

INDIRECT DETERMINATION OF NEOMYCIN BY DERIVATIVE SPECTROPHOTOMETRY

BALAZS SZANISZLO, CRISTINA IUGA, MARIUS BOJIȚĂ

Department of Drug Analysis, Faculty of Pharmacy,
University of Medicine and Pharmacy „Iuliu Hațieganu” Cluj-Napoca

Abstract

Neomycin is an aminoglycosidic antibiotic, widely used against Gram-negative bacteria. Because of its advantages (simplicity, high speed, low cost and accessible instruments), ultraviolet-visible (UV-Vis) spectrophotometry represents a suitable method for the quantitative determination of pharmaceutical active ingredients in raw materials. Since neomycin presents weak absorbance on the UV-Vis domain, direct determination by UV-Vis spectrophotometry would not assure adequate detection and quantification limits.

The aim of this study was to develop an indirect spectrophotometric method based on the capacity of neomycin to form in the presence of Cu^{2+} ions complex combinations with increased UV-Vis light absorbing capacity.

Optimum complex formation was found to take place in the presence of $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$ 1 mg/mL when 20% methanol in bidistilled water was used as solvent. All readings were performed at 277 nm on the first derivative of the absorbance spectrum (dA). The method was validated, and proved to be linear on the 0.102-0.510 $\mu\text{g/mL}$ concentration range.

The developed method is easy-to-use, fast, accessible, and can be used for the determination of neomycin in raw materials.

Keywords: Neomycin; spectrophotometry; Metal complexes; Chemical analysis.

DETERMINAREA INDIRECTĂ DE NEOMICINĂ PRIN SPECTROFOTOMETRIE DERIVATIVĂ

Rezumat

Neomicina este un antibiotic aminoglicozidic de uz topic, larg utilizat în infecții locale cauzate de germeni Gram-negativi. Spectrofotometria în ultraviolet-vizibil (UV-Vis) reprezintă o metodă utilă în determinarea cantitativă a substanțelor farmaceutice din materii prime, prezentând ca avantaje simplitatea, rapiditatea și costul scăzut al analizelor, respectiv accesibilitatea instrumentelor necesare. Deoarece neomicina prezintă absorbantă slabă în domeniul spectral UV-Vis, dozarea ei prin spectrofotometrie nu asigură atingerea unor limite de detecție și cuantificare necesare. În vederea valorificării avantajelor spectrofotometriei, ne-am propus realizarea unei metode spectrofotometrice indirecte, bazată pe capacitatea neomicinei de a forma în prezența ionilor de Cu^{2+} complecși care au o absorbantă mai bună. Formarea complecșilor a fost optimă în prezența $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$ 1 mg/mL și folosind ca solvent 20% metanol în apă bidistilată.

Determinările au fost realizate la 277 nm, folosind derivata de ordin I al spectrului de absorbție (dA). Metoda a fost validată, astfel ea s-a dovedit a fi liniară pe domeniul 0.102-0.510 mg/mL.

Metoda realizată este o metodă rapidă, simplă, accesibilă și poate fi aplicată cu succes la determinarea cantitativă a neomicinei din materii prime.

Cuvinte cheie: Neomicină, spectrofotometrie, complexe metalice, analiză chimică.

Introduction

Neomycin sulphate is an aminoglycosidic antibiotic of biosynthetic origin, widely used in topic infections caused by Gram-negative microorganisms. It is a mixture of the neomycins A, B and C, neomycin B being the major component [1] (Fig. 1).

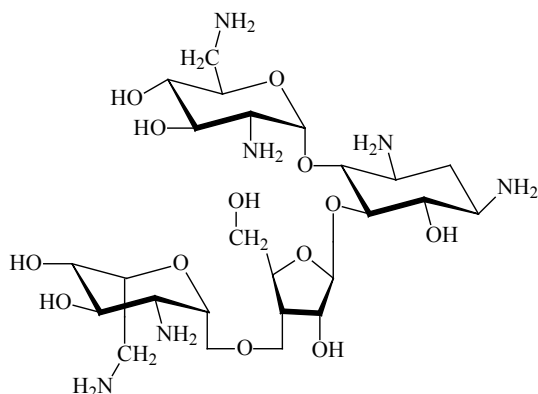


Fig. 1. Chemical structure of neomycin B.

Ultra-violet (UV-Vis) spectrophotometry represents a suitable method for the routine analysis of active ingredients in raw materials, since it is fast, easy to perform and does not require expensive instruments. Because its use is limited to the analysis of substances able to absorb light on the UV-Vis domain, spectrophotometry is not useful for the direct determination of aminoglycosidic antibiotics, substances with weak light-absorbing capacity (Fig. 2). Although the lack of chromophores in the chemical structure of aminoglycosides makes direct detection and quantification impossible, indirect spectrophotometry can be used in their case.

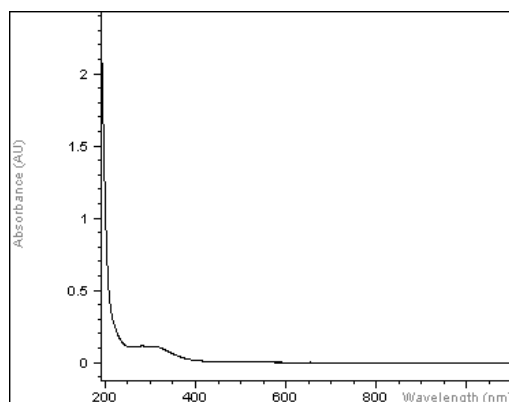


Fig. 2. Non-derivated spectrum of neomycin.

Since in the presence of metallic ions, such as Cu^{2+} , aminoglycosides form complex combinations with improved light-absorbing capacity [2-5], in this study our

aim was to develop a validated indirect spectrophotometric assay for the quantitation of neomycin in active pharmaceutical ingredients (API) via its complexes with Cu^{2+} .

Materials and Method

Equipments

The absorbances were read using an Agilent 8453 model spectrophotometer, equipped with a 1024 element PDA (Agilent Technologies, Germany). All spectra were recorded using the 200-800 nm UV-Vis wavelength domain. Acquired data was processed with a Chemstation software (Agilent Technologies, Germany).

Kinetic study of the complex formation and degradation was performed using a Spectronic Genesys 5 spectrophotometer (Milton Roy).

Weighings were performed using a four-digit Mettler-Toledo analytical balance.

Reagents

Chemicals used in the study were of analytical grade:

- Neomycin sulphate (Fluka)
- Methanol (Chimopar)
- $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$ (Merck)
- Purified Milli-Q water

Method

It is known that electron-donor solvents increase the stability of metallic complexes [6]. Because neomycin sulphate is not soluble in the majority of these, a water-methanol (4:1) mixture was used for all the spectrophotometric readings. Optimum $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$ amount was found to be 0.1 mg/mL. This amount proved to be large enough to form a stable complex combination throughout the linearity interval of the study, but small enough not to interfere the quantification of the neomycin sulphate.

Since the signal-to-noise ratio proved to be the highest on the first derivative (dA) of the spectrum at 277 nm, quantification was done at this wavelength (Fig. 3).

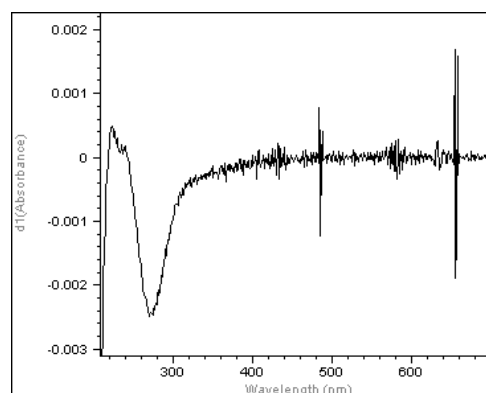


Fig. 3. Derivated UV-Vis spectrum of the neomycin - Cu^{2+} complex.

In order to establish the right frame time interval

for the spectrophotometric readings, a kinetic study was performed, investigating the formation and potential degradation of a 0.510 mg/mL neomycin sulphate solution. For the 0-30 minutes after the preparation no significant differences between absorbances were found (relative standard deviation RSD=0.095%), therefore all subsequent readings were performed 10-20 minutes after the solutions were prepared.

Neomycin stock solutions (2.04 mg/mL) were prepared by dissolving in water an exactly weighed amount of neomycin sulphate of around 51.0 mg in a glass 25 mL volumetric flask. Fresh stock solutions had to be prepared daily.

Cu²⁺ stock solutions (0.5 mg/mL) were prepared by weighing an exact amount of CuCl₂·6H₂O of around 12.5 mg in a 25 mL glass volumetric flask and dissolving it using methanol. Because of the volatility of the methanol, fresh stock solutions had to be prepared daily.

Working standard solutions were prepared in 10 mL volumetric flasks using the neomycin stock solution (2.04 mg/mL) and adding 2 mL of Cu²⁺ stock solution (0.5 mg/mL). Working standard solutions were prepared for the 0.102-0.510 mg/mL linearity domain and contained 0.1 mg/mL of CuCl₂·6H₂O in water-methanol (4:1).

Results

Linearity

The method was linear on the 0.102-0.510 mg/mL concentration range. The calibration curve was constructed using five calibration points (C₁=0.102 mg/mL, C₂=0.204 mg/mL, C₃=0.306 mg/mL, C₄=0.408 mg/mL and C₅=0.510 mg/mL), the regression equation being $dA=4.7E-03-9E-06 \cdot C$ (C - neomycin concentration in mg), and the correlation coefficient $r=0.99905$.

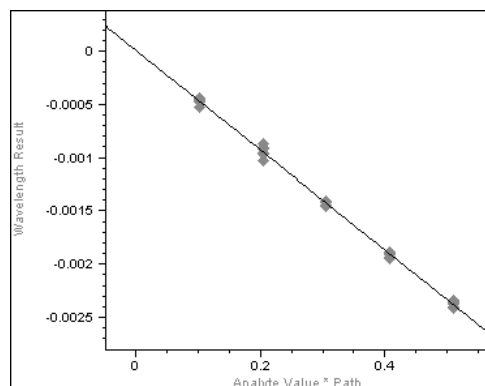


Fig. 4. Calibration curve.

Accuracy

Accuracy of the method was established across the entire linearity range, for all the five calibration levels. Recoveries were found to be between 99.89%-100.84% and are presented in Table I.

Table I. Accuracy.

Calibration level	Mean Recovery (%)	Confidence interval (%)
C ₁ =0.102mg/mL	100.38	98.68-101.31
C ₂ =0.204mg/mL	99.89	98.16-101.84
C ₃ =0.306mg/mL	100.40	98.94-101.06
C ₄ =0.408mg/mL	100.84	99.32-100.68
C ₅ =0.510mg/mL	100.25	99.46-100.53

Precision

Repeatability and intermediate precision were assessed by running measurements on 3 different days. Each day 6 replicates of the C₃=0.306 mg/mL calibration level were performed. RSD and confidence intervals for repeatability and intermediate precision are presented in Table II.

Table II. Repeatability and intermediate precision for C₃=0.306 mg/mL level.

Type of precision	RSD (%)	Confidence interval (%)
Repeatability - day 1	2.3266	98.13-101.86
Repeatability - day 2	1.3955	98.88-101.12
Repeatability - day 3	1.5152	98.79-101.21
Intermediate precision	1.8984	98.48-101.52

Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantification (LOQ) of the method were evaluated based on the signal-to-noise ratio. We found that LOD=0.025 mg/mL, while LOQ=0.075 mg/mL.

Conclusions

In the presented study a derivative spectrophotometric method was developed for the quantification of neomycin sulphate in bulk material samples. The method is based on the capacity of neomycin to form UV-Vis light-absorbing complex combinations in the presence of Cu²⁺ ions. Although the complex combination formed in the described conditions was not chemically characterised, it proved to be fairly stable and obeyed Lambert-Beer's law, therefore can be used for the quantitative determination of neomycin sulphate.

The described method proved to be linear on the 0.102-0.510 mg/mL concentration range; it was validated, and since it presents the advantages of spectrophotometry – being fast, easy to perform and accessible – it can be used for routine quantitative determinations of neomycin sulphate in active pharmaceutical ingredients.

References

- Negreş S. Aminoglicozide. În: Cristea AN, Tratat de Farmacologie, Ediția I, Editura Medicală, București 2005, 1006-1017
- Szczepanik W, Czarny A, Zaczynska E, Jezowska-Bojczuk M. Preferences of kanamycin A towards copper (II). Effect of the resulting complexes on immunological mediators' production by human leukocytes. J Inorg Biochem 2004; 98[2]:254-253

-
3. Kozłowski H, Kowalik-Jankowska T, Jeżowska-Bojczuk M. Chemical and biological aspects of Cu²⁺ interactions with peptides and aminoglycosides. *Coord Chem Rev* 2005; 249: 2323-2334
 4. Zaher M, Baussanne I, Ravelet C, Halder S, Haroun M., Fize J, Décout J-L, Peyrin E. Copper(II) complexes of lipophilic aminoglycoside derivatives for the amino acid enantiomeric separation by ligand-exchange liquid chromatography. *J Chromat A* 2008, 1185: 291-295
 5. Agrawala JK, Harmalkarb SG, Vijayavargiyac R. Spectrophotometric studies of neomycin-copper complex and determination of neomycin sulfate using an auxiliary ligand. *Microchem J* 1976, 21[2]:202-208
 6. Arya DP, Aminoglycoside Antibiotics. *From Chemical Biology to Drug Discovery*. Wiley-Interscience 2007.