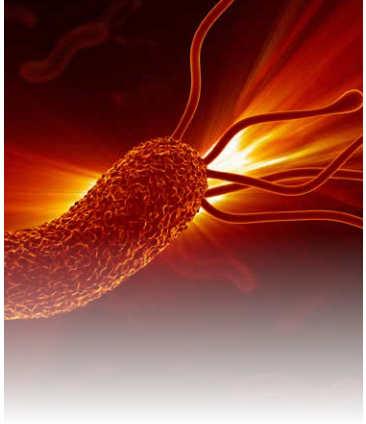
Application Note

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

Spectinomycin in Pharmaceutical Preparations

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

Introduction

Spectinomycin is an aminoglycoside- like antibiotic produced by Streptomyces spectabilis. In solution, spectinomycin will undergo a ring opening and closing of the hemiketal function, resulting in an equilibrium mixture of four possible anomers. Hydrolysis with acid produces actinamine and in basic solutions actinospectinoic acid (ASA) is formed. Important fermentation impurities are dihydrospectinomycin and dihydroxyspectinomycin [1, 2].Because of the presence of glycoside groups in Spectinomycin and by-products, LC with pulsed amperometric detection (PAD) has been applied for analysis [3]. Conditions are to a large extent in correspondence with the EP requirements [4,5].

Summary

The ALEXYS® 'Aminoglycosides analyzer' is a dedicated LC solution for the analysis of Spectinomycin, which matches the EP requirements for peak resolution and repeatability of the principal peak. The European Pharmacopoeia, 6.0, (2008), 2947-2949 was used as a basis to set-up this method. In this application note typical results obtained with the Aminoglycosides analyzer are reported demonstrating its performance for the analysis of impurities in Spectinomycin bulk drugs.

Figure 1: ALEXYS Aminoglycosides analyzer for Spectinomycin.

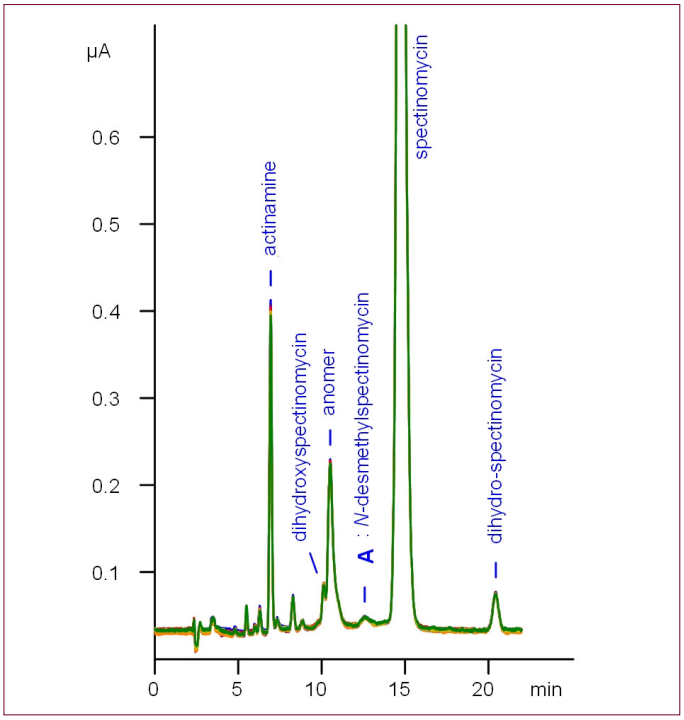


Figure 2: Overlay (n=6) of 20 µL injections of 80 mg/L Spectinomy- cin-HCl in mobile phase (diluted from 800 mg/L Spectinomycin in water, standing time 68 h).

Method

Solutions and standards are prepared as described in the EP method [4,5]. Assay validation was done with special attention to EP requirements.

Table 1

Conditions	
HPLC	ALEXYS Aminoglycosides analyzer (part no.180.0050A)
Flow rate	1 mL/min, post-column: 0.5 mL/min
Flow cell	Flexcell™ with Au WE and HyREF™
Temperature	35 DC for separation and detection
Range	2 µA/V
ADF	0.5 Hz
I-cell	about 2 µA

EP requirements

In the EP monographs for Spectinomycin [4.5] two system suitability requirements are specified:

[1] Peak resolution: between impurity E and the principle peak (Spectinomycin), R > 1.5.
[2] Repeatability: maximum RSD(%) for the principle peak, n=6 injections, RSD > 3%.

In Table 2 the criteria of the EP are compared with the typical results obtained with the ALEXYS Aminoglycosides analyzer.

Table 2

EP system suitability requirement		
Parameter	EP criteria	Result
RSD of principal peak	< 3.0 %	1.0 %
Resolution, peak 'A'	> 1.5	2.5

Table 2. Performance ALEXYS aminoglycosides analyzer versus EP system suitability requirements.

It is evident from table I that the EP requirements for both peak resolution and repeatability are met by the ALEXYS aminoglyco- sides analyzer.

Conclusion

The ALEXYS Aminoglycosides analyzer provides a reliable solution for the routine analysis of Spectinomycin in Pharmaceutical Preparations. It meets the EP system suitability requirement for resolution and repeatability.

References

1. J. Szunyog, E. Adams, K. Liekens, E. Roets, J. Hoogmartens, *Journal of Pharmaceutical and Biomedical Analysis,* 29 (2002) 213–22.
2. D. Debremaeker, E. Adams, E. Nadal, B. Van Hove, E. Roets, J. Hoogmartens, *Journal of Chromatography A*, 953 (2002) 123–132
3. W.R. LaCourse, "Pulsed Electrochemical Detection in High Performance Liquid Chromatography", *John Wiley & Sons, New York,* 1ed,1997.
4. "Spectinomycin Dihydrochloride Pentahydrate", *European Pharmacopoeia*, 6.0, (2008) 2947-2949
5. "Spectinomycin Sulphate Tetrahydrate for veterinary use", *European Pharmacopoeia*, 6.0, (2008) 2949 -2951

PART NUMBERS AND CONFIGURATIONS

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

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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