



The most reliable LC-EC applications for Antibiotics analysis

**Macrolide antibiotics**

Azithromycin  
Azaerythromycin  
Clarithromycin  
Erythromycin  
Roxithromycin

**Aminoglycoside drugs**

Amikacin  
Framycetin sulphate  
Gentamicin sulphate  
Kanamycin  
Netilmicin  
Neomycin sulfate  
Spectinomycin  
Lincomycin  
Tobramycin

## Amikacin and Kanamycin

- **HPAEC-PAD analytical method**
- **Method according USP38 monograph**
- **Assay of main substituent**

### Summary

Kanamycin and amikacin are closely related water soluble, broad spectrum aminoglycoside antibiotics. The United States Pharmacopeia (USP) describes monographs with very similar methods based on High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD). The ALEXYS HPAEC-PAD Analyzer is a dedicated LC solution for the analysis of both antibiotics, which gives results that meet the USP system suitability requirements (peak resolution, tailing and reproducibility). In this note typical results obtained with the ALEXYS Analyzer are shown to demonstrate its applicability.



# Amikacin and Kanamycin

## Introduction

Kanamycin and amikacin are closely related, water soluble, broad spectrum aminoglycoside antibiotics (Fig. 1, ref. [1]). Kanamycin is obtained from *Streptomyces kanamyceticus*. Amikacin is synthesized by acylation of an amino group of kanamycin A with L(-)-g- amino- $\alpha$ - hydroxybutyric acid (LHABA). Both antibiotics can be analyzed using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) [2-5].

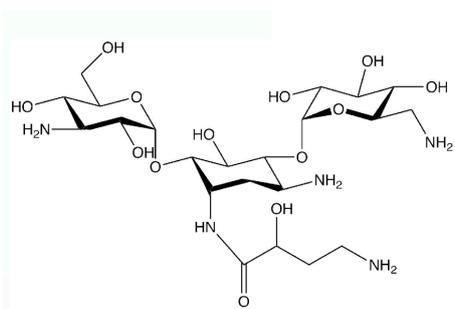
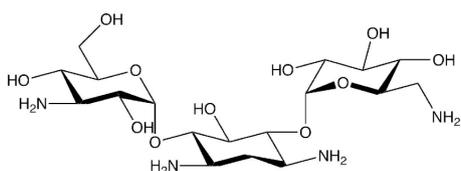


Figure 1: Structural formulas of kanamycin (top) and amikacin (bottom)

The United States Pharmacopoeia (USP) describes various amikacin and kanamycin related monographs that share very similar analytical methods and system suitability requirements for their Assay [6 - 11]. This application note presents typical results as can be obtained with the ALEXYS® HPAEC-PAD Analyzer, demonstrating its performance and suitability for the analysis of kanamycin and amikacin.

## Method

The analysis was performed using an ALEXYS HPAEC-PAD Analyzer (Fig. 2). A summary of the applied LC-ECD conditions is given in Table 1 (ECD settings as given for amikacin-related monographs)

Table 1

LC-ECD conditions	
HPLC*	ALEXYS Antibiotics base system - Isocratic
Columns	Hamilton RCX-10 analytical column 250 x 4.6 mm ID, 7 $\mu$ m* (Hamilton) + guard, USP L47 phase
Mobile phase**	100 mM NaOH
Flow rate	0.5 mL/mL
Pressure	Ca. 35 bar
Temperature	30 °C for separation and detection
V <sub>injection</sub>	20 $\mu$ L
Flow cell	FlexCell™ with Au and Ag/AgCl REF, 50 $\mu$ m spacer
Potential waveform (3-step)	E1, E2, E3: +0.04, +0.80, -0.80 V t1, t2, t3, ts: 0.5, 0.19, 0.19 s, 60 ms
Range	5 $\mu$ A/V
ADF	0.5 Hz
I-cell	Ca. 1 - 4 $\mu$ A

\*) Note that the presented data are obtained with an older version of the ALEXYS system than shown in figure 2.

\*\*) The USP monographs related to kanamycin and amikacin [6 - 11] actually prescribe a slightly stronger mobile phase concentration of 115 mM NaOH



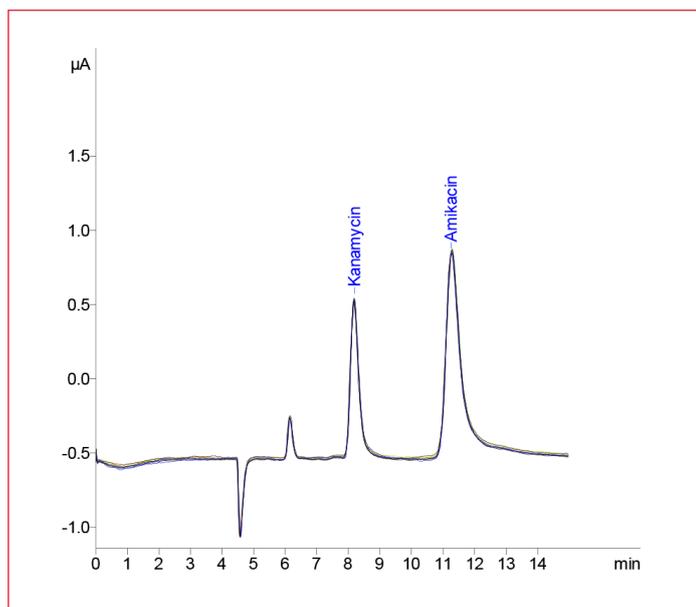
Figure 2: The ALEXYS analyzer for Kanamycin & Amikacin, consisting of the ALEXYS Antibiotics base system - Isocratic, and dedicated flow cell and bottles. The base system consists of a P6.1L pump with, an AS6.1L autosampler, an ET 210 Eluent tray and the DECADE Elite electrochemical detector.



## Results

### System suitability test

Figure 3 shows an overlay of the chromatograms obtained with the USP system suitability solution of 8 mg/L kanamycin and 20 mg/L amikacin, which is the same for all amikacin and kanamycin related USP monographs [6 - 11]. The monographs specify a set of tests to check the system suitability. The chromatograms shown in Figure 3 were used to compare the results against the system suitability criteria. It is evident that all system suitability requirements are met (Table 2).



**Figure 3:** Overlay of 10 chromatograms from 8 mg/L kanamycin and 20 mg/L amikacin in water (USP system suitability solution).

### Linearity

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

**Table 2**

### USP system suitability parameters and test results

Parameter	USP criterium	Measured
Peak resolution (kanamycin - amikacin)	> 3	5.2
Tailing factor (amikacin)	<2	1.8
Tailing factor (kanamycin)	<2	1.5
RSD peak area (amikacin)	<3%	2.7%
RSD peak area (kanamycin)	<2%	0.6%

## Conclusion

The ALEXYS HPAEC-PAD Analyzer provides a sensitive and reliable solution for the analysis of kanamycin and amikacin. The results obtained with this analyzer meet the system suitability requirements for peak resolution, tailing and reproducibility as stated in the kanamycin and amikacin-related USP monographs.



## References

1. Ramirez, M. S., & Tolmasky, M. E. (2017). Amikacin: uses, resistance, and prospects for inhibition. *Molecules*, 22(12), 2267.
2. 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.
3. 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
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5. W.R. LaCourse, Pulsed Electrochemical Detection in High Performance Liquid Chromatography., John Wiley & Sons, New York, 1997.
6. United States Pharmacopeia (USP), Kanamycin Injection, USP38-NF33S1, page 4002
7. United States Pharmacopeia (USP), Kanamycin sulfate, USP38-NF33S1, page 4000
8. United States Pharmacopeia (USP), Kanamycin sulfate Capsules, USP38-NF33S1, page 4001
9. United States Pharmacopeia (USP), Amikacin, USP38-NF33S1, page 2167
10. United States Pharmacopeia (USP), Amikacin sulfate, USP38-NF33S1, page 2168
11. United States Pharmacopeia (USP), Amikacin sulfate injection, USP38-NF33S1, page 2169

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## Ordering information

<b>Detector only</b>	
176.0035A	DECADE Elite SCC electrochemical detector
102.4325*	Flexcell Au HyREF
<b>Recommended ALEXYS analyzer</b>	
180.0058W	ALEXYS Antibiotics base system - Isocratic
102.4325*	Flexcell Au HyREF
184.0205	PPCO bottle assembly, 2L, Helium
102.2218	flattening/polishing kit for metal WE

\*) Although the work was performed using a FlexCell with Ag/AgCl saltbridge reference electrode (RE), Antec recommends to use a FlexCell with the maintenance-free Hydrogen Palladium RE (HyREF) for this application.

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