Application Note
Aminoglycoside Antibiotics

The most reliable LC-EC applications for Antibiotics analysis

Aminoglycosides
Amikacin
Framycetin Sulphate
Gentamicin Sulphate
Kanamycin Sulphate
Lincomycin
Neomycin
Spectinomycin
Tobramycin

Macrolide antibiotics
Azithromycin
Azaerythromycin
Clarithromycin
Erythromycin
Roxithromycin

Neomycin and Framycetin Sulphate in Bulk Drugs

- **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 (EP) has two monographs describing the analysis of Neomycin and Framycetin [4,5] using LC-PAD. The ALEXYS analyzer based on the antibiotics base system with post-column addition kit is a dedicated LC solution for the analysis of Neomycin and Framycetin, which matches the EP requirements for peak resolution and signal-to-noise ratio of the principal peak (Neomycin B). In this application note typical results obtained with the ALEXYS analyzer are reported demonstrating its performance for the analysis of impurities in bulk drugs.

Introduction

Neomycin is an antibiotic complex consisting of a mixture of the aminoglycosides Neomycin A, B and C, obtained from Streptomyces fradiae, were Neomycin B is the main constituent. It is a widely-used broad spectrum water-soluble antibiotic useful primarily in infections involving aerobic bacteria. It is available as skin ointment (e.g., creams, gels, lotions, etc.) and eye drops. Framycetin (also known as Neomycin B sulphate) is an aminoglycoside antibiotic similar to Neomycin and commonly sold under the brand name Soframycin. Impurities in neomycin and framycetin preparations are analyzed using reversed phase HPLC, with post-column NaOH addition and pulsed amperometric detection (LC-PAD) [1-3].

Figure 1: ALEXYS Analyzer for Neomycin and Framycetin

Method

The ALEXYS analyzer is applied 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 0.5M NaOH to facilitate PAD detection of the aminoglycosides [2,3]. For post-column mixing a low dead volume Tee connector was used and a PEEK mixing coil with a volume of 375 µL between the Tee and the flow cell.

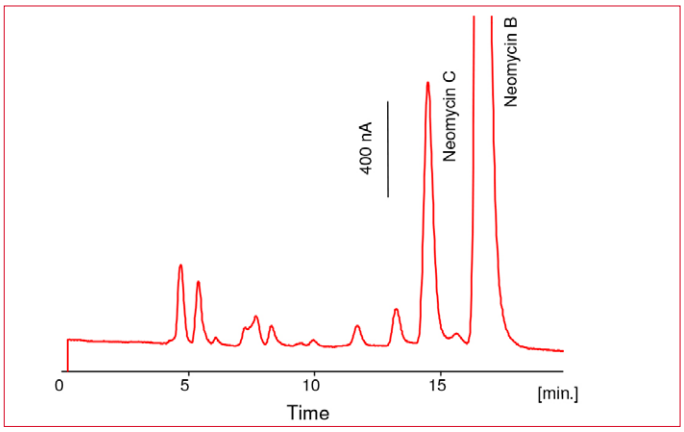

Figure 2: Chromatogram of a 0.5 mg/mL solution of commercial Neomycin sulphate formulation, 10 µL injected. Neomycin B is the main constituent and neomycin C the main impurity.

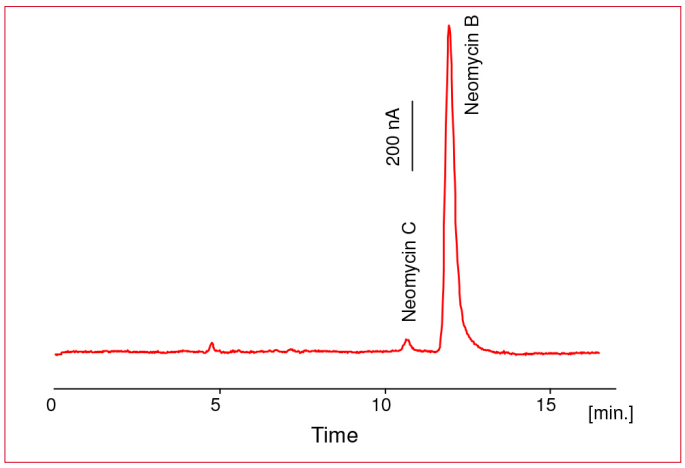

Figure 3: Example chromatogram of a 75 µg/mL Framycetin sulphate reference standard solution (EP BP190 –F67029), 10 µL injection (slightly different LC conditions compared to fig.2).

The mobile phase was prepared as described in the European Pharmacopoeia monographs [4,5]. The optimal mobile phase consisted of 2% trifluoroacetic acid (20 mL/L) and 8 mL/L of a commercial 50% carbonate-free NaOH solution.

Results

According to the EP the pH may be changed to optimize the resolution between Neomycin C and the principal peak (Neomycin B) if necessary. The effect of pH on the separation of Neomycine was investigated with the aminoglycosides Analyzer by varying the amount of 50% NaOH solution in the mobile phase.

In Fig. 4 two chromatograms are shown recorded with a mobile phase with 6 mL/L 50% NaOH (blue curve, pH 1.18) and 8 mL/L 50% NaOH (red curve, pH 1.21), respectively. The retention time for Neomycin C and B shifted significant with increasing pH, and a change in resolution of 1.7 to 2.3 was observed. It is evident from that the pH of the mobile phase is an effective parameter to optimize the LC separation of the aminoglycosides and its impurities.

Table 1

Conditions	
HPLC *	ALEXYS analyzer for Neomycin/Framycetin
Oven temperature	32 °C (column and detection)
Flow rate	0.7 mL/min, 0.5 mL/min post column
Flow cell	FlexCell™ with Au WE and HyREF™
ADF™	0.5 Hz
Range	10 µA/V

* See last page for more details. Note that the presented data are obtained with an older version of the ALEXYS LC system than shown in fig 1.

EP criteria

In the EP monographs for Neomycin and Framycetin two system suitability requirements are specified for peak resolution and signal-to-noise ratio of the principal peak. In Table 2 the criteria of the EP are compared with the typical results obtained with the ALEXYS analyzer.

An example chromatogram of reference solution (c) for the calculation of the signal-to-noise ratio of Neomycin B is shown in Fig. 5. The EP requirements for both peak resolution and S/N ratio are met with the ALEXYS analyzer.

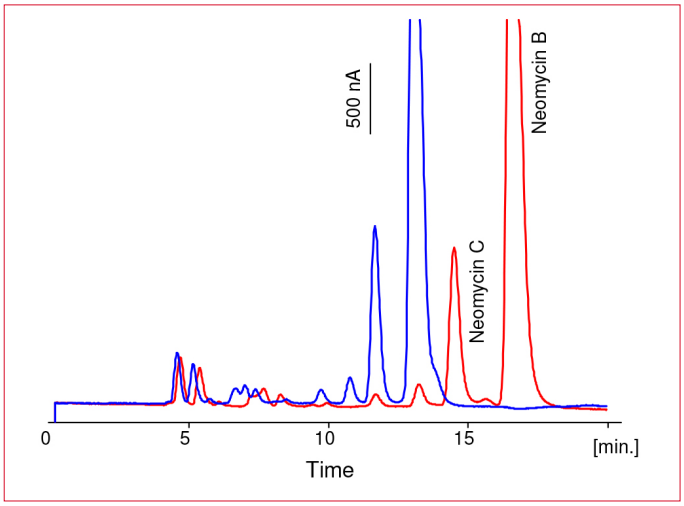

Figure 4: Effect of pH on the separation. Blue: mobile phase with 6 mL/L 50% NaOH (pH 1.18), Red: mobile phase with 8 mL/L 50% NaOH (pH 1.21).

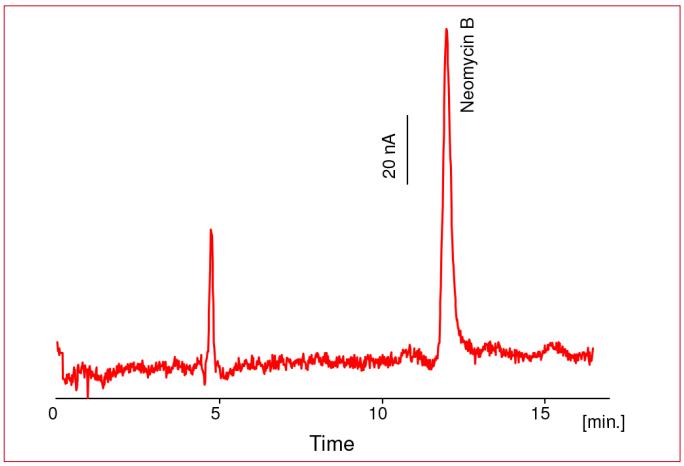

Figure 5: EP system suitability: chromatogram of a 10 µL injection of 5 µg/mL framycetin (reference solution c) for signal-to-noise ratio calculation (Neomycin B). Actual S/N = 25.

The C_{LOD} for Neomycin B is approximately 0.6 µg/mL. The C_{LOD} defined as the concentration that gives a signal that is three times the peak-to-peak noise.

Table 2

EP system suitability requirement

Parameter	EP criteria	Result
Peak resolution	> 2	2.3
S/N principle peak	> 10	25

Repeatability

The repeatability of the method was evaluated by executing 11 repetitive injections (10 µL) of a 0.5 mg/mL Framycetin and 0.5 mg/mL Neomycin solution. The relative standard deviation (RSD%) for retention time, peak area and height are listed in table II.

Table 3

Peak table

	%RSD tR	%RSD H	%RSD A
Neomycin			
Neomycin B	0.11	1.06	0.62
Neomycin C	0.08	1.42	2.42
Framycetin			
Neomycin B	0.04	2.48	1.92
Neomycin C	0.10	1.06	1.75

For Neomycin B and C, RSD's smaller then 2.5% (n=10) were found for both peak area and peak height.

Conclusion

The ALEXYS® analyzer provides a sensitive and reliable solution for the analysis of impurities in Neomycin and Framycetin bulk drugs. It meets the EP requirements for peak resolution and signal-to-noise ratio.

References

1. David A. Stead, "Current methodologies for the analysis of aminoglycosides", J. Chromatogr. B, 747 (2000) 69–93
2. W.R. LaCourse, "Pulsed Electrochemical Detection in High Performance Liquid Chromatography", John Wiley & Sons, New York, 1ed,1997.
3. E. Adams, R. Schepers, E. Roets, J. Hoogmartens, "Determination of neomycin sulfate by liquid chromatography with pulsed electrochemical detection", J. Chromatogr. A, 741 (1996) 233 - 240
4. "Neomycin sulphate", European Pharmacopoeia, 6.0, (2008) 2487-3489
5. "Framycetin sulphate", European Pharmacopoeia, 6.0, (2008) 1947-1949

Ordering information

Recommended ALEXYS analyzer + parts	
180.0058W	ALEXYS Antibiotics base system - Isocratic
180.0605EP	Post Column Kit EP
102.4325EP	Flexcell Au HyREF with stainless steel AUX
250.1045	Flattening/polishing kit for metal WE
184.0209	Glass bottle assembly, 1L, Helium
Column	
00G-4252-E0*	Luna® C18 column, 250 x 4.6 mm ID, 5 µm

*) The column is manufactured and sold by Phenomenex Inc., Torrance, California, USA. Luna is a registered trademark of Phenomenex Inc.

LC-EC conditions (full)

Table 1a

LC-EC Conditions	
HPLC	ALEXYS analyzer
Flow rate	0.7 mL/min, post-column: 0.5 mL/min
Mobile phase	2 % (v/v) trifluoroacetic acid, 8 mL/L 50% (w/w) NaOH carbonate-free, pH 1.2
Addition	0.5 mol/L NaOH, post column
Flow cell	Flexcell™ with Au WE and HyREF™
Temperature	32 °C for separation and detection
E-cell	E1, E2, E3: 0.0, 0.8, -0.6 V ts, t1, t2, t3: 0.06, 0.5, 0.13, 0.12s
I-cell	-800 - -300 nA

Antec Scientific (USA)
info@AntecScientific.com
www.AntecScientific.com
T 888 572 0012

Antec Scientific (worldwide)
info@AntecScientific.com
www.AntecScientific.com
T +31 71 5813333