

INSTALLATION REQUIREMENTS FOR THE ALEXYS[®] OMD MONOAMINES ANALYZER

This document lists the requirements for the installation of the ALEXYS[®] OMD Monoamines analyzer (p/n 180.0081C). It also summarizes all relevant conditions, chemicals and consumables to run the OMD monoamines application.

The customer must arrange the listed necessities before the start of the installation. Therefore, this document should be send to the customer well in advance of the installation date in order for them to be able to take the necessary actions.

Computer

The minimal PC hardware and software requirements for the installation of the ALEXYS[®] system in combination with the Clarity[®] HPLC data software are listed in document 195.7000.

Laboratory facilities

- For the preparation of the LC solutions and standards, access to the proper facilities is a prerequisite (microbalance, pH meter and relevant pH standards, analytical pipettes, pipette tips, tubes, glassware such as measuring cylinders, etc.).
- An ultra sonic bath is required for degassing the mobile phase, the piston backwash solution and the auto sampler wash liquid before installation in the ALEXYS[®] system.

Do not use vacuum filtering units for degassing or filtering because it can introduce electrochemically active contaminations. The ALEXYS[®] system is equipped with in-line filters and degassers.

System consumables

The following items should be available for flow cell cleaning:

- Soft paper tissue (for instance Kleenex facial tissues)
- A squeeze bottle with acetone
- A squeeze bottle with deionised water

CHEMICALS

General

- All relevant chemicals should be available at the lab at the moment of installation to make mobile phase, standards, reagent etc.
- For LC-ECD only chemicals of sufficient specific quality should be used to be able to set up an optimal system with good performance.

Note: chemicals that are highly purified for application with UV detection may contain electrochemically active impurities! Therefore, HPLC grade water that was tested for UV-active impurities is not recommended for use with EC detection. Instead, use **deionised water with a resistivity of at least 18 MOhm-cm and TOC free.**

- See the appendix for more detailed descriptions of the chemicals that have been used in the Antec R&D laboratory, as an example.

System chemicals

The following chemicals are necessary for general system performance (piston wash solution of the pump, check valve cleaning, column cleaning, column storage, and flow cell cleaning).

- Iso-propanol (check valve cleaning)
- Demineralised water with a resistivity of at least 18 MOhm-cm
- Acetone (flow cell cleaning)
- Methanol
- Acetonitril (column storage)

Application-specific chemicals

For the analysis of NA, DA, 5-HT, L-DOPA and the acidic metabolites, the following chemicals are necessary:

- Phosphoric acid (we recommend the commercially available solution of 85% w/v in water)
- Citric acid, monohydrate
- Potassium chloride
- Ethylenediaminetetraacetic acid (EDTA)
- Octane sulfonic acid, sodium salt (OSA)
- Methanol
- Demineralised water with a resistance of at least 18 MOhm-cm
- 50% w/w NaOH in water (commercially available solution)
- Standards of the components of interest in high purity grade
- Appropriate chemicals to prepare standard stock solutions

Solutions

The following solutions should be available at the start of the first installation day. These solutions should thus be arranged/prepared in advance by the customer.

- A small bottle with about 50 mL of 15% HNO₃ is needed once during the installation for passivation of the metal parts of the ALEXYS system.
- 1 L demineralised water, degassed
- 250 mL 20% (v/v) MeOH in demineralised water, degassed

Mobile phase for the analysis of the catecholamines and metabolites

Two different mobile phases are required for the analysis of neurotransmitters and the metabolites. One mobile phase composition is specific for the analysis of DA, 5-HMT and 5-HT. The second mobile phase will result in the separation of L-DOPA, NA 3-OMD and the acidic components.

Table 1. Mobile phase composition for the analysis of catecholamines and metabolites

	Flow path 1	Flow path 2
Analysis	Neutral components	NA and acidic components
Columns	ALF-105	ALF-115
Mobile phase composition	50 mM phosphoric acid 0,1 mM EDTA 12,5% methanol 500 mg/L OSA pH 6.0	50 mM citric acid 50 mM phosphoric acid 0,1 mM EDTA 7.5 % methanol 600 mg/L OSA pH 3.1

Preparation of 1 L mobile phase

- Dissolve 0.0292 g EDTA in about 20 mL demineralised water, with 2-3 drops of 50% w/w NaOH solution, in a small glass beaker.
- In a large glass beaker containing about 0.75 L demineralised water, add 3.45 mL 85% w/v phosphoric acid solution. Also dissolve 10.51 g citric acid monohydrate at this point when preparing the mobile phase of flow path 2.
- Transfer the dissolved EDTA solution to the mobile phase.
- Set the pH of the mobile phase to the correct pH (see Table 1) using 50% NaOH solution.

- Add 75 or 125 mL methanol (see Table 1).
- Fill up to 1 L with demineralised water.
- Dissolve 500 or 600 mg OSA in the mobile phase (see Table 1).
- Degas the mobile phase.

APPENDIX

A list of chemicals is shown below as a guideline for the purchase of chemicals at the customer site. The listed brands/purities are not necessarily the best chemicals, but the application was developed at the Antec R&D laboratory using these specific brands/purities. If for any reason alternative chemicals need to be purchased use the following guidelines:

- The chemicals should have at least the same purity or better than the chemicals listed in the table below
- Do not purchase ultra dry grade or anhydrous chemicals

Table 2. Brands and purities of chemicals used for application development at Antec.

Component	Purity	Brand
1-Octane sulphonic acid, sodium salt (OSA)	HPLC grade	Acros
Acetone	General purpose grade	Fisher
Citric acid, monohydrate	p.a.	Acros
Ethylenediamine-tetraacetic acid (EDTA)	99%	Acros
Isopropanol	pure (>99.99%)	Acros
Methanol	HPLC gradient grade	Baker
NaOH, 50% w/v in water	puriss., p.a., for HPLC; 50%	Fluka
Phosphoric acid, 85% w/v in water	p.a.	Acros
Water	TOC free (<10 pbb) and deionised to resistivity >18 MOhm-cm (Barnstead Easypure II)	

Manufacturers

ACROS Organics

JT-Baker

Fluka

Fisher Scientific

Barnstead

Sigma-Aldrich