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Requirements

for Kanamycin / Amikacin analysis

using the ALEXYS® analyzer for Kanamycin/Amikacin

according to USP method

180.7060, Edition 3, 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 **Kanamycin Amikacin 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 (see this document)

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

C H A P T E R 2

Laboratory requirements

For Kanamycin-Amikacin analysis, keeping the mobile phase free of carbonate is one of the key factors towards reproducible retention times. The reason is that carbon dioxide gas present in air will dissolved as CO_3^{2-} ions in the strong alkaline eluent. The dissolved carbonate ions will increase the ionic strength of the mobile phase, resulting in a shortening of the retention times of the signals. To prevent this, He-degassing of the mobile phase is strongly advised.

The ALEXYS Analyzer for Kanamycin-Amikacin contains the ET 210 Helium eluent tray and necessary PPCO bottle and tubing assemblies for Helium sparging (mobile phase preparation) and blanketing (analysis) the mobile phase with Helium gas.

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 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.

C H A P T E R 3

Chemicals

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

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.

Prepare the solutions according to the USP document. Below is a list of guidelines for the required chemicals.

Mobile phase

The mobile phase that is applied for the separation of Kanamycin Amikacin is a solution of NaOH in water.

Do not prepare the mobile phase in glass bottles, as NaOH is a strong etching agent: the glass will release silicates and borates into the mobile phase in such case. Use the dedicated PPCO plastic bottle assemblies delivered with your system (Antec pn. 184.0205).

Chemicals

- 50% w/w NaOH in water, carbonate free (commercial solution)
- Water with resistivity of 18 MOhm.cm and TOC<10ppb

Preparation

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

1. Pour the required volume of water in the PPCO mobile phase bottle.
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. Pipette the required volume of NaOH solution from the top part of the commercial 50% NaOH solution and add to the degassed solution under gentle stirring.
5. Sparge the mobile phase for another 10 min under slow continuous He sparging before use.
6. Install the bottle with Helium headspace pressure in the ET210 as described in the ET210 manual.

Do not store this solution, but prepare when needed.

By any means do <u>not</u> filtrate the mobile phase. Filters can be a source of contamination.

System wash solutions

The system wash solutions should not contain any alcohols, as these will be detected when entering the analytical flow path. Therefore, pure water is used as wash solvent for both pistons and injector.

Chemicals

- Water with resistivity of 18 MOhm.cm and TOC<10ppb

Column regeneration solution (250 mM NaOH, 1M sodium acetate)

When retention times are shorter than expected or become shorter over time, the column may be regenerated by flushing it with about 30 bed volumes of strong eluting solution. This regeneration solution will wash off adsorbed components from the active sites of the ion-exchange column.

Equilibrate the column with 30 bed volumes of mobile phase after running the regeneration solution.

Chemicals

- 50% w/w NaOH in water, carbonate free (commercial solution)
- Water with resistivity of 18 MOhm.cm and TOC<10ppb
- Sodium acetate trihydrate (high purity grade)

Preparation

1. Dissolve 34 g sodium acetate trihydrate in 243 mL of water with high resistivity/low TOC
2. Pour the solution in an PPCO bottle.
3. Degas the solution for 15 min in a sonic bath.
4. Pipette 6.5 mL NaOH solution from the top part of the commercial 50% NaOH solution and add to the degassed solution under gentle stirring.
5. Sparge the mobile phase for another 10 min under slow continuous He sparging before use.
6. Install the bottle with Helium headspace pressure in the ET210 as described in the ET210 manual.

Do not store this solution, but prepare when needed.

A P P E N D I X

A list of 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 'HPLC grade' water is not recommended for use with EC detection:

- choose chemicals with the same purity or better
- do not choose ultra-dry grade or anhydrous chemicals
- do not make the NaOH solution from pellets as an alternative, as these contain high concentration of absorbed sodium carbonate on the surface.

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

Component	Purity	Brand	Order no	Mw	kg/L
Sodium hydroxide*					
Sodium hydroxide (NaOH), approx. 50% in water	Pro analyse, carbonate free	Boom	80011912	40.00	D:1.57
Sodium Hydroxide Solution (50% w/w/Certified)	Certified grade	Fisher Scientific	SS254500	40.00	D:1.56
Sodium hydroxide solution, 50-52%	Eluent for IC	Sigma Aldrich	72064	40.00	D:1.53
Sodium Acetate					
Sodium acetate trihydrate (CH3OONa.3H2O)	HPLC grade for EC detection	Fisher Scientific	S/2052/50	136.08	
Sodium acetate trihydrate (CH3OONa.3H2O)	>=99%, BP, Ph.Eur grade	Fisher Scientific	S/2000/60	136.08	
Sodium acetate trihydrate ($CH_3OONa.3H_2O$)	HPLC grade	Baker	0393	136.08	
Water	TOC <10 ppb and deionised, resistivity >18 MOhm-cm (Barnstead Easypure II)				

* The contents of Hydroxide in commercial hydroxide solutions specified on the bottles are always by approximation. Always use the actual contents of hydroxide as stated in the certificate of analysis to calculate the amount of solution needed to make mobile phases. The certificate of analysis may be delivered with the bottle or can be requested/downloaded from the manufacturer (web site).

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