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Research Article

SPECTROPHOTOMETRIC DETERMINATION AMIKACIN IN REACTION WITH *1*,2-NAPHTHOQUINONE-4-SULFONIC ACID SODIUM SALT

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Abstract

A new spectrophotometric method for the quantitative determination of amikacinin pharmaceutical formulations has been developed. This method is based on the measurement of aqueous amikacin solutions absorption at 530 nm. The proposed method is actual according to the validation requirements of Ukrainian Pharmacopeia. The analytical method was optimized and validated by establishing the linearity (the correlation coefficient *r* = 1,000), precision and the accuracy. According to the experimental data, the technique can be correctly reproduced and it is suitable for using in laboratories of the State Inspection for Quality Control of Medicines and QCD of the chemicopharmaceutical enterprises.

Keywords: amikacin, quantitative determination, spectrophotometric method, drug dosage forms.

Introduction

According to British Pharmacopoeia and US Pharmacopoeia amikacin is determined by liquid chromatography [BP, 2000; USP, 2003]. Polish scientists conducted a quantitative determination of some aminoglycosides, such as amikacin, kanamycin, neomycin and tobramycin, by thin-layer chromatography. This antibiotics determination was held on thin-layer plates with a layer of silica gel without fluorescent detector in methanol – 25 % ammonia – chloroform (3: 2: 1) system. As the developer a 0,2% solution of nihydrin in ethanol was used [Hubicka U., et al., 2009].

But researchers from the US quantitatively determined amino glycosides, including amikacin, by liquid chromatography method with mass detection, after previous interactions with searched antibiotics with phenyl isocyanate [Turnipseed S. B., et al., 2009]. The literature provided information regarding high quality liquid chromatography for amino acid quantitative determination. The method is based on the previous column derivatization with 2,4,6-trynitrobenzensulphate acid as reagent and X-ray detection at 350 nm [Zhou M. J.,et al., 2004]. As for spectrophotometric techniques, missBorowiecka B. (Poland), quantitatively determined amikacin, kanamycin for the formation of a colored reaction antibiotic product with ortsynol solutions and iron chloride and subsequent spectrophotometric determination [Borowiecka B., 1976].

Ryan J.A. held colorimetric determination of gentamicin, kanamycin, tobramycin and amikacin based on their interaction with 2,4-dynitrofluorobenzen and further absorption measurement in the visible spectrum [Ryan J. A., 1984]. Described spectrophotometric method of quantitative determination of amikacin, kanamycin, neomycin and tobramycin in substances and in dosage forms based on the formation of colored products by Ganci reaction [Gupta V. D., et al., 1983].

Some of the offered methods require usage of expensive equipment, unobtainable reagents; some are hard in implementing, or are offered only for active pharmaceutical ingredients. Therefore, the expediency of development of new simple valid methods of quantitative determination of amikacin specifically in pharmaceutical formulations is unquestionable.

Materials and Methods

Objects of study, reconstitution solutions and equipment

Study subject – medications«Amicil» 0,50g and 1,0 gof amikacin sulfate (ARTERIUM, Ukraine), series120314 and 520912 respectively; «Lorikacin» 0,50 g/2 ml (Exir Pharmaceutical, Iran) series 0020513. As reagents and solution, sodium salt of 1,2-naphthoquinone-4-sulfonic acid with purity qualification CP (chemically pure), 0,2 M solution of NaOH and purified water were used. As a standard, a working reference sample of amikacin was used. Analytical equipment used: spectrophotometer Specord 200, electronic scales ABT-120-5DM, volumetric glass ware of class A.

General technology of amikacin quantitative determination

An aliquot part of amikacin liquor (0,0016 g) was placed in a measuring bottle with 25,00 ml volume, treated with 1,00 ml of a 1% liquor of the sodium salt of 1,2-naphthoquinone-4-sulfonic acid, add 3,00 ml 0,2 M NaOH liquor, mixing. The resulting reaction mix was kept during 10 minutes, heated for 3 min in a water-bath at 85°C, cooled and adjusted with purified water to the line. Optical density was measured at compensation solution background, not containing the test substance, at 530 nm. The definition with 1,00 ml amikacin standard liquor was parallel conducted. Standard amikacin liquor (0,16%) was prepared by the sample weightresolving in purified water. Reagent liquor (1%) was prepared by dissolving the sample weight in purified water.

Amikacin definitionin drug dosage forms

At determining of the amikacin in pharmaceutical formulation “Amicil”, the exact weighted amount of a powder (0,0150 – 0,0650 g) is put into a 25,00 ml volumetric flask and filled to the mark with water. 0,60 ml of the solution for injections «Lorikacin» is put into a 100,0 ml volumetric flask and filled to the mark with water.The amount of 1,00 ml of the resulted solutions is poured into a 25,00 ml volumetric flask, then treated with 1,00 ml of 1,0% aqueous solution of the sodium salt of 1,2-naphthoquinone-4-sulfonic acid, 3,00 ml of 0,2 M solution of NaOH, and mixed up. The received reaction mixture is kept during 10 minutes, heated up on the water bath at 85°C for 3 minutes, then cooled down and filled to the mark with the purified water. In parallel, the experiment with a working reference solution is carried out.

Results and Discussion

For the development of a method of quantitative determination of amikacin based upon its reaction with the sodium salt of 1,2-naphthoquinone-4-sulfonic acid, there have been studied the factors affecting the character of the absorbance spectrum and the value of absorbance, in particular – the nature of the solution medium, the amount of added reagents, pH of the reaction mixture, conduction period of the reaction and the reaction products resistance in time. When choosing a solution medium, a solubility of the testing substances, reagents and the maximum value of absorbance of the resulted solution were taken into account. It was experimentally established that there agent interacts with amikacin in the aqueous medium to form a colored composition with maximum light absorbance at530 nm (*Fig. 1*). Given that, the optimum amount of 1% reagent needed to form there action resultant with maximum absorbance is 1,00ml.

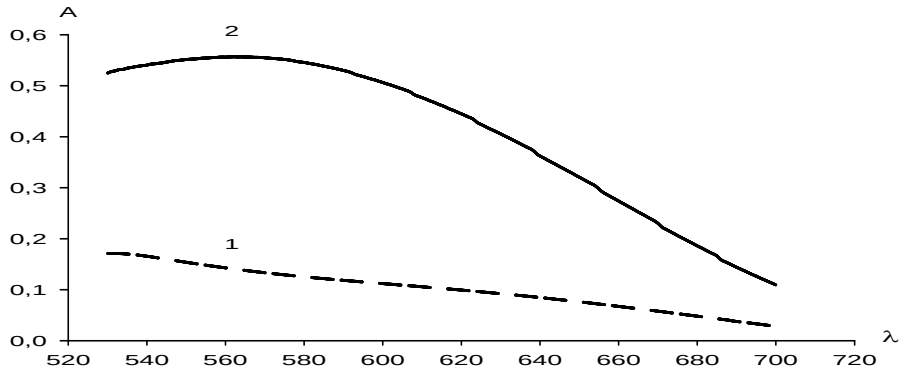

Fig. 1.Absorption spectra of: 1 – aqueous solution of the 1,2-naphthoquinone-4-sulfonate, 2 – reaction resultant of amikacin with 1,2-naphthoquinone-4-sulfonate

Formore complete conduction period of reaction it is necessary to prepare an alkaline medium and heat it on the water bath. It was experimentally established that the maximum absorbance was reached with adding of 3,00 ml of 0,2 M NaOH solution and heating the reaction mixture for 3 min at 85°C. Targetvaluesofthedetectionthresholdandmolarabsorptioncoefficient are indicative of a high sensitivity of the reaction (*Table 1*).

Determination of the main validation characteristics

Linearity was determined within the range of concentrations, in which a subjection to the Beer's law was observed. The solutions with the established concentration obtained by diluting of the reference solution of amikacin were determined by the introduced general procedure. On the basis of the obtained data, a dependence diagram of absorbance and concentration of a testing substance was drawn (*Fig. 2*), and the parameters of linear dependency were calculated (*Table 1*).

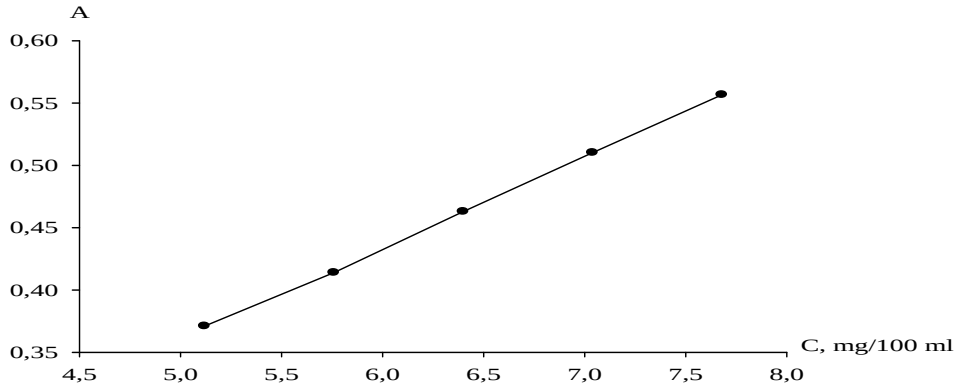

Fig. 2.Absorbance–amikacin concentration dependency diagram

Table1 Optical specifications and basic parameters of linear dependency of 1,2-naphthoquinone-4-sulfonate and amikacin reaction

Molar absorbance rateε	7902,2
Sendel factorW_S	0,0989
Detection thresholdC_{min}(mcg/ml)	4,95
Equation of linear regression	Y = bX + a
Slope coefficientb±(s_b)	0,0726±(0,0003)
Intercept term of a linear regression a±(s_a)	0,00120±(0,0006)
Residual standard deviation$S_{x.0}$%	0,108
Correlation coefficientr	1,000

The numeric indicators of linear dependency, obtained in line with regulations of SPU (State Pharmacopoeia of Ukraine),showed that all requirements as to the parameters to linear dependency have been met; there by the technique linearity can be confirmed across the range of the selected concentrations[SPU, 2008]. *Precision* was determined at convergency level. To this for each of drug dosage forms were conducted on nine determinations covering a range of application techniques (three concentrations / three determinations for each). According to SPU technique is accurate at the convergency level if the relative confidence interval *(*Δ*x)* is not more than the maximum relative uncertainty of the quantitative results determination *(*Δ*As%)*. Based on the data, shown in Table 2, the technique is accurate.

Table 2 Definition of convergency results of the amikacin drug dosage forms quantitative determination

Drug dosage form	$\overline{X}$	*S*	*RSD%*	Δ_x	Δ*As*Δ_{As} *%*
«Amicil», powder 1,0g	0,998	$1,06 \cdot 10^{-2}$	1,06	1,98	3,20
«Amicil», powder 0,50g	0,501	$3,95 \cdot 10^{-3}$	0,788	1,46	3,20
«Lonkacin», solution for injections 0,50 g/2 ml	0,505	$8,32 \cdot 10^{-3}$	1,64	3,	

Accuracy was set for 3 drug dosage forms by the addition method (Table 3). The results of the determinations are correct, if there is no meaningful systematic mistake, i.e. the true value of the determined amount is getting in a setting confidence interval ($\Delta\overline{Z}$).

Table 3 Accuracy definition of amikacin quantitative determination results in drug dosage forms

Drug dosage form	$\overline{Z}$	*RSD%*	$\Delta\overline{Z}$	$\overline{Z}-100$
«Amicil», powder 1,0g	99,96	0,0629	0,0390	0,0370
«Amicil», powder 0,50g	99,88	0,830	0,519	0,110
«Lorikacin», solution for injections 0,50 g/2 ml	98,94	1,70	1,06	1,06

The application range of the analytical procedure is the interval between the minimum and maximum concentrations of the testing substance, for which it was shown that the procedure exhibits a required linearity, validity and accuracy. Based on the results of the undertaken study, the application range for the developed methodology is 38–162 % and ranges within the performance period for the procedures of quantitative determination according to SPU requirements (80 – 120 %) [SPU, 2008].

Robustness assessment was carried out at the stage of technique development, as well as there were determined the stability of testing solutions in time and the influence of the quantity of the added reagents on the results of the determination. It was established that the test colored solutions are stable for no less than 30 min, and variations in the quantity of added reagent (a solution of the sodium salt of 1,2-naphthoquinone-4-sulfonic acid) within ± 10% do not affect the value of the absorbance.

Conclusion

It was set that amikacin reacts with the sodium salt of 1,2-naphthoquinone-4-sulfonic acid in in the aquatic environment presence of 0,2 M NaOH liquor (3,00 ml) by heating the reaction mixture at 85°C (3 min). Studied reaction is highly sensitive, identification limit is 4,95 mcg/ml. Developed sensible, the economic technique of amikacin analysis in 3 drug dosage forms.It was proved that the developed technique for quantitative determination, according to validated characteristics as linearity, precision, accuracy, application range and robustness are valid, diggers is simple execution, not toxicity, availability, and may be recommended for drug dosage forms quality control.

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