Eurasian Journal of Analytical Chemistry

Eurasian J Anal Chem 10(3): 180-186, 2015

New Extractive Colorimetric Method for the Assay of Gentamicin and Streptomycin Using Ion-Pair Association Complexes

Safwan M. Fraihat*
Department of Chemistry, College of Science, Aljouf University Sakaka-Saudi Arabia

Received: *03/09/2015****; Accepted:*** *01/10/2015*

Abstract

Gentamicin and streptomycin aminoglycozide antibiotics, were determined using new selective spectrophotometric methods based on the formation of stable ion association complexes with methyl red dye in alkaline medium. These complexes can be extracted with chloroform at (pH = 8.5) after that the absorbance of the colored complexes was measured at 490 nm. The methods were linear in the concentration ranges of 15-60 and 18-75 with Limit of detection (LOD) of 9 and 13 μgmL^{-1} for gentamicin and streptomycin respectively. The stoichiometry of the two ion pair complexes was determined by the continuous variation method.

Keywords:

Gentamicin, Streptomycin, ion-association, methyl red, spectrophotometry

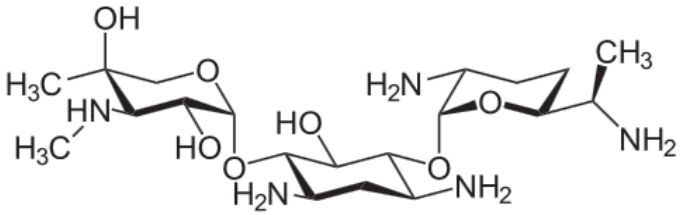
1. Introduction

Gentamicin and Streptomycin are broad spectrum aminolycoside antibiotics, which are widely used for human as well as veterinary medications. They have wide spectrum activity against many types of bacterial infections [1]. The chemical structure of these drugs contains many amino and hydroxyl groups as shown in Fig. 1.

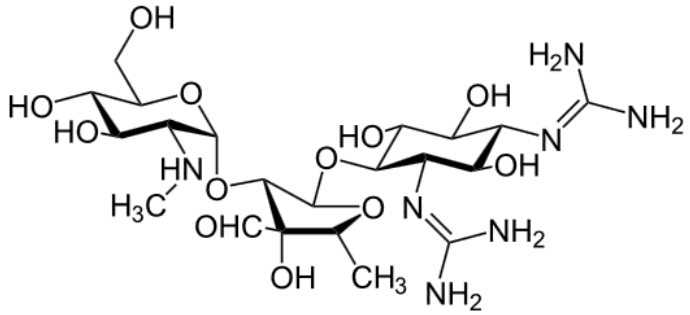
Gentamicin and streptomycin have been determined in different food and pharmaceutical samples using sophisticated liquid Chromatography methods which require derivatization steps and highly sensitive detection systems [2-9], Spectrofluorimetric [10-12], electrochemical [13] and spectrophotometric methods [14-19]. Most of the spectrophotometric methods used depend on using nonselective reagents and require heating steps.

Because of a limited number of simple and selective methods used for determination of either streptomycin or gentamicin in pharmaceutical preparations, we aimed to develop simple and selective methods for determination of these antibiotics based on the formation of selective ion pair association complex that can be extracted with organic solvents. All the conditions of the methods were studied, including the type of suitable dye, optimum pH, type and volume of organic solvent. Validation of the methods was investigated for linearity, sensitivity, accuracy and precision. The proposed methods were applicable for the determination of Gentamicin and Streptomycin in pharmaceutical formulations and the results were compared with that of standard methods [20].

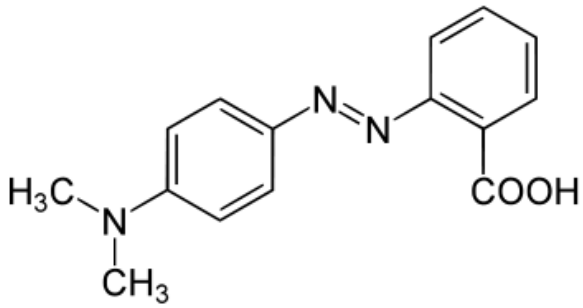
* **Corresponding Author**
E-mail: safwanf@yahoo.com
ISSN: 1306-3057



(I)



(II)



(III)

Fig. 1: Chemical structure of (I) Gentamicin, (II) Streptomycin and (III) Methyl Red dye

Streptomycin was the first aminoglycoside antibiotic used in chemotherapy. It was derived from streptomyses genus bacteria, then other aminoglycoside antibiotics, including gentamicin were derived from the same genus.

2. Experimental

2.1. Instrument

A double beam UV-VIS spectrophotometer (UVD-3500, LABOMED. INCO) was used with a spectral bandwidth of 1.0 NM, wavelength accuracy ± 0.3 NM, (automatic wavelength correction), wavelength range (190-1100 nm), reproducibility ± 0.2 nm, a pair of 1-cm quartz cells were used for absorbance measurements.

2.2. Materials and Reagents

Chemicals and reagents used were all of reagent grade, Standards of Gentamicin sulfate (%) and streptomycin sulfate (99 %) were purchased from Fischer Scientific. Methyl Red was purchased from LOBAchemie (99.9%). Gentamicin and streptomycin eye drops were purchased from a local pharmacy, and claimed to contain (1.0 mg mL^{-1})

2.3. Solution preparation

Stock solutions of 200 μgmL^{-1} of Gentamicin and streptomycin were prepared daily in double distilled water, and were diluted as appropriate. Sodium tetraborate buffer solutions were prepared by dissolving appropriate amounts of the salt and either HCl or NaOH solutions,

2.4. General procedure

2.4.1. Constructing calibration curve

Aliquots of stock solutions of both Gentamicin and Streptomycin ranging from (0.1-1.0) mL were pipette Into a series of 100 mL separatory funnels, then 0.2 ml of Methyl Red solution and 4 ml of borate buffer were added, the volume was completed to 10.0 mL with distilled water. The ion association complexes being formed were extracted with 10 mL chloroform, shacked for one minute, then allowed to stand for complete layer separation. The lower organic layer was passed through anhydrous sodium sulfate into 10 ml volumetric flask, the absorbance of the colored sample was measured at 490 nm against a reagent blank prepared by the same procedure. All measurements were taken at at 25°C ±1, the calibration curves were constructed using the corresponding results. The regression data are shown in (Table 1).

Table 1. Regression results for the developed methods

Parameter	Gentamicin	Streptomycin
Wavelength (nm)	490	490
Slope	0.016	0.022
Intercept	0.132	0.041
R^2	0.98	0.99
Dynamic range (μgmL^{-1})	15-60	18-75
LOD (μgmL^{-1})	9	13
LOQ (μgmL^{-1})	15	22

2.5. Procedure for Drug formulations

For Gentamicin eye drops: An accurately measured volume of gentamicin eye drop equivalent to 200 μgmL^{-1} was dissolved in 100 mL volumetric flask with distilled water.

For Streptomycin eye drops: An accurately measured volume of Streptomycin eye drop equivalent to 200 μgmL^{-1} was dissolved in 100 mL volumetric flask with distilled water. Aliquots of the above solutions were used for the analysis as described in the above procedure for the calibration curve. The results of the proposed methods were validated by comparison with standard methods [21] using the statistical t-test, as shown in (Table 2).

Table 2. Comparison of the suggested methods with standard methods (6 determinations)

Drug taken	Gentamicin	Streptomycin	Standard method
Amount taken (μgmL^{-1})	30	30	30
% recovery	97%	98%	98%
t-value	1.8	1.4	1.7

* t-value for six replicates is 2.45

3. Results and Discussion

Aminoglycosides usually present in positively charged forms in acidic and even in slightly basic medium, according to this fact, we aimed to use it as counter ions in ion-pair association complexes with anionic dyes that can be extracted with organic solvents and the colored association complex can be determined by measuring its absorbance at a certain wavelength. For this purpose, we tried several dyes and we found that the most suitable results of Gentamicin and streptomycin drugs were obtained with Methyl red (MR) dye considering the sensitivity of the method. Several factors were studied, including the type and volume of solvent, the amounts of the dye and pH. We select chloroform as a suitable after testining other organic solvent such as cyclohexan and dichloroethane solvent, the effect of the volume of

(MR) dye was critical, the optimum volume was determined by fixing all other variables such as the concentration of the drugs, pH at 8.5 and 10.0 mL volume of chloroform, the concentration of the dye was selected as low as possible, so that to obtain a lower limit of detection of the drugs and keeping appropriate values of absorbance to maintain accurate application of beer's law. The pH of the medium was a critical factor, in acidic medium (pH≤7.5) protonation of the dye takes place so that the ion-pair complexes with the drugs will be minimal. In a relatively alkaline medium (pH≥8.5) the drugs are present in a neutral form, so that the ion pair complexation doesn't reach its maximum level. Hence the effect of pH was varied and the absorbance of the extracted ion-pair complexes was recorded in order to obtain the optimum pH as shown in Fig. 2 for Gentamicin. The effect of the variation the of concentration of both Gentamicin and Streptomycin on the spectrum using the developed extractive spectrophotometric method are shown in Fig. 3 and Fig.4 respectively.

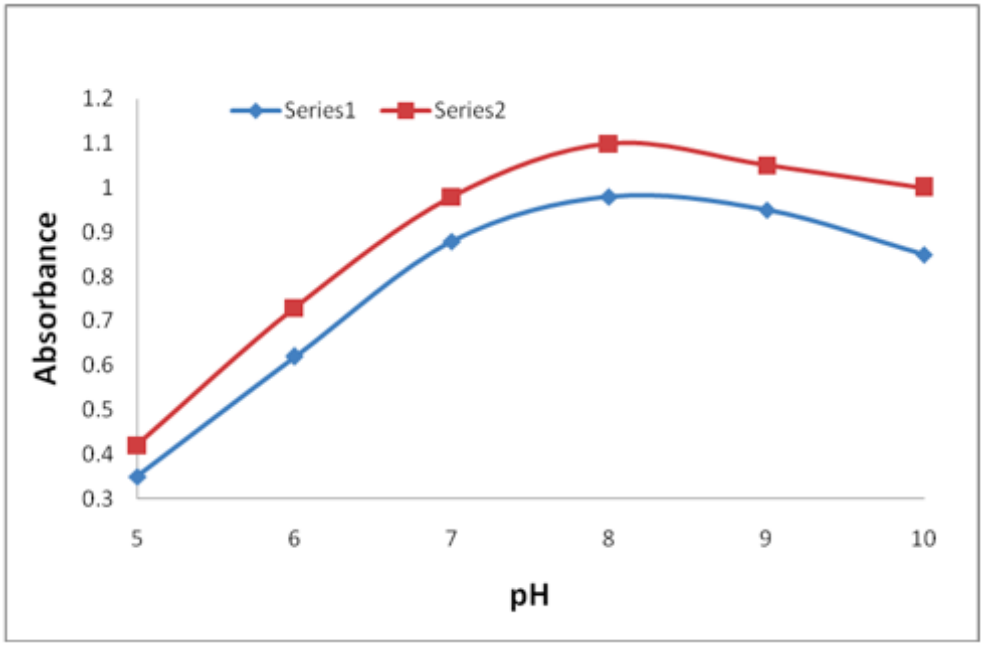


Fig. 2. Effect of variation of pH on the absorbance of the ion-pair complexes
Series 1: Gentmicin, series 2: Streptomycin.

3.1. Analytical Parameters

Calibration curves were plotted using Beer's law, the results show a wide dynamic range of (15-60) and (18-75) with high correlation factor (R^2) of 0.98 and 0.99 for Gentamicin and streptomycin respectively. The sensitivities were investigated using LOD and LOQ using (3σ/S) and (10σ/S), where (σ) is the standard deviation and (S) is the slope of the calibration equation.

3.2. Recovery studies

Recovery studies were carried out using six replicate determinations using the calibration curve obtained from the general procedure, using eye drops containing the corresponding drugs. To validate the new proposed methods, t-values were calculated and they were less than the critical value (t=2.45). The results in (Table 2) show that the proposed methods are accurate compared to the standard methods used.

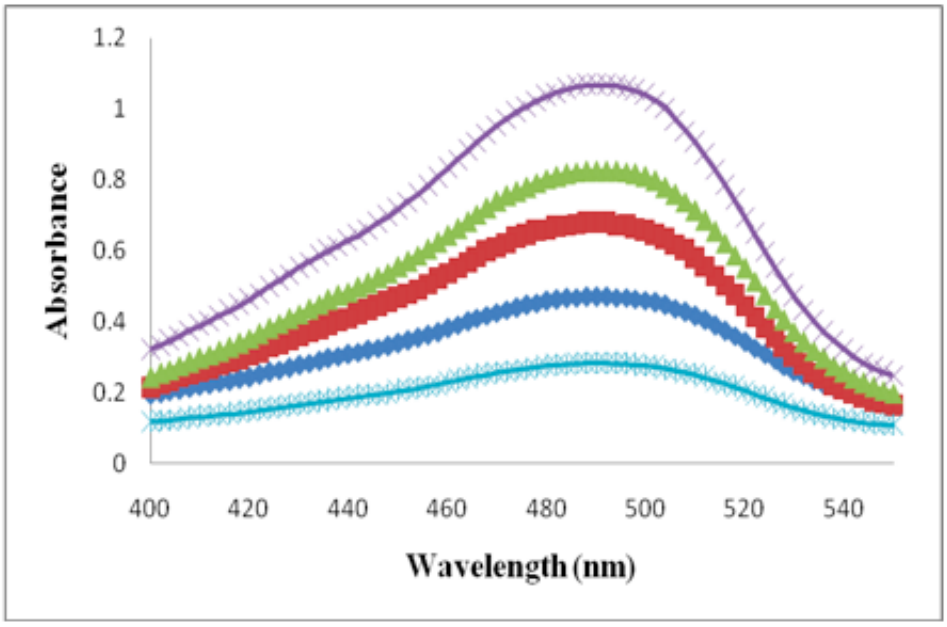


Fig. 3. Effect of Gentamicin concentration on the absorbance of the extracted complex

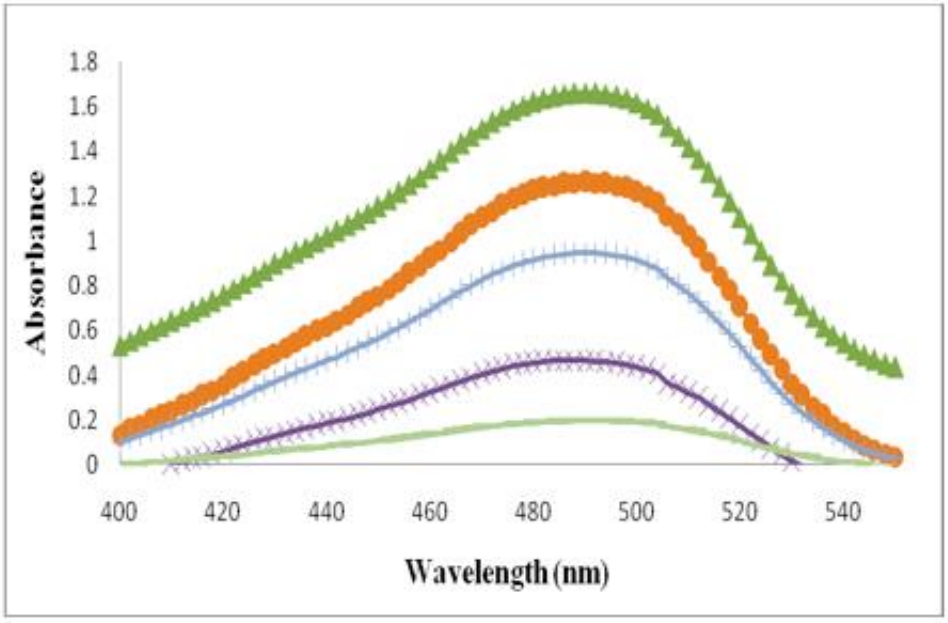


Fig 4. Effect of concentration of Streptomycin on the absorbance of the extracted

3.3 Stoichiometry studies

The molecular composition of the ion pair complexes of both Gentamicin and Sterptomycin with (MR) dye was investigated using the method of continuous variation the results show that the mole ratio of Gentamicin and Streptomycin to the Dye was 1:5 and 1:4 for the two complexes respectively.

4. Conclusion

Rapid, selective and sensitive methods for the determination of Gentamicin and Streptomycin in bulk and drug samples were developed. The methods require no heating steps and complicated procedures and applied at room temperature. The methods are considered as selective because of the formation of stable and extractable ion-pair complexes at certain pH,

which enable the application in complicated mixtures. The developed methods have the advantages of using simple chemical reagents, thus they considered as cost effective methods.

Conflict of interests

The Author declares no conflict of interest.

References

1. Jiame ND and William AR. (1998) Text book of organic, medicinal and pharmaceutical chemistry 10th ed; Wilson's and Gisvold's Lippincott-Raven; Philadelphia ;USA, p 291.
2. Gremilogianni AM, Megoulas NC and Koupparis MA (2010) Hydrophilic interaction versus ion pair liquid chromatography for the determination of streptomycin and dihydrostreptomycin residues in milk based on mass spectrometric detection. J Chromatogr A. 217:6646.
3. Vucicevic PK, Cservenak R and Radulovic N (2011) Development and validation of liquid chromatography tandem mass spectrometry methods for the determination of gentamicin, lincomycin and spectinomycin in the presence of their impurities in pharmaceutical formulations. J Pharm Biomed Anal 56: 736.
4. Kaufmann A, Butcher P and Maden K. (2012) Determination of aminoglycoside residues by liquid chromatography and tandem spectrometry in a variety of matrices. Anal Chim Acta 711: 46.
5. Lauser G and BergnerLang B. (1995) Determination of aminoglycoside antibiotics by RP-HPLC based on an automated pre-column derivatization procedure with 9-fluorenyl-methylchloroformate. Deutsche Lebensmittel-Rundschau 91: 390.
6. Granja RH, Niño AM, Zucchetti RA, Niño RE, Patel R and Salerno AG (2009) Determination of streptomycin residues in honey by liquid chromatography-tandem mass spectrometry. Anal Chim Acta 637:64.
7. Larsen NE and Heilsen KAM (1980) Determination of gentamicin in serum using liquid column chromatography. J Chrom B 221:182.
8. Isoherranen, N and Soback S (1999) Chromatographic methods for analysis of aminoglycoside antibiotics. J AOAC Int 82:1017.
9. Lecaroz C, Campanero MA, Gamazo C, Blanco PM. (2006) Determination of gentamicin in different matrices by a new sensitive high-performance liquid chromatography-mass spectrometric method. J Antimicrob Chemother 58:557.
10. Belal F, El-Ashry SM, El Kerdawy MM, El Wasseef DR (2001) Spectrofluorimetric determination of Streptomycin in dosage forms and in spiked plasma using 9,10-phenanthraquinone. J Pharm and Biomed Anal 26: 435.
11. Habbal ZM (1979) Spectrofluorometric assay of gentamicin in serum. Clin Chim Acta 95:301
12. Rizk M, Elshabrawy YZ, Zakhari NA, Toubar SS and Carreira LA. (1995) Fluorimetric determination of Aminoglycoside antibiotics using lanthanide probe ion spectroscopy. Talanta 42: 1849.
13. Ayad MM, Yousef M (1985) D.C. Polarographic determination of certain aminoglycosides. Analyst (London) 110:963.

14. El-Didamony AM, Amin AS, Ghoneim A, Ahmed K and Telebany AM (2006) Indirect spectrophotometric determination of gentamicin and vancomycin antibiotics based on their oxidation by permanganate. Cent Eur J Chem 4:708.
15. Omar MA, Nagy DA, Hammad MA and Aly AA (2013) Validated spectrophotometric methods for determination of certain aminoglycosides in pharmaceutical formulations. J App Pharm Sci 3:151.
16. Fraihat S (2015) Kinetic spectrophotometric methods for the determination of Gentamicin in pharmaceutical forms. Int J pharmacy and pharm sci 7:302.
17. Gao L and Lin-Xiao. (2008) A novel method for the determination of Streptomycin using sodium nitroprusside as a chromagenic reagent by spectrophotometry. Anal lett 41:2595.
18. Aman T, Rashid A, Kulsoom R and Khokhar I (1995) Spectrophotometric determination of streptomycin. Anal Lett. 28:881.
19. Wang HY, Ren JC and Zhang YC. (1993) Dimethylaminobenzaldehyde as a colored reagent for the determination of gentamicin. Talanta 40:851.
20. The united states pharmacopoeia and NF XXV; American Pharmaceutical Association: Washington, DC, 2007, Electronic version.