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Colorimetric Microdetermination of Some Aminoglycosides in Pure and in their Pharmaceutical Formulations

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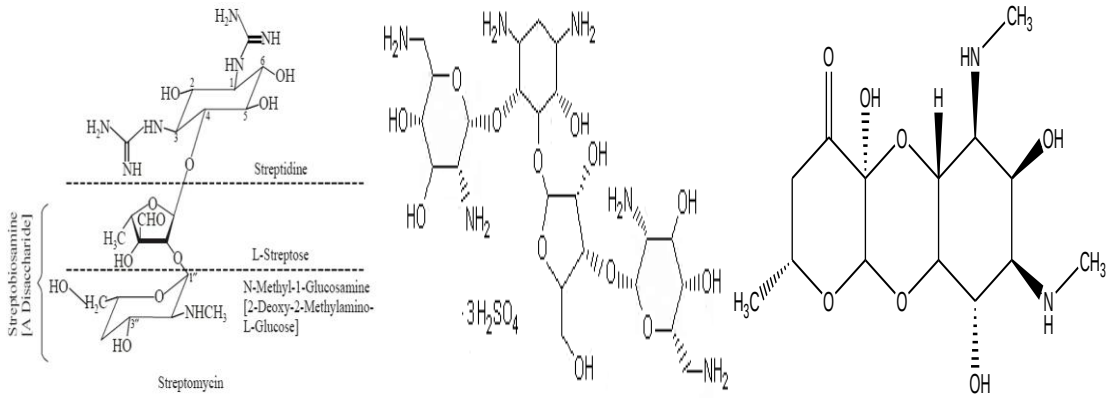
Abstract: *Two simple, selective and sensitive spectrophotometric methods are described for the determination of some aminoglycosides antibiotics: Streptomycin sulphate (STR), neomycin sulphate (NMS) and spectinomycin (SPC). The methods are based on the reaction of the studied drugs with either bromocresol green (BCG), bromocresol purple (BCP), to give yellow species. The coloured products are quantified spectrophotometrically at 430, 477, and 441, for STR, NMS, and SPC, using BCG, respectively, whereas on using BCP at 436, 440, and 466, respectively. The optimization of the different experimental conditions is described. No interferences from different additives and common degradation products were observed in the determination. The BCP method was better than the BCG method, because of its wider range of determination (0.4 - 12 μg mL^{-1} vs. 0.6 - 18 μg/ mL on using BCP), higher molar absorptivity and Sandell sensitivity (8.26 x 10^4 L mol^{-1} cm^{-1} and 0.007 μg cm^{-2} vs. 5.62 × 10^4 L mol^{-1} cm^{-1} and 0.011 μg cm^{-2}), greater stability (24 and 18 days on using BCG and BCP, respectively) and better selectivity. The stoichiometry of the formed ion-pairs was found to be 1: 1 for all complexes. The proposed methods have been applied successfully for the analysis of the studied drugs in pure forms and pharmaceutical formulations.*

Keywords: *Aminoglycosides determination, spectrophotometry, ion pair complexation, Pharmaceutical formulations*

1. INTRODUCTION

Aminoglycosides are a group of highly potent and wide spectrum antibiotics that are active against both gram-positive and gram-negative bacterial infections [1]. They are a large class of antibiotics that are characterized by two or more amino sugars linked by glycosidic bonds to an aminocyclitol component [2]. They bind to various RNA molecules such as ribosomal RNA,50-untranslated region of thymidylate synthase mRNA, both the Revresponse element and the transactivating response element RNA motifs of HIV-1, and a variety of catalytic RNA molecules such as group Iintrons ribonuclease P RNA hairpin ribozyme, Hammerhead ribozyme and hepatitis delta virus ribozyme [3-10]. The mechanism of action of aminoglycoside antibiotics are irreversible binding to the 30S ribosomal subunit, thereby inhibiting bacterial protein synthesis. In addition, these drugs damage the cytoplasmic membrane of bacteria sensitive to them [11]. STR is having chemical name 1,-(4-(4-(3,5- dihydroxy-6-(hydroxymethyl)-4-(methylamino)tetrahydro-2H-pyran-2-yloxy)-5-formyl-5-hydroxy-3-methyl tetra hydrofuran-2-yloxy)-2,5,6-trihydroxy cyclohexane-1, 3-diyl)diguanidine is an aminoglycosidic antibiotic with three components: streptidine, streptose and N-methyl-L-glucosamine [12]. STR is bactericidal antibiotic drug under aminoglycosides category and derived from Streptomyces griseus. It is also used to control bacteria, fungi, and algae in crops [13]. NMS is [(1R,2R,3S,4R,6S)-4,6-diamino-2-{[3-O-(2,6-diamino-2,6-dideoxy-β-Lidopyranosyl)-β-Dribofuranosyl]oxy}-3hydroxycyclohexyl 2,6-diamino-2,6-dideoxy-α-D-glucopyranoside] [14]. NMS is produced by the growth of *Streptococcus fradiae* [15]. SPC is (1*R*,3*S*,5*R*,8*R*,10*R*,11*S*,12*S*,13*R*,14*S*)-8,12,14-trihydroxy-5-

methyl-11,13-*bis* (methyl amino)-2,4,9-trioxatricyclo tetradecan-7-one.SPC is an aminoglycoside antibiotic produced by *Streptomyces spectabilis* [15].



[1] [2] [3]

Scheme1. *The chemical structure of the studied aminoglycosides [1] STR, [2] NMS and [3] SPC.*

Several methods have been reported for the determination of Aminoglycosides either in pure forms, dosage forms, or biological fluids like chromatography [16-46] and conductometry [47, 48]. The spectrophotometric technique continues to be the most preferred method for the assay of different classes of drugs in pure, pharmaceutical formulations and in biological samples, for its simplicity and reasonable sensitivity with significant economical advantages. Spectrophotometric methods are reported for the assay of STR [49–52], NMS [53-54], and SPC [55]. These methods were associated with some major drawbacks such as decreased selectivity due to measurement in ultraviolet region and/or decreased simplicity of the assay procedure (e.g., tedious precipitation, heating, or liquid-liquid extraction steps in the ion-pair formation-based methods). For these reasons, it was worthwhile to develop a new simple and selective spectrophotometric method for the determination of the studied drugs in their pharmaceutical dosage forms.

In the present work, the development of accurate and precise extractive spectrophotometric methods based on the chloroform soluble ion-pair complexes between the studied Aminoglycosides antibiotics (STR,NMS and SPC) and some acid dyes (BCG and BCP) were reported. The absorbance measurements were measured at optimum wavelengths. The proposed methods were applied successfully for the determination of the studied drugs in pure and in dosage forms. No interference was observed from the additives. The methods provide rapid, economic procedures and more sensitive compared to the previously reported spectrophotometric methods. These methods were validated by the statistical data.

2. EXPERIMENTAL

2.1. Apparatus

All absorption spectra were made using Kontron Unikon 930 (UV-Visible) spectrophotometer (German) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells. The pH values of different buffer solutions were checked using a Hanna pH-meter instrument (pH 211) (Romania) equipped with a combined glass-calomel electrode.

2.2. Materials and Reagents

All reagents and chemicals used were of analytical or pharmaceutical grade and all solutions were prepared fresh daily.

Pharmaceutical grade Streptomycin sulphate (STR) was supplied by Al-Obour Pharmaceutical and Chemical Industries Company, Egypt. Neomycin sulphate (NMS) reference standard was provided by Sabaa, Kahira Company, Egypt. Spectinomycin (SPC) was kindly provided by Pharma Swede, Egypt (AVICO).

All pharmaceutical preparations were obtained from commercial sources in the local markets. Neomycin tablets were obtained from Memphis for Pharmaceuticals & Chemical Industries, Cairo, Egypt, labeled to contain 350 mg NMS per tablet; Streptomycin injectable (were obtained

from Amriya Pharm. Ind. Co. for Nile Co. for Pharm. & Chem. Ind.) was labeled to contain 1.0 mg STR.

2.3. Preparation of Stock Standard Solutions

Stock standard solutions of STR, NMS, and ENR (100 μg/mL and 2×10^{-3} M), were prepared by dissolving an exact weight of pure drugs in bidistilled water in a 100 mL measuring flask. The standard solutions were found stable for at least one week without alteration when kept in an amber coloured bottle and stored in a refrigerator when not in use. Further dilutions were made to prepare different concentrations containing.

2.4. Reagents

Bromocresol green (BCG), and bromocresol purple (BCP) (BDH Chemicals Ltd., and Poole, England) were used without further purification. Stock solutions (2×10^{-3} M) of BCG and BCP were prepared by dissolving the appropriate weight of each reagent in 10 mL of acetone and diluted to 100 mL with bidistilled water. These solutions are stable for at least one week if kept in the refrigerator.

A series of buffer solutions of universal pH (2.0-12), acetate buffer pH (2.6-5.6), Borate; pH (5.6-9.5) and phthalate; (pH=2.0-7.0) buffers were prepared by following the standard methods [56].

2.5. General Procedures

Using BCG

An aliquot of the solution containing 6.0-120, 10-150 or 10-180 μg/mL of STR, NMS, and SPC were transferred into a 50 mL separating funnel. A 0.6 mL for NMS or 1.2 mL (for STR and SPC) of BCG solutions (1.0×10^{-3} M) and 3.0 mL of universal buffer of pH 3.0 using BCG were added. The total volume of each solution was completed to 10 mL with bidistilled water. The formed ion pair complex was extracted with 10 mL of dichloromethane for STR and/or chloroform for NMS and SPC. The solution was shaken for 3.0 min; the two phases were allowed to separate and the organic layer was passed through anhydrous sodium sulphate into a 10-mL volumetric flask. The absorbance of the yellow coloured species was measured at 430, 477 and 441 nm for STR, NMS and SPC, respectively, against the corresponding reagent blank solution prepared in the same manner. All measurements were made at room temperature (25 ± 1 ˚C). A calibration graph was plotted.

Using BCP

An aliquot of the solution containing 4.0-80, 10-100, and 4.0- 120 μg/mL of STR, NMS, and SPC, respectively, were transferred into a 50 mL separating funnel. 1.2 (for STR), or 1.8 (for NMS and SPC) mL of BCP reagent solutions (1.0×10^{-3} M) and 1.0 mL of pH (3.2 and 3.0) for (NMS and SPC) or 2.0 mL pH 3.0 for STR of universal buffer were added. The total volume of each solution was completed to 10 mL with bidistilled water. The formed ion-pair complex was extracted with 10 mL of chloroform, carbon tetrachloride, and dichloromethane for STR, MNS, and SPC, respectively. The solution was shaken for 3.0 min; the two phases were allowed to separate and the organic layer was passed through anhydrous sodium sulphate into a 10 mL measuring flask. The absorbance of the yellow coloured species was measured at 436, 440 and 466 nm, respectively, against the corresponding reagent blank solution prepared in the same manner. All measurements were made at room temperature (25 ± 1 ^{o}C). A calibration graph was plotted.

2.6. Applications to Pharmaceutical Formulations

Procedure for tablets

The content of ten tablets (Neomycin) labeled to contain 350 mg NMS per tablet were crushed, powdered, and weighted out and the average weight of one tablet was determined. An accurate weight equivalent to 10 mg NMS was dissolved in 20 mL bidistilled water with shaking for 5.0 min and filtered. The filtrate was diluted to 100 mL with bidistilled water in a 100 mL measuring flask to give 100 μg/mL stock solution. An aliquot of the diluted drug solution was treated as described previously.

Procedure for injection

Accurate weight of streptomycin equivalent to 10 mg were dissolved in 100 mL bidistilled water into 100 mL volumetric flasks. Aliquots of these solutions were transferred into a series of 10 mL calibrated flasks, and the analysis was completed as previously mentioned.

2.7. Stoichiometric Relationship

The stoichiometric ratios of the ion-pairs formed between the drugs and reagents under investigation were determined by applying the continuous variation [57] and the molar ratio [58] methods at the wavelengths of maximum absorbance. In continuous variation method, equimolar solutions were employed: 2.0×10^{-3} M standard solutions of drug and reagents were used. A series of solutions were prepared in which the total volume of the studied drugs and the reagent was kept at 1.0 mL. The drug and reagent were mixed in various complementary proportions (0.1: 0.9, 0.2:0.8,…,0.9:0.1) and completed to volume at the optimum conditions in a 10 mL calibrated flask with the appropriate solvent for extraction following the above mentioned procedure. In the molar ratio method, the concentrations of STR, NMS, and SPC are kept constant (0.5 mL of 2.0×10^{-3} M) while that of reagents (2.0×10^{-3} M) are regularly varied (0.1–2.0 mL). The absorbance of the prepared solutions optimum is measured at optimum conditions at wavelength for each complex.

3. RESULTS AND DISCUSSION

3.1. Absorption Spectra

The absorption spectra of the ion-pairs formed between STR, NMS or SPC and sulphonphthalein dyes were measured at 350-550 nm against the corresponding reagent blank solution. The extracts showed maximum absorbance at 430, 477 and 441 nm using BCG and at 436, 440 and 466 nm using BCP method, respectively.

Investigations were carried out to establish the most favourable conditions to give a highly intense colour and achieve maximum colour development in the quantitative determination of STR, NMS or SPC. The influence of each of the following variables on the reaction was tested.

3.2. Effect of pH

The effect of pH was studied by extracting the coloured complex in the presence of various buffers such as acetate (pH = 1.99-5.56), borate (pH = 6.75-9.5) and phthalate (pH = 2.2-4.6) and universal buffer solutions (2.2-12). It was noticed that the maximum colour intensity and constant absorbance were observed in universal buffer of pH 3.0 for all ion pairs except for MNS with BCP complex, which has maximum absorbance at pH 3.2. Moreover, the volume of buffer solution was also examined and found that 2.0 mL was sufficient on using BCG for all drugs and STR with BCP, whereas, 1.0 mL only was sufficient for NMS and SPC using BCP reagent.

3.3. Choice of Organic Solvent

The effect of the extracting solvent used both on extraction efficiency and colour intensity was examined. Chloroform, dichloromethane, dichloroethane, toluene and carbon tetrachloride proved useful solvents; chloroform was selected for NMS and SPC using BCG and dichloromethane was selected for STR-BCG SPC-BCP ion pairs, whereas, carbon tetrachloride was selected for NMS-BCP ion pairs due to the greater stability of the extracted coloured product (24 h) and considerably lower extraction abilities of the reagent blank. Consequently a single extraction with 10 mL of chloroform, dichloromethane or carbon tetrachloride met optimum conditions

3.4. Effect of Shaking Time

The extraction was studied by shaking different samples on a shaker and varying the shaking time for 0.5-5.0 min for the ion pair complexes. It was found that the absorbance remained constant over this time period for all systems. A shaking time of 3.0 min was adopted for all extractions. It was further observed that the yellow extracts remained stable for at least 18 h. The intensity of ion-pairs extraction were found to be stable in the temperature range 20-70 ^{o}C. Hence room temperature, (25 ± 1.0 ^{o}C), was used.

3.5. Effect of Reagent Concentrations

When various concentrations of BCG, or BCP were added to a fixed concentration of 8.0 µg/mL of STR, NMS or SPC. 0.6 mL for NMS-BCG and 1.2 mL for STR with BCG and BCP, and SPC-BCG ion pairs were found to be enough to develop the colour to its full intensity, whereas, 1.8 was the optimum for NMS and SPC using BCP ion –pairs.

3.6. Sequence of Additions

The optimum sequence was defined by following to color intensity and maximum absorbance on changing the sequences of addition of drug, reagent and buffer. The best condition was "drug-reagent-buffer-solvent" for the highest absorbance and stability. Other sequences needed longer time in addition to lower stability.

3.7. Composition of Ion-Pair Extraction

Job's method of continuous variations and the molar ratio methods using both a variable reagent concentration and a variable drug concentration established the composition of the ion pair. The results obtained with these methods showed that the composition of the associate was equimolar (1:1) and the shapes of the curves indicated that the ion pair was labile. Hence, a large excess of reagent must be used to enhance the stability of the ion pair.

3.8. Interferences

A systematic quantitative study was undertaken by measuring the absorbance's of solutions containing 1.0 mL of 1×10^{-3} M drug together with varying excess of different additives and excipients which may be present in the dosage forms using the recommended methods of such reagents for STR, NMS and KET. No significant interference was observed from the excipients commonly used such as glucose, lactose, starch, fructose and magnesium stereate. This shows that the method is applicable in case of dosage forms of the investigated drugs.

3.9. Analytical Data

Under the above mentioned experimental conditions, standard calibration graphs were constructed for the reaction at 430, 477 and 441 nm using BCG and at 436, 440 and 466 nm using BCP, respectively. Beer's law was evident for drugs analyzed in the concentration range mentioned (Table 1). For more accurate analysis, Ringbom optimum concentration ranges were calculated and the molar absorptivities, the detection and quantification limits, Sandell sensitivities, linear regression equations and the correlation coefficient are recorded in Table 1. The reproducibility of the proposed method was determined by running six replicate samples, containing 8.0 µg/mL DEX or KET with acid dyes. The relative standard deviation (≤ 1.108% using BCG and ≤ 0.9357% using BCP) and the percentage range of the error at a 95% confidence level (≤ 1.163% for BCG and ≤ 0.9819% for KET) can be considered be satisfactory considered to be satisfactory.

Table1. *Analytical features of the proposed methods*

Parameters	BCG method			BCP method		
	STR	NMS	SPC	STR	NMS	SPC
pH	3.0	3.0	3.0	3.0	3.2	3.0
Amount of buffer	2.0	3.0	2.0	2.0	1.0	1.0
[R] × 10^{-4} M	1.2	0.6	1.0	1.2	1.9	1.8
Extracting solvent	CH_2Cl_2	CH_3Cl	CH_3Cl	CH_3Cl	CCl_4	CH_2Cl_2
Shaking time; min	2.0	3.0	4.0	3.0	4.0	5.0
λ_{max} (nm)	430	477	441	436	440	466
Beer's range; µg/mL	0.6 -12	0.8 -15	0.8-18	0.4 -8.0	0.8 -10	0.4 -12
Ringbom range; µg/mL	1.5 -10.5	1.5 -13	2.0 -16	0.8 -7.0	2.0 -9.0	1.0 -10.8
Molar absorptivity ($L\ mol^{-1}\ cm^{-1}$) x 10^4	5.62	2.42	3.51	8.26	5.89	1.19
Sandell sensitivity; ng/cm^2	0.011	0.025	0.009	0.007	0.010	0.028
Regression equation [a]						
Slope	0.169	0.155	0.115	0.066	0.096	0.031

Intercept	-0.013	0.027	-0.021	0.242	0.009	0.009
Correlation coefficient (*r*)	0.9988	0.9992	0.9995	0.998	0.999	0.999
RSD [a] (%)	0.77	0.96	0.82	0.325	0.980	0.907
Detection limits; ng/m)	0.18	0.25	0.26	0.12	0.25	0.13
Quantification limits; ng/mL	0.62	0.0.79	0.83	0.39	0.81	0.44

[a] *A = a + bC, where C is the concentration in µg/mL; and A is the absorbance units.*

3.10. Accuracy and Precision

In order to determine the accuracy and precision of the proposed method, solutions containing six different concentrations of the examined drugs were prepared and analyzed in quintuplicate. The analytical results obtained from this investigation are summarized in Table 2. The relative standard deviations and the percentage range of error at a 95% confidence level were calculated. The results can be considered to be satisfactory, at least for the level of concentrations examined.

Table 2. *Evaluation of the accuracy and precision of the proposed methods*

Method	Drug taken µg ml^{-1}	Drug found µg ml^{-1}	Recovery (%)	RSD[a] (%)	RE (%)	Confidence [b] Limits
BCG-STR	3.0	3.02	100.67	1.339	1. 808	2.02 ± 0.0218
	6.0	6.10	101.67	1.229	1.246	6.10 ± 0.0712
	12	11.88	99.00	0.859	0.891	11.88 ± 0.0326
BCP-STR	2.0	1.97	98.50	1.060	1.629	1.97 ± 0.0124
	4.0	4.02	100.50	1.218	1.283	4.02± 0.0332
	8.0	7.95	99.38	0.746	0.811	7.95 ± 0.0426
BCG-NMS	10	10.10	101.00	1. 208	1.369	10.10 ± 0.0352
	5.0	5.02	100.04	0.994	1.945	5.02 ± 0.0472
	15	14.88	99.20	1.319	1.4246	14.88 ± 0.0296
BCP-NMS	3.0	2.99	99.67	1.023	1.132	2.99 ± 0.0362
	6.0	5.97	99.50	1.182	1.251	5.97 ±0.0487
	9.0	8.94	99.33	1.436	1532	8.94 ± 0.0269
BCG-SPC	6.0	6.03	100.50	0.896	0.963	6.03 ± 0.0307
	12	11.90	99.17	1.009	1,148	11.90 ± 0.0511
	18	17.90	99.44	1.428	1,505	17.90 ± 0.0159
BCP-SPC	3.0	2.97	99.00	1.341	1.414	2.97 ± 0.0237
	6.0	5.95	99.16	1.135	1.203	5.95 ± 0.0411
	10	9.92	99.20	0.987	1.007	9.92 ± 0.158

** a= Relative standard deviation for six determinations. *b=95% confidence limits and five degrees of freedom.*

3.11. Analytical Applications

The validity of the proposed procedures are tested to determine STR, or NMS in dosage forms (tablets, or injection). For further confirmation, the standard addition technique was applied to test the reliability and recovery of the proposed procedures in which variable amounts of STR or NMS were added to the previously analyzed portion of pharmaceutical preparations, since the ion-pair complexes are stable for at least 18 h. The results are recorded in Table 4 are compared statistically with the official methods (Table 3). The student's t- and F-values obtained at a 95% confidence level [59] did not exceed the theoretical tabulated values indicating no significant difference between the proposed and official method. The proposed methods are sensitive, therefore they could be used easily for routine analysis in pure form and in pharmaceutical preparations.

Table4. *Statistical treatment of data obtained for determination of (CAND) applying the proposed methods in comparison with the reference methods.*

Parameters	Official method	BCP	BCG
Pure solution 12 µg m L^{-1} X ± SD n t-value*	99.05± 0.036	98.6±30.04 6 1.05	100.58 ±0.04 6 1.83

F-value		2.03	3.77
Neomycin tablets 350 mg / per tablet 12 µg/mL^{-1} X ±SD n t-value* F-value	101.00 ± 0.038	99.90 ± 0.17 6 0.81 1.56	101.25 ± 0.04 6 1.78 1.22
Streptomycin injectable 50 µg m L-1 X ± SD n t-value* F-value	98.58 ± 0 .036	100.05 ± 0.04 6 1.83 1.78	

**: theoretical value at 95% confidence level.*

n: number of replicates

4. CONCLUSION

Extractive colorimetric procedure for the determination of STR, NMS or SPC in pure form and pharmaceutical formulations was developed based on the formation of extractive ion-pair complexes with acid dyes. The proposed procedure is highly sensitive, simple, accurate, precise, less time consuming in handling and with higher tolerance limits. Student t- and f- values gave lower values relative to the theoretical ones indicating high accuracy and precision with no significant differences compared to the official one.

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