Agilent Amino Acid (AA)

Datasheet

General Description

The Amino Acid (AA) column contains a silica based reversed phase material having “C-18 functionality”. The packing material is fully endcapped to optimize efficiency and resolution for the separation of amino acid derivates.

Packing used in the AA column is specially designed and tested for the analysis of amino derivates, but is also generally suitable for the analysis of moderately polar solutes, acids or neutrals and lipophilic compounds. The reversed phase packing material is based on fully porous spherical silica particles with a pore size of 120Å, a pore volume of 0.65 mL/g, and a surface area of 170m^2/g. The columns are loaded to a stable, uniform bed density using Agilent's proprietary packing technique to give maximum column efficiency, longevity and column reproducibility.

A technical note describing the use of this column for amino acid analysis in protein hydrolysates is available on the Agilent website. Please go to www.agilent.com/chem and enter 5968-5658E in the search field to locate this publication.

Shipping Solvents

All columns are shipped with the solvent used for the final test of the column. For detailed specification see the individual test chromatogram on the Column Performance Report enclosed with your column.

Unpacking

Inspect the column immediately upon its arrival. If there are any signs of damage, notify your local Agilent representative at once. Record the column type and serial number, purchase date and operating limits. Keep a record of column usage along with your test chromatogram. This record will be invaluable in diagnosing chromatographic problems.

Installation

Before you install the column in your LC system, make sure that the solvent lines are cleaned and filled with HPLC-grade solvent. When the solvent is flowing freely from the capillary outlet, connect the capillary to the column. Attach the column outlet to the detector. Set the solvent flow according to the i.d. of the column. You must wash out the shipping solvent and condition the column with the required mobile phase prior to using it. Flushing with a minimum of 10 column volumes is recommended.

Typical wash volumes for different column dimensions:

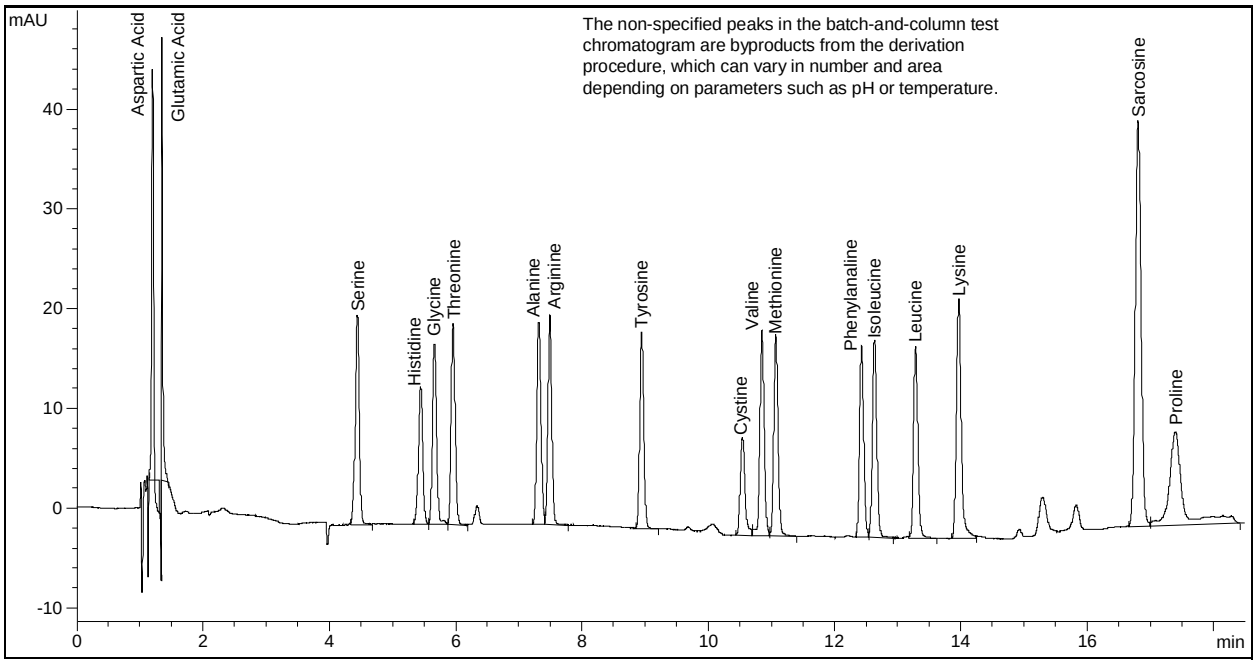
i.d. length	2.0mm 2.1mm	3.0mm	4.0mm	4.6mm
50 mm			3.5 mL	
60 mm				10 mL
75 mm			5 mL	
100 mm	4 mL			17 mL
125 mm	5 mL	9 mL	16 mL	
200 mm	7 mL			33 mL
250 mm	9 mL	18 mL	35 mL	41 mL

Column Characteristics

Figure 1 shows a typical batch QA chromatogram performed with a 2.1 x 100 mm column. Each batch of AA packing material is thoroughly QA tested with respect to selectivity, capacity factor and stability. These data have to be comparable with a mother batch to ensure maximum batch-to-batch reproducibility. The individual column QC test chromatogram provided with your column gives you information about the packing quality of each single separation column. Each individual column has to meet the specifications for plate numbers and tailing factor.

Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the toxicity or flammability of their mobile phases.
- Because of the small particle size, dry AA packings are respirable. Columns should only be opened in a well-ventilated area.



Operational Guidelines

- The direction of flow is marked on the column.
- While generally not harmful to the column, reverse flow should be avoided except to attempt removal of inlet pluggage (see "Maintenance" section).
- A new column contains a mixture of methanol and water. Initially, care should be taken not to pass any mobile phase through the column that might cause a precipitate.
- Agilent AA columns are compatible with water and all common organic solvents.
- The use of an AA guard column is recommended to protect the Agilent AA column and extend its useful lifetime.
- Avoid use of this column below pH 2.0 or above pH 8.
- Maximum operating pressure for columns is 400 bar (6000 psi).
- Maximum operating temperature is 60°C.
- **NOTE:** All silica-based packings have some solubility in pH > 6 aqueous mobile phases. Therefore, when using silica-based columns under conditions of pH > 6, maximum column lifetime is obtained by operation at low temperatures (< 40°C) using low buffer concentrations in the range of 0.01 to 0.02M. Column stability at pH > 6 is also enhanced by avoiding phosphate and carbonate buffers [ref.: H.A. Claessens, M.A. van Straten, and J.J. Kirkland, *J. Chromatogr.*(A), 728 (1996) 259].

Maintenance

If the column is to be in use on a daily basis, it can be left in buffer/organic solvent overnight by continually flushing the column with a low flow rate. If the column is not going to be used for several days, wash out any buffers with a compatible water-methanol or water-acetonitrile mixture. Remove the column from the system and replace the end-plugs.

After the column has been in use for an extended period of time, the column may show reduced efficiency together with increased backpressure. This may be caused by impurities in sample or solvents. A column can often be restored by pumping a strong solvent through. Solvents such as methanol, acetonitrile, or a 95/5 mixture of dichloromethane and methanol should remove most highly retained compounds. Sometimes diluted acids (hydrochloric acid or nitric acid) may be of a great help.

When switching between solvents with vastly different polarities, it may be necessary to first purge the column with a mutually miscible solvent such as isopropanol under reduced flow.

Reversing the direction of flow can be helpful to reduce backpressure. If this does not help, the inlet filter might be blocked and has to be replaced. We recommend that you test the column periodically, either with your own standard or preferably according to the conditions specified in the test chromatogram.

To All Agilent AminoQuant Series II Users

Lysine Peak Symmetry

When doing amino acid analysis according to the AminoQuant instructions, as described in the Operator's Handbook, it has been reported that in a few cases Lysine shows a tailing peak. If required, the symmetry of the Lysine peak is improved by adding ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) to the mobile phase for channel A. The instructions for preparing mobile phase A with EDTA are described below.

Preparation of Mobile Phase for Channel A EDTA

See also page 1 - 3 for preparing the chemicals in your Agilent AminoQuant Series II Operator's Handbook. (part number 01090-90025)

The mobile phase for channel A is a 20 mM sodium acetate buffer containing 0.018 % (v/v) triethylamine (TEA), adjusted to pH 7.2 with 1 - 2 % acetic acid, and with 0.3 % tetrahydrofuran (THF) and 50 µl EDTA stock solution added. The following instructions are for 500 ml of channel A buffer.

NOTE: This solution does not keep for long periods of time. To avoid contamination, do not prepare more mobile phase than you will need for two days work. Discard any unused buffer.

I. Preparing EDTA stock solution Dissolve 4 g ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) in 100 ml water.

II. Preparing mobile phase for channel A

1. Weigh 1.36 ± 0.025 g of sodium acetate tri-hydrate and transfer it into the 800 ml beaker.
2. Add 500 ml of purified water.
3. Add 50 µl of EDTA stock solution (I).
4. Add stirring bar and stir gently on mechanical stirrer.
5. When all the salt crystals are completely dissolved, pipette 90 µl of TEA and add it to the solution in the beaker.
6. Continue stirring the solution until the TEA is homogeneously mixed.
7. Rinse the tip of your pH electrode and place it in the solution.
8. Adjust the pH to 7.2 ± 0.05 by adding a few drops of 1 - 2 % acetic acid with a clean Pasteur pipette.

NOTE: When the pH of the solution gets below 8.0, add the acetic acid more slowly to avoid overshooting.

9. Remove the pH electrode from the beaker, rinse the tip with distilled water and store it according to the manufacturer's instructions.
10. Pipette 1.5 ml of THF and add it to the solution in the beaker.

NOTE: To pipette the THF satisfactorily, you may find it necessary to draw up and release the contents of the pipette back into the bottle before measuring the 1.5 ml.

11. Continue stirring the solution until the THF is homogeneously mixed.
12. Transfer the contents of the beaker to an AminoQuant buffer reservoir and stopper it. Label the reservoir Channel A.

Agilent Ordering Information

For more information on our products, visit our Agilent Technologies home page on the World Wide Web at: www.agilent.com/chem/supplies
For Technical Support in the US and Canada, call 1-800-227-9770 or call your local Agilent sales office.

NOTE

The serial number of our columns consists of the letters "US" and 8 digits. Do not use the letters "US" to program the column identification module (P/N 5062-8588). For fulfilment of GLP and GMP needs the column is already fully identified by 8 digits in conjunction with the product number.

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Printed in USA June 29, 2005 Part No. 820105-001 A