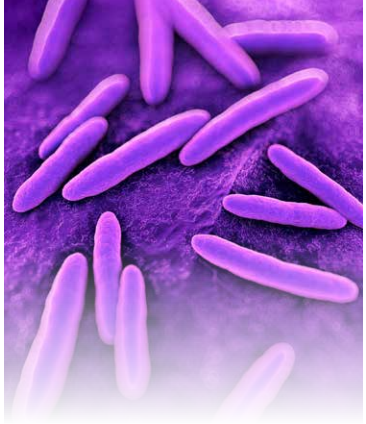
Application Note

Aminoglycosides Antibiotics



Pharmaceutical & Biotech analysis

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

Gentamicin Sulphate in Pharmaceutical Preparations

- **European Pharmacopoeia 6.0 (2008) used as basis for this application**
- **Analysis of main substituent and impurities**
- **Reproducible & robust**

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].

Summary

In the European Pharmacopoeia 6.0 (2008) 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.

Figure 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.

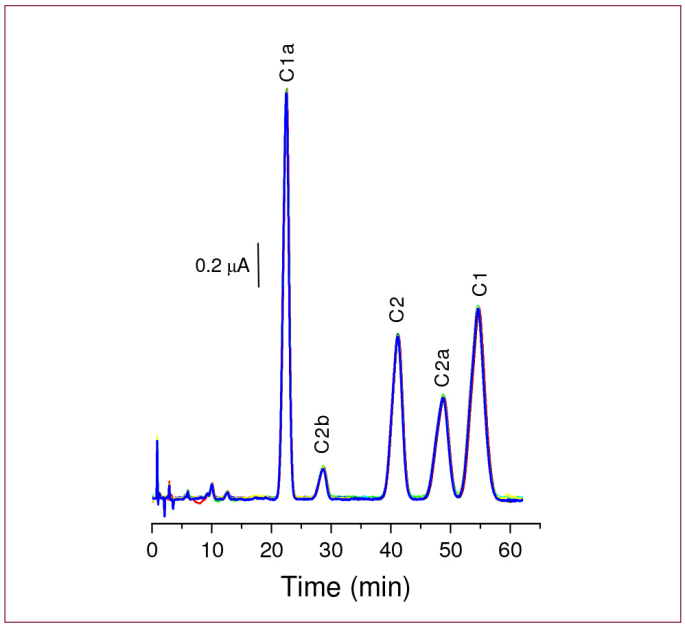


Figure 2: Gentamicin sample (400 µg/ml, 20 µl injected). Overlay of 7 chromatograms. Peak identities were derived from paper [2] and based on peak area percentages.

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 then 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-tovalley ratio is specified as Hp/Hv , where Hp = height above the baseline of the peak due to gentamicin C2a, and Hv = 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 Hp/Hv > 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 Hp/Hv	> 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

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

PART NUMBERS AND CONFIGURATIONS

180.0056C	ALEXYS Aminoglycosides analyzer, including column, flow cell, and post-column addition kit
250.1068	ALA-510 C18 column, 100x4.6mm, 5um

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system. The application was developed with the European Pharmacopoeia, 6.0, (2008) as a basis and conditions may vary slightly from the EP method. The actual performance may be affected by factors beyond Antec Leyden's control. Specifications mentioned in this application note are subject to change without further notice.