

The most reliable LC-EC applications for Antibiotics analysis

Aminoglycosides

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

Macrolide antibiotics

Azithromycin
Azaerythromycin
Clarithromycin Erythromycin
Roxithromycin

Gentamicin Sulphate in Pharmaceutical Preparations

- **European Pharmacopoeia 6.0 (2008) used as basis for this application**
- **Analysis of main substituent and impurities**
- **Reproducible & robust**

Summary

In the European Pharmacopoeia 6.0 (2008) the use of a reversed-phase polymeric column is prescribed for this application [1]. In literature it is shown that such a column may result in very wide and tailing peaks [3]. We have confirmed this and found much better separation using a C18 silica-based column.

In this application note typical results are reported for the ALEXYS[®] analyzer with a method based on a C18 column, demonstrating its performance for the analysis of gentamicin.



Gentamicin sulphate in pharmaceutical preparations

Introduction

Like neomycin and tobramycin, gentamicin belongs to the group of aminoglycoside antibiotics. It is manufactured by a fermentation process and the main constituents are gentamicin C1, C1a, C2 and C2a. Usually also other minor aminoglycosides are found in a pharmaceutical gentamicin preparation. The number of impurities and components possible makes the chromatographic analysis not quite straightforward. Because of the presence of a sugar moiety in these analytes the selectivity and inherent sensitivity of pulsed amperometric detection (PAD) is a very attractive approach [2].

Method

The ALEXYS analyzer, equipped with a second pump for the post-column addition of NaOH was used (Fig. 2). The mobile phase was prepared as described in the EP monograph [1] and the applied conditions are summarized in Table 1.

The pH of the acidic column effluent was increased to a final pH of about 13 before entering the detector cell. For the preparation of the NaOH solution, a commercially available stock solution of 50% w/w NaOH was used. For the preparation of the mobile phase it is necessary to use 'stabilized' THF to assure low cell currents.

Table 1

LC-ECD conditions (EP)

HPLC*	ALEXYS Antibiotics base system - Isocratic + Post Column Kit EP
Column	Thermo Scientific™ HyPURITY™ C18 HPLC Column 100 x 4.6 mm, 5 µm
Mobile phase	60 g/L Na ₂ SO ₄ (water free), 1.75 g/L octane sulphonic acid, sodium salt, 3 mL/L tetrahydrofuran (THF) stabilized, 50 mL/L 0.2 M KH ₂ PO ₄ (pH = 3).
Post-column NaOH addition	0.76 mol/L NaOH
Flow rate	Mobile phase: 1.5 mL/mL; NaOH addition: 0.6 mL/min
Temperature	45 °C for separation and detection
V _{injection}	20 µL
Pump piston wash	Water (refresh weekly)
Flow cell	FlexCell™ with Au WE and HyREF™
Potential waveform (3-step)	E1, E2, E3: +0.1, +0.75, -0.15 V ts, t1, t2, t3, t4: 0.1, 0.32, 0.2, 0.4 s
Range	10 µA/V
ADF	0.5 Hz
I-cell	About 2 µA

*) Note - the presented data are obtained with an older version of the ALEXYS LC system than shown in figure 2.

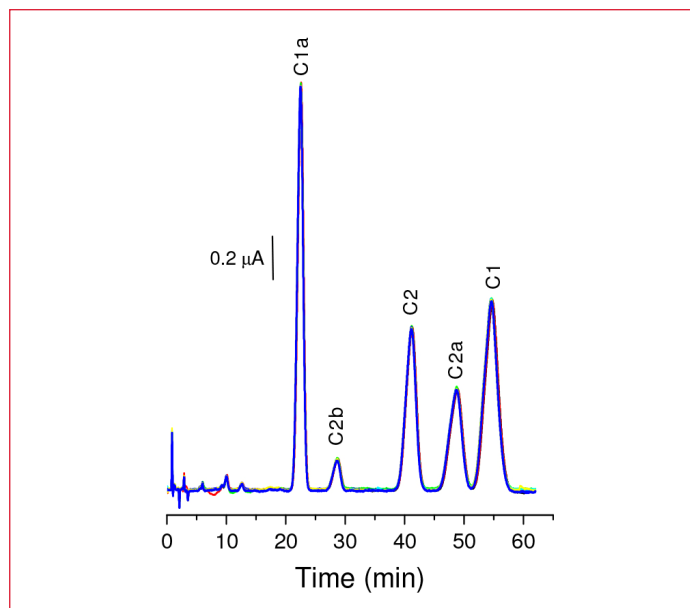


Figure 1: Gentamicin sample (400 µg/ml, 20 µl injected). Overlay of 7 chromatograms. Peak identities were derived from reference [2] and based on peak area percentages.

Results

Figure 1 shows a typical chromatogram of gentamicin, as obtained with the method settings summarized in Table 1.

Linearity & Repeatability

Linearity of gentamicin was investigated in the concentration range of 50 – 500 µg/mL. For all gentamicin derivatives the correlation coefficients were better than 0.998 for peak areas and peak heights. The relative standard deviation (RSD) in peak area for 10 replicate injections for gentamicin was ranging between 0.9 and 2.5% for gentamicin C1 and C2b, respectively. The RSD for the retention times was better than 0.2%. Peak resolution between gentamicin C2a and C1 was 1.6.

System suitability test

In the EP monographs for gentamicin sulphate a system suitability requirement is specified for the peak-to-valley ratio. The peak-to-valley ratio is specified as Hp/Hv, where Hp =

Table 2

EP system suitability requirement

Parameter	EP specification	Measured
Peak-to-valley ratio Hp/Hv	> 2.0	100



height above the baseline of the peak due to gentamicin C2a, and H_v = height above the baseline of the lowest point of the curve separating this peak from the peak due to gentamicin C2. In Table 1 this EP requirement is compared with a typical result as obtained with the ALEXYS Aminoglycosides Analyzer.

It is evident from Fig. 1 that gentamicin C2 and C2a are well baseline separated and therefore the peak-to-peak ratio requirement is easily met by when using the ALEXYS Aminoglycosides Analyzer

References

1. Gentamicin sulphate, European Pharmacopoeia, 6.0, (2008) 1965-1967
2. W.R. LaCourse, "Pulsed Electrochemical Detection in High Performance Liquid Chromatography", John Wiley & Sons, New York, 1ed,1997.
3. E. Adams, W. Roelants, R. De Paepe, E. Roets, J. Hoogmartens, J. Pharm. Biomed. Anal., 18, 689-698 (1998).

Conclusion

The ALEXYS analyzer for Gentamicin is a reliable solution for the routine analysis of gentamicin in pharmaceutical preparations. The results meet the EP system suitability requirement for peak- to-valley ratio between gentamicin C2 and C2a.



Gentamicin sulphate in pharmaceutical preparations



Ordering information

Detector only	
176.0035A	DECADE Elite SCC electrochemical detector
102.4325EP	Flexcell Au HyREF with stainless steel AUX
250.1045	Flattening/polishing kit for metal WE
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

Figure 2. The ALEXYS analyzer for Gentamicin, consisting of the ALEXYS Antibiotics base system - isocratic, post-column addition kit (NaOH) and FlexCell with gold working electrode, HyREF and stainless steel auxiliary electrode. The ALEXYS analyzer is controlled by DataApex™ Clarity™ software.

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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