

Requirements

for Gentamicin analysis

using the ALEXYS[®] Gentamicin Analyzer (180.0056E)

180.7058, Edition 3, 2016



Warning Symbol



The warning sign denotes a warning. It calls attention to a procedure or practice which, if not adhered to, could result in costs, damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

For research purposes only. The ALEXYS system is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.

Observe safety

Operation of an electrochemical detector can involve the use of hazardous materials including corrosive fluids and flammable liquids. The instrument should only be operated by users with the following expertise:

- Completed degree as chemical laboratory technician or comparable vocational training
- Fundamental knowledge of liquid chromatography
- Knowledge and experience in the safe handling of toxic and corrosive chemicals and knowledge of the application safety measures prescribed for laboratories.
- Participation in an end-user training (daily use of system and chromatography software) performed by the manufacturer or a company authorized by the manufacturer.



Unskilled, improper, or careless use of the instrument and the related chemicals can create fire hazards, or other hazards which can cause death, serious injury to personnel, or severe damage to equipment and property.

Observe all relevant safety practices at all times.

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C H A P T E R 1

Introduction

Thank you for ordering an ALEXYS LC-ECD system. For a successful on-site installation of the Gentamicin analysis on the ALEXYS system, please arrange the following requirements at your location in advance:

- a computer (see document 195.7000 for the PC requirements)
- general laboratory conditions and facilities, consumables and chemicals for use with the ALEXYS system (see document 180.7070C)
- application specific chemicals and consumables listed in this document



Arrange these requirements well in advance before the installation to prevent (costly) delays.

This document lists the application specific chemicals for the analysis of Gentamicin using the following hardware:

ALEXYS® Gentamicin Analyzer (180.0056E)

CHAPTER 2

Chemicals

For LC-ECD applications, only chemicals of sufficient specific quality should be used to be able to have an optimal system with good performance. The appendix shows detailed descriptions of some of the chemicals that have been used in the Antec R&D laboratory, as an example of what works.



Have the chemicals and solutions ready at the start of the installation.

This document describes the preparation of the following mobile phase and post column solution composition, :

Mobile phase composition	60 g/L Na ₂ SO ₄ 1.75 g/L OSA 3 mL/L THF 10 mmol/L KH ₂ PO ₄ pH 3.0
Post-column solution	0.76 mol/L NaOH

Mobile phase and post-column solution

Chemicals

- Water (Resistivity >18MΩm.cm, TOC<10ppb)
- Sodium sulfate (Na₂SO₄)
- 1-Octane sulphonic acid, sodium salt (OSA)
- Tetrahydrofuran (THF), stabilised with 250 mg/L 2,6-di-tert.-butyl-4-methylphenol
- Potassium dihydrogen phosphate (KH₂PO₄)
- Phosphoric acid, 85% w/v in water
- 50% w/w NaOH in water (commercial solution)

Preparation

0.2 M phosphate buffer pH 3.0

1. Dissolve 27.22 g KH₂PO₄ (MW 136.09) in about 0.9 L demineralised water.
2. Set the pH to 3.0 with a solution of 85% H₃PO₄ in water.
3. Add demineralised water to a final volume of 1 L.

This solution can be stored in the fridge at 4°C for about one week

Mobile phase

1. Dissolve 120 g Na₂SO₄ in 1.8 L water with high resistivity/low TOC.
2. Add 3.5 g 1-octane sulphonic acid, sodium salt and stir till dissolve
3. Add 100 mL 0.2 M phosphate buffer, pH 3.0
4. Add 6 mL THF
5. Add demineralised water to a final volume of 2 L.
6. Degas the mobile phase in a sonic bath for about 15 minutes.

The mobile phase can be stored for 1 week at 4 °C and should be refreshed frequently when it is in the system at room temperature.



Daily check the mobile phase bottle in the system for microbial growth. Refresh the mobile phase **at least once every 3 days** or more often if bacterial growth is observed earlier!

Post-column NaOH solution

1. Pour 1.92 L of water with high resistivity/low TOC in an HDPE bottle.
2. Degas the water for 15 minutes in a sonic bath.
3. Add a clean stir bar.
4. Pipette 80 mL from the top part of the commercial 50% NaOH solution and add to the degassed water under gentle stirring.



Do not filter the solutions by any means. The 0.2 µm inline Whatman filters present in the low pressure solvent lines will take care of filtering.

System wash solutions

Chemicals

- Isopropanol
- Acetonitrile
- Water (Resistivity >18MΩ·cm, TOC<10ppb)

Preparation

Autosampler needle wash

- 250 mL water, degassed
Cap and store at room temperature until use (max 1 week)

Pump piston wash

- 500 mL 20% isopropanol in water, degassed
Cap and store at room temperature until use (max 1 month)

Column flushing solution

- 250 mL 20% acetonitrile, degassed
Cap and store at room temperature until use (max 1 month)

Column flushing/storage solution

- 250 mL 50% acetonitrile, degassed
Cap and store at room temperature until use (max 1 month)

A P P E N D I X

A list of the application specific chemicals with purity and purchase details is shown below as a guideline. The listed brands/purities are not necessarily the best chemicals, but these have been giving good results at the Antec R&D laboratory.

If for any reason alternative chemicals need to be purchased, be aware that chemicals that have a specification of high purity may have been tested for UV-active impurities, which can mean that they may still contain electrochemically active impurities. This is one of the reasons why general 'HPLC grade' water is not suitable for use with EC detection:

- choose chemicals with the same purity or better
- do not choose ultra dry grade chemicals

Table 1. Brands and purities of chemicals used for application development at Antec.

Component	Purity	Brand	Order no:	Mw	Kg/L
Ortho-Phosphoric acid, 85% w/v in water	p.a.	Fluka	79620	98.00	D:1.68
Potassium dihydrogen phosphate	>99%	Fisher	p/4806/60	136.09	
Disodium sulphate, anhydrous	99.0%	Baker	0313	142.04	
Acetonitrile	HPLC grade, 99.9%	Acros	268260025	41.05	D:0.781
NaOH, 50% w/v in water	puriss., p.a., for HPLC; 50%	Fluka	71686	40.00	D:1.54
Tetrahydrofuran, stabilised with 250 mg/L 2,6-di-tert.-butyl-4-methylphenol	Puriss. p.a. stabilised	Riedel-de-Haen	33709	72.11	0.887
1-Octane sulphonic acid, sodium salt (OSA)	HPLC grade	Acros	384771000	216.28	
NaOH, 50% w/v in water	puriss., p.a., for HPLC; 50%	Fluka	71686	40.00	D:1.54
Water	TOC <10ppb and deionised, resistivity >18 MOhm-cm (Barnstead Easypure II)				

Manufacturers/vendors

JT-Baker	http://www.avantormaterials.com
Sigma-Aldrich	http://www.sigmaaldrich.com
Fluka	http://www.sigmaaldrich.com
Fisher Scientific	http://www.fishersci.com
Barnstead	http://www.thermoscientific.com
LC Tech	http://www.lctech.de