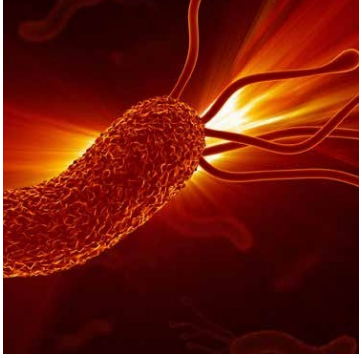
Application Note

Aminoglycoside Antibiotics



The most reliable LC-EC applications for Drugs & Pharmaceuticals analysis

Antipsychotic drugs
Clozapine
Olanzapine
Risperidone

PET imaging tracer
Fluorodeoxyglucose (FDG)
FDG impurities

Pharmaceuticals, API
Acetaminophen
Artemether
Artemisinin
Dihydro- artemisinin
Betadex sulfobutyl ether sodium
Etoposide
Epinephrine
Heparin
mesna BNP7787
8-OH-DPAT
Vincristine

Sulfides
Glutathione
Aminothiols
Disulfides

Aminoglycoside drugs
Amikacin
Framycetin sulphate
Gentamicin sulphate
Kanamycin
Netilmycin
Neomycin sulfate
Spectinomycin
Lincomycin
Tobramycin

Spectinomycin in Pharmaceutical Preparations

- **Method based on European Pharmacopoeia 10.0 (2019)**
- **Flow cell with exchangeable Au working electrode and stainless steel AUX**
- **Analysis of main substituent and impurities**
- **Reproducible and robust**

Summary

The ALEXYS® analyzer based on the antibiotics base system with post-column addition kit is a dedicated LC solution for the analysis of Spectinomycin, which meets the EP requirements for peak resolution and repeatability of the principal peak. The European Pharmacopoeia 10.0 (2019) monograph for Spectinomycin was used to set-up the method. In this application note typical results obtained with the ALEXYS system are reported, demonstrating its performance for the analysis of impurities and the assay of Spectinomycin in bulk drugs.

Introduction

Spectinomycin (Fig. 1) is an aminoglycoside 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 dihydroxy-spectinomycin [1, 2].

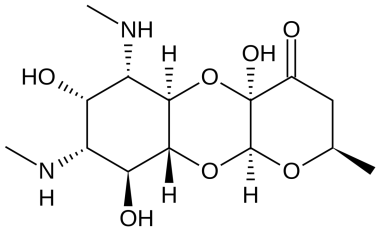


Figure 1: Structural formula of spectinomycin

Due to the presence of glycoside groups in spectinomycin and its by-products, LC with pulsed amperometric detection (PAD) is a suitable method for analysis [3]. Conditions reported in this application note are to a large extent in correspondence with the EP monographs for spectinomycin [4,5].

Method

Solutions and standards were prepared as described in the EP monograph [4,5]. The assay validation was done with special attention to the EP requirements.

Potential waveform

The EP monographs [4, 5] prescribe the use of a 3-step potential waveform. The potentials of the waveform are defined in the monograph, but the time duration of the applied potentials are free to be set 'according to the instrument used'. The pulse durations (t1,t2,t3 and ts) were optimized for the FlexCell equipped with a stainless steel auxiliary (AUX) electrode. The optimized pulse waveform is given in Table 1. This will result in meeting the EP monograph system suitability requirement for repeatability and a stabile response over at least 24 hours (steady between 97 and 103%).

Flow cell maintenance

A slow wear of the gold electrode over time is a normal phenomenon for analyses that are based on a 3-step pulsed

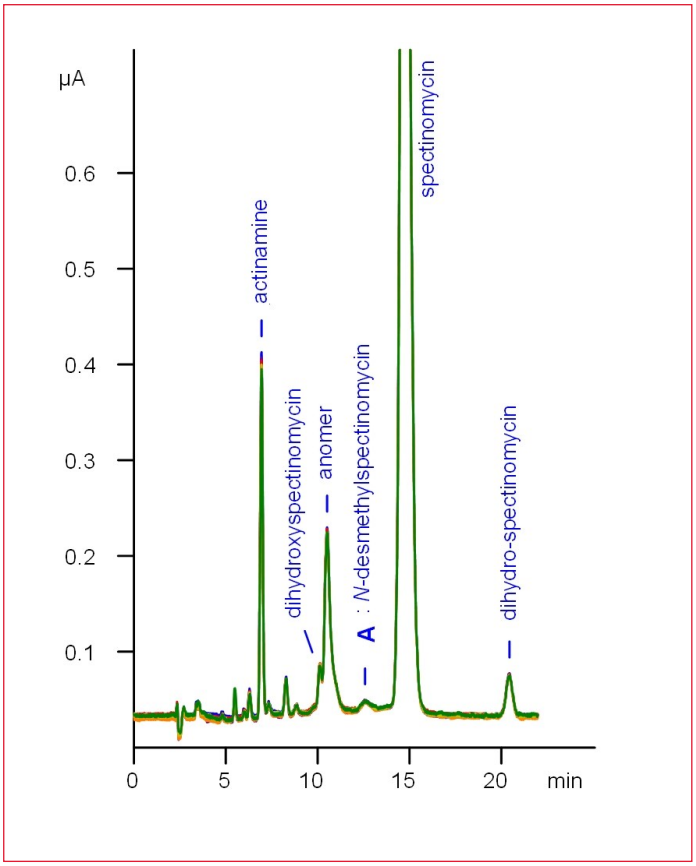
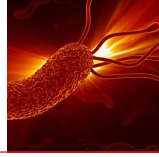


Figure 2: Overlay (n=6) of 20 µL injections of 80 mg/L spectinomycin-HCl in mobile phase (diluted from 800 mg/L spectinomycin in water, with a standing time of 68 h). LC-ECD conditions as in Table 1.

Table 1

LC-ECD conditions	
HPLC	ALEXYS aminoglycosides Analyzer
Column	Luna ® 5 µm C18 (2) 250 x 4.6 mm HPLC Column (Phenomenex ™)
Mobile phase	47 mM oxalic acid, 15 mM heptafluorobutyric acid, set to pH 3.2 with 19 M NaOH, 10% (v/v) acetonitril
Post-column addition	540 mM NaOH
Flow rate	1 mL/min, post-column addition: 0.5 mL/min
Mixing coil volume	375 µL
Temperature	35 °C for separation, mixing and detection
$V_{injection}$	20 µL
Flow cell*	FlexCell™ with Au, HyREF and stainless steel AUX, 50 µm spacer
Potential waveform* (3-step)	E1, E2, E3: +0.12, +0.7, -0.6 V t1, t2, t3, ts: 0.22, 0.2, 0.2 s, 20 ms
Range	20 µA/V
ADF	0.5 Hz
I-cell	About 6 µA

* Original work done with VT-03 and pulse times t1, t2, t3, ts: 0.4, 0.2, 0.4 s, 200 ms



amperometric detection on gold electrodes. The gold electrode can be flattened and polished when needed [6]. The FlexCell has a removable electrode and is designed for easy service. After polishing the gold electrode, the signal is quickly stabile again (disregard at least the first chromatogram after gold electrode service). Polishing and flattening is advised when the overall response and/or sensitivity have decreased below a certain threshold. If the sensitivity of the system has dropped below the disregard limit as mentioned in the EP monograph, then it is due time for service.

The contact surface of a new stainless steel AUX inlet block needs some time to equilibrate under the analytical conditions of Table 1. The background current will quickly stabilize around 6 µA, but peak heights become reproducible after 5 - 10 hrs of running a calibration standard repeatedly. It is normal for the peak heights to first drop and then increment to a stabile response with good reproducibility.

The contact surface of a used stainless steel AUX will look a bit discolored (light blue or light beige) and it must not be polished (otherwise it will show the long stabilization pattern again as for a new block). The AUX contact surface may be cleaned with water and wiped with acetone before using it and reproducible results are obtained more quickly.

Results

The EP monographs for Spectinomycin [4.5] specifies two system suitability requirements as given in Table 2. In this table, the criteria of the EP are compared with the typical results (Fig. 2) as obtained with the ALEXYS analyzer.

Table 2

EP system suitability requirement and results from chromatograms shown in Fig. 2

Parameter	*EP criterium*	*Result*
RSD of principal peak (n=6)	RSD < 3%	1.0
Resolution, between the principal peak and impurity E (peak 'A' in Fig. 2)	R > 1.5	2.5

It is evident from Table 2 that the EP system suitability requirements for both peak resolution and repeatability are met by the ALEXYS analyzer.

References

1. J. Szúnyog, E. Adams, K. Liekens, E. Roets, J. Hoogmartens, Journal of Pharmaceutical and Biomedical Analysis 29:213-220 (2002)
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, 10.0, (2019) 3873 - 3875
5. "Spectinomycin Sulphate Tetrahydrate for veterinary use", European Pharmacopoeia, 10.0, (2019) 3875 - 3877
6. Antec Scientific, Flattening & Polishing kit for metal WE: User Guide, part number 250.7010

Conclusion

The ALEXYS 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.

Figure 3. The ALEXYS Aminoglycosides Analyzer is the recommended instrument configuration for the analysis of Spectinomycin. It consists of an ALEXYS base system and an additional pump for post column NaOH addition.

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-4252E0#	Luna ® C18 column, 250 x 4.6 mm ID, 5 µm

#) The column is manufactured and sold by Phenomenex Inc., Torrance, California, USA

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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