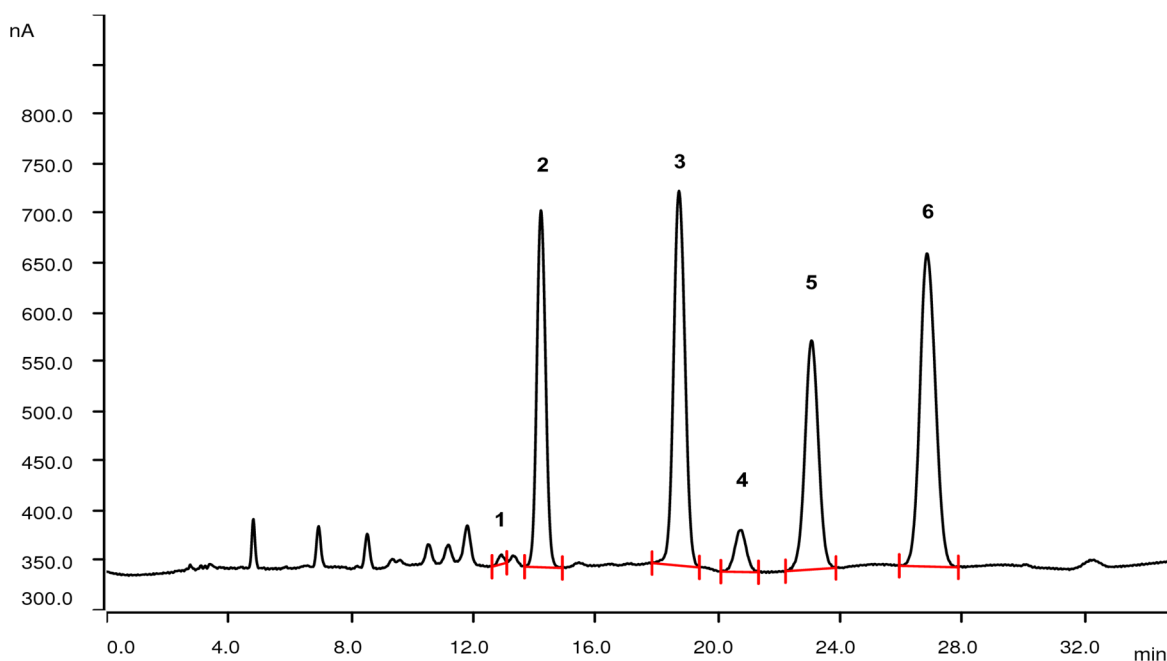


# Content of gentamicin according to USP applying pulsed amperometric detection



Gentamicin is an aminoglycoside antibiotic and is composed of a number of related gentamicins. It is applied for several types of infections. For the determination of the major components, USP asks for chromatographic separation with pulsed amperometric detection using a gold working electrode. A post-column addition of NaOH is performed prior to the detection.

## Results

Component	Acceptance Criteria NMT* [%]	Conc. measured [%]
1 Sisomicin	-	-
2 Gentamicin C <sub>1a</sub>	10–35	19
3 Gentamicin C <sub>2</sub>	25–55	44
4 Gentamicin C <sub>2a</sub>		
5 Gentamicin C <sub>2b</sub>	25–50	32
6 Gentamicin C <sub>1</sub>		

\* Not more than

## Sample

Gentamicin sample

## Sample preparation

Direct injection of a 20 mg/100 mL gentamicin solution.

## Columns

Phenomenex – Gemini 5  $\mu\text{m}$  C18 110Å

## Solutions

Eluent  
900 mL ultrapure water  
7 mL trifluoroacetic acid  
250  $\mu\text{L}$  pentafluoropropionic acid  
4 mL NaOH (12.5 mol/L, carbonate free)  
Adjust to pH = 2.5 with 0.5 mol/L NaOH (carbonate free)  
Add 15 mL acetonitrile  
Fill up to 1 L with ultrapure water (carbonate free)

Post column addition solution  
260 mmol/L NaOH

## Parameters

Flow rate 1.0 mL/min

Post column addition 0.3 mL/min

Injection volume 20  $\mu\text{L}$

Recording time 35 min

Column temperature 35 °C

## PAD Parameters

Cell Wall-Jet cell

Working electrode Gold

Reference electrode Ag/AgCl

Spacer 50  $\mu\text{m}$

Temperature 35 °C

Mode PAD

## Analysis

Pulsed amperometric detection

## Instrumentation

940 Professional IC Vario ONE/HPG 2.940.1140

IC Amperometric Detector 2.850.9110

889 IC Sample Processor – cool 2.889.0020

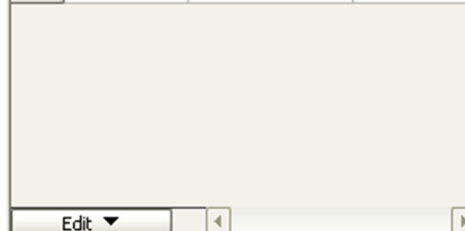
Au working electrode 6.1257.210

Ag/AgCl reference electrode 6.1257.720



## Potential profile

	Duration [ms]	Sum Duration [ms]	Potential [V]
▶ 1	400	400	0.05
2	150	550	0.75
3	450	1000	-0.15
*			

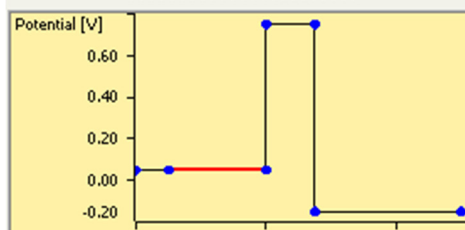


Cycle duration 1000 ms

## Measurement

Duration 300 ms

Range 200  $\mu\text{A}$



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