User guide

Amino acid trap column

# Column care and use

## Introduction

Amino acids are electrochemically active and can be detected using pulsed amperometric detection (PAD) at high PH on gold working electrodes. Therefore, in HPAEC-PAD carbohydrate analysis, the presence of amino acids in samples might negatively affect the quantification in case they coelute with the sugars of interest. Moreover, the presence of amino acids (AA) might also attenuate the detector response for the sugars due to incomplete removal of the formed AA oxidation products (fouling) under the applied 4-step PAD waveform.

The Antec Scientific 2.1 x 50 mm amino acid trap, 5 µm (pn 260.0041) is a precolumn specifically designed to temporarily trap amino acids which might be present in sugar samples to avoid interference during the analysis of monosaccharides. For example, the analysis of monosaccharides released after acid hydrolysis of glycoprotein or heparin samples. Proteins can be glycosylated in a variety of ways and a monosaccharide compositional analysis can often reveal the nature of the carbohydrate attachments. In addition, monosaccharide compositional analysis is performed to judge the consistency of glycosylation of a purified glycoprotein produced through recombinant techniques for use as a human therapeutics. The release of the glycans from the protein backbone and subsequent break up into its component monosaccharides is accomplished by acid hydrolysis with a volatile acid, like trifluoroacetic acid (TFA). Besides the sugars also some amount of amino acids and small peptides will be generated during hydrolysis. If the concentration of amino acids, in particular lysine, is high enough compared to that of the released mono-saccharides, they will interfere with the monosaccharide quantification. See Antec application note 220.024.

The amino acid trap column is installed in series before the analytical column instead of a regular precolumn. The trap column will enable the delayed elution of the amino acids assuring optimal separation and quantification of the monosaccharides (see figure 2, note that in this example a chromatogram obtained with a 4 x 50 mm version of the amino acid trap column is shown, however these data are also representative for a 2.1 x 50 mm trap column in combination with a SweetSep™ AEX20 2.1 x 200 mm).

Subsequently, the amino acids are eluted during the wash/ regeneration step at high pH, without fouling the Au electrode surface. Please read and follow the guidelines below carefully to ensure optimal column performance and maximum lifetime.

## Requirements

To assure a optimum lifetime & performance of the amino acid trap column take care of the following:

- The amino acid trap should never be exposed to pure water because it will damage the column irreversibly.
- Always use high purity chemicals and DI water (> 18.2 Ohm.cm, TOC < 5 ppb) to prepare mobile phases and solutions used in sample preparation. Do not use bottled HPLC water.
- Borosilicate glass bottles should be avoided at all time in HPAEC-PAD analysis, because borate can leach out of the glassware at high pH.

## Shipping & storage eluent

The amino acid trap columns are shipped with 20 mM NaOH as storage solution. Columns are securely sealed with end-plugs on inlet and outlet, which needs to be re-placed when the column is disconnected from the system to prevent column drying out. For storage flush the column for 15 minutes with the storage solution and seal the column securely with the supplied end-plugs.

## Precautions

Columns should be handled with care, as every drop or shock can potentially damage the column or the column bed. SweetSep™ columns are stabile over the full pH range (0 - 14), but are typically operated in the pH range 12 - 14 in carbohydrates analysis. The maximum pressure limit of the amino acid trap column is 275 bar/ 4000 psi. Furthermore, the columns can be used in the temperature range of 5 - 60°C. Do not operate the columns beyond the rated pressure limit and temperature limits, because it can lead to loss of performance or damage. Do not expose the column to sudden system pressure drops.

# Amino acid trap column care and use

## Installation & start-up

Connect the column with suitable PEEK one-piece finger-tights for use up to 300 bar (4350 psi) , such as Vici JR-5518 in combination with tightening tools for 1/16” Hex-Head Fittings (ZNFT). These parts can be ordered from Antec Scientific under pn 250.1572A and 250.0094, respectively.

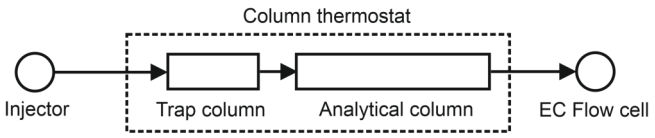


*Fig 1. Column configuration.*

Do not use stainless steel nuts and ferrules because they might damage the column threading of inlet and outlet. Use a short length of 7 cm of 0.005” ID 1/16” OD PEEK tubing (red-striped) to connect the trap column before the analytical column in the flow path according the flow direction indicated on the column label. The typical back pressure of the trap column at 0.18 mL/min will be about 13 to 23 bar using 10 mM NaOH as mobile phase at 30°C. It is normal for the amino acid trap column to cause slightly more band broadening (i.e., the monosaccharide peaks will get wider) than a typical precolumn.

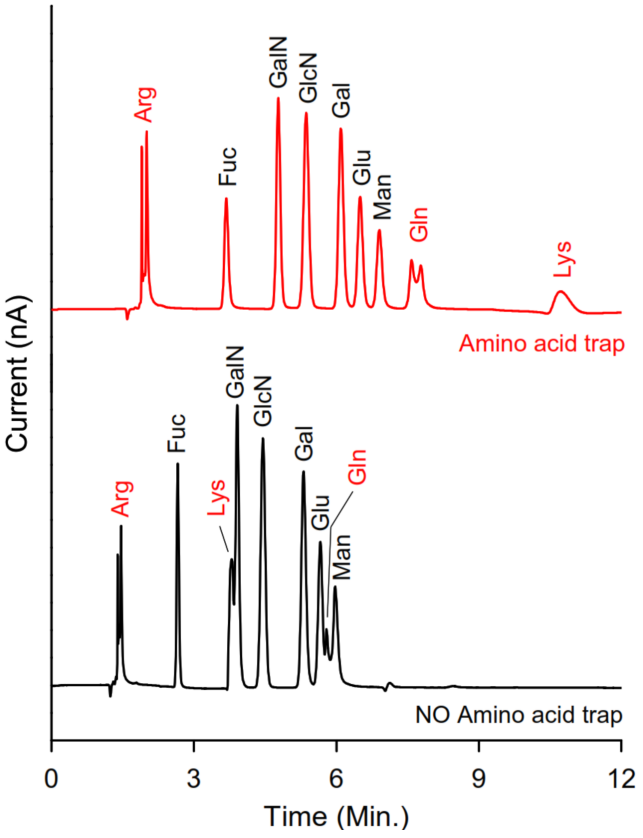


*Fig 2. Effect of the amino acid trap on the elution of interfering amino acids during monosaccharide analysis. Conditions: separation on SweetSep™ AEX20 4 x 200 mm column using a 20 mM NaOH eluent (30°C, 0.7 mL/min). Injected sample: 10 µL of a standard mix in DI water of 10 µM monosaccharides (fucose, galactosamine, glucosamine, galactose, glucose and mannose) and 1 mM amino acids (arginine, lysine and glutamine). Top (red): with amino acid trap; Bottom (black): without amino acid trap. Amino acids marked in red.*

## Troubleshooting

The malfunction of the amino acid trap column is frequently mistaken for a failure of the analytical column. If you are observing a significant loss in performance (resolution, efficiency, asymmetry) of monosaccharide peaks, first remove the amino acid trap column to determine if that improves the chromatography of a standard. Verify the performance with the data & criteria in the QAR delivered with your analytical column. In the case of a significant loss of performance (resolution, efficiency, asymmetry) compared to values reported in the QAR, follow the trouble shooting guidelines in the column care documentation. If this does not help replace the analytical column. Replace the amino acid trap column in the case that good performance of the analytical column is restored after removal of the trap column.

In the case of clogging (high pressure), systematically check and determine which part is the cause of the high back pressure (trap column, analytical column or other part of the LC system). Replace the amino acid trap column when clogged.

**A column is considered a LC consumable and limited warranty applies. Optimal column performance cannot be guaranteed when the above-mentioned requirement and precautions are not met.**

In case of any further questions do not hesitate to contact us using the contact information below or visit our website:

https://antecscientific.com/products/columns/amino-acid-trap/

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