

## TOBRAMYCIN 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

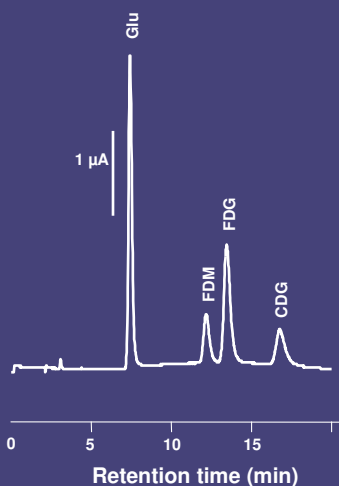
- FDG

### Macrolide antibiotics

- Azithromycin
- Azaerythromycin
- Clarithromycin
- Erythromycin
- Roxithromycin

### Bioanalysis of pharmaceuticals

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



### INTRODUCTION

Tobramycin belongs to the group of the aminoglycoside antibiotics. Like the other aminoglycosides, it binds to bacterial ribosomes and causes non-functional proteins to accumulate within the cell leading to cell death. It is often effective against bacterial strains that prove resistant to other aminoglycosides like gentamicin. The production is mainly achieved by fermentation resulting in several minor by-products. The analysis of the by-product contribution in bulk tobramycin and preparations is important as to insight in stability, quality control and authenticity. A number of qualitative and quantitative methods has been published so far [1] but the focus is mainly on tobramycin and not on the by-products. Because of the presence of sugar groups in both tobramycin and by-products LC with pulsed amperometric detection (PAD) is a highly selective and sensitive analytical tool [2].

- European Pharmacopoeia, 6.0, (2008) used as a basis for this application
- Flexcell with exchangeable gold electrode
- Analysis of main substituent and impurities
- Reproducible & Robust

### Summary

The European Pharmacopoeia describes a method for the analysis of Tobramycin and its impurities based on LC-PAD [3]. The ALEXYS Aminoglycosides analyzer is a dedicated solution for the analysis of aminoglycoside antibiotics using a silica-based C18 column.

In this application note results are shown for the analysis of Tobramycin and its impurities using the ALEXYS Aminoglycosides analyzer.



Fig. 1. ALEXYS Aminoglycosides Analyzer.

## Method

The Aminoglycosides analyzer (see figure 1) is a versatile solution, it contains all LC hardware and the analytical column for the analysis of several aminoglycosides including Neomycin, Tobramycin and Spectinomycin. The analyzer is equipped with a second pump for the post-column addition of NaOH. Addition of NaOH is necessary to make the mobile phase strongly alkaline (pH > 12), in order to allow PAD detection of the aminoglycosides using an Au electrode [2]. The mobile phase was prepared as described in the European Pharmacopoeia monograph [3].

Table 1

Conditions	
HPLC	ALEXYS Aminoglycosides 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	0.01 Hz
Range	10 µA/V

## Results

In an example chromatogram is shown of a 20 µL injection of 100 µg/mL Tobramycin dissolved in mobile phase. The chromatogram is zoomed in on the baseline to show the impurities (Kanamycin B, Neamine). The impurity Kanamycin B and Tobramycin are sufficiently separated with a resolution of 3.07.

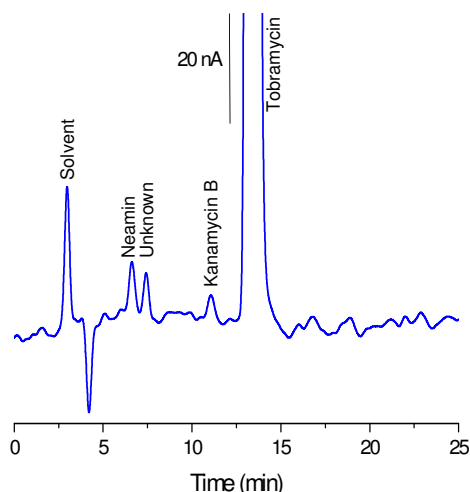


Fig. 2. Tobramycin sample (100 µg/mL, 20 µl injected). The peak height of the Tobramycin peak is 2.32 µA. Impurities as percentage % of the main peak are: Neamin 0.38%, unknown 0.29%, Kanamycin B 0.19%.

*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. Column type and actual conditions differ 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.

The EP requires a resolution > 3. In the EP monograph the concentration of octane sulphonic acid in the mobile phase is designated as a variable to optimize the resolution between Kanamycin B and the principle peak (Tobramycin). This parameter can be used to increase the resolution if necessary.

Another EP system suitability requirement is the signal-to-noise ratio of the principle peak of a 20 µL injection of reference standard B (2.5 µg/mL Tobramycin CRS solution). The S/N ratio of the principle peak in that case should be larger than 10.

The signal-to-noise ratio for the principle peak using the ALEXYS aminoglycosides analyzer was estimated to be around 15 under the specified conditions (estimation based on the noise and peak height of the principle peak in the chromatogram shown in figure 2, the value for peak height was divided by 40 to reflect a concentration of 2.5 µg/mL Tobramycin).

## Conclusion

The ALEXYS® Aminoglycosides analyzer is a suitable solution for the analysis of Tobramycin and its impurities in bulk drugs.

## References

- David A. Stead, "Current methodologies for the analysis of aminoglycosides", J. Chromatogr. B, 747 (2000) 69–93
- W.R. LaCourse, "Pulsed Electrochemical Detection in High Performance Liquid Chromatography", John Wiley & Sons, New York, 1ed, 1997.
- "Tobramycin", European Pharmacopoeia, 6.0, (2008) 3085-3086

## PART NUMBERS

180.0050C	ALEXYS Aminoglycosides analyzer, including column, flow cell, and post-column addition kit
250.1070	ALA-525 C18 column, 250x4.6mm, 5µm



## LC-EC conditions

Table 2

LC-EC Conditions	
HPLC	ALEXYS Aminoglycoside analyzer (part no. 180.0050A)
Flow rate	1 mL/min, post-column: 0.6 mL/min
Cell	Flexcell™ with Au WE and HyREF™
Sample	20 µL
Mobile phase	52 g/L Na <sub>2</sub> SO <sub>4</sub> , 1.5 g/L OSA, 3 mL/L THF, 10 mmol/L KH <sub>2</sub> PO <sub>4</sub> , pH 3
Addition	0.76 mol/L NaOH post column
Temperature	45 °C for column, mixing and flow cell
E-cell	E1, E2, E3: 0.1, 0.75, -0.15 V ts, t1, t2, t3: 0.1, 0.32, 0.2, 0.4 s
I-cell	ca. 2 µA