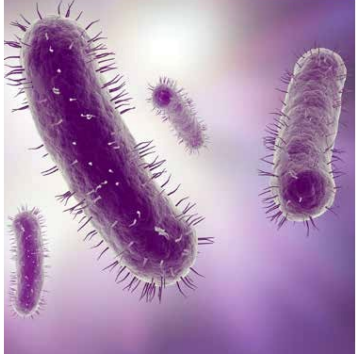
Application Note

Aminoglycoside Antibiotics



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-α- hydroxybutyric acid (LHABA). Both antibiotics can be analyzed using High Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) [2-5].

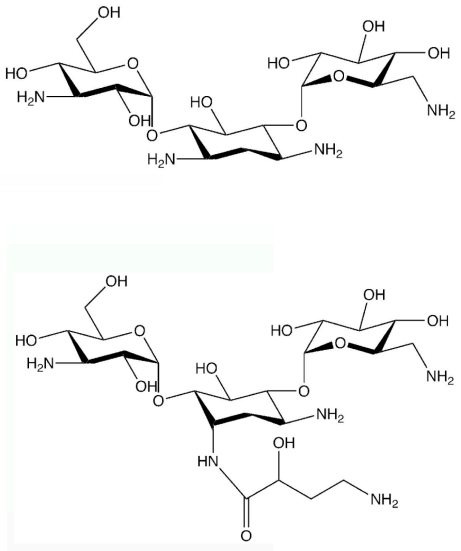


Figure 1: Structural formulas of kanamycin (top) and amikacin (bottom)

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 HPAEC-PAD Analyzer
Columns	RCX-10, guard + 250 x 4.6 mm ID, 7 µm* (Hamilton)
Mobile phase	100 mM NaOH**
Flow rate	0.5 mL/mL
Pressure	Ca. 35 bar
Temperature	30 °C for separation and detection
$V_{injection}$	20 µL
Flow cell	FlexCell™ with Au and Ag/AgCl REF, 50 µm 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 µA/V
ADF	0.5 Hz
I-cell	Ca. 1 - 4 µA

* USP classification: USP L47

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

Figure 2: ALEXYS HPAEC-PAD Analyzer.

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).

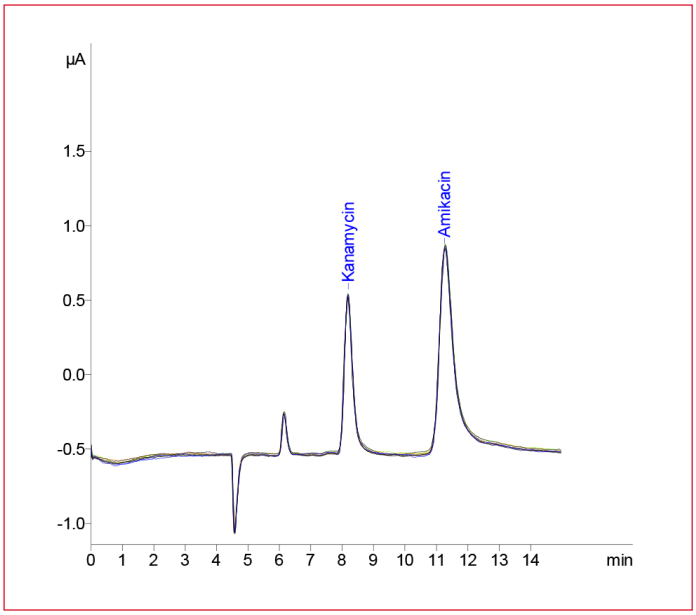


Figure 3: Overlay of 10 chromatograms from 8 mg/L kanamycin and 20 mg/L amikacin in water (USP system suitability solution).

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 a 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
4. David A. Stead, Current methodologies for the analysis of aminoglycosides, J. Chromatogr. B, 747 (2000) 69-93
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

Ordering information

Detector only	
176.0035A	DECADE Elite SCC electrochemical detector
102.4125	FlexCell Au sb
102.0913	HyREF for F/R
250.1035	sb REF tool
Recommended ALEXYS analyzer	
180.0057W	ALEXYS HPAEC-PAD Analyzer
102.4125	FlexCell Au sb
102.0913	HyREF for F/R
102.2218	flattening/polishing kit for metal WE
250.1035	sb REF tool
Software	
195.0035#	Clarity CDS single instr. incl LC, AS module

#) optional: Antec ECD drivers are available for use with OpenLAB™ CDS, OpenLAB™ Chemstation™ CDS, and Thermo Scientific™ Chromeleon™ CDS.

For research purpose only. The information shown in this communication is solely to demonstrate the applicability of the ALEXYS system and DECADE Elite detector. The actual performance may be affected by factors beyond Antec's control. Specifications mentioned in this application note are subject to change without further notice.

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