Indirect Determination of Gentamicin by Derivative Spectrophotometry

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Introduction: Gentamicin sulfate is an aminoglycosidic antibiotic, used in severe infections caused by Gram-negative bacteria. UV-Vis spectrophotometry, a simple, high speed and accessible analytical method, is generally suitable for the quantitative determination of pharmaceutical active ingredients in raw materials. In the case of gentamicin, a drug that presents low absorbances on the UV-Vis domain, direct determination by UV-Vis spectrophotometry would not assure adequate detection and quantitation limits.
The **aim** of this study was to develop a new indirect spectrophotometric method, based on the capacity of gentamicin to form in the presence of Cu^{2+} ions complex combinations with increased UV-Vis light absorbing capacity.
Material and method: Optimised experimental conditions which assure formation of a stable complex was found to take place in the presence of $CuCl_2.6H_2O$ 0.1 mg/mL, while 1 mM NaOH was used as solvent. All readings were performed at 291 nm on the first derivative of the absorbance spectrum.
Results: The developed method was validated, and proved to be linear on the 0.051–0.261 mg/mL concentration range (r = 0.99935).
Conclusions: The developed method is fast, accessible and can be used for the determination of gentamicin in raw materials.

Keywords: gentamicin sulfate, spectrophotometry, metal complexes, chemical analysis

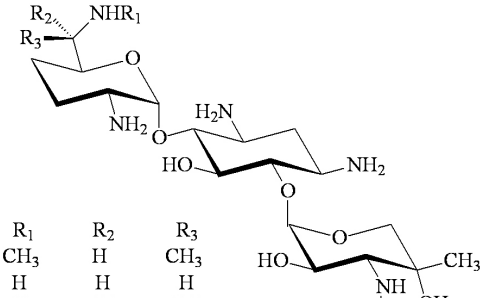
Introduction

Gentamicin sulfate is an aminoglycosidic antibiotic, widely used in topic infections caused by Gram-negative bacteria. Like many drugs of biosynthetic origin, it is a mixture of closely related substances. Its main components are gentamicin C1, gentamicin C1a and gentamicin C2 [1] (Figure 1).

Ultraviolet-visible (UV-Vis) spectrophotometry represents a suitable method for the routine analysis of pharmaceutical active ingredients in raw materials, since it is fast, easy to perform and does not require expensive instruments. Because aminoglycosidic antibiotics present low absorbances on the UV-Vis domain, direct determination by UV-Vis spectrophotometry would not assure adequate detection and quantitation limits, making direct quantification impossible (Figure 2).

However, since in the presence of metallic ions, such as Cu^{2+}, aminoglycosides form complex combinations with improved light-absorbing capacity [2–5], indirect spectrophotometry can be used in their case (Figure 3).

In this study our aim was to develop a validated, indirect spectrophotometric assay for the quantitation of gentamicin in active pharmaceutical ingredients (API) via its complex combinations with Cu^{2+}. Because the stability of complex combinations can be strongly influenced by the solvents used, the pH value of the solution or the presence of co-ligands [1], we proposed to also identify the optimum conditions for complex formation.



	R_1	R_2	R_3
Gentamicin C1	CH_3	H	CH_3
Gentamicin C1a	H	H	H
Gentamicin C2	H	H	CH_3
Gentamicin C2a	H	CH_3	H
Gentamicin C2b	CH_3	H	H

Fig. 1. Chemical structure of gentamicin

Material 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).

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

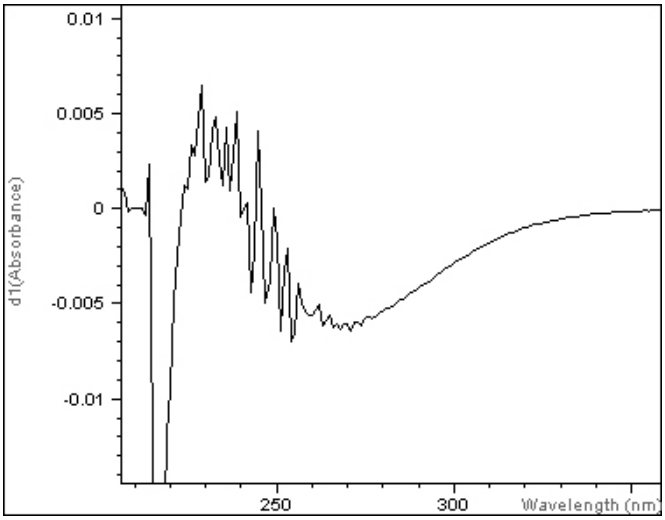


Fig. 2. Derivated spectrum of gentamicin

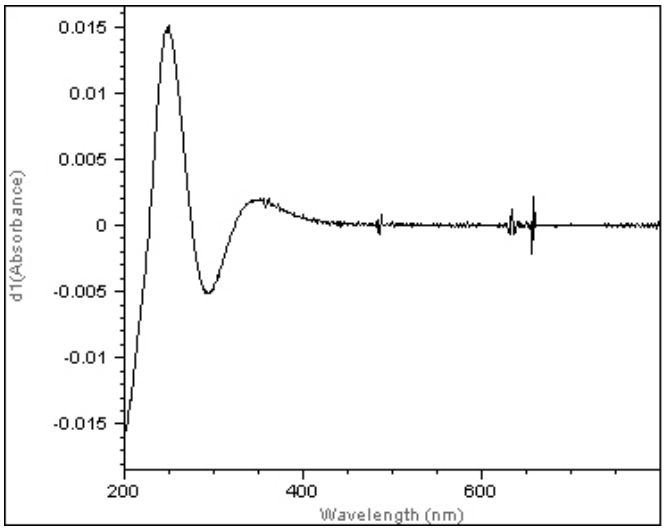


Fig. 3. Derivated spectrum of gentamicin-Cu^{2+} complex

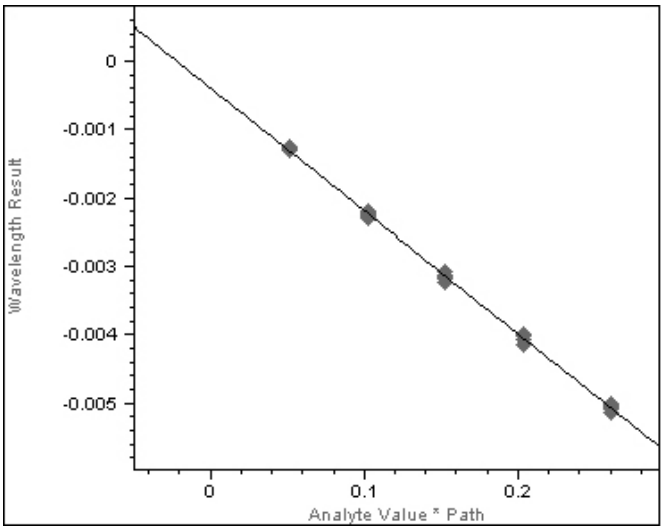


Fig. 4. Calibration curve

Reagents

Chemicals used in the study were of analytical grade:

- Gentamicin sulfate (Sigma Aldrich);
- NaOH 0.1 M (Merck);
- $CuCl_2.6H_2O$ (Merck);
- Purified Milli-Q water.

Method

For all the standard and sample solutions NaOH 1 mM (pH = 11.0) was used as solvent. As a source of Cu^{2+} 0.1 mg/mL of $CuCl_2.6H_2O$ was used.

Since the signal-to-noise ratio proved to be the highest on the first derivative (dA) of the spectrum at 291 nm, quantitation was done at this wavelength.

Gentamicin stock solutions (1.02 mg/mL) were prepared by dissolving in water an exactly weighed amount of gentamicin sulfate of around 20.4 mg in 20 mL volumetric flask. Gentamicin sulfate stock solutions had to be prepared daily.

Cu^{2+} stock solutions (1.0 mg/mL) were prepared by weighing an exact amount of $CuCl_2.6H_2O$ of around 20.0 mg in a 20 mL glass volumetric flask and dissolving it using purified water. Fresh Cu^{2+} stock solutions had to be prepared daily.

Working standard solutions were prepared in 10 mL volumetric flasks using the gentamicin stock solution (1.02 mg/mL). Every solution contained 0.1 ml of 0.1 M NaOH, 1 mL of Cu^{2+} stock solution (1.0 mg/mL) and was brought to volume using purified water. Working standard solutions were prepared for the 0.051–0.261 mg/mL gentamicin sulfate domain and contained 0.1 mg/mL of $CuCl_2.6H_2O$ in 1 mM NaOH.

Results

Linearity

The method was linear on the 0.051–0.261 mg/mL concentration range. The calibration curve was constructed using five calibration points (C1 = 0.051 mg/mL, C2 = 0.102 mg/mL, C3 = 0.153 mg/mL C4 = 0.204 mg/mL and C5 = 0.261 mg/mL), the regression equation being dA = -0.021609–55.547*C (C – gentamicin concentration in mg), and the correlation coefficient r = 0.99935 (Fig.4.).

Accuracy

Accuracy of the method was established across the entire linearity range (9 readings for each level). Recoveries were found to be between 99.53%–101.11% and are presented in Table I.

Precision

Repeatability and intermediate precision were assessed by running measurements on 3 different days (6 replicates of C3 = 0.153 mg/mL each day). Relative standard deviations (RSD) along with the confidence intervals are presented in Table II.

Limit of detection and limit of quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were evaluated based on the signal-to-noise ratio.

Table I. Accuracy

Calibration level	Mean Recovery (%)	Confidence interval (%)
C1 = 0.051mg/mL	97.26	99.98–100.02
C2 = 0.102mg/mL	101.12	99.98–100.02
C3 = 0.153mg/mL	100.50	99.98–100.02
C4 = 0.204mg/mL	100.61	99.98–100.02
C5 = 0.261mg/mL	99.53	99.99–100.01

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

Type of precision	RSD (%)	Confidence interval (%)
Repeatability – day 1	-1.0006	99.20–100.80
Repeatability – day 2	-1.2543	99.00–101.00
Repeatability – day 3	-1.6367	98.69–101.31
Intermediate precision	-1.2704	98.98–101.02

We found that LOD = 6.16 μg/mL, while LOQ = 20.28 μg/mL.

Discussions

The presented method is based on the capacity of gentamicin to form UV-Vis light-absorbing complex combinations in the presence of Cu^{2+} ions. Although this complex combination formed under 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 determination of gentamicin sulphate [6].

Conclusions

In the presented study a new derivative spectrophotometric method was developed for the quantitation of gentamicin sulfate in bulk material samples. The described method proved to be linear on the 0.051–0.261 mg/mL concentration range; it was accurate and precise and it can be used for routine quantitative determinations of gentamicin sulfate in active pharmaceutical ingredients.

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