

GENTAMICIN SULPHATE IN PHARMACEUTICAL PREPARATIONS



THE MOST RELIABLE LC-EC APPLICATIONS FOR PHARMACEUTICAL & BIOTECH ANALYSIS EVER FORMULATED

Aminoglycosides

- Amikacin
- Framycetin Sulphate
- Gentamicin Sulphate
- Kanamycin Sulphate
- Lincomycin
- Neomycin
- Spectinomycin
- Tobramycin

PET imaging tracer

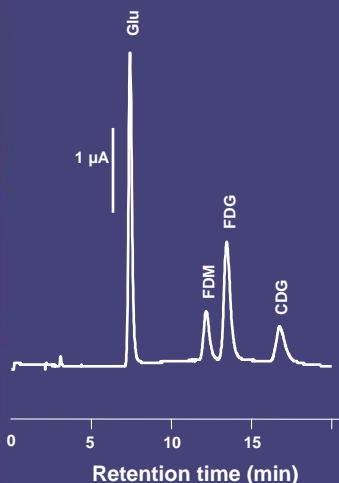
- FDG

Macrolide antibiotics

- Azithromycin
- Azaerythromycin
- Clarithromycin
- Erythromycin
- Roxithromycin

Bioanalysis of pharmaceuticals

- Artemisinin
- Dihydro-artemisinin
- Artemether
- Etoposide
- 8-OH-DPAT
- mesna BNP7787
- Vincristine



INTRODUCTION

Like neomycin and tobramycin, gentamicin belongs to the group of aminoglycoside antibiotics. It is manufactured by a fermentation process and the main constituents are gentamicin C1, C1a, C2 and C2a. Usually also other minor aminoglycosides are found in a pharmaceutical gentamicin preparation. The number of impurities and components possible makes the chromatographic analysis not quite straightforward. Because of the presence of a sugar moiety in these analytes the selectivity and inherent sensitivity of pulsed amperometric detection (PAD) is a very attractive approach [2].

- In compliance with the European Pharmacopoeia
- Flexcell with exchangeable gold electrode
- Analysis of main substituent and impurities
- Reproducible & Robust

Summary

In the European Pharmacopoeia (EP) the use of a reversed-phase polymeric column is prescribed for this application [1]. In literature it is shown that such a column may result in very wide and tailing peaks [3]. We have confirmed this and found much better separation using a C18 silica-based column.

In this application note typical results obtained with the ALEXYS[®] gentamicin analyzer based on a C18 column are reported, demonstrating its performance for the analysis of gentamicin.



Fig. 1. ALEXYS Aminoglycosides Analyzer.

Method

The ALEXYS 100 system equipped with a second pump for the post-column addition of NaOH was used. The mobile phase was prepared as described in the EP monograph [1]: 60g/L Na₂SO₄ (water free), 1.75 g/L octane sulphonic acid, sodium salt, 3 mL/L tetrahydrofuran (THF), 50 mL/L 0.2 M KH₂PO₄ (pH = 3). The flow rate was 1.5 mL/min. A 0.76 mol/L NaOH solution (prepared from a 50 % stock solution) was added post-column with a flow rate of 0.6 mL/min, leading to a final pH of about 13. The cell current was about 2 µA with the PAD settings selected. Note: only use stabilized THF solvents in the mobile phase to assure low cell currents.

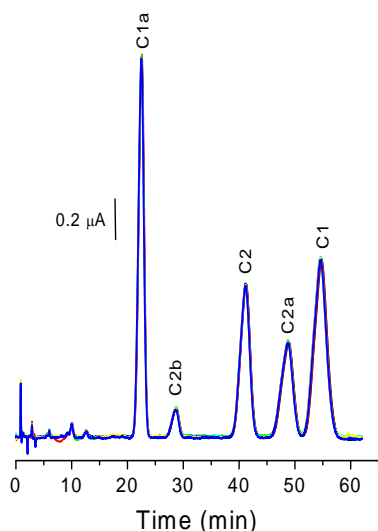


Fig. 2. Gentamicin sample (400 µg/ml, 20 µl injected). Overlay of 7 chromatograms. Peak identities were derived from paper [2] and based on peak area percentages.

Table 1

Conditions	
HPLC	ALEXYS Gentamicin Analyzer
Temperature	45 °C for separation and detection
Flow rate	1.5 mL/min, post-column: 0.6 mL/min
Flow cell	Flexcell™ with Au WE and HyREF™
ADF	0.5 Hz
Range	10 µA/V

Linearity & Repeatability

Linearity of gentamicin was investigated in the concentration range of 50 – 500 µg/mL. For all gentamicin derivatives the correlation coefficients were better than 0.998 for peak areas and peak heights. The relative standard deviation (RSD) in peak area for 10

replicate injections for gentamicin was ranging between 0.9 and 2.5% for gentamicin C1 and C2b, respectively. The RSD for the retention times was better than 0.2%. Peak resolution between gentamicin C2a and C1 was 1.6.

EP requirements

In the EP monographs for gentamicin Sulphate a system suitability requirement is specified for the *peak-to-valley ratio*. The peak-to-valley ratio is specified as H_p/H_v , where H_p = height above the baseline of the peak due to gentamicin C2a, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to gentamicin C2. The peak-to-valley ratio $H_p/H_v > 2.0$. In Table 1 this EP requirement is compared with the typical results obtained with the ALEXYS gentamicin analyzer.

Table 2

EP system suitability requirement		
Parameter	EP criteria	Result
peak-to-valley ratio H_p/H_v	> 2.0	100

It is evident from Fig. 2 that gentamicin C2 and C2a are well baseline separated and therefore the peak-to-peak ratio requirement is easily met by the gentamicin analyzer.

Conclusion

The ALEXYS Gentamicin Analyzer provides a reliable solution for the routine analysis of gentamicin in Pharmaceutical Preparations. It meets the EP requirement for peak-to-valley ratio between gentamicin C2 and C2a.

References

- Gentamicin sulphate, *European Pharmacopoeia*, **6.0**, (2008) 1965-1967
- W.R. LaCourse, "Pulsed Electrochemical Detection in High Performance Liquid Chromatography", *John Wiley & Sons, New York*, 1st, 1997.
- E. Adams, W. Roelants, R. De Paepe, E. Roets, J. Hoogmartens, *J. Pharm. Biomed. Anal.*, **18**, 689-698 (1998).

PART NUMBERS

180.0056A	ALEXYS Aminoglycosides analyzer, including column, flow cell, and post-column addition kit
250.1068	ALA-525 column, 250x4.6mm, 5µm