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Validated spectrophotometric methods for determination of certain aminoglycosides in pharmaceutical formulations

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ABSTRACT

Two simple and selective spectrophotometric methods have been proposed for the determination of amikacin sulfate, gentamicin sulfate, kanamycin sulfate, streptomycin sulfate, neomycin sulfate and tobramycin in pure forms and in their pharmaceutical formulations. Both methods are based on the proton transfer from the Lewis acid such as 2,4,6-trinitrophenol (picric acid; PA) or 2,4-dinitrophenol (2,4-DNP) to the primary amino group of aminoglycosides as Lewis base with formation of yellow ion-pair complexes. Different variables and parameters affecting the reactions were studied and optimized. Beer's plots were obeyed in a general concentration range of 2.5-140 and 2.5–100 µg mL^{-1} with 2,4-DNP and PA, respectively, with correlation coefficients not less than 0.9991. The proposed methods were successfully applied to the analysis of the cited drugs in their dosage forms. The proposed methods were validated according to ICH and USP guidelines with respect to specificity, linearity, accuracy, precision, robustness and ruggedness.

INTRODUCTION

Aminoglycosides are a group of highly potent and wide spectrum antibiotics that are active against both gram-positive and gram-negative bacterial infections (Jiame, 1998). The chemical structures of the studied drugs are shown in table 1.

Various spectrophotometric (Kalashnikov *et al.,* 2000, Jiang *et al.*, 2003, Jiang *et al.*, 2003, Jiang *et al.,* 2004, Mitic *et al.*, 2006, Ahmad *et al.*, 2006, Sunaric *et al.,* 2007, Li *et al.,* 2008, Al-Sabha *et al.,* 2010), Spectrofluorimetric (Mai *et a*l.,1990, Meng *et al*., 1993, Rizk *et al*., 1995, Gilmartin *et al*., 2000, Belal *et al.*, 2001, El-Shabrawy *et al.*, 2002, Sanchez-Martinez *et al*., 2004), Chemiluminescence (Koerner *et al*., 1987, Deng *et a*l., 1993, Halvatzis *et al*., 1994, Yang *et al.*, 1995, Yang *et al*., 2010), Thin layer chromatographic (Szabo *et al.*, 2005, Hubicka *et al.*, 2009, Kaya *et al.,* 2010), Capillary electrophoresis (Lin *et al.*, 2008, Huidobro *et al.,* 2009, Yu *et al*., 2009), High performance liquid chromatographic (Kajita *et al.*, 2008, Turnipseed *et al.*, 2009, Clarot *et a*l., 2009, Bohm *et al.*, 2010, Gremilogianni *et al.*, 2010), polarographic (Ayad *et al*., 1985, Liang *et al.*, 1991), Immunoassay (Sanchez-Martinez *et al*., 2007, Sánchez-Martínez *et al.*, 2009) and Microbiological methods (Stahl *et al.*, 1984, Stahl *et al.*, 1989, Yamamoto *et al*., 1996) for the determination of aminoglycosides in different matrices have been reviewed.

Two simple colorimetric methods for the analysis of pharmaceutical formulation containing aminoglycosides were developed.

These methods have been successfully applied for the analysis of drugs in pharmaceutical formulation depending on the presence of a primary amine moiety in these drugs.

GENERAL EXPERIMENTAL

Apparatus

- Spectronic ™ Genesys ™ 2PC. Ultraviolet/Visible spectrophotometer (Milton Roy Co, USA) with matched 1 cm quartz cell connected to IBM computer loaded with winspec™ application software was used for all measurements.

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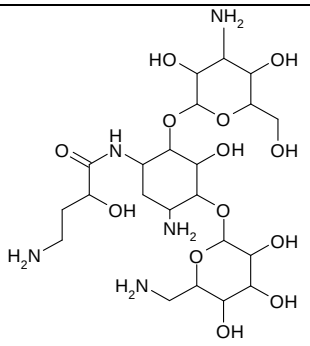
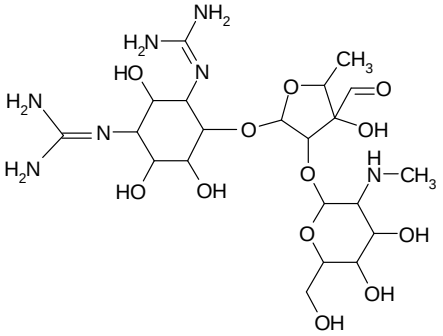
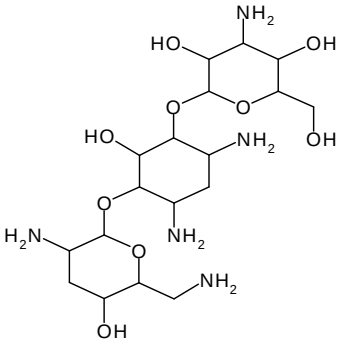
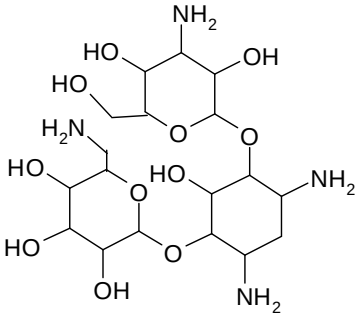
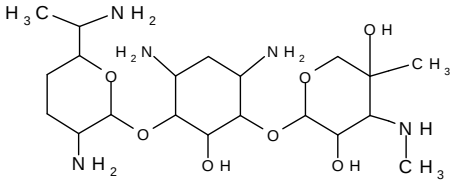
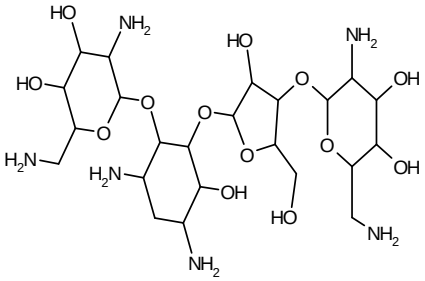
Table. 1: The structure of studied drugs:

Drug	Structure	Drug	Structure
Amikacin		Streptomycin	
Tobramycin		Kanamycin	
Gentamicin		Neomycin	

Materials and reagents

All the materials were of analytical reagent grade. Samples of aminoglycosides were generously supplied by their respective manufacturers and were used without further purification; Amikacin sulfate and Tobramycin (Egyptian International Pharmaceutical Industries Co.; E.I.P.I.CO., El Asher Ramadan City, Cairo, Egypt), Neomycin sulfate and Gentamicin sulfate (Memphis Co. For Pharmaceutical & Chemical Industries, Cairo, Egypt), Kanamycin sulfate (Miser Co. for Pharm. Ind. S.A.E., Cairo, Egypt) while Streptomycin sulfate (The Nile Co. for Pharmaceutical & Chemical Industries, Cairo, Egypt.). DNP (13 Courtyard Workshops, Bath Street, Market Harborough, Leicestershire, LE 169 EJ. U. K). PA (Packed under license from Biolab Kirk Quebec Canada, Factory 10th Ramadan City.)

All solvents used were analytical grade and obtained (El Nasr chemical co., Abu Zabbal, Egypt).

Pharmaceutical formulations

The following available commercial preparations were analyzed; Amikin® vials (Smith Kline Beecham; an affiliated co. to Glaxo SmithKline, Egypt) labeled to contain 100 mg amikacin sulfate in 2 ml aqueous solution, Neomycin® tablets (Memphis Co. For Pharmaceutical & Chemical Industries, Cairo, Egypt.), labeled to contain 500 mg Neomycin sulfate per tablet, Tobrin® eye drops and Tobrin® eye ointments (Egyptian International Pharmaceutical Industries Co.; E.I.P.I.CO., El Asher Ramadan City, Cairo, Egypt) , labeled to contain 0.3 % w/v and w/w tobramycin, respectively. Tobradex® eye drops (Alcon-Couvreur), labeled to contain 0.3 % tobramycin and 0.1 % dexamethasone. Streptomycin® vial (Manufactured by Amriya Pharm. Ind. Co. for the Nile Co. For Pharmaceutical & Chemical Industries, Cairo, Egypt.), labeled to contain 1 g Streptomycin sulfate equivalent to 1 g Streptomycin. Diakan-M® syrup (Misr Co. for Pharm. Ind. S.A.E., Cairo, Egypt), labeled to contain 100 mg Kanamycin as sulfate. Garamycin® ampoules (Manufactured by Memphis Co. For Pharmaceutical & Chemical Industries, Cairo – A.R.E. under authority of Schering-Plough Corporation / U.S.A.), labeled to contain 40 mg/ml Gentamicin sulfate and Garamycin® ointment (Manufactured by Memphis Co. For Pharmaceutical & Chemical Industries, Cairo – A.R.E. under authority of Schering-Plough Corporation / U.S.A.), each gram ointment labeled to contain 1 mg Gentamicin sulfate.

Preparation of reagents

Preparation of stock standard solutions

An accurately weighed 25 mg of each drug was carefully transferred to a 100-ml separating funnel contained about 20 ml distilled water. Then the aqueous layer was rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated aminoglycoside base was extracted with three portions of 15 ml chloroform. The chloroform extract was filtrated through anhydrous Na_2SO_4 supported on filter paper. The obtained filtrate was evaporated to dryness. The residue lifted was dissolved in about 2 ml methanol. The resultant solution was transferred quantitatively into 50-ml volumetric flask and completed to volume with acetonitril. The final solution was diluted quantitavely with acetonitril to obtain working standard solutions in the general concentration ranges 25 - 1400 µg mL^{-1}.

Picric acid and 2,4-Dinitrophenol reagents

A 1.0 mg mL^{-1} for DNP and PA solutions were prepared separately in dichloromethane for use in both methods.

General analytical procedure:

An aliquot volume of the working standard solutions of the studied drugs were accurately transferred into a series of 10 mL volumetric flasks, the final concentration was in the range of 2.5–140.0 µg mL^{-1}, then a 1.5 mL of DNP solution or 1.0 ml of picric acid solution was added. The contents were mixed well and completed to volume with acetonitril. The absorbance of the resulting solutions was measured against a reagent blank treated similarly at 406 and 418 nm after 10 min for DNP and PA, respectively.

Preparation of sample solution

Procedures for tablets

Twenty tablets of Neomycin were weighted accurately, finely powdered and mixed thoroughly. An accurate weighted amount from the powdered tablets equivalent to 25 mg was transferred to a 100-ml separating funnel contained about 20 ml distilled water. Then the aqueous layer was rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated aminoglycoside base was extracted with three portions of 15 ml chloroform. The chloroform extract was filtrated through anhydrous Na_2SO_4 supported on filter paper. The obtained filtrate was evaporated to dryness. The residue lifted was dissolved in about 2 ml methanol. The resultant solution was transferred quantitatively into 50-ml volumetric flask and completed to volume with acetonitril. The final solution was diluted quantitavely with acetonitril to obtain working standard solutions in the general concentration ranges 150 - 1200 µg mL^{-1} and then the general procedures were followed.

Procedures for Ophthalmic ointment

An equivalent amount of 6 mg of tobramycin in Tobrin® ophthalmic ointment was vigorously shaked with 5 ml chloroform. The investigated drug was extracted with 5 ml of distilled water with vigorous shaking, shake vigorously for 1 minute and centrifuge for 15 minutes. The clear upper aqueous layer contain drug was transferred to a 100-ml separating funnel and was rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated tobramycin base which was extracted with three portions of 15 ml chloroform. The chloroform extract was filtrated through anhydrous Na_2SO_4 supported on filter paper. The obtained filtrate was evaporated to dryness. The residue lifted was dissolved in about 2 ml methanol, transferred quantitatively into 25-ml volumetric flask and completed to volume with acetonitril. The final solution was diluted quantitavely with acetonitril to obtain working standard solutions in the general concentration ranges 25 – 200 µg mL^{-1} and then the general procedures were followed.

Procedures for Ophthalmic drops

The solution of Tobrin® and Tobradex® drops was diluted with distilled water and transferred to a 100-ml separating funnel and was rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated tobramycin base which was extracted with three portions of 15 ml chloroform. The chloroform extract was filtrated through anhydrous Na_2SO_4 supported on filter paper. The obtained filtrate was evaporated to dryness. The residue lifted was dissolved in about 2 ml methanol, transferred quantitatively into 50-ml volumetric flask and completed to volume with acetonitril. The final solution was diluted quantitavely with acetonitril to obtain working standard solutions in the general concentration ranges 25 – 200 µg mL^{-1} and then the general procedures were followed.

Procedures for Amikin® vial and Garamycin ® ampoule

The solution of Amikin ® vial and Garamycin ® ampoules were diluted with distilled water transferred to a 100-ml separating funnel and was rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated bases were extracted with three portions of 15 ml chloroform. The chloroform extracts were filtrated through anhydrous Na2SO4 supported on filter paper. The obtained filtrates were evaporated to dryness. The residues lifted were dissolved in about 2 ml methanol, Further dilution were made with acetonitril to obtain working solutions have a final concentration (100 - 1000 µg mL-1). Then the general procedures were followed.

Procedures for Streptomycin® vial

An amount of Streptomycin® vial content equivalent to 25 mg streptomycin was dissolved in 10 ml distilled water, transferred to a 100-ml separating funnel and was rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated tobramycin base which was extracted with three portions of 15 ml chloroform. The chloroform extract was filtrated through anhydrous Na_2SO_4 supported on filter paper. The obtained filtrate was evaporated to dryness. The residue lifted was dissolved in about 2 ml methanol, further dilution was made with acetonitril

to obtain working solutions have a final concentration (100 - 1400 µg mL^{-1}) and then the general procedures were followed.

Procedures for syrup

A certain volume of suspension contain 25 mg kanamycin was transferred into a 100-ml separating funnel contained about 20 ml distilled water. Then the aqueous layer was rendered alkaline through addition of 33 % w/v aqueous ammonia solution and the liberated kanamycin base was extracted with three portions of 15 ml chloroform. The chloroform extract was filtrated through anhydrous Na_2SO_4 supported on filter paper. The obtained filtrate was evaporated to dryness. The residue lifted was dissolved in about 2 ml methanol, further dilution was made with acetonitril to obtain working solutions have a final concentration (50 - 1000 µg mL^{-1}), and then the general procedures were followed.

RESULTS AND DISCUSSION

Absorption Spectra

The reaction of PA or 2,4-DNP as Lewis acids with aminoglycosides as Lewis base results in the formation of an intense yellow colored products. The absorption spectra of the yellow colored products were recorded at 350–500 nm against the corresponding blank solutions. The resulted yellow colored ion-pair complexes showed maximum absorbance at 418 and 406 nm for aminoglycoside-PA and aminoglycoside-2,4-DNP, respectively, (Figure 1).

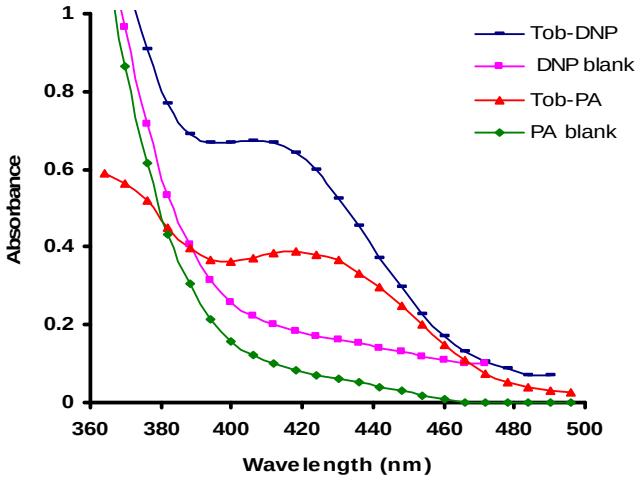


Fig. 1: Absorbance spectra of reaction product of (tobramycin 5 µg mL^{-1} with picric acid 1 mg mL^{-1}) and (tobramycin 7.5 µg mL^{-1} with 2,4-Dinitrophenol 1mg mL^{-1}).

Optimization of variables

Effect of dye volume

The effect of dye volume on the intensity of the color developed at the selected wavelength and constant drugs concentrations was critically examined using different volumes of DNP or PA dyes solution (1 mg mL^{-1}).

For DNP method, the results indicated that the maximum absorbance was found upon using 1.5 mL of DNP. Fig. (2). For PA method, the results indicated that the maximum absorbance was found upon using 1.0 ml 0f PA. Fig. (3).

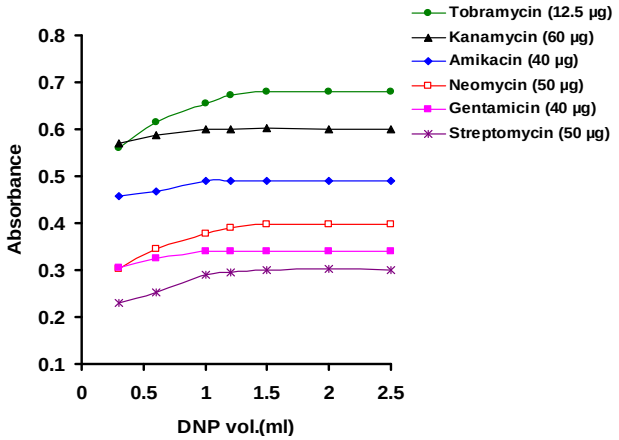


Fig. 2: Effect of DNP volume on the absorbance of the formed complexes of the studied drugs with DNP (1 mg / ml).

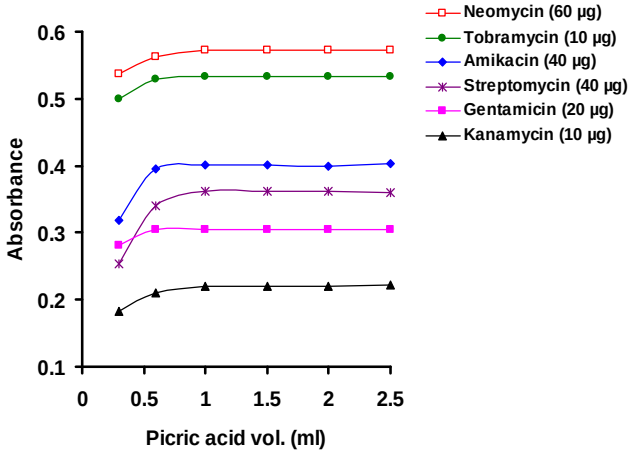


Fig. 3: Effect of PA volume on the absorbance of the formed complexes of the studied drugs with PA (1 mg / ml).

Effect of reaction time

Different time intervals were tested before dilution with acetonitril. It was found that maximum absorbance was attained after 10 min. for both DNP and PA. **Fig (4, 5)**

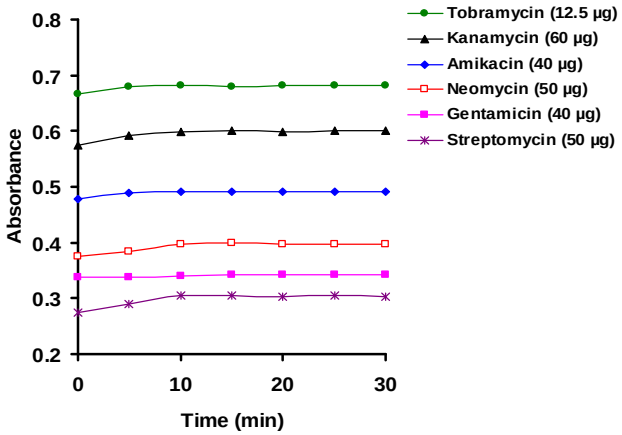


Fig. 4: : Effect of time on the reaction between studied drugs and DNP (1 mg / ml).

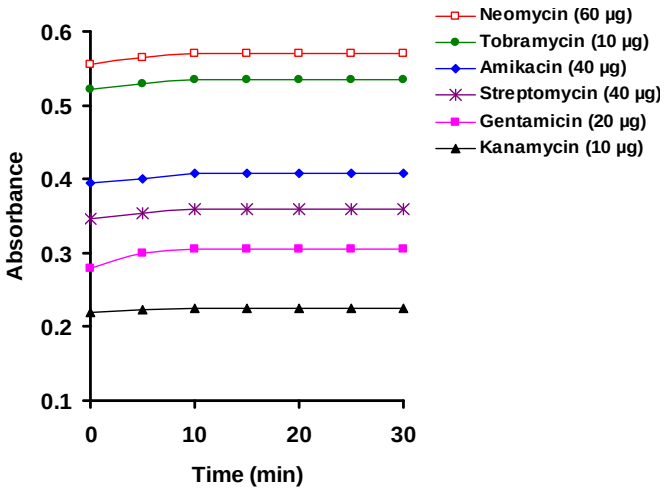


Fig. 5: Effect of time on the reaction between studied drugs and PA (1 mg / ml).

Stability of the complex

The stability of the complexes was studied after completion to volume with acetonitril. It was found that the absorbance remained stable for at least 30 minutes; this indicates good stability of the formed complexes. **Fig (6, 7)**

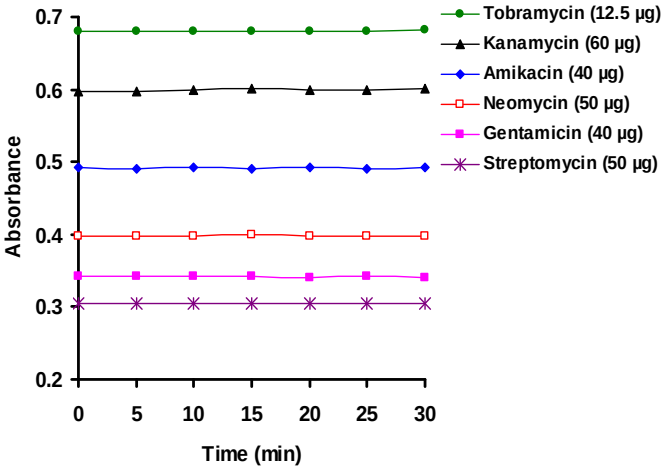


Fig. 6: Stability of complex of the studied drugs with DNP (1 mg / ml).

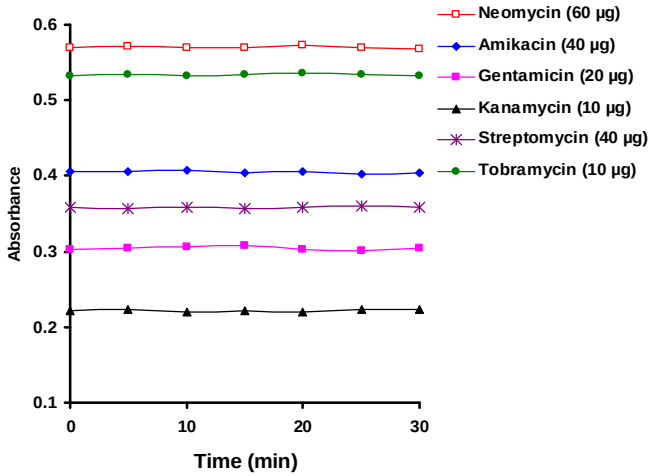


Fig. 7: Stability of complex of the studied drugs with PA (1 mg / ml).

Effect of diluting solvent

In order to select the most appropriate solvent for dilution, different solvents were tested; acetonitrile, acetone, 1,2-dichloroethane, methylene chloride and chloroform. Acetonitril was found to be an ideal diluting solvent.

Stoichiometry and Mechanism of the reaction

The stoichiometry of the reaction mechanism was studied through the job's method (Job, 1964) of continuous variation. The molar ratio of DNP or PA to each of investigated drugs was 1:1. The reaction pathway was proposed in Figure (8).

Validation of the proposed method

The proposed methods were validated according to ICH and USP guidelines with respect to specificity, linearity, accuracy, precision, robustness and ruggedness.

Linearity

Linearity was indicated by high correlation coefficient obtained. The correlation coefficients (r) of the formed products were in the range from 0.9991 to 0.9999 indicating good linearity, as shown in table (2).

The limit of detection (LOD) and limit of quantification (LOQ) for the proposed methods were calculated using the following equations (USP, 2007):

$LOD = 3.3\ \sigma / S, \qquad LOQ = 10\ \sigma / S$

Where σ is the standard deviation of intercept. S is the slope of calibration curve.

The results are summarized in table (2). The calculated detection limits for all the studied drugs were less than 3.73 µg mL^{-1} while the quantitation limits for all the studied drugs were all less than 11.3 µg mL^{-1} indicating good sensitivity of the proposed method.

Accuracy (USP, 2007)

Accuracy was checked at five concentration levels within the specified range. Three replicate measurements were recorded at each concentration level. The results were recorded as percent recovery ± standard deviation as shown in table (3, 4). The results obtained show the close agreement between the measured and true values. Meanwhile, comparison of the obtained results from the analysis of the drug products by the proposed procedure with those obtained from the reported methods (Gupta *et al.*, 1983, Sampath *et al.*, 1990, Aman *et al.*, 1995) revealed that their is no significant difference between them with respect to accuracy as indicated by t- and F- tests as shown in table (9, 10)

Precision (USP, 2007)

Precision was checked at three concentration levels, three replicate measurements were recorded at each concentration level; the results are summarized in table (5, 6). The calculated relative standard deviations were below 2.2 % indicating excellent precision of the proposed procedures at both level of repeatability and intermediate precision.

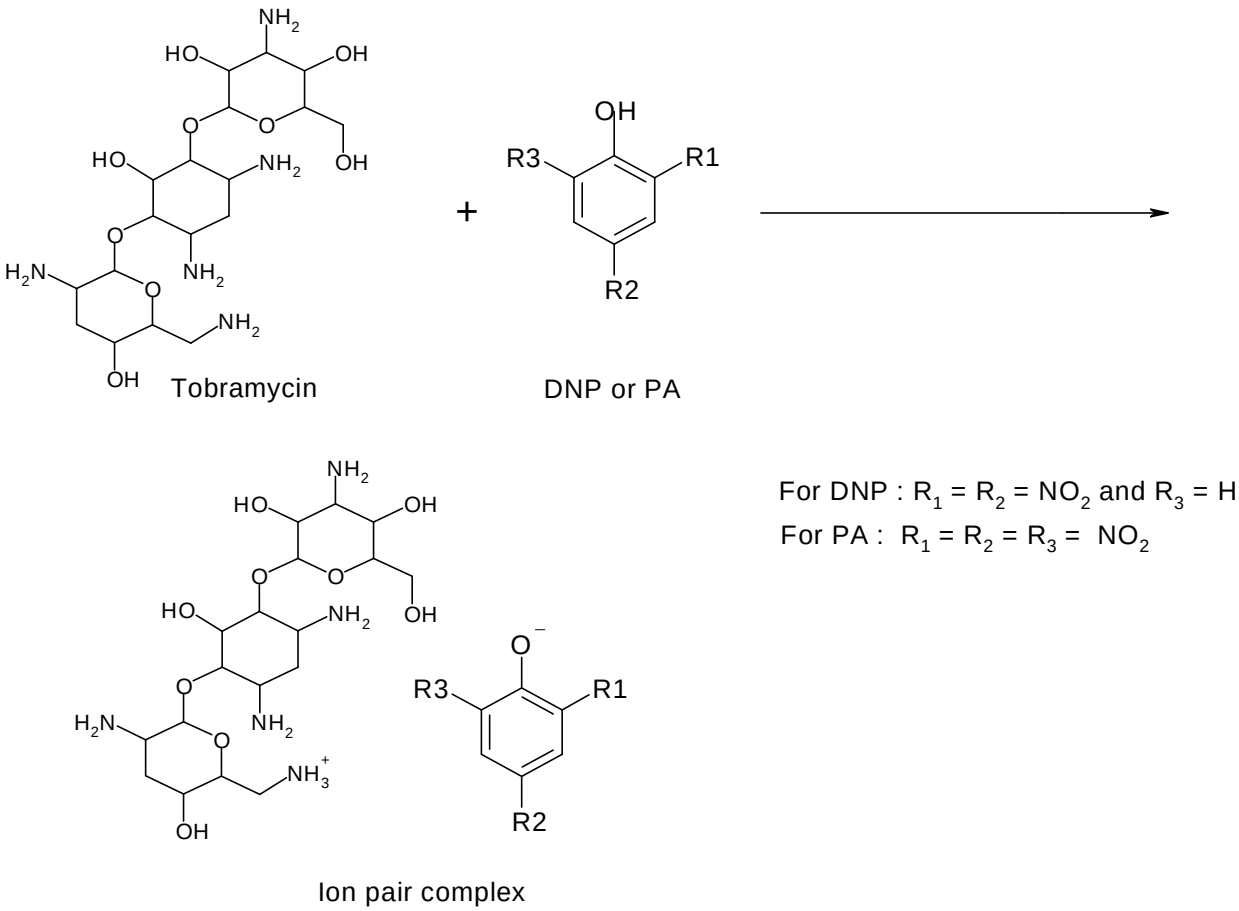


Fig. 8: Proposal of the reaction pathway between tobramycin and DNP or PA.

Table. 2: Analytical parameters for the analysis of the studied aminoglycosides by DNP method.

Parameter	DNP					
	Amik.	**Tobra.**	**Gent.**	**Neo.**	**Kana.**	**Strep.**
Λ_{max}				406 nm.		
Linear range (µg mL^{-1})	10-100	2.5-20	10-100	15-120	5-100	10-140
Intercept (a)	0.174	0.176	0.092	0.115	0.093	0.0644
SE of intercept (S_a)	0.0059	0.0089	0.0045	0.0063	0.0041	0.00474
Slope (b)	0.0077	0.041	0.0062	0.0056	0.0083	0.0048
SE of slope (S_b)	0.000098	0.00076	0.000073	0.000091	0.000072	0.000059
Correlation Coefficient (r)	0.9997	0.9991	0.9997	0.9992	0.9998	0.9995
Determination coeff. (r^2)	0.9994	0.9983	0.9994	0.9984	0.9996	0.9990
SD of residuals ($S_{y.x}$)	0.0076	0.0112	0.00577	0.00894	0.0064	0.0074
LOD (µg mL^{-1})	2.55	0.722	2.39	3.734	1.606	3.23
LOQ (µg mL^{-1})	7.72	2.189	7.25	11.317	4.865	9.773

LOD : Limit of detection (µg mL^{-1})
LOQ : Limit of quantitation (µg mL^{-1})

Follow table. 2: Analytical parameters for the analysis of the studied aminoglycosides PA method.

Parameter	PA					
	Amik.	**Tobra.**	**Gent.**	**Neo.**	**Kana.**	**Strep.**
Λ_{max}				418 nm.		
Linear range (µg mL^{-1})	5-100	2.5-15	5-80	10-90	5-80	10-100
Intercept (a)	0.119	0.0454	0.143	0.048	0.14	0.074
SE of intercept (S_a)	0.00295	0.0064	0.0042	0.0083	0.0036	0.0074
Slope (b)	0.0072	0.0493	0.0087	0.0086	0.0075	0.0074
SE of slope (S_b)	0.000052	0.00066	0.000094	0.000145	0.000081	0.00012
Correlation Coefficient (r)	0.9999	0.9996	0.9998	0.9994	0.9998	0.9995
Determination coeff. (r^2)	0.9997	0.9993	0.9995	0.9989	0.9995	0.9989
SD of residuals ($S_{y.x}$)	0.0047	0.0069	0.0062	0.0104	0.0054	0.0095
LOD (µg mL^{-1})	1.34	0.429	1.595	3.214	1.603	3.284
LOQ (µg mL^{-1})	4.1	1.299	4.834	9.738	4.856	9.953

LOD : Limit of detection (µg mL^{-1})
LOQ : Limit of quantitation (µg mL^{-1})

Table. 3 : Evaluation of the accuracy of the proposed DNP method.

Sample number	Amikacin			Tobramycin			Gentamicin		
	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery
1	20	19.64	98.2	2.5	2.537	101.5	10	10.2	102.0
2	40	40.16	100.4	5	5.115	102.3	20	20.22	101.1
3	60	60.96	101.6	7.5	7.46	99.5	40	39.8	99.5
4	80	82.32	102.9	10	10.1	101.0	60	58.8	98.0
5	100	99.6	99.6	15	15.21	101.4	80	79.76	99.7
Mean			**100.54**			**101.14**			**100.06**
±S.D			**1.808**			**1.031**			**1.544**
±R.S.D			**1.798**			**1.019**			**1.543**

*Average of three determinations SD: Standard deviation RSD: Relative standard deviation

Follow table. 3 : evaluation of the accuracy of the proposed DNP method

Sample number	Neomycin			Kanamycin			Streptomycin		
	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery
1	20	20.04	100.2	10	9.97	99.7	20	19.74	98.7
2	40	39.84	99.6	20	19.78	98.9	40	39.44	98.6
3	60	58.98	98.3	40	39.68	99.2	60	59.04	98.4
6	80	80.64	100.8	60	60.6	101	80	80.88	101.1
5	100	99.7	99.7	80	80.16	100.2	100	99.6	99.6
Mean			**99.72**			**99.8**			**99.28**
±S.D			**0.9257**			**0.834**			**1.12**
±R.S.D			**0.928**			**0.835**			**1.13**

*Average of three determinations SD: Standard deviation RSD: Relative standard deviation

Table. 4: Evaluation of the accuracy of the proposed picric acid method.

Sample number	Amikacin			Tobramycin			Gentamicin		
	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery	Taken µg mL^{-1}	Found* µg mL^{-1}	% Recovery
1	5	4.93	98.6	2.5	2.49	99.5	5	4.98	99.6
2	10	10.18	101.8	5	4.95	99.0	10	9.84	98.4
3	20	19.88	99.4	7.5	7.6	101.3	40	40.68	101.7
4	40	39.84	99.6	10	9.96	99.6	60	59.76	99.6
5	60	60.84	101.4	12.5	12.46	99.7	80	79.68	99.6
Mean			**100.16**			**99.82**			**99.78**
±S.D			**1.374**			**0.870**			**1.192**
±R.S.D			**1.372**			**0.871**			**1.194**

*Average of three determinations SD: Standard deviation RSD: Relative standard deviation

Follow table. 4: evaluation of the accuracy of the proposed **picric acid** method.

Sample number	Neomycin			Kanamycin			Streptomycin		
	Taken µg mL-1	Found* µg mL-1	% Recovery	Taken µg mL-1	Found* µg mL-1	% Recovery	Taken µg mL-1	Found* µg mL-1	% Recovery
1	10	10.13	101.25	5	4.94	98.71	10	10.11	101.11
2	20	20.24	101.19	20	19.84	99.18	40	39.68	99.20
3	40	40.1	100.26	40	39.91	99.78	60	59.73	99.55
4	60	59.84	99.73	60	59.7	99.50	80	80.3	100.38
5	90	89.61	99.57	80	79.66	99.58	100	99.65	99.65
Mean			100.4			99.35			99.97
±S.D			0.791			0.418			0.764
±R.S.D			0.788			0.421			0.765

*Average of three determinations SD: Standard deviation RSD: Relative standard deviation

Table. 5: Evaluation of the precision of the proposed DNP method.

Parameter		Amikacin *(%found)**			Tobramycin *(%found)**			Gentamicin *(%found)**		
		20 µg mL^{-1}	60 µg mL^{-1}	100 µg mL^{-1}	5 µg mL^{-1}	10 µg mL^{-1}	15 µg mL^{-1}	10 µg mL^{-1}	40 µg mL^{-1}	80 µg mL^{-1}
Intraday	1	99.79	99.63	101.54	99.31	99.38	98.56	100.83	99.63	98.1
	2	99.42	99.21	99.71	99.53	98.24	98.27	99.73	99.63	99.5
	3	98.51	100.9	99.32	99.17	99.07	98.34	101.34	99.21	99.3
	Mean	99.24	99.91	100.19	99.34	98.89	98.39	100.63	99.49	98.97
	S.D	0.658	0.879	1.185	0.182	0.589	0.151	0.822	0.242	0.757
	R.S.D	0.662	0.880	1.827	0.183	0.595	0.153	0.817	0.243	0.765
Interday	1	98.83	101.11	99.82	99.22	99.11	99.8	99.9	99.40	98.8
	2	101.34	100.28	99.33	101.3	98.97	99.54	99.2	98.87	98.7
	3	98.96	101.01	100.86	99.4	98.16	98.31	101.18	99.30	99.44
	Mean	99.71	100.80	100.03	99.97	98.75	99.22	100.09	99.19	98.98
	S.D	1.413	0.453	0.781	1.152	0.513	0.795	1.004	0.282	0.402
	R.S.D	1.417	0.449	0.780	1.153	0.519	0.802	1.003	0.284	0.406

*Average of three determinations SD: Standard deviation RSD: Relative standard deviation

Follow table. 5: evaluation of the precision of the proposed **DNP** method.

Parameter		Neomycin *(%found)**			Kanamycin *(%found)**			Streptomycin *(%found)**		
		20 µg mL^{-1}	60 µg mL^{-1}	100 µg mL^{-1}	10 µg mL^{-1}	40 µg mL^{-1}	80 µg mL^{-1}	20 µg mL^{-1}	60 µg mL^{-1}	100 µg mL^{-1}
Intraday	1	99.5	100.7	99.7	98.5	100.2	99.5	99.1	99.5	99.7
	2	99.2	101.0	99.4	100.3	98.3	98.8	100.4	99.0	100.2
	3	100.4	100.3	100.2	99.9	99.2	99.5	99.7	100.4	100.6
	Mean	99.70	100.67	99.77	99.57	99.23	99.27	99.73	99.63	100.17
	S.D	0.624	0.351	0.404	0.945	0.950	0.404	0.651	0.709	0.451
	R.S.D	0.626	0.348	0.405	0.949	0.957	0.407	0.652	0.712	0.450
Interday	1	0.361	0.203	0.233	0.545	0.549	0.233	0.376	0.409	0.260
	2	101.1	99.9	99.6	99.6	98.8	100.8	98.6	100.9	99.4
	3	99.7	100.8	99.2	100.7	100.4	99.3	100.2	101.2	98.9
	Mean	99.1	100.5	99.5	99.0	100.7	100.2	100.6	99.3	100.5
	S.D	99.97	100.40	99.43	99.76	99.96	100.10	99.80	100.47	99.60
	R.S.D	1.026	0.458	0.208	0.862	1.021	0.755	1.058	1.031	0.818

*Average of three determinations SD: Standard deviation RSD: Relative standard deviation

Table. 6 : Evaluation of the precision of the proposed **Picric acid** method.

Parameter		Amikacin *(%found)**			Tobramycin *(%found)**			Gentamicin *(%found)**		
		20 µg mL^{-1}	60 µg mL^{-1}	100 µg mL^{-1}	2.5 µg mL^{-1}	5 µg mL^{-1}	15 µg mL^{-1}	5 µg mL^{-1}	40 µg mL^{-1}	80 µg mL^{-1}
Intraday	1	100.3	99.1	100.4	99.2	99.6	99.7	100.8	99.6	101.5
	2	99.1	99.6	100.7	99.7	99.4	98.9	99.1	100.4	99.7
	3	99.8	100.4	100.9	98.8	98.9	100.4	98.1	99.3	99.3
	Mean	99.73	99.70	100.67	99.23	99.30	99.67	99.33	99.67	100.17
	S.D	0.602	0.656	0.252	0.451	0.361	0.751	1.365	0.568	1.172
	R.S.D	0.604	0.657	0.250	0.454	0.363	0.753	1.374	0.569	1.170
Interday	1	100.9	99.3	99.7	100.7	100.3	98.90	99.6	98.7	99.9
	2	99.6	100.7	99.1	99.4	99.6	99.40	100.3	98.9	99.2
	3	99.4	100.6	100.2	101.5	98.9	98.60	99.8	100.9	98.8
	Mean	99.97	100.20	99.67	100.53	99.60	98.67	99.90	99.50	99.3
	S.D	0.815	0.781	0.551	1.060	0.700	0.404	0.360	1.217	0.557
	R.S.D	0.814	0.779	0.553	1.054	0.702	0.408	0.361	1.223	0.561

*Average of three determinations SD: Standard deviation RSD: Relative standard deviation

Follow table . 6: evaluation of the precision of the proposed Picric acid method.

Parameter		Neomycin *(%found)**			Kanamycin *(%found)**			Streptomycin *(%found)**		
		10 µg mL^{-1}	50 µg mL^{-1}	90 µg mL^{-1}	5 µg mL^{-1}	40 µg mL^{-1}	80 µg mL^{-1}	20 µg mL^{-1}	60 µg mL^{-1}	100 µg mL^{-1}
Intraday	1	101.07	101.74	100.89	99.51	99.70	100.91	99.12	100.73	99.61
	2	101.80	100.65	101.08	99.82	100.54	101.10	99.51	100.24	98.43
	3	100.42	100.94	100.92	100.13	100.98	100.44	98.91	99.87	99.42
	Mean	101.09	101.11	100.96	99.82	100.41	100.82	99.18	100.28	99.15
	S.D	0.690	0.564	0.351	0.310	0.650	0.339	0.305	0.431	0.634
	R.S.D	0.682	0.557	0.347	0.311	0.647	0.336	0.307	0.429	0.639
Interday	1	99.63	101.03	100.56	100.35	99.33	101.19	98.37	100.61	99.11
	2	99.83	100.44	99.82	101.04	98.86	100.17	99.22	100.80	99.75
	3	100.24	101.27	99.33	100.91	99.63	100.22	98.81	99.88	98.94
	Mean	99.90	100.91	99.90	100.76	99.27	100.53	98.80	100.43	99.27
	S.D	0.311	0.427	0.619	0.367	0.388	0.575	0.425	0.486	0.427
	R.S.D	0.310	0.423	0.619	0.364	0.390	0.572	0.430	0.484	0.430

Table . 7: Robustness of the proposed spectrophotometric methods.

%Recovery * ± SD											
Amikacin		Tobramycin		Gentamicin		Neomycin		Kanamycin		Streptomycin	
DNP volume (mL)											
1.5 mL		1.5 ml		1.5 ml		1.5 ml		1.5 ml		1.5 ml	
1.2	100.7 ± 0.50	**1.2**	100.6± .567	**1.2**	99.65 ± 0.87	**1.2**	100.6 ± 0.785	**1.2**	100.8 ± 1.34	**1.2**	98.55 ± 1.56
2.0	99.9 ± 0.987	**2.0**	100.3 ± 1.21	**2.0**	99.12 ± 0.43	**2.0**	99.13 ± 0.25	**2.0**	99.41 ± 1.1	**2.0**	99.11 ± 1.32
Picric acid volume (mL)											
1.0 ml		1.0 ml		1.0 ml		1.0 ml		1.0 ml		1.0 ml	
0.8	97.9 ± 0.79	**0.8**	98.78 ± 1,32	**0.8**	99.34 ± o.45	**0.8**	100.12 ± 1.2	**0.8**	101.3 ± 0.56	**0.8**	99.2 ± 0.687
1.2	98.2 ± 0.98	**1.2**	98.9 ± 0.765	**1.2**	98.35 ± 1.64	**1.2**	101.05 ± 1.3	**1.2**	100.98 ± 0.7	**1.2**	98.97 ± 1.23

Table . 8: Ruggedness of the proposed spectrophotometric methods.

Drug	Instrument			
	Jenway®, ultraviolet-visible spectrophotometer		Spectronic™genesys™, ultraviolet-visible spectrophotometer®	
	%Recovery * ± SD			
	DNP	*Picric acid*	*DNP*	*Picric acid*
Amikacin	99.54 ± 1.11	100.32 ± 1.44	99.14 ± 1.54	99.73 ± 1.21
Tobramycin	98.54 ± 0.87	97.98 ± 0.88	98.99 ± 0.45	98.82 ± 0.93
Gentamicin	99.34 ± 0.95	99.6 ± 0.744	99.78 ± 0.77	99.15 ± 1.46
Neomycin	101.1 ± 1.14	101.3 ± 0.87	100.81 ± 1.37	100.99 ± 0.33
Kanamycin	99.63 ± 0.83	100.5 ± 0.29	99.48 ± 0.49	99.39 ± 1.43
Streptomycin	100.4 ± 0.35	99.2 ± 1.38	99.86 ± 0.71	98.14 ± 1.55

Table. 9: Comparison between the proposed DNP and the reported methods for the determination of the studied Aminoglycosides in their pharmaceutical dosage forms.

Pharmaceutical dosage forms	*% Recovery ± SD**		*t-value* [b]	*F-value* [b]
	Proposed method	*Reported method*		
Amikacin sulfate				
Amikin 100 mg® vials	100.17 ± 0.764	99.13 ± 1.026	1.399	1.806
		Neomycin sulfate		
Neomycin 500 mg ® tablets	99.47 ± 0.907	98.73 ± 1.55	**0.729**	2.924
		Tobramycin		
Tobradex ® eye drops	100.83 ± 1.26	**102**.23 ± 0.75	1.64	2.73
Tobrin ® eye drops	100.57 ± 1.69	**100.33**± 1.53	0.177	1.23
Tobrin ® eye ointment	100.5 ± 1.32	101.43 ± 1.102	0.944	1.44
		Gentamicin		
Garamycin ® 80 ampoule	100.17 ± 0.764	**99**.9 ± 1.114	0.342	2.126
Garamycin ® ointment	100.53 ± 1.168	**100**.21 ± 1.203	0.337	1.062
		Kanamycin		
Diakan M ® syrup	99.63 ± 0.611	**99**.11 ± 0.885	0.843	2.098
		Streptomycin		
Streptomycin ® 1gm vial	99.2 ± 1.054	**99**.33 ± 0.585	0.187	3.243

* Average of five determinations.
[b] Tabulated values at 95% confidence limit are t=2.306, F=6.338.

Table. 10: Comparison between the proposed PA and the reported methods for the determination of the studied Aminoglycosides in pharmaceutical dosage forms.

Pharmaceutical dosage forms	*% Recovery ± SD**		*t-value* [b]	*F-value* [b]
	Proposed method	*Reported method*		
Amikacin sulfate				
Amikin 100 mg® vials	100.07 ± 0.666	99.13 ± 1.026	1.321	2.376
Neomycin sulfate				
Neomycin 500 mg ® tablets	100.1 ± 0.656	98.73 ± 1.55	**0.979**	1.915
Tobramycin				
Tobradex ® eye drops	101.2 ± 0.721	**102**.23± 0.75	1.695	1.116
Tobrin ® eye drops	99.37 ± 1.097	100.33 ± 1.53	1.031	2.38
Tobrin ® eye ointment	100.53 ± 1.5	101.43 ± 1.102	0.842	1.856
Gentamicin				
Garamycin ® 80 ampoule	99.3 ± 1.179	**99**.9 ± 1.114	0.641	1.121
Garamycin ® ointment	99.53 ± 1.16	**100**.21 ± 1.203	0.698	1.078
Kanamycin				
Diakan M ® syrup	99.5 ± 1.159	**99**.11 ± 0.885	0.506	1.715
Streptomycin				
Streptomycin ® 1gm vial	99.67 ± 1.35	**99**.33 ± 0.585	0.396	5.33

* Average of five determinations.
[b] Tabulated values at 95% confidence limit are t=2.306, F=6.338.

Robustness

Robustness of the procedures was assessed by evaluating the influence of small variation in experimental variables on the analytical performance of the method (DNP or picric acid volume and reaction time).

In these experiments, one experimental parameter was changed while the other parameters were kept unchanged, and the recovery percentage was calculated each time (Table 7). The small variations in any of the variables did not significantly affect the results. This gave an indication for the reliability of the proposed method during routine work.

Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, different instruments, different lots of reagents, different assay temperatures, different elapsed assay times, etc. To examine ruggedness of the procedure, the analysis was done using different instruments, and then the results were evaluated as shown in Table (8). The precision of the proposed method was fairly high, as indicated by the low values of the (% RSD) which did not exceed 2% for the studied drugs.

Application to pharmaceutical dosage forms

The proposed method was applied for determination of investigated drugs in commercial pharmaceutical dosage forms. The results were statistically compared with those of reported methods (Gupta et al., 1983, Sampath et al., 1990, Aman et al., 1995), in respect to accuracy and precision. The obtained mean recovery values were 98.73-102.23 ± 0.585-1.69 %, as shown in table (9, 10). According to t- and F- tests, no significant difference was found between the calculated and theoretical values of both the proposed and the reported methods at 95% confidence level. This indicates good level of precision and accuracy.

CONCLUSION

In conclusion, the proposed spectrophotometric procedures are simple and time saving. Moreover, they could be applied to the quality control analysis of the investigated drugs.

REFERENCES

Ahmad A. S., Hoda M. D. N., Ahmad M., Islam F., Qureshi S. Z. A simple and selective kinetic spectrophotometric method for the determination of kanamycin using acetylacetone-formaldehyde reagent in N,N '-dimethylformamide medium. Journal of Analytical Chemistry. 2006; 61(9): 870-874.

Al-Sabha T. N. Spectrophotometric Determination of Amikacin Sulphate Via Charge Transfer Complex Formation Reaction Using Tetracyanoethylene and 2,3-Dichloro-5,6-Dicyano-1,4-Benzoquinone Reagents. Arabian Journal for Science and Engineering. 2010; 35(2A): 27-40.

Ayad M. M., Yousef M. D.c. polarographic determination of certain Aminoglycosides. Analyst (London). 1985; 110(8): 963-965.

Aman T., Rashid A., Kulsoom R., Khokhar I. Spectrophotometric determination of streptomycin. Anal Lett. 1995; 28(5): 881-892.

Belal F., El-Ashry S. M., El Kerdawy M. M., El Wasseef D. R. Spectrofluorimetric determination of streptomycin in dosage forms and in spiked plasma using 9,10-phenanthraquinone. Journal of Pharmaceutical and Biomedical Analysis. 2001; 26(3): 435-441.

Bohm D. A., Stachel C. S., Gowik P. Confirmatory method for the determination of streptomycin in apples by LC-MS/MS. Anal Chim Acta. 2010; 672(1-2):103-106.

Clarot I., Storme-Paris I., Chaminade P., Estevenon O., Nicolas A., Rieutord A. Simultaneous quantitation of tobramycin and colistin sulphate by HPLC with evaporative light scattering detection. Journal of Pharmaceutical and Biomedical Analysis. 2009; 50(1): 64-67.

Deng A., Yang X., Yang Y., Yang H. Study on determination of gentamycin by enhanced chemiluminescent immunoassay. Hua Xi Yi Ke Da Xue Xue Bao. 1993; 24(1): 101-103. 1993.

El-Shabrawy Y. Fluorimetric determination of aminoglycoside antibiotics in pharmaceutical preparations and biological fluids. Spectroscopy Letters.2002; 35(1): 99-109.

Gilmartin M. R., McLaren J., Schacht J. Confounding factors in lanthanide ion probe spectrofluorimetric assay of aminoglycoside antibiotics. Anal Biochem. 2000; 283(1): 116-119.

Gremilogianni A. M., Megoulas N. C., Koupparis M. A. Hydrophilic interaction vs ion pair liquid chromatography for the determination of streptomycin and dihydrostreptomycin residues in milk based on mass spectrometric detection. J Chromatogr A. 2010; 1217(43):6646-51.

Gupta V. D., Stewart K. R., Gunter J. M. Quantitation of amikacin, kanamycin, neomycin, and tobramycin in pharmaceutical dosage forms using the Hantzsch reaction. Journal of pharmaceutical sciences.1983, 72 (12):1470-1471.

Hubicka U., Krzek J., Woltynska H., Stachacz B. Simultaneous Identification and Quantitative Determination of Selected Aminoglycoside Antibiotics by Thin-Layer Chromatography and Densitometry. J AOAC Int. 2009; 92(4): 1068-1075.

Halvatzis S. A., Mihalatos A. M., Palilis L. P., Calokerinos A. C. Continuous-Flow Chemiluminometric Determination of Amiloride and Streptomycin by Oxidation with N-Bromosuccinimide. Analytica Chimica Acta. 1994; 290(1-2): 172-178.

Huidobro A. L., Garcia A., Barbas C. Rapid analytical procedure for neomycin determination in ointments by CE with direct UV detection. Journal of Pharmaceutical and Biomedical Analysis. 2009; 49(5): 1303-1307.

ICH Harmonized Tripartie Guidline Q2B (R1): " Validation of Analytical Proceudures: Text and Methodology". (available at http://www.ich.org): Incorporated in November 2005.

Jiame N. D., William A. R. " Wilson's and Gisvold's text book of organic, medicinal and pharmaceutical chemistry"; 10 th ed.;1998; pp 291, 292.

Jiang H., Liu S.P., Hu X.L., Yang J.D. Colour reaction of pontamine sky blue with aminoglycoside antibiotics and their application in analysis. Fenxi Ceshi Xuebao.2003; 22(3): 72-74.

Jiang H., Liu S.P., Hu X. L. Color reactions of trypan blue with aminoglycoside antibiotics and their analytical applications. Fenxi Shiyanshi. 2003; 22(3): 59-61.

Jiang H., Hu X. L., Liu S. P., Liu Z. F., Zhan H. L., Qin Z. H. Spectrophotometric determination of several antibiotics in human serum and urine liquid by lanthanum(III)-pontamine sky blue-antibiotics system. Chinese Journal of Analytical Chemistry.2004; 32(8): 1043-1046.

Job P.; Advanced Physicochemical Experiments, 2nd ed., Oliner and Boyd, Edinburgh, P. 54 (1964), Ann. Chem., 16, 97 (1936).

Kalashnikov V. P., Dolotova T. M., Mynka A. F. Spectrophotometric determination of streptomycin sulfate. Farm Zh (Kiev). 2000; 3: 61-63.

Kajita H., Akutsu C., Hatakeyama E., Komukai T. Simultaneous determination of aminoglycoside antibiotics in milk by liquid chromatography with tandem mass spectrometry. Journal of the Food Hygienic Society of Japan. 2008; 49(3): 189-195.

Kaya S. E., Filazi A. Determination of Antibiotic Residues in Milk Samples. Kafkas Universitesi Veteriner Fakultesi Dergisi. 2010; 16: *S31-S35*.

Koerner P. J., Nieman T. A. Luminol chemiluminescence HPLC reaction detector for amino-acids and other ligands. Mikrochim Acta. 1987; II (1-3): 79-90.

Liang Y., Zhang T. Quantitative determination of streptomycin by first-derivative high-speed pulse polarography. Zhongguo Kangshengsu Zazhi. 1991; 16(2): 102-105.

Li Q. M., Gao L. X. A Novel Method for the Determination of Streptomycin Using Sodium Nitroprusside as a Chromogenic Reagent by Spectrophotometry. Analytical Letters. 2008; 41 (14): 2595-2607.

Lin Y. F., Wang Y. C., Chang S. Y. Capillary electrophoresis of aminoglycosides with argon-ion laser-induced fluorescence detection. Journal of Chromatography A. 2008; 1188(2): 331-333.

Mai E. Spectrofluorimetric method for the determination of amikacin. Zhongguo Yaoxue Zazhi. 1990; 25(10): 597-599.

Meng L., Wang A., Li L., Zhang J. Spectrofluorimetric method for amikacin. Zhongguo Yaoxue Zazhi.1993; 28(7): 417-418.

Mitic S. S., Sunaric S. M., Tosic S. B. Determination of streptomycin in a pharmaceutical sample based on its degradation by hydrogen peroxide in the presence of copper(II). Analytical Sciences. 2006; 22(5): 753-756.

Rizk M., ElShabrawy Y., Zakhari N. A., Toubar S. S., Carreira L. A. Fluorimetric determination of aminoglycoside antibiotics using lanthanide probe ion spectroscopy . Talanta. 1995; 42(12): 1849-1856.

Sampath S.S., Robinson D.H. Comparison of new and existing spectrophotometric methods for the analysis of tobramycin and other Aminoglycosides. J Pharm Sci.1990; 79(5):428-431.

Sanchez-Martinez M.L., Aguilar-Caballos M.P., Gomez-Hens A. Long-wavelength fluorescence polarization immunoassay:

Determination of amikacin on solid surface and gliadins in solution. Analytical Chemistry, 2007; 79 (19):7424-7430.

Sánchez-Martínez M.L., Aguilar-Caballos M.P., Gómez-Hens A. Long wavelength homogeneous enzyme immunoassay for the determination of amikacin in water samples. Talanta. 2009; 78(1):305-309.

Sanchez-Martinez M. L., Aguilar-Caballos M. P., Gomez-Hens A. Selective kinetic determination of amikacin in serum using long-wavelength fluorimetry. J Pharm Biomed Anal. 2004; 34(5):1021-1027.

Stahl G. L., Kratzer D. D. Microbiological determination of neomycin in feeds and formulated products. J Assoc Off Anal Chem. 1984; 67(5):863-865.

Stahl G. L., Dalkratzer D., Kasson C. W. Microbiological Determination of Neomycin in Feeds - Collaborative Study. Journal of the Association of Official Analytical Chemists. 1989; 72(2):237-241.

Sunaric S. M., Mitic S. S., Miletic G. Z., Kostic D. A., Trutic N. V. Spectrophotometric initial-rate method for determination of neomycin in ophthalmic drops. Chemia Analityczna. 2007. 52(5): 811-821.

Szabo A., Erdelyi B., Salat J., Mate G. Densitometric determination of some bioactive guanidinium compounds without post-derivatization. Jpc-Journal of Planar Chromatography-Modern Tlc. 2005; 18(103): 203-206.

Turnipseed S. B., Clark S. B., Karbiwnyk C. M., Andersen W. C., Miller K. E., Madson M. R. Analysis of aminoglycoside residues in bovine milk by liquid chromatography electrospray ion trap mass spectrometry after derivatization with phenyl isocyanate. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877(14-15):1487-1493.

The United States Pharcopoeia XXVIIII and NF XXV; American Pharmaceutical Assiciation: Washington, DC, 2007, Electronic version.

Yamamoto C. H., Pinto T. J. Rapid determination of neomycin by a microbiological agar diffusion assay using triphenyltetrazolium chloride. Journal of Aoac International. 1996; 79 (2):434-440.

Yang X., Wu L., Deng A., Zhang J., Zhu L. Study on determination of gentamycin in serum by enhanced chemiluminesent immunoassay based on immobilized antibody and biotin-avidin system. Hua Xi Yi Ke Da Xue Xue Bao. 1995; 26(2):234-236.

Yang C. Y., Zhang Z. J., Wang J. L. New luminol chemiluminescence reaction using diperiodatoargentate as oxidate for the determination of amikacin sulfate. Luminescence. 2010; 25(1): 36-42.

Yu C. Z., He Y. Z., Fu G. N., Xie H. Y., Gan W. E. Determination of kanamycin A, amikacin and tobramycin residues in milk by capillary zone electrophoresis with post-column derivatization and laser-induced fluorescence detection. J Chromatogr B Analyt Technol Biomed Life Sci. 2009; 877(3):333-338.

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