Antec Scientific
Hoorn 131
2404 HH Alphen a/d Rijn
The Netherlands

Requirements

for Aminoglycoside analysis

using the ALEXYS® analyzer for Aminoglycosides

180.7056, Edition 4, 2022

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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CHAPTER 1

Introduction

Thank you for ordering an ALEXYS LC-ECD system. For a successful on-site installation of 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 various aminoglycosides using the following hardware:

ALEXYS® analyzer for Aminoglycosides

The instrumentation can be used to analyze the following aminoglycosides using post-column addition of NaOH and Pulsed Amperometric Detection: Neomycin, Framycetin, Gentamicin, Tobramycin, Netilmicin, Spectinomycin and Lincomycin.

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.

In most EP monographs for aminoglycosides the use of carbonate free mobile phases is described (both mobile phase for separation as well as the post-column NaOH solution). The ALEXYS system solutions for the analysis of aminoglycosides are delivered with the ET 210 Helium eluent tray and dedicated glass and plastic (PPCO) bottle assemblies to prepare and keep your mobile phases carbonate free. The ET 210 can be used for Helium sparging during mobile phase preparation and blanketing of the mobile phase with Helium gas during analysis.

A Helium 5.0 laboratory gas supply regulated to a pressure of 2 – 3 bar is required for operation. Red the manual of the ET 210 (pn 192.0010) carefully for more details about the requirements etc.. **Please make sure that a Helium 5.0 gas supply with the appropriate connections is available in your lab prior to installation.**

Laboratory equipment

- □ Magnetic stirring plate and stir bars
- □ Helium 5.0 laboratory gas supply (see previous section).

Fig. 1. ET 210 with connected PPCO mobile phase bottles.

Fig. 1. ET 210 glass bottle assembly for use with the acidic mobile phases for separation.

See the ET 210 manual (pn 192.0010) chapter 4 for details about how to prepare carbonate free mobile phases using the ET 210.

The general procedure is:

1. Pour the required volume of water in the glass or PPCO mobile phase bottle for preparation of the acidic mobile phase or alkaline post-column solution, respectively.
2. Degas the water for 15 min in a sonic bath.
3. Add a clean stir bar and sparge with Helium 5.0 for 15 min under gentle stirring.
4. Add the required mobile phase constituents to the degassed solution under gentle stirring and He sparging.
5. Install the bottle with Helium headspace pressure in the ET210 as described in the ET210 manual.

Note that in the next sections the preparation of the specific mobile phases for the different aminoglycosides are described without taking Helium sparging into account.

CHAPTER 2

General system wash solutions

Chemicals

- Isopropanol
- Acetonitrile
- Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparations

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)

Use the sonic bath for degassing.

Do not filter any of the solutions by any means. This also holds for the mobile phase and post-column solutions of every application mentioned in this document. The 0.2 µm inline Whatman filters present in the low pressure solvent lines of the system will take care of filtering.

C H A P T E R 3

Neomycin & Framycetin analysis

This chapter describes the preparation of the mobile phase and post column solution for the analysis of Neomycin and Framycetin:

Mobile phase	2% (v/v) TFA 8 mL/L 50% (w/w) NaOH pH 1.2
Post-column solution	0.5 mol/L NaOH

Mobile phase

Chemicals

- Trifluoroacetic acid (TFA), HPLC grade
- Water (Resistivity >18MOhm.cm, TOC<10ppb)
- 50% w/w NaOH in water (commercial solution)

Preparation

1. Add 40 mL Trifluoroacetic acid to about 1.8 L demineralized water.
2. Add 16 mL of 50% (w/w) NaOH solution
3. Add demineralized water to a final volume of 2 L.
4. Degas the mobile phase for 15 minutes in a ultrasonic bath.

Post-column solution

Chemicals

- Water (Resistivity >18MOhm.cm, TOC<10ppb)
- 50% w/w NaOH in water (commercial solution)

Preparation

1. Fill the HDPE bottle with 1.947 L demineralized water.
2. Degas the water for 15 minutes in a sonic bath.
3. Add a clean stir bar.
4. Pipette 53 mL from the top part of the commercial 50% NaOH solution and add to the degassed water under gentle stirring.

Prepare this solution shortly before use

C H A P T E R 4

Tobramycin analysis

This chapter describes the preparation of the mobile phase and post column solution for the analysis of Tobramycin:

Mobile phase	10 mmol/L KH_2PO_4 pH 3.0 52 g/L Na_2SO_4 1.5 g/L OSA 3 mL/L THF
Post-column solution	0.76 mol/L NaOH

0.2 M phosphate buffer pH 3.0 for mobile phase

Chemicals

- Potassium dihydrogen phosphate
- Water (Resistivity >18MOhm.cm, TOC<10ppb)
- Phosphoric acid, 85% w/v in water

Preparation

1. Dissolve 27.22 g KH_2PO_4 (MW 136.09) in about 0.9 L demineralized water.
2. Set the pH to 3.0 with a solution of 85% H_3PO_4 in water.
3. Add demineralized water to a final volume of 1 L.

Store the solution at 4°C for max 1 week

Mobile phase

Chemicals

- Disodium sulphate
- 1-Octane sulphonic acid, sodium salt (OSA)
- Water (Resistivity >18MOhm.cm, TOC<10ppb)
- Tetrahydrofurane (THF), stabilised with 250 mg/L 2,6-di-tert.-butyl-4-methylphenol.
  Do not take other qualities of THF, because it may negatively affect the background current and noise level.

Preparation

1. Dissolve 104 g Na_2SO_4 in about 1.8 L demineralized water.
2. Add and dissolve 3.0 g OSA
3. Add 100 mL 0.2 M phosphate buffer, pH 3.0

4. Add 6 mL THF
5. Add demineralized water to a final volume of 2 L.
6. Degas the mobile phase for 15 minutes in a sonic bath.

Post-column solution

Chemicals

- Water (Resistivity >18MOhm.cm, TOC<10ppb)
- 50% w/w NaOH in water (commercial solution)

Preparation

1. Fill the HDPE bottle with 1.92 L demineralized water.
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.

Prepare this solution shortly before use

C H A P T E R 5

Spectinomycin analysis

This chapter describes the preparation of the mobile phase and post column solution for the analysis of Spectinomycin:

Mobile phase	47 mM oxalic acid 15 mM heptafluorobutyric acid set to pH 3.2 with NaOH 10% (v/v) acetonitrile
Post-column solution	0.54 mol/L NaOH

Mobile phase

Chemicals

- Oxalic acid
- Heptafluorobutyric acid
- Acetonitrile, HPLC grade
- Water (Resistivity >18MOhm.cm, TOC<10ppb)
- 50% w/w NaOH in water (commercial solution)

Preparation

1. Add 8.4 gram oxalic acid to about 1.75 L demineralized water.
2. Add 4 mL heptafluorobutyric acid
3. Adjust the pH to 3.2 with a 50% NaOH solution
4. Add 200 mL acetonitrile
5. Add demineralized water to a final volume of 2 L.
6. Degas the mobile phase for 15 minutes in a ultrasonic bath.

Post-column solution

Chemicals

- Water (Resistivity >18MOhm.cm, TOC<10ppb)
- 50% w/w NaOH in water (commercial solution)

Preparation

1. Fill the HDPE bottle with 1.947 L demineralized water.
2. Degas the water for 15 minutes in a sonic bath.
3. Add a clean stir bar.
4. Pipette 53 mL from the top part of the commercial 50% NaOH solution and add to the degassed water under gentle stirring.

Prepare this solution shortly before use

C H A P T E R 6

Spectinomycin & Lincomycin analysis

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

Mobile phase	A	B
	100 mM phosphoric acid 100 mM citric acid pH 3.0 25 mg/L OSA 1 mL/L THF	100 mM phosphoric acid 100 mM citric acid pH 3.0 25 mg/L OSA 20 mL/L THF
Post-column solution	0.76 mol/L NaOH	

Mobile phase A and B

Chemicals

- □ Water (Resistivity >18MOhm.cm, TOC<10ppb)
- □ Phosphoric acid (commercial solution of 85% w/v in water)
- □ Citric acid, monohydrate
- □ 1-Octane sulphonic acid, sodium salt (OSA)
- □ Tetrahydrofuran (THF), stabilized with 250 mg/L 2,6-di-tert.-butyl-4-methylphenol
- □ 50% w/w NaOH in water (commercial solution)

Preparation

7. Dissolve 42.0 g citric acid (monohydrate) in about 1.8 L demineralized water.
8. Add 13.7 mL 85% phosphoric acid.
9. Set the pH to 3.0 with a solution of 50% NaOH in water.
10. Add and dissolve 50 mg OSA.
11. Add demineralized water to a final volume of 1.9 L.
12. Equally split the solution to two glass cylinders (950 mL each).
13. Add 1 mL THF to one cylinder and fill up to 1 L with demineralized water (mobile phase ‘A’).
14. Add 20 mL THF the other cylinder (add slowly while gently stirring to prevent the formation of an irreversible white cloud) and fill up to 1 L with demineralized water (mobile phase ‘B’).
15. Degas the mobile phases for 15 minutes in a sonic bath..

Prepare this solution shortly before use

Post-column solution

Chemicals

- □ Water (Resistivity >18MOhm.cm, TOC<10ppb)
- □ 50% w/w NaOH in water (commercial solution)

Preparation

5. Fill the HDPE bottle with 1.9 L demineralised water.
6. Degas the water for 15 minutes in a sonic bath.
7. Add a clean stir bar.
8. Pipette 100 mL from the top part of the commercial 50% NaOH solution and add to the degassed water under gentle stirring.

Prepare this solution shortly before use

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
Acetonitrile	HPLC grade, 99.9%	Acros	268260025	41.05	D:0.781
Disodium sulphate, anhydrous	99.0%	Baker	0313	142.04	
Heptafluorobutyric acid	99%	Acros	172800250	214.04	D:1.640
NaOH, 50% w/v in water	puriss., p.a., for HPLC; 50%	Fluka	71686	40.00	D:1.54
1-Octane sulphonic acid, sodium salt (OSA)	HPLC grade	Acros	384771000	216.28	
Ortho-Phosphoric acid, 85% w/v in water	p.a.	Fluka	79620	98.00	D:1.68
Oxalic acid, anhydrous	>99%	Fluka	75688	90.04	
Potassium dihydrogen phosphate	>99%	Fisher	p/4806/60	136.09	
Tetrahydrofurane, stabilised with 250 mg/L 2,6-di-tert.-butyl-4-methylphenol	Puriss. p.a. stabilised	Riedel-de-Haen	33709	72.11	D:0.887
Trifluoro acetic acid	HPLC grade	Fisher	T/3258/PB05	114.02	D:1.489
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