INSTALLATION REQUIREMENTS FOR THE ALEXYS® MONOAMINES ANALYZER

In this document the requirements for the installation of the ALEXYS® Monoamines analyzer (p/n 180.0088A) are listed. It summarizes all relevant conditions, chemicals and consumables that are required at the customer site for a successful installation and to run the monoamines application. This document should be send to the customer well in advance of the installation date in order to be able to take the necessary actions. The customer must arranged these necessities before the start of the installation.

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 of the mobile phase and auto sampler wash liquid before use in ALEXYS® system. Do not use vacuum filtering units because it can introduce electrochemical contaminations.

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

We recommend the following vials in combination with air-caps, which are available at Grace Alltech. Any equivalent from an other supplier can also be used.

Table 1. Recommended autosampler vials. These are available at Grace Alltech.

Sample	Vial type & dimensions	p/n (Grace Alltech)
Standards	1.5 mL Snap Ring Vials, 32 x 11.6mm, clear	98030
Small volumes (microdialysates)	Topsert (tm) TPX-short thread Vial, 32 x 11.6mm with 2 mL glass micro inserts	AV061890

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Note that the ALEXYS monoamines analyzer comes with a set of aluminium adapters for fraction collector vials. In that case fraction collector vials (35 x 5.5 mm OD) can be directly transferred to the autosampler without loss of sample. Do not use fraction collector vial caps that are deeper than 4 mm (see vials and caps on the right-side on the photos shown below), these caps cannot be penetrated by the AS 100 pre-puncturing needle.

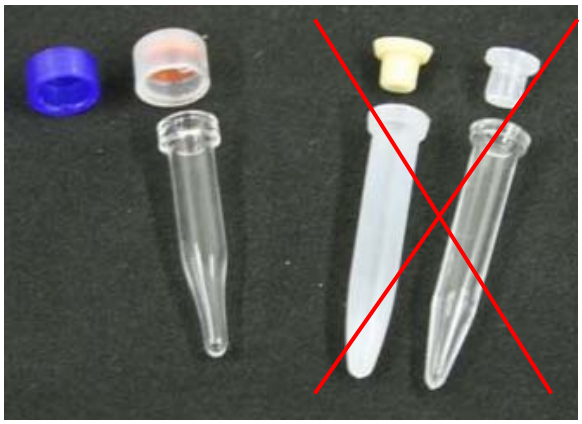

Table 2. Recommended fraction collector vial and caps. These are available at VWR-Omnilabo. See vial and caps on the leftt-side on the photos shown above.

Part	P/N (VWR-Omnilabo)
Vial conic glass (0.3 mL)	548-0078
Cap blue	548-0810
Cap re-pierceable	548-0374

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-EC only chemicals of sufficient specific quality should be used to be able to set up an optimal system with good performance. Note that chemicals that are highly purified for the use with UV detection may contain electrochemically active impurities! For example, HPLC grade water (tested for UV-active impurities) is not recommended for EC detection. Instead, use deionised water with a resistivity of at least 18 MOhm-cm.

- See the appendix for more detailed descriptions of the chemicals that are use in the Antec R&D laboratory.

System chemicals

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

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

Application-specific chemicals

For the analysis of NA, DA, 5-HT 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 (only necessary if interested in the acidic metabolites)
- Sodium 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
- Perchloric acid (as a matrix for standard stock solutions)

Solutions

At the start of the installation the following solutions are necessary on day 1. These solutions should be arranged/prepared in advance by the customer. The customer will also be asked to prepare mobile phase on day 1.

- About 50 mL of 15% HNO_3 is needed once during the installation for passivation of the metal parts of the ALEXYS system.
- 500 mL 20% (v/v) MeOH in demineralised water, degassed
- 250 mL 20% (v/v) isopropanol in demineralised water, degassed
- 1 L demineralised water, degassed

Mobile phase for the analysis of the catecholamines and metabolites (optionally)

Two different mobile phases are required for the analysis of NA, DA, 5-HT with or without the metabolites. One mobile phase composition is specific for the analysis of DA and 5-HT. For the second mobile phase a choice can be made between a composition that will result in the detection of NA only (pH 6.0), or NA and the acidic metabolites (pH 3.25). The choice to analyse only NA on the second flow path will reduce the run time significantly.

Table 3. Mobile phase composition for the analysis of catecholamines and metabolites (optionally).

Flow path	Flow path 1, column ALF-105	Flow path 2, column ALF-115	
Analysis	DA and 5-HT	NA (no metabolites)	NA and metabolites
Mobile phase composition	50 mM phosphoric acid 8 mM NaCl 0,1 mM EDTA 12,5% methanol 500 mg/L OSA pH 6.0	50 mM phosphoric acid 8 mM NaCl 0,1 mM EDTA 7.5 % methanol 500 mg/L OSA pH 6.0	50 mM phosphoric acid 50 mM citric acid 8 mM NaCl 0,1 mM EDTA 10 % methanol 500 mg/L OSA pH 3.25

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, dissolve 0.468 g NaCL and add 3.45 mL 85% w/v phosphoric acid solution. Also dissolve 10.51 g citric acid at this point if interested in the acidic metabolites.
- Transfer the dissolved EDTA solution to the mobile phase.
- Set the pH of the mobile phase to the correct pH (see Table 3) using 50% NaOH solution.
- Add the required volume of methanol (see Table 3).
- Fill up to 1 L with demineralised water.
- Degas the mobile phase.
- Dissolve 0.500 g OSA in 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 GABA/Glu 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 then the chemicals listed in the table below
- Do not purchase ultra dry grade or anhydrous chemicals

Table 4. 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
Perchloric acid (PCA)	p.a.	Acros
Phosphoric acid, 85% w/v in water	p.a.	Acros
Sodium chloride	Baker Analyzed, > 99.5%	Baker
Water	Deionised, resistivity >18 MOhm-cm (Elga UHQ apparatus)	

Manufacturers

ACROS Organics
JT-Baker
Fluka
Fisher Scientific