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Requirements

for Monoamines/metabolites analysis

using the ALEXYS® Neurotransmitters system (180.0091UA)

180.7050u, Edition 7, 2025

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 **Monoamines analysis** on the ALEXYS system, please arrange the following requirements at your location in advance:

- a computer (see document 195.7000 ‘PC requirements and settings’)
- general laboratory conditions and facilities, consumables and chemicals for use with the ALEXYS system (see document 180.7070C ‘General requirements for installation of ALEXYS systems’)
- application specific chemicals and consumables (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 monoamines using the following combination of hardware:

ALEXYS® Neurotransmitters system (180.0091UA)

SenCell 2 mm GC sb (116.4120)

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.

C H A P T E R 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.

Standards

Chemicals

- Standards of the components of interest in high purity grade
- Perchloric acid
- Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

- 10 mL 1 mM of each individual standard in 0.1 M perchloric acid
  Store at 4 °C until use (max 1 month)

Calibrator solutions

Chemicals

- Chemicals for preparation of real sample background (e.g. homogenization solution or microdialysis perfusion solution)
- Acetic acid
- Water (Resistivity >18MOhm.cm, TOC<10ppb)

Preparation

- 100 mL 100 mM acetic acid
  Store at 4 °C until use (max 3 days)
- 10 mL microdialysis perfusion fluid, 10 x concentrated
  Store at 4 °C until use (max 1 month)

Suggestion for perfusion fluid composition:

Component	Concentration (mM)
NaCl	147
KCl	3
$MgCl_2.6H_2O$	1.2
$CaCl_2$	1.2

For a 10x concentrated solution, dissolve the salts in a volume 10 x smaller as calculated

- □ 1 mL of each: 0, 2, 4, 6, 8 and 10 nM of the mixed standards in a background of homogenization solution, or perfusion fluid acidified with 10 mM HAc.
  Store at 4 °C until use (max 1 day)

Mobile phase

Depending on the analysis requirement for monoamines and/or acidic metabolites, two different mobile phase starting conditions are available to run with the listed column.

Table 1. Starting conditions for the analysis of monoamines and acidic metabolites using ALEXYS Neurotransmitter Analyzer.

Analysis	**Monoamines (incl. NA)**	**DA & 5-HT**	**Monoamines & metabolites**
Column	Acquity UPLC BEH C18, 100 x 1 mm ID, 1.7µm (Waters pn. 186002346)	Acquity UPLC BEH C18, 50 x 1 mm ID, 1.7µm (Waters pn. 186002344)	Acquity UPLC BEH C18, 100 x 1 mm ID, 1.7µm (Waters pn. 186002346)
Flow rate	50 uL/min	50 uL/min	50 uL/min
Ecell	460 mV vs. salt bridge	460 mV vs. salt bridge	800 mV vs. salt bridge
Mobile phase	100 mM phosphoric acid 100 mM citric acid 0,1 mM EDTA pH 6.0 600 mg/L OSA 8% v/v acetonitrile	100 mM phosphoric acid 100 mM citric acid 0,1 mM EDTA pH 6.0 600 mg/L OSA 8% v/v acetonitrile	100 mM phosphoric acid 100 mM citric acid 0,1 mM EDTA pH 3.0 600 mg/L OSA 8% v/v acetonitrile

Note 1: once used for monoamines, that column should be kept dedicated to this particular analysis. The reason is that the ion-pairing agent is difficult to wash off a column and will affect subsequent analyses when run without ion-pairing agent in the mobile phase.

Note 2: these conditions are suggested starting conditions and not guaranteed to give best results for all kinds of unknown samples. Condition optimization may be necessary.

Chemicals

- □ Water (Resistivity >18MOhm.cm, TOC<10ppb)
- □ Phosphoric acid (commercial solution of 85% w/v in water)
- □ Citric acid, monohydrate
- □ Di sodium ethylenediaminetetraacetic acid (EDTA. Na_2)
- □ Octane sulphonic acid, sodium salt (OSA)
- □ Acetonitrile
- □ 50% w/w NaOH in water (commercial solution)

Preparation of the 2x concentrated stock solution for mobile phase

1. Add 0.4 liter water and a clean stir bar to a clean wide glass beaker on a stirring plate.
2. Add 0.0372 g $Na_2EDTA.2\ H_2O$ and stir till dissolved.
3. Add 6.86 mL 85% w/v phosphoric acid solution.
4. Add 21.02 g citric acid and stir till dissolved.
5. Set the pH of the mobile phase to the correct pH (see Table 1), using the 50% NaOH solution.
6. Fill up to 0.5 liter with water

Store at 4 °C until use (max 1 month)

Preparation of the mobile phase

7. Add 100 mL of the mobile phase stock solution to a glass bottle.
8. Add 120 mg of OSA
9. Add 16 mL acetonitrile
10. Add water to a total volume of 0.2 liter.
11. Degas the mobile phase for 15 minutes in a sonic bath.

Store at 4 °C until use (max 1 week)
Refresh within 3 days if kept at room temperature on the system.

Do not filter the mobile phase; the 0.2 µm inline Whatman filters present in the low pressure solvent lines of the ALEXYS system will take care of filtering.

When making a larger batch to last a week, a small batch of the mobile phase can be poured in a smaller bottle and connected to the system; visually inspect the mobile phase daily for microbial growth. Refresh mobile phase that is standing in room temperature **at least every 3 days** or more often if microbial growth is observed.

The mobile phase that is connected to the system (at room temperature) should be refreshed at least every 3 days

System wash solutions

Chemicals

- □ Isopropanol
- □ Acetonitrile
- □ Water

Preparation

- □ 250 mL water, degassed (autosampler needle wash solution)
- □ 1 L 20% isopropanol in water, degassed (piston wash)
- □ 250 mL 20% acetonitrile, degassed (column flushing)
- □ 250 mL 50% acetonitrile, degassed (column flushing/storage)

A P P E N D I X

A list of general use 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

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
Citric acid, monohydrate	p.a.	Acros	124910010	210.14	
$Na_2EDTA.\ 2\ H_2O$	SigmaUltra, 99%	Acros	147855000	372.23	
1-Octane sulphonic acid, sodium salt (OSA)	HPLC grade	Acros	384771000	216.28	
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
Perchloric acid	p.a.	Acros	223310010	100.46	1.66
Acetic acid	99.8%, for analysis	Acros	222140010	60.05	1.048
Water	TOC <10 ppb 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