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

Amikacin and Kanamycin in Bulk Drugs

- **United States Pharmacopeia USP30–NF25 used as basis for this application**
- **Flexcell with exchangeable gold electrode**
- **Analysis of main substituent and impurities**

Introduction

Kanamycin and amikacin are closely related, water soluble, broad spectrum aminoglycoside antibiotics. Kanamycin is obtained from Streptomyces kanamyceticus. Amikacin is synthesised by acylation of an amino group of kanamycin A with L-(-)-g- amino-α- hydroxybutyric acid (LHABA). Both antibiotics can be analysed using ionexchange chromatography in combination with pulsed amperometric detection [1-4].

Summary

The United States Pharmacopeia (USP) has two monographs describing the analysis of both Kanamycin [5] and Amikacin [6] using LC-PAD. The ALEXYS Kanamycin and Amikacin analyzer is a dedicated LC solution for the analysis of both antibiotics which matches the USP requirements for peak resolution, tailing and reproducibility. In this note typical results obtained with the analyzer are shown to demonstrate its performance.

Figure 1: ALEXYS Kanamycin and Amikacin analyzer

Table 1

Conditions	
HPLC	ALEXYS Kanamicin and Amikacin Analyzer
Temperature	32 °C for separation and detection
Flow rate	0.5 mL/min
Flow cell	Flexcell™ with Au WE and Ag/AgCL REF
ADF	0.5 Hz
Range	2 µA/V

Results

USP requirements

The results listed in the table below are based on an average of ten 20 µL injections of a mixture of 8 mg/L Kanamycin and 20 mg/L Amikacin in water.

Table 2

USP system suitability requirement		
Parameter	USP criteria	Result
Peak resolution	> 3	> 5
Tailing factor	< 2	< 1.6
Reproducibility, area (%RSD)	< 3	1.5% (n=10)

For both Kanamycin and Amikacin a RSD smaller then 1.5% in area was achieved for 10 replicate injections. (USP requires < 3%). Peak resolution between amikacin and kanamycin was > 5 (better than 3 is required for USP). The peak tailing factor for both components was better then 1.6 (USP requires smaller than 2).

Linearity of kanamycin was investigated in the range of 1.6-8mg/L. Linearity of amikacin was investigated in the range of 4 - 20 mg/L. In all cases correlation coefficients were better than 0.998 for peak areas and peak heights.

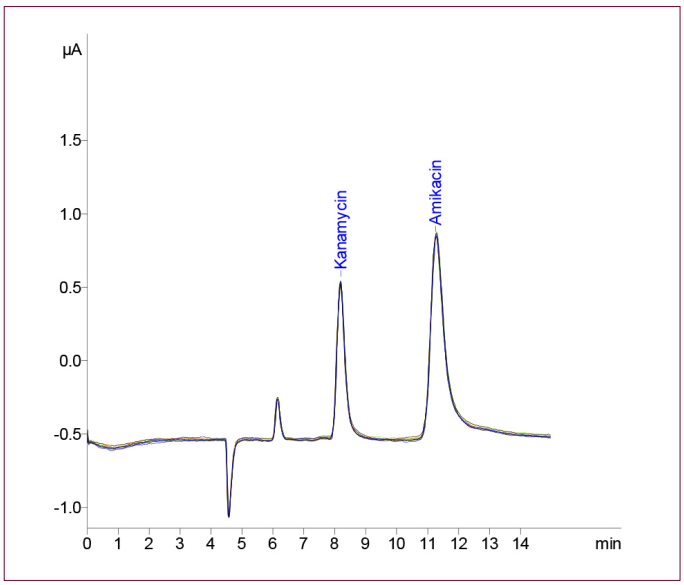

Figure 2: Overlay of 10 injections of 20 µL of 8 mg/L Kanamycin and 20 mg/L Amikacin in water.

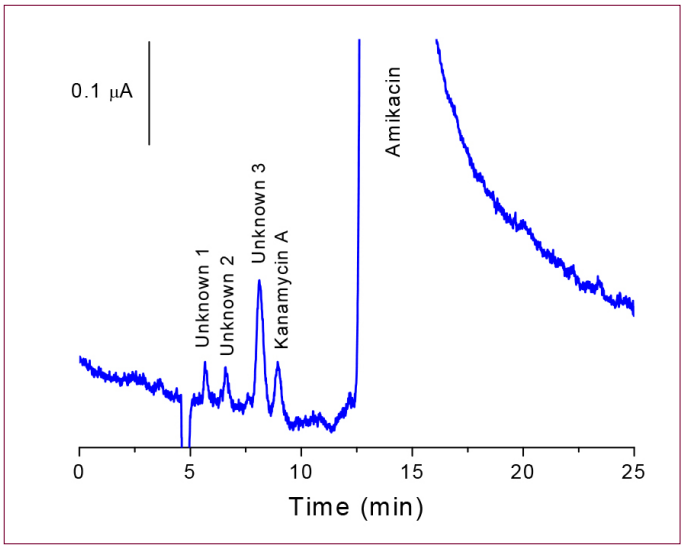

Figure 3: Impurities in a solution of 200 mg/L Amikacin.

Conclusion

The ALEXYS Kanamycin and Amikacin analyzer provides a sensitive and reliable solution for the analysis of Kanamycin and Amikacin bulk drugs. It matches the USP requirements for peak resolution, tailing and reproducibility.

References

1. E. Adams, J. Dalle, E. De Bie, I. De Smedt, E. Roets, J. Hoogmartens, *Analysis of kanamycin sulfate by liquid chromatography with pulsed electrochemical detection,* J. Chromatogr. A, 766 (1997) 133-139.
2. E. Adams , G. Van Vaerenbergh, E. Roets, J. Hoogmartens, *Analysis of amikacin by liquid chromatography with pulsed electrochemical detection,* J. Chromatogr. A, 819 (1998) 93-97
3. David A. Stead, *Current methodologies for the analysis of aminoglycosides*, J. Chromatogr. B, 747 (2000) 69-93
4. W.R. LaCourse, *Pulsed Electrochemical Detection in High Performance Liquid Chromatography.*, John Wiley & Sons, New York, 1ed,1997.
5. United States Pharmacopeia (USP), *Kanamycin Sulfate,* USP30-NF25 Page 2434
6. United States Pharmacopeia (USP), *Amikacin Sulfate,* USP30-NF25 Page 1372

PART NUMBERS AND CONFIGURATIONS

180.0058C	ALEXYS Kanamycin and Amikacin analyzer, including column, flow cell, and kit
250.1080	ALC-525 anion exchange column, 250x4.6mm, 7um
250.1082	ALC guard column starter kit

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.

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