PRODUCT MANUAL

for

AminoTrap™ Columns

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Product Manual

for

AMINOTRAP COLUMN

(4 x 50 mm P/N 046122)
(3 x 30 mm P/N 060146)
(0.4 x 35 mm P/N 076200)

Document No. 031197
Revision 06
June 2011

TABLE OF CONTENTS

SECTION 1 – INTRODUCTION

The AminoTrap column is used to retain amino acids present in carbohydrate samples and thereby prevent them from interfering with the carbohydrate analysis. This manual describes the use of the AminoTrap column.

The AminoTrap is packed with a 10 µm, high capacity anion exchange resin which has high selectivity for hydrophobic amino acids relative to monosaccharides, using hydroxide eluents. The maximum operating pressure is 4,000 psi (27.57 MPa) and the recommended maximum flow rate is 4 mL/min. The normal operating pressure is 450 - 600 psi (2.07 - 2.76 MPa) at 1.0 mL/min.

Hydrophobic amino acids such as lysine are known to foul the gold electrode used in the amperometric detection of monosaccharides, within the time frame of a standard chromatogram. Samples containing lysine, such as glycoprotein hydrolysates or those from food sources, may show diminished response for monosaccharides that elute after lysine.

The AminoTrap column is used immediately before the CarboPac PA10 or CarboPac PA20 columns in place of a CarboPac Guard column, and serves to delay the elution of the amino acids until after the monosaccharides have eluted. The gold electrode is therefore protected from contamination. The amino acids are eluted during the regeneration step, at high hydroxide concentration, when the electrode is less susceptible to fouling.

Retention of monosaccharides on AminoTrap is very low such that only small changes in retention times may be seen.

1.1. Recommended System Configurations

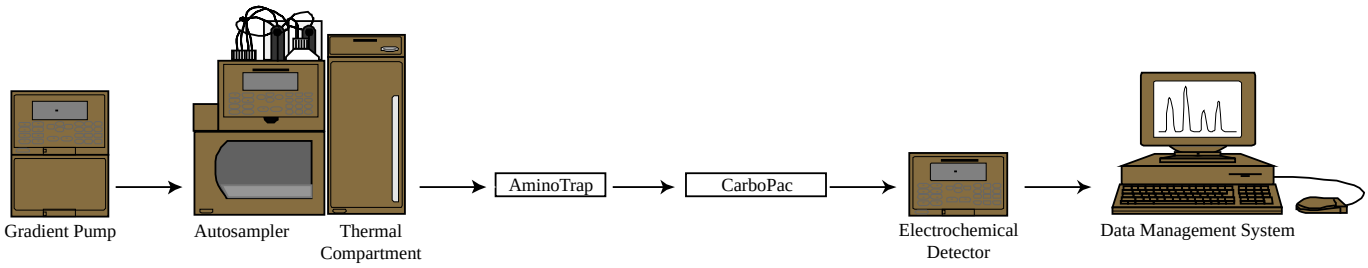

Figure 1
Recommended System Configuration

SECTION 2 – EXAMPLE APPLICATIONS

2.1. 4-mm AminoTrap with the CarboPac PA10

Sample Volume:	10 µL
Analytical Column:	See Chromatogram
Eluent:	18 mM NaOH
Eluent Flow Rate:	1.5 mL/min
Detector:	Pulsed electrochemical detection, Au electrode
Waveform:	Quadruple potential
Storage Solution:	18 mM NaOH

Peaks
1. Fucose
2. Galactosamine
3. Glucosamine
4. Galactose
5. Glucose
6. Mannose
7. Lysine

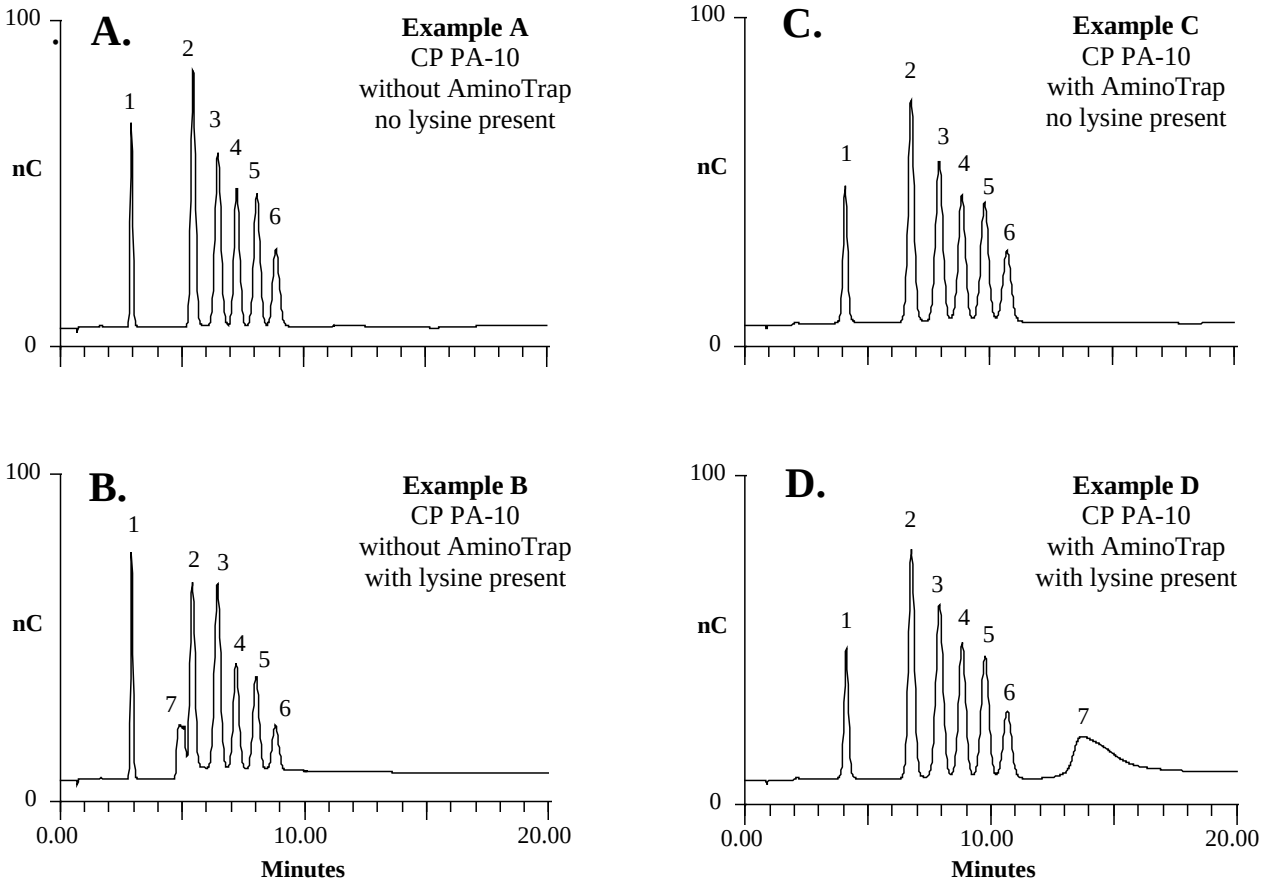

Figure 2
Isocratic Elution of Mix of Six Monosaccharides Using a
CP PA-10 with AminoTrap Column in the Presence of Lysine

2.2. 3-mm AminoTrap with the CarboPac PA20

If the samples are glycoprotein hydrolysates that have a high ratio of amino acids to carbohydrate, the AminoTrap 3-mm (P/N 060146) column is the guard column of choice and replaces the standard guard column. The AminoTrap column will remove problematic amino acids from the carbohydrate elution window, reduce the working electrode poisoning, give cleaner chromatography of monosaccharides, and greatly reduce the need for correction factors.

Monosaccharide detection can be compromised by fouling of the working electrode from amino acids. This is especially apparent with amine-containing glycoconjugates with low levels of glycosylation. Lysine, which is eluted before galactosamine when the AminoTrap is not employed, tails on the gold electrode. The slow release of lysine's oxidation products inhibits detector response for later eluting monosaccharides. The AminoTrap resolves the quantitation problem by retaining lysine until after the monosaccharides have been eluted.

Sample Volume:	20 µL
Analytical Column:	CarboPac PA20 Analytical Column
Eluent:	12 mM NaOH
Eluent Flow Rate:	0.5 mL/min
Detector:	Pulsed electrochemical detection, Au electrode
Waveform:	Quadruple potential
Storage Solution:	18 mM NaOH

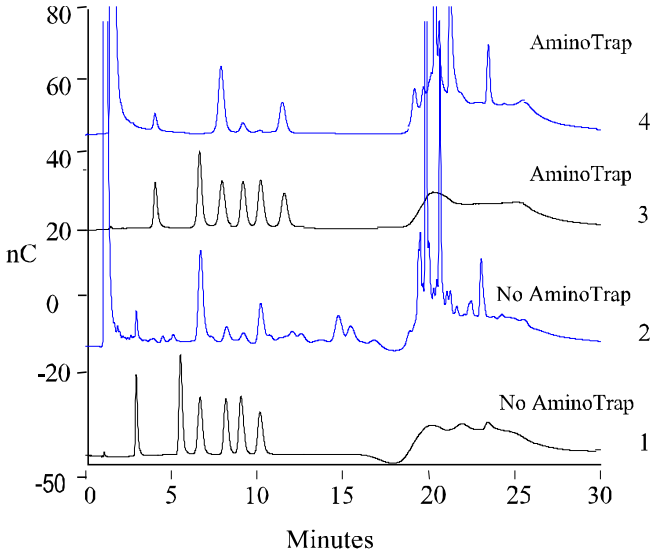

Figure 3
Profiling Mab Hydrolysate on CarboPac PA20 Column with and without an AminoTrap

2.3. 0.4 mm AminoTrap for Use with the Capillary CarboPac PA20

Sample Volume:	0.4 µL
Analytical Column:	CarboPac PA20 Analytical Column (0.4 x 150 mm)
Eluent:	12 mM KOH (15 min) / 100 mM KOH (15 min) / 12 mM KOH (20 min) (EG)
Eluent Flow Rate:	9 µl / min
Detector:	Pulsed electrochemical detection, Au electrode
Waveform:	Quadruple potential
Storage Solution:	18 mM NaOH

Peaks
1. Fucose
2. DeoxygLucose
3. Galactosamine
4. Glucosamine
5. Galactose
6. Glucose
7. Mannose

* Amino acids

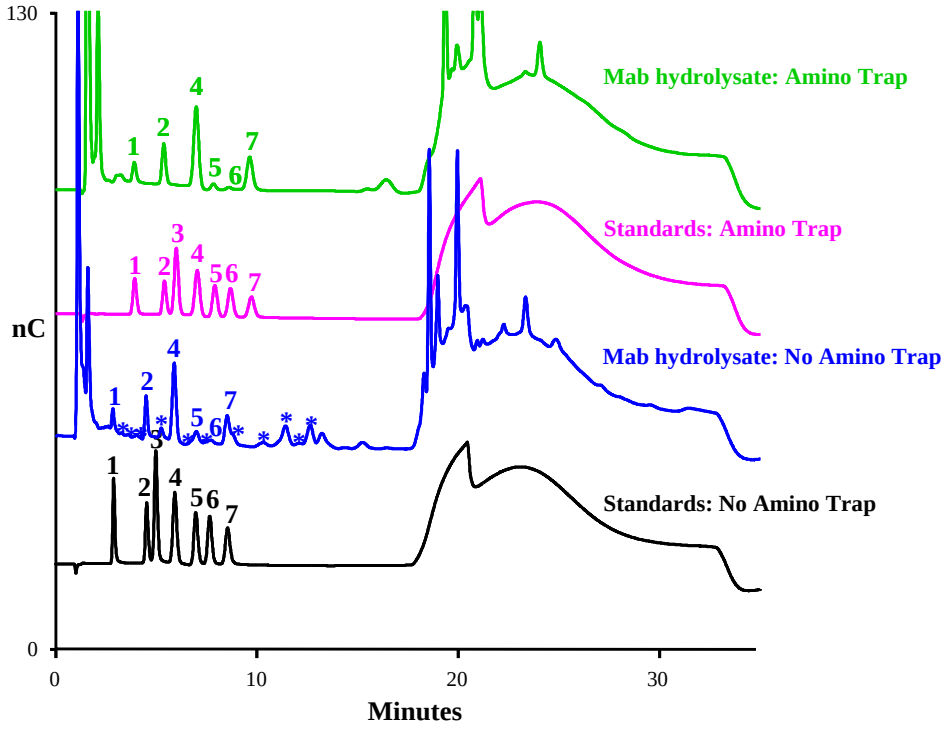

Figure 4
Analysis of Mab hydrolysate using capillary CarboPac PA20 and capillary version of AminoTrap column

SECTION 3 – TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the AminoTrap Column. For more information on problems that originate with the Ion Chromatograph, refer to the Troubleshooting Guide in the appropriate operator's manual. If you cannot solve the problem on your own, contact the DIONEX North America Technical Call Center at 1-800-DIONEX-0 (1-800-346-6390) or the nearest DIONEX Office (see, "DIONEX Worldwide Offices").

3.1. High Back Pressure from a Contaminated Inlet Bed Support

If the AminoTrap displays high back pressure, the bed support in the column inlet may be contaminated. Follow the instructions below to change the bed support assembly using one of the two spare bed support assemblies included in the ship kit provided with the column.

A. Disconnect the column from the system.

B. Carefully unscrew the inlet (top) column end fitting using two open-end wrenches.

C. **Remove the old bed support.** Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you **do not scratch the walls of the end fitting**. Discard the old assembly.

D. **Place a new bed support assembly in the end fitting.** Use the end of the column to carefully start the bed support assembly into the end fitting.

	4 mm P/N	**3 mm P/N**
Bed Support Assembly	042955	056823
End Fitting	052809	052809

E. **Screw the end fitting back onto the column.** Tighten it finger-tight and then using two open-end wrenches, tighten it an additional 1/4 turn (25 in x lb). Tighten further only if leaks are observed.

NOTE ***If any of the column packing becomes lodged between the end of the column and the bed support washer assembly, no amount of tightening will seal the column. Make sure that the washer and the end of the column are clean before screwing the end fitting back onto the column.***

F. Reconnect the column to the system and resume operation.

