Analysis of Aminoglycoside Antibiotics

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Introduction

The aminoglycoside antibiotics form a large family of compounds, which structure contains two or more amino sugars linked via glycoside bonds to an aminocyclitol [1]. The aminoglycosides are a broad-spectrum antibiotics which are widely used against gram-positive and gram-negative bacillary infections in both clinical and veterinary medicine.

Here we present a simple and sensitive analysis method for the determination of Neomycin, Tobramycin and Gentamicin in pharmaceutical preparations, using reversed-phase Liquid Chromatography (LC) and Pulsed Amperometric Detection (PAD) on a gold working electrode after post-column addition of sodium hydroxide.

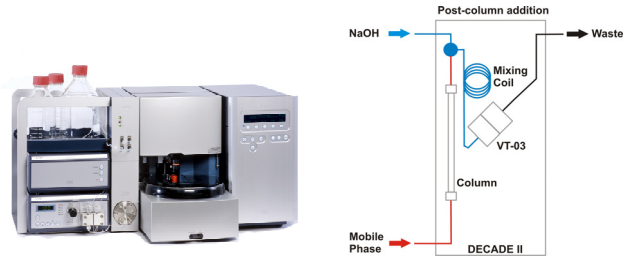

Fig 1. ALEXYS 100 Aminoglycosides I (part no 180.0050) with post-column addition.

Neomycin

Flow rate	0.7 mL/min, post-column: 0.5 mL/min
Mobile phase	2 % (v/v) trifluoroacetic acid, 2 mL/L 50% (w/w) NaOH, pH 0.85 – 1.0
Addition	0.5 mol/L NaOH, post column
Temperature	35 °C for column, mixing coil and flow cell
E-cell	E1, E2, E3: 0.1, 0.6, -0.15 Volt
	ts, t1, t2, t3: 0.1, 0.32, 0.2, 0.4 seconds
I-cell	100 - 800 nA

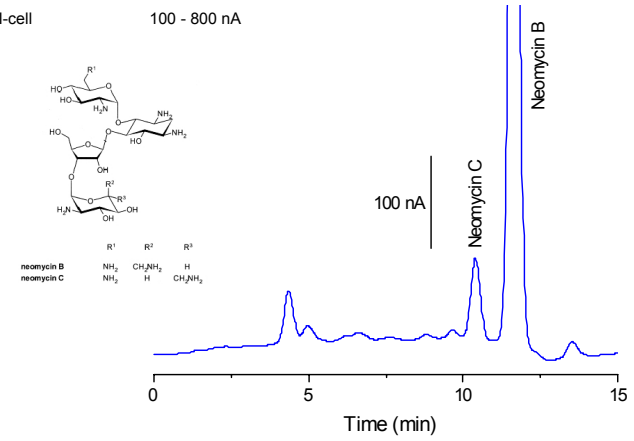

Figure 2. Chromatogram of 100 µg/ml Neomycin (10 µl injected), ADF 0.01 Hz. Resolution of neomycin B vs.C is 2.1. Theoretical plate number of Neomycin C and B is 21444 and 22956 plates/meter, respectively.

Reproducibility, Linearity & LOD (Neomycin)

The response for Neomycin C and B was linear in the concentration range up to 250 µg/mL (R^2= 0.998 and 0.996 for peak area, respectively). A Limit of Detection (LOD) of 85 ng/mL (~ 0.14 µmol/L) for Neomycin B was determined. For the calculation of the LOD (S/N =3) the peak-to-peak noise was used. These results were obtained with a 360 µm (stacked) spacer. Reproducibility in peak area and height was 1% for Neomycin C and B. Resolution of both peaks is 0.8.

Gentamicin

Flow rate	1 mL/min, post-column: 0.6 mL/min
Mobile phase	60 g/L Na_2SO_4, 1.75 g/L OSA, 8 mL/L THF, 10 mmol/L KH_2PO_4, pH 3.0
Addition	0.76 moL/L NaOH post column
Temperature	45 °C for column, mixing coil and flow cell
E-cell	E1, E2, E3: 0.1, 0.75, -0.15 V
	ts, t1, t2, t3: 0.1, 0.32, 0.2, 0.4 s
I-cell	~ 3 µA

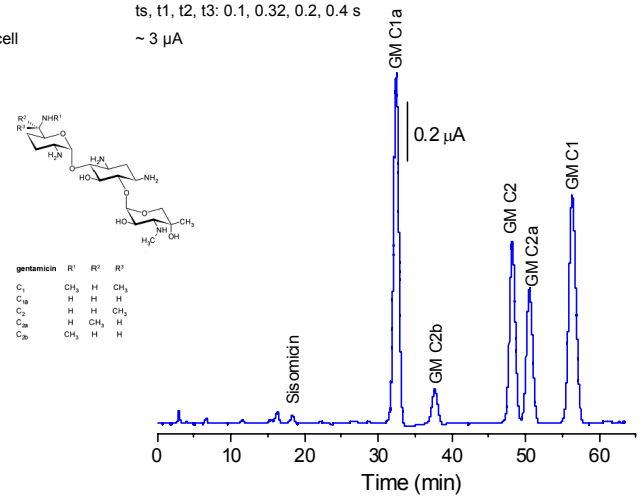

Figure 3. Gentamicin sample (500 µg/ml, 20 µl injected). Peak identities of Gentamicin C1, C1a, C2, C2a and C2b were derived from reference [2] and based on relative peak area percentages.

Tobramycin

Mobile phase	52 g/L Na_2SO_4, 1.5 g/L OSA, 3 mL/L THF, 10 mmol/L KH_2PO_4, pH 3.0
	Other settings identical as for the Gentamicin analysis.
I-cell	~ 2 µA

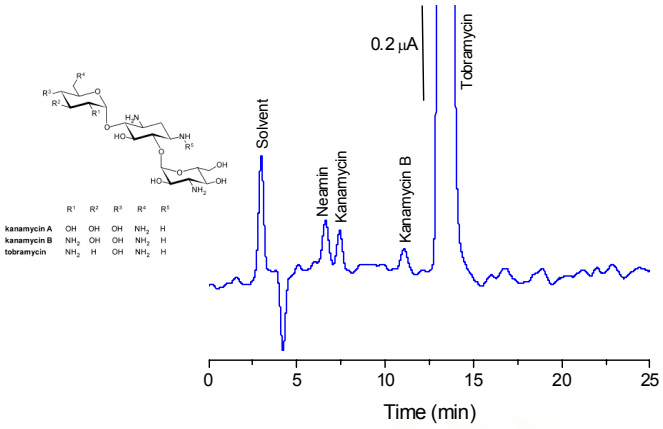

Figure 4. Tobramycin sample (100 µg/ml, 20 µl injected). The peak height of the Tobramycin peak is 2.32 µA. Impurities as percentage % of the main peak are: Neamin 0.38%, unknown 0.29%, Kanamycin B 0.19%.

References

[1] D.A. Stead, J. Chromatogr. B., 747, 69-93 (2000)

[2] E. Adams, J. Hoogmartens et al., J. Pharm. Biomed. Anal., 18, 689-698 (1998)