



## The Analysis of Spectinomycin and Lincomycin

### ALEXYS® LC-EC system

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#### Introduction

Spectinomycin is an aminoglycoside-like antibiotic produced by *Streptomyces spectabilis*. The European Pharmacopoeia (EP) describes a specific LC-EC method to detect the impurities [1]. Spectinomycin may be used in combination with Lincomycin to prevent respiratory infections in poultry. The simultaneous analysis of both antibiotics in formulations is complicated due to the large difference in chromatographic retention behaviour [2].

Here we present a simple and sensitive analysis method for the analysis of Spectinomycin and a mixture of Spectinomycin & Lincomycin using reversed-phase Liquid Chromatography (LC) and Pulsed Amperometric Detection (PAD) on a gold working electrode after post-column addition of sodium hydroxide [3].

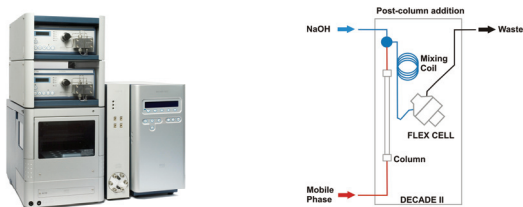


Fig. 1. ALEXYS Aminoglycosides system with post-column NaOH addition.

#### Spectinomycin

The system configuration and settings of the EP method [1] were used, with a few system dependent modification.

#### Method

HPLC	ALEXYS 'Aminoglycosides' system + kit
Flow cell	Flexcell™ with Au electrode and HyREF™ reference electrode
V <sub>injection</sub>	20 µL
T <sub>oven</sub>	35 °C for separation and detection
Flow rate	1 mL/min; post-column: 0.5 mL/min

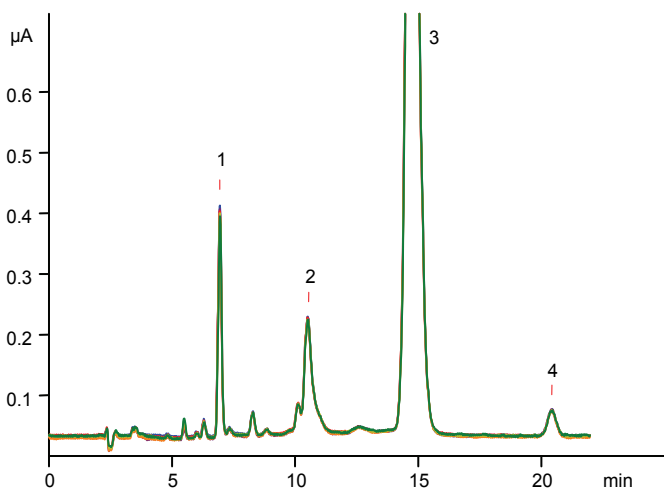


Fig. 2. Overlay of 6 chromatograms of 80 mg/L Spectinomycin (3). Other peaks are anomer (1), unknown (2), and dihydro-spectinomycin (4).

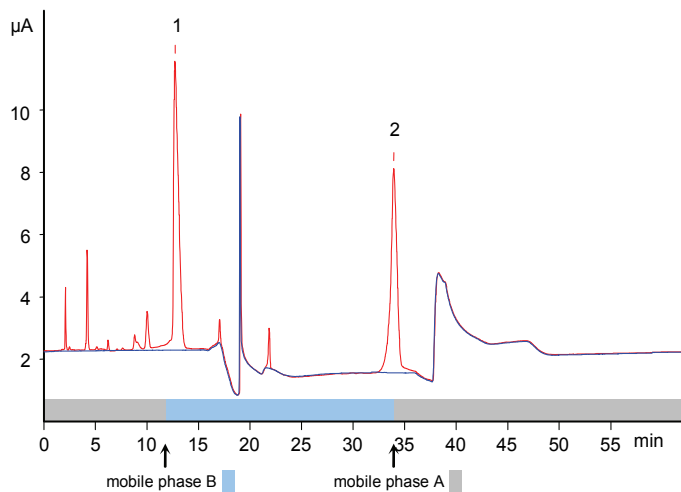


Fig. 3. Overlay of baseline (dark blue) and chromatogram (red) of 100 mg/L Spectinomycin (1) and Lincomycin (2). The light blue and grey scheme represents the step gradient program.

#### Spectinomycin and Lincomycin

To separate Spectinomycin from its impurities and considerably reduce the retention time of Lincomycin, a step gradient was applied by means of an additional solvent selector in the LC-EC system. Additionally a column with a smaller ID was used to reduce the consumption of mobile phase.

#### Method

HPLC	ALEXYS 'Aminoglycosides' system + kit
Flow cell	Flexcell with Au electrode and HyREF reference electrode
V <sub>injection</sub>	20 µL
T <sub>oven</sub>	35 °C for separation and detection
Flow rate	0.4 mL/min; post-column: 0.2 mL/min

#### Reproducibility and linearity

Using the ALEXYS LC-EC system, the RSD for 6 replicate injections is better than 0.2% for retention time and better than 2% for peak signal in both applications. Also a good linear response with a correlation coefficient better than 0.999 is observed in both applications.

#### Conclusion

The ALEXYS LC-EC system has been successfully used for the analysis of Spectinomycin and a mixture of Spectinomycin & Lincomycin. The methods are reproducible, robust and in correspondence with the EP requirements for the analysis of Spectinomycin.

#### References

1. Spectinomycin, *European Pharmacopoeia*, 7 (04/2007:1358)
2. J. Szunyog, E. Adams, K. Liekens, E. Roets, J. Hoogmartens, *Journal of Pharmaceutical and Biomedical Analysis*, 29 (2002), 213-22
3. W. R. LaCourse, *Pulsed Electrochemical Detection in High-Performance Liquid Chromatography*, Wiley, New York, 1997