, which show resistant to GEN and TOB. Acinetobacter, Flavobacter and Providencia produce the resistant strains towards the AMK. Gram +ve bacteria of anaerobic origin are effectively treated with AMK. Tuberculosis is also treated with AMK including STP-resistant strains, atypical mycobacteria and atypical mycobacterial infection in acquired immunodeficiency syndrome (AIDS) patients.

Amikacin and other aminoglycosides are reacted with suitable reagents due to lack of chromophoric group so it is difficult to analyze colorimetric without derivatiztion with any chromophoric group containing compound like vanillin. Aldehydes produce shiff base by reacting with primary amino group present in aminioglycosides. Vanillin was chosen due to its water solubility and drug is also soluble in water. This method is useful to analyze amikacin from pharmaceutical preparations. Amikacin sulfate is quantitatively determined through various analytical techniques such as spectrophotometry,[4-8] spectroflourimetry[9-13] and HPLC methods.[14-25] The aminoglycoside antibiotic activity can be increased by association with Herbs like activity by *Pityrogramma calomelanos* (L.).[26] The spectroscopic method for assay of amikacin sulfate is not official in any pharmacopoeia.

MATERIAL AND METHOD

MATERIALS

The pharmaceutical grades of the chemical and reagents were used in this study. During the current study glass distillation assembly was used collect the required distilled water. Pure amikacin sulfate Bosch Pharmaceuticals (Pvt) and vanillin (VAN) from Merck were utilized. The different solutions of buffers were used with pH range of 1-14. These aqueous buffers were prepared as 0.1Msolution of acetic acid with sodium acetate, potassium chloride with hydrochloric acid, Sodium chloride with sodium hydroxide, sodium tetra borate with sodium hydroxide and boric acid.

INSTRUMENTATION

The present method was performed using 1cm cells of fused silica on double beam spectrophotometer UV-1601 (Shimadzu Corporation, Japan).

ANALYTICAL METHOD

Preparation of standard solution

Accurately weighed 50 mg of amikacin sulfate test standard was transferred to a 100 mL volumetric flask and water was added to dissolve it. Finally water was added up to the mark. The 2% solution of vanillin was prepared with water and alcohol mixture in 9:1 ratio. The 1M standard buffer solutions were prepared with water.

Analytical method for determination of AMK

The 0.1-0.5mL of aqueous solution (500 µg/mL) of amikacin sulfate was taken in (05 milliliter) volumetric flasks followed by addition of 1.5 milliliter of 2% vanillin and 0.5 milliliter of buffer (borate) with pH 12. The solution was heated for 15 minutes at 95^{0}C in boiling water bath. After cooling the contents at room temperature water was added up to the mark. Finally an absorbance of solution at 400 nm was observed using reagent blank arranged in a parallel manner without adding analyte.

Reference UV procedure to determine pure AMK (standard)

The 0.2-1 mL of amikacin sulfate (pure) solution (10 milligram per milliliter) was taken in volumetric flasks (05 milliliters) and water was added quantity sufficient to produce the required volume. Then the absorbance was observed against water (solvent) at 191 nm. The molar absorptivity was calculated as $5.172x10^2$ Liter/mole/centimeter.

RESULT AND DISCUSSION

Amino group in amikacin sulfate reacts with vanillin (aldehyde) to produce an imine base (chromogen) which showed a maximum absorbance at on 400 nm with molar absorptivity was $5.27x10^3$ L/mole/cm. Therefore Vanillin was used and studied as derivatizing reagent to determine amikacin spectrophotometerically. The different other analytical parameters were optimized i.e., Concentration of reagent, pH effect, Effect of heating time and temperature to form a stable (AMK-VAN) derivative.

To optimize the analytical parameters

Selection of optimum wavelength using absorption spectra (λ_{max}). For maximum absorbance of 10 µg/mL of derivative of amikacin with vanillin was noted on spectrophotometer at wavelengths ranges from 200 to 500 nm following heat for 15 minutes at 95°C with pH 12 borate buffer. The derivate showed maximum absorbance at 400 nm against reagent blank and that wavelength i.e., 400 nm was considered as maximum absorbance wavelength. It is necessary that the derivative and reagent should not have close absorbance range. In quantitative evaluation the derivatizing reagent is added in excess which may result in inaccurate absorption of the drug. So it is necessary to choose that wavelength at which the new formed derivative absorbed maximally and the agent used for derivatization absorbed minimally.

Effect of reagent Concentration

The effect of reagent concentration was studied by the addition of different amounts of vanillin solutions on absorbance of 10 µg/ mL of amikacin sulfate. The reagent amount was checked using 0.5-02 mL and 1.5 ml was selected due to optimum results (Figure 1).

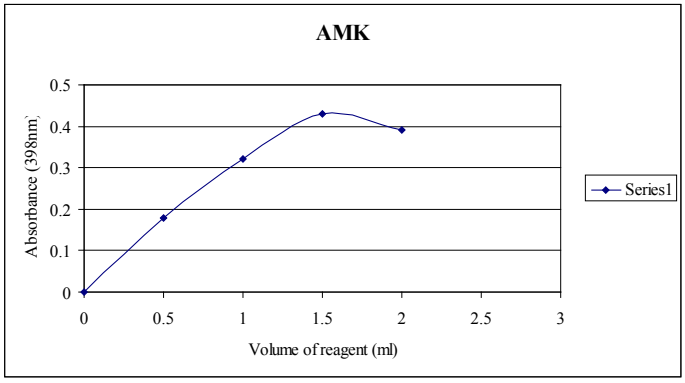

Figure 1: Volume of Reagent for Amikacin

Impact of mixing order

Derivatization process showed the order of adding reagents plays very vital part in precision of result & achievement of absorbance at optimized parameters. Initially the borate buffer (0.5 mL) was added to different concentrations of amikacin sulfate followed by the addition of reagent (2% VAN) showed decrease in absorbance value. Then the reagent was added followed by the addition of borate buffer and solution of amikacin sulfate also resulted in decreased absorbance. Finally when the reagent was added after adding drug solution and buffer showed the maximum absorbance. After that the heating was applied using water bath at 95°C to the mixture which was cooled and water was added to make up the volume before checking absorbance.

Effect of heating time and temperature

Selection of optimum heating time required for heating with optimum temperature for the formation of stable derivative to achieve the maximum absorbance at other optimized conditions. The 10 µg/mL of amikacin sulfate sample with Vanillin and borate buffer were heated with a space of five minutes from zero to thirty minutes at 95°C. The temperature variation was also studied from 50 to 100°C with a space 10°C. The maximum absorbance recorded was following 15 minutes heating time with a temperature of 95°C so that temperature of 95°C and 15 minutes heating time is considered optimum for derivatization (Figure 2).

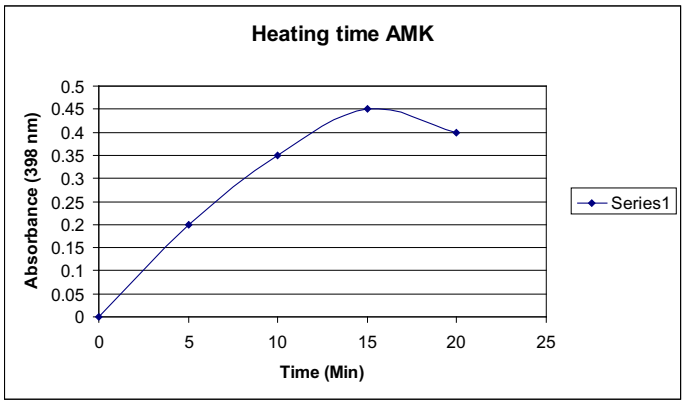

Figure 2: Heating Time of Amikacin

Solvent addition effect

To observe the impact of different solvents such as toluene, acetone, ethyl alcohol, chloroform, cyclohexane, ethyl acetate, isopropanol, acetonitrile, 1-propanol, methanol, 1-butanol, Water on absorbance was observed. When toluene, cyclohexane, acetone,chloroform, ethyl acetate and butanol were added they showed turbidity. From all of above mentioned solvents one and two ml was added after adding all the components for derivatization and the contents were heated at 95°C for 15 minutes. The water was chosen due to maximum absorbance and small LOD (Table 1).

Table 1: Effects of solvents

S. no	*Solvents*	Volume (ml) added	Average Effect % age
1.	Water	.....	**0.0**
2.	Ethanol	**0.5**	2.2
		01	2.9
3.	Methanol.	**0.5**	1.0
		01	2.2
4.	1-Propanol.	**0.5**	1.3
		01	1.1
5.	2-propanol.	**0.5**	2.0
		01	1.1
6.	Acetonitrile.	**0.5**	1.0
		01	1.8

Effect of pH

The impact of buffers was studied after addition of 0.5 milliliters of 0.1 molar solutions with a pH series from 1-14 on the absorbance of derivative of 10 µg mL^{-1} of AMK at optimum conditions were noted.

The results showed that the AMK-VAN derivative showed maximum absorbance by using borate buffer with pH 12 (Figure 3).

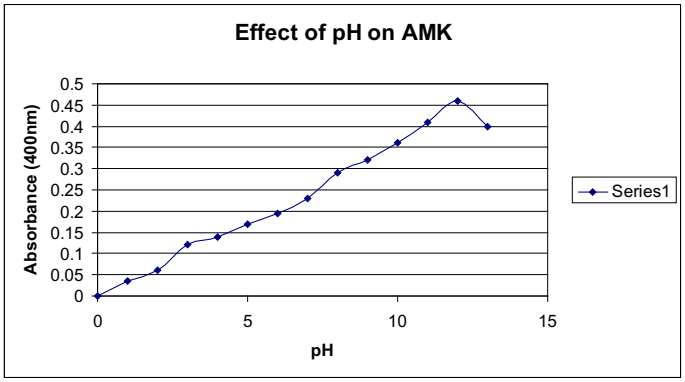

Figure 3: Effect of pH on Amikacin

Effect of excipients

The possible effect of additives/excipients was studied in same, double and 10 times the concentration of amikacin sulfate. The additives used were lactose, methyl paraben, propyl paraben, sodium metabisulfite, sodium citrate, white Petrolatum These additives produce a change of less than ± 5% in absorbance of AMK-VAN derivative (Table 2).

Table 2: Interference study

S.no	*Chemical added*	*Absorbance (nm) and Relative error ± (%). AMK*
	----------	0.44
1.	Lactose	0.42 (4.5)
2.	Methyl Paraben	0.43(2.2)
3.	Propyl Paraben	0.45(2.2)
4.	Sodium Metabisulfite	0.43(2.2)
5.	Sodium Citrate	0.42(4.5)
6.	White Petrolatum	----------

Determination of Stability

The study to determine the stability of derivative i:e AMK-VAN was conducted and results were expressed as absorbance at the concentration of 50 µg ml^{-1} Amikacin sulfate. It was observed that the change was not observed more than ± 5% in absorbance of derivative during a period of 72 hrs.

Calibration graph (Beer's law)

The calibration curve of AMK-VAN derivative was constructed using absorbance versus concentration of 10-50 µg ml^{-1} of drug concentration followed the beer's law. The coefficient of determination of Amikacin was r^2 0.999.

The sandells sensitivity was calculated as 0.004 at 0.45 µg mL^{-1} AMK-VAN. The calibration curve was validated using standard solution of AMK and relative deviation from the labeled values were found 0.002-0.005 %, for parenteral preparation. The six samples of AMK were purchased from local market and were analyzed using this method and results showed the percentage of recovery was 103-106.4% with RSD of

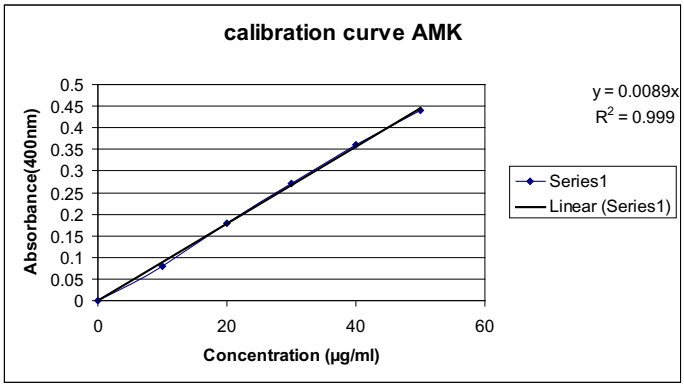

Figure 4: Calibration graph of Amikacin sulfate

0.004-0.005 (Figure 4).

Analysis of AMK from pharmaceutical preparations: After optimization of all above parameters. Amikacin sulfate is only available as injection. Six samples of different pharmaceutical companies containing 500 mg/2 mL of AMK were collected from local market and analyzed by following above mentioned procedure.

Amikacin	Zafa Pharmaceutical Laboratories (Pvt) Ltd.
Amikin	Glaxosmithkline (Pvt) Ltd.
Amkay	Bosch Pharmaceuticals (Pvt) Ltd.
Grasil	Sami Pharmaceuticals (Pvt) Ltd.
Kovex	S.J. & G. Fazul Ellahie (Pvt) Ltd.
Mikan	AGP (Private) Limited

100 mL of distill water was used for dissolving an equivalent amount of 0.05 zg of AMK. Then for dilution 0.5 mL of each solution was transferred to volumetric flask (05 mL) and the analytical procedure was repeated as mentioned above for derivatiztion (Table 3).

Table 3: Analysis of AMK from different

S.no	*Drug*	*Amount labeled (g)/ sample*	*Quantity in sample (g)/ sample*	*Relative Standard deviation ±(%)*	*Relative Deviation ± (%)*	*Recovery ± (%)*
1.	Amikacin	0.5	0.53	(0.004)	6.4	106.4
2.	Amikin	0.5	0.53	(0.005)	6.2	106.2
3.	Amkay	0.5	0.53	(0.005)	5.8	105.8
4.	Grasil	0.5	0.52	(0.002)	05	105
5.	Kovex	0.5	0.52	(0.005)	4.8	104.8
6.	Mikan	0.5	0.51	(0.004)	03	103

Table 4: Optimization, precision and accuracy results

S.No	Parameter (s)	Values
1.	Wave length	max (nm) 400
2.	Beer's law limits (µg mL-1)	10-50
3.	Molar absorptivity (L mol-1 cm-1)	$5.27x10^3$L/mole/cm.
4.	Sandells sensitivity (µg /mL/ cm2 0.004 unit absorbance)	0.45
5.	Slope of regression equation (b)	0.0089
6.	Coefficient of determination (r2)	0.999
7.	Standard deviation	± 0.002-0.005

CONCLUSION

The newly developed procedure is simple, easy, selective, specific, money and time saving. In this method vanillin is used which is water soluble aldehyde and drug is also water soluble so water is used as solvent which is inexpensive and non toxic. In this method no any process of drug extraction is used. The additives did not produce any interference because they may absorb in the UV region. This method is useful for determination of Amikacin sulfate in pure as well as from pharmaceutical preparations.

SUMMARY

- The bulk and pharmaceutical analysis was carried out of different brands with formation of slight yellow colored imine base. The product showed absorbance at 400 nm wi t h molar absorptivity of 5.27x103 L/ mole/cm. In a concentration of 10-50µg ml-1 a linear relationship was established wi t h absorbance which follow the beer's law with coefficient of determination r2 0.9991-0.9998. The procedure was valid because it did not show change in absorbance of the derivative up to 3 days. The sand¬ells sensitivity was calculated as 0.004 at 0.45 µg mL-1 of amikacin sulfate with vanillin. The percentage of recovery was 103-106.4 % with RSD of 0.004-0.005.

ABBREVIATIONS USED

VAN= Vanillin ; Pvt= Private ; GEN= Gentamicin ; TOB= Tobramycin ; AMK= Amikacin ; AIDS= Acquired immunodeficiency syn¬drome ;UV= Ultraviolet HPLC= High Performance Liquid Chromatography ; LOD= Loss on drying

ABOUT AUTHOR

Ubed-Ur-Rehman Mughal is a Ph.D scholar at Department of Pharmaceutics, Faculty of Pharmacy, University of Sindh Jamshoro, Pakistan. He is also working as Assistant Professor in same department and University. The research paper is from his own Ph.D topic.

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