PRODUCT MANUAL

AMINOPAC™ PA1 GUARD

(P/N 037023)

AMINOPAC™ PA1

(P/N 037022)

QUICKSTART STEPS AND LINKS
Click blue text below to get started.

1. See Section 3, “Operations”. Note operation precautions and chemical purity requirements.

2. See Section 3.3, “Preparation of Eluents”. Make the required stock and working solutions for eluents.

3. See “Quality Assurance Report”. Run the Production Test Chromatogram as a system check.

4. See Section 4, "Example Applications” for example applications.

5. See “Column Care” for column cleanup and long-term storage recommendations.

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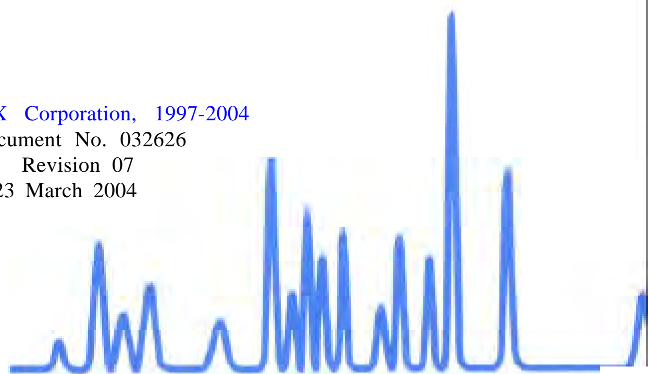

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SECTION 1 - INTRODUCTION

1.1 Theory of Operation

The AminoPac PA1 separator column was developed for the separation of amino acids as anionic species. Amino acids are anions in the presence of alkaline pH eluents (pH 10.0). Separation, therefore, is effected by the relative differences in the dissociation constants of the amino, carboxylic, and R groups of each amino acid. For example, the protons of the amino acid glutamic acid dissociate as follows:

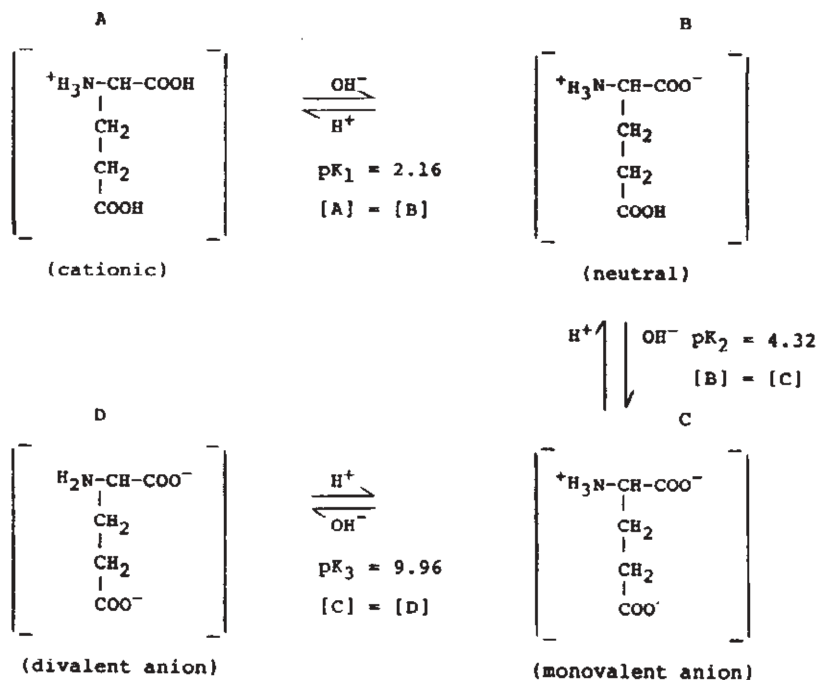


Figure 1
Dissociation of Protons of Glutamic Acid

Therefore, at a pH above 11.0, glutamic acid is present primarily as a divalent anion. Some amino acids will be separated primarily as monovalent anions and others as divalent anions.

Another separation mode is based on the relative hydrophilic or hydrophobic nature of the R groups associated with each amino acid.

Figure 2
Relative Hydrophobicity of R-groups

The more basic amino acids, including arginine and lysine, generally elute before more acidic amino acids, such as glutamic and aspartic acids. The more hydrophilic amino acids, such as serine, elute before phenylalanine, which is more hydrophobic.

The AminoPac PA1 anion exchange column is designed for the high resolution separation of amino acids. In this manual, detection of the amino acids is with visible spectroscopy following post-column derivatization with ninhydrin. However, other detection schemes, such as OPA, are possible.

The packing material is composed of 180 nm quaternary amino functionalized MicroBeads bound to a 10 µm non-porous substrate.

Resin Characteristics:

Particle size:	10 µm
Pore size:	non-porous
Cross-linking:	55%
Ion Exchange Capability:	90 µeq

Latex Characteristics:

Functional group:	Quaternary ammonium ion
Latex diameter:	180 nm
Latex Cross-linking	55%

Typical Operating Parameters:

pH:	0-14
Temperature:	60°C
Pressure:	3000 psi
Organic Solvent:	≤ 10%
Typical eluents:	Hydroxide, borate, acetate
Typical Flow Rate:	1 mL/min

SECTION 2 - INSTALLATION

The boric acid, sodium tetraborate, and sodium acetate used in the eluents for the AminoPac PA1 column are not very soluble in organic solvents. To prevent the precipitation of these eluents (especially Eluent 4) when flushing the column, use water as Reagent 2 instead of any organic solvent.

2.1 System Requirements

The amino acid separations with the AminoPac PA1 columns are optimized for use with Dionex 4-mm systems, whether they are the 4-mm Dionex DX-500, DX-600 or BioLC. All of these systems are metal-free.

Each of the possible configurations offers multiple sampling options; however, a consistently reproducible quantitation and an absence of disturbing artifacts are achieved best using the “full loop” mode and in conjunction with a 25 µL loop (P/N 042857). Good reproducibility of retention times requires the use of temperature controlled modules from Dionex and applications of the exact settings described in the following sections of this manual.

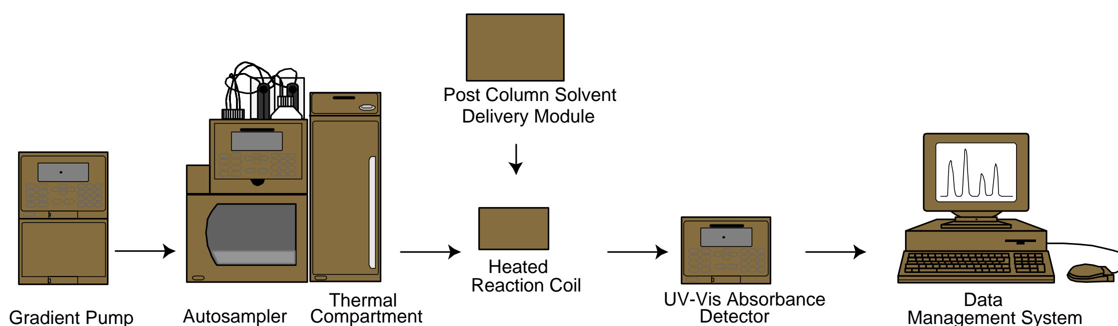


Figure 3
Amino Acid System Configuration

2.2 System Operation Requirements

The Dionex Amino Acid systems should be configured to comply with the following key requirements:

1. Mobile phase components are kept under helium or nitrogen at all times
2. On-line degassing of eluents
3. Accurate and precise flow rates at 1.0 mL/min
4. Post-column solvent delivery pump at 0.66 mL/min
5. Heated reaction coil P/N 039349
6. Minimized contribution to the background signal by contaminants from the system and reagents

2.3 AminoPac PA1 Column Operational Parameters

pH range:	pH = 0–14
Temperature limit:	60 °C
Pressure limit:	3,000 psi
Organic Solvent Limit:	10% Acetonitrile, methanol, acetone, if required for cleaning
Typical Eluents:	High purity water (18.2 megohm-cm), sodium hydroxide, sodium acetate, sodium tetraborate

SECTION 3 - OPERATIONS

3.1 Purity Requirements for Chemicals

Obtaining reliable, reproducible and accurate results requires eluents that are free from impurities and prepared only from the chemicals recommended below. Dionex cannot guarantee proper column performance when alternate suppliers of chemicals or lower purity water are utilized. It is very important that the eluents used with the AminoPac PA1 column be as free of iron impurities as possible. Thus, the chemicals and water used to prepare them should be of the highest purity available.

- All reagents used with the AminoPac PA1 column must be certified ACS reagent grade or better. Reagents must contain no more than 5 ppm iron. Levels of iron higher than this will cause poor efficiency and selectivity changes. Section 5 illustrates the effects of metal contamination on the chromatography.

- To prevent damaging the column resin, do not use more than a 10% wt/wt level of any organic solvent when preparing the eluents.

- Use deionized water with a resistance of 18.2 megohm-cm.

3.1.1 Deionized Water

The deionized water used to prepare eluents should be Type I reagent grade water with a specific resistance of 18.2 megohm-cm. The water should be free from ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. The availability of UV treatment as a part of the water purification unit is recommended. Follow the manufacturer's instructions regarding the replacement of ion exchange and adsorbent cartridges. Expanding their period of use beyond the recommended time may lead to bacterial contamination and as a result, a laborious cleanup may be required. Use of contaminated water for eluents can lead to high background signals and gradient artifacts.

3.1.2 Sodium Hydroxide

Use 50% w/w sodium hydroxide (Certified Grade, Fischer Scientific P/N UN 1824) for preparation.

3.1.3 Sodium Acetate

Dionex highly recommends the use of Dionex Sodium Acetate Reagent (P/N 059326) for amino acid analysis. However, anhydrous sodium acetate from Fluka Biochemika (MicroSelect, P/N 71183) is also adequate. Dionex cannot guarantee proper detection performance when different grades or alternate suppliers of sodium acetate are utilized.

3.1.4 Inorganic Chemicals

Reagent grade inorganic chemicals should be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These inorganic chemicals will detail the purity by having an actual ion analysis on the label.

3.1.5 Post-Column Ninhydrin

Purchase from Pickering and follow the instructions from Pickering.

3.2 Solution Preparation

NOTE
Always sanitize the entire analyzer with 2M NaOH prior to initial start-up and after idle periods.

Obtaining reliable, consistent and accurate results requires eluents that are free from ionic and UV-Vis wavelength active impurities. Chemicals and deionized water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also help to protect your ion exchange columns and system components. Dionex cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents is substandard.

3.2.1 Degassing Deionized Water

Vacuum degas the water by placing the eluent reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump. Vacuum degas the reservoir for 5–10 minutes while sonicating. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere. On-line degassing is supported through the use of the GP40, GP50 and GS50 gradient pumping systems and the IS20 and IS25 isocratic pumping systems.

3.2.2 Stock Solutions

0.5 M Sodium Hydroxide

A. The first step in the preparation of sodium hydroxide eluent is to degas an aliquot (typically 974 mL) of the deionized water, as described above.

B. To make 0.5 M NaOH, add 40 g (26.1 mL) of 50% (w/w) NaOH to the degassed deionized water by removing the NaOH aliquot from the middle of the stock solution where sodium carbonate is least likely to have formed.

C. Do not pipet from the bottom where sodium carbonate precipitate may have fallen, and always discard the bottle of 50% sodium hydroxide when it reaches about 2/3 empty.

D. Place the tip of the pipet containing the aliquot of NaOH about 1 inch below the surface of the water and dispense the NaOH. If done properly, without stirring, most of the concentrated sodium hydroxide will stay at the lower half of the container and the rate of carbon dioxide adsorption will be much lower than that of a homogenous solution.

E. Seal the container after the sodium hydroxide transfer is complete.

F. Remember to replace the cap to the 50% hydroxide bottle immediately as well.

G. Mix the contents of the tightly sealed container holding the 1M hydroxide.

NOTE
DO NOT prepare NaOH eluents from sodium hydroxide pellets! The pellets are coated with a layer of carbonate. Always degas and store NaOH eluents in plastic eluent bottles blanketed with helium or nitrogen to avoid carbon dioxide contamination from the air. Carbonate in the eluent can significantly reduce retention times.

The eluents can be prepared by either weight or by volume. Using a volumetric pipet is more effective in preventing contamination than the weight method, but is less precise. For applications requiring less than or equal to 100 mM hydroxide, an on-line eluent generator may be used. Thus the preparation of caustic eluents may be avoided altogether. Table 1, next page, lists the mass or volume, of NaOH (50% w/w) required in 1 L to make the listed concentrations. Decide which technique to use, mass or volume, and then always use the same methodology to ensure consistent chromatographic results.

The sodium hydroxide eluents used with the AminoPac PA1 column will readily absorb carbon dioxide, producing sodium carbonate. The presence of variable amounts of carbonate will lead to inconsistent retention times, therefore always degas the water prior to use, discard the 50% sodium hydroxide once it is 2/3 empty and keep the prepared eluent blanketed under an inert gas.

Table 1
Mass or Volume of 50% NaOH required to make 1 L of common eluents.

Eluent Concentration	NaOH (g)	NaOH (mL)
0.1M	8.0	5.2
0.2M	16.0	10.5
0.3M	24.0	15.7
0.4M	32.0	20.9
0.5M	40.0	26.1
0.6M	48.0	31.4
0.7M	56.0	36.6
0.8M	64.0	41.8
0.9M	72.0	47.1
1.0M	80.0	52.3

1.0 M Sodium Acetate

A. To make one (1) liter of 1.0 M sodium acetate, dispense approximately 800 mL of DI water into a 1 L volumetric flask.

B. Vacuum degas for approximately 5 minutes. Add a stir bar and begin stirring.

C. Weigh out 82.04 g anhydrous, crystalline sodium acetate.

D. Add the solid acetate steadily to the briskly stirring water to avoid the formation of clumps which are slow to dissolve.

E. Once the salt has dissolved, remove the stir bar with a magnetic retriever.

F. Add DI water to the flask to bring the volume to the 1 L mark.

G. Vacuum filter the solution through a 0.45 µm nylon filter. This may take a while as the filter may clog with insoluble material from the sodium acetate.

H. The eluent should be kept blanketed under helium at 34 to 55 kPa (5–8 psi) at all times, and last about 1 week.

NOTE
Dionex recommends the use of dedicated glassware, pipets and filtration apparatus for exclusive use in the preparation of amino acid eluents

0.1 M Sodium Tetraborate

A. Fill a one liter volumetric flask about two-thirds full with 18.2 megohm-cm deionized water.

B. Carefully add 38.14 grams of $Na_2B_4O_7 \cdot 10H_2O$ to the flask.

C. Mix thoroughly until the $Na_2B_4O_7 \cdot 10H_2O$ is dissolved.

D. Fill the flask to the mark with 18.2 megohm-cm deionized water.

E. Filter the solution through a 0.45 micron filter.

3.3 Preparation of Eluents

There are four eluents required for the ion exchange separation of amino acids with post-column visible detection with ninhydrin. E1 is 560 mM sodium hydroxide with 640 mM boric acid. E2 is sodium hydroxide/sodium tetraborate and 2% methanol. E3 is sodium hydroxide/sodium tetraborate and 10% methanol. E4 is sodium acetate, hydroxide and methanol.

Except for E1, all these are prepared from the eluent concentrates described in Section 3.2.2.

3.3.1 Eluent Preparation

Eluent E1: 560 mM NaOH / 640 mM H_3BO_3

A. Fill a 1 liter volumetric flask about two-thirds full with filtered 18.2 megohm-cm DI water.

B. Carefully add 44.8 g of 50% NaOH (P/N 033465) to the flask.

C. Carefully add 39.6 g of H_3BO_3 to the same flask.

D. Mix thoroughly until the NaOH and H_3BO_3 go into the solution.

E. Fill the flask to the mark with filtered 18.2 megohm-cm DI water and mix thoroughly.

F. Degas the solution with vacuum and sonication.

Eluent E2: 23 mM NaOH / 7 mM $Na_2B_4O_7$

A. Place 46 mL of 500 mM NaOH concentrated solution (prepared freshly) and 70 mL of 100 mM $Na_2B_4O_7$ concentrated solution in a 1 L volumetric flask.

B. Fill the flask to the mark with filtered 18.2 megohm-cm DI water and mix thoroughly.

C. Degas the solution with vacuum and sonication.

Eluent E3: 80 mM NaOH / 18 mM $Na_2B_4O_7$ + 10% MeOH

A. Place 160 mL of 500 mM NaOH concentrated solution and 180 mL of 100 mM $Na_2B_4O_7$ concentrated solution in a 1 L volumetric flask.

B. Fill the flask to the mark with filtered 18.2 megohm-cm DI water and mix thoroughly.

C. Degas the solution with vacuum and sonication. Add 100 mL of methanol.

Eluent E4: 400 mM NaOAc/1 mM NaOH/2% MeOH

A. Place 400 mL of 1000 mM sodium acetate concentrated solution and 2.0 mL of 500 mM NaOH concentrated solution in a 1 L volumetric flask.

B. Fill the flask to the mark with filtered 18.2 megohm-cm DI water and mix thoroughly.

C. Degas the solution with vacuum and sonication. Add 20 mL of methanol.

3.4 Amino Acid Standards

Amino Acid Standard H (2.5 mM 17 amino acid standard) can be obtained from Pierce Chemicals (P/N 20088ZZ), as 1 mL ampules. To prepare this standard for injection (4 nmol per solute injected using a 20 µL loop), weigh out the solution contained in 1 ampule. Multiply this weight by 12.5. Using 18.2 megohm-cm DI water, dilute the amino acid standard to the weight calculated, in grams. Mix the solution thoroughly and refrigerate until ready to use.

3.5 Post-Column Reagent Preparation

The post-column reagent is Trione ninhydrin reagent which comes ready to use from Pickering. No further preparation is necessary.

SECTION 4 - EXAMPLE APPLICATIONS

4.1 Introduction to the Methods

In earlier editions of this manual, the solvent concentration was limited to 2% MeOH. Greater retention time reproducibility is observed with 10% MeOH.

4.1.1 Introduction to the Gradient Method

The initial selection of gradient method depends on the specific analytes to be separated. The gradient below provides a good compromise between resolution and run time for the 17 amino acids in the the Pierce Amino Acid Standard H. If the early eluting amino acids are not present in your sample, a stronger initial eluent concentration can be used to shorten the run time. In the gradient method listed in Table 2, "Gradient Table for Elution of Amino Acids, " the column clean up and re-equilibration occurs at the start of the method with the injection occuring at 26 minutes.

Table 2
Gradient Table for Elution of Amino Acids

Time	% E1	% E2	% E3	% E4	V5	V6
0.0	0	100	0	0	Inject	Off
4.0	0	100	0	0	Inject	Off
4.1	100	0	0	0	Inject	Off
13.9	100	0	0	0	Inject	Off
14.0	0	100	0	0	Inject	Off
25.8	0	100	0	0	Load	On
26.0	0	100	0	0	Inject	Off
36.0	0	100	0	0	Inject	Off
40.0	0	0	100	0	Inject	Off
46.0	0	0	100	0	Inject	Off
46.1	0	0	90	10	Inject	Off
56.0	0	0	0	100	Inject	Off
62.0	0	0	0	100	Inject	Off
62.1	0	100	0	0	Inject	Off
66.1	0	100	0	0	Inject	Off

NOTE

Table 2, "Gradient Table for Elution of Amino Acids," as shown above is for the DX-300. For newer instruments, instead of V5 and V6, use appropriate commands.

4.1.2 Introduction to the Detection Method

Post column detection with ninhydrin employs visibile detection at 500 nm. To ensure the reaction goes to completion, a heated reaction coil is required after the column. The sample and post-column ninhydrin are mixed after the column and go through the reaction coil which is heated to 130 °C before entering the detector and appearing as peaks.

4.1.3 Operating Conditions

Column Temperature:	Ambient
Eluent flow rate:	1.00 mL/min.
Ninhydrin flow rate:	0.66 mL/min.
Reaction coil temperature:	130 °C
Injection loop:	20 µL
Detection:	Visible at 500 nm

4.2 Production Test Chromatogram

Eluent:	E1: 0.56 M NaOH/0.64 M H_3BO_3
	E2: 0.023 M NaOH/0.007 M $Na_2B_4O_7$
	E3: 0.080 M NaOH/0.018 M $Na_2B_4O_7$/10% Methanol
	E4: 0.4 M NaOAc/0.001 M NaOH/2% Methanol
Eluent Flow Rate:	1.0 mL/Min.
Detector Scale:	VIS at 500 nm (AD25)
Standard:	Pierce hydrolyzate H = 2.5 µmol/mL
	Cystine, 1.25 µmol/mL in 0.1 M HCl
Diluted standard (with DI water)	200 nmol/mL
Inj. Loop:	20 µL
PCR Flow Rate:	0.66 mL/min

Gradient Program:

Time	% E1	% E2	% E3	% E4	V5	V6
0.0	0	100	0	0	Inject	Off
4.0	0	100	0	0	Inject	Off
4.1	100	0	0	0	Inject	Off
13.9	100	0	0	0	Inject	Off
14.0	0	100	0	0	Inject	Off
25.8	0	100	0	0	Load	On
26.0	0	100	0	0	Inject	Off
36.0	0	100	0	0	Inject	Off
40.0	0	0	100	0	Inject	Off
46.0	0	0	100	0	Inject	Off
46.1	0	0	90	10	Inject	Off
56.0	0	0	0	100	Inject	Off
62.0	0	0	0	100	Inject	Off
62.1	0	100	0	0	Inject	Off
66.1	0	100	0	0	Inject	Off

	Peaks	nmol/20 µL
1.	Arginine	4
2.	Lysine	4
3.	Threonine	4
4.	Alanine	4
5.	Glycine	4
6.	Serine	4
7.	Valine	4
8.	Proline	4
9.	Isoleucine	4
10.	Leucine	4
11.	Methionine	4
12	Histidine	4
13	Phenylalanine	4
14	Glutamic Acid	4
15	Aspartic Acid	4
16	Cystine	4
17	Tyrosine	4

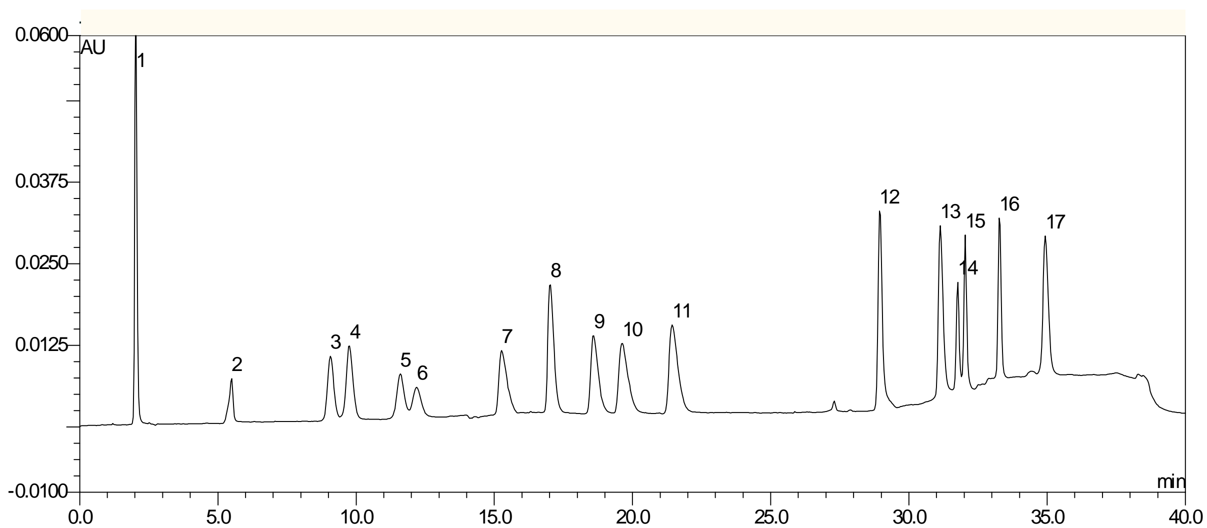


Figure 4
Production Test Chromatogram

SECTION 5 - TROUBLESHOOTING GUIDE

5.1 Effect of Borate Concentration in Eluent 1

The effect of borate concentration on the resolution of THR-ALA and GLY-SER is illustrated below. In general, increasing the borate concentration in eluent 1 improves the resolution of THR-ALA; decreasing the borate concentration improves the resolution of GLY- SER.

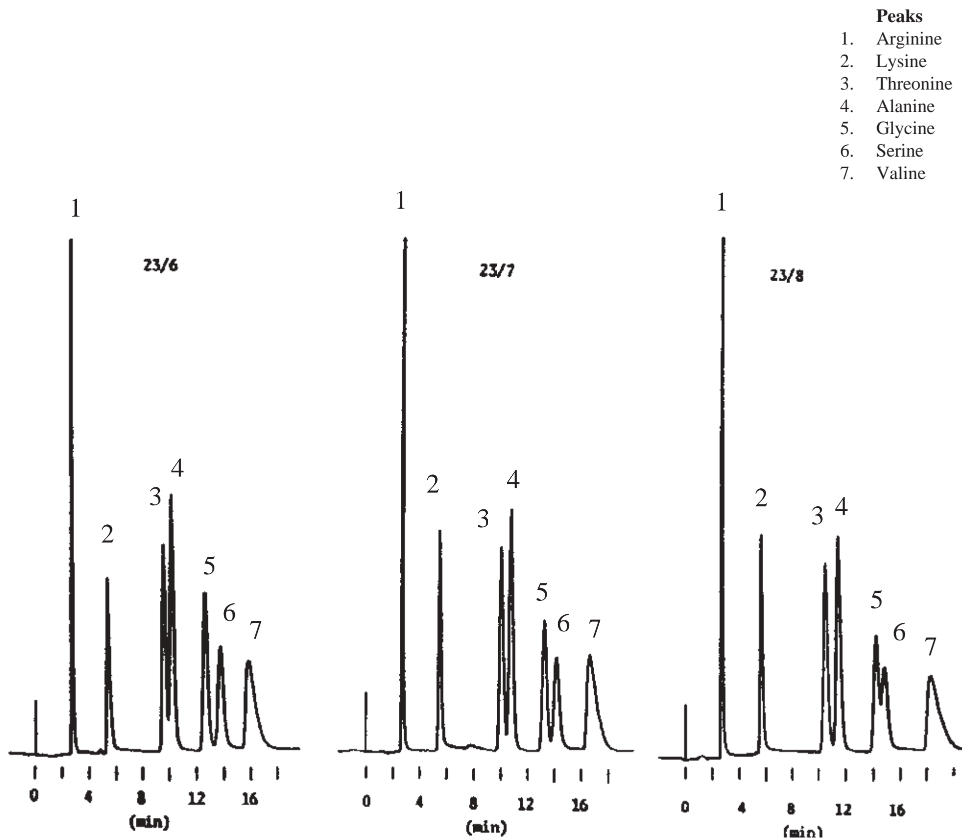


Figure 5
Effect of Borate Concentration in Eluent 1

5.2 Effect of Metal Contamination

Consult these chromatographic examples of metal contamination to ensure that changes in the eluents or gradient program are not initiated for inappropriate reasons.

A metal trap column, IonPac MFC-1 (Metal-Free Trap Column P/N 037017), may be put into the system between the pump and the injection valve to remove metals found in eluents.

Peaks
1. Arginine
2. Lysine
3. Threonine
4. Alanine
5. Glycine
6. Serine
7. Valine

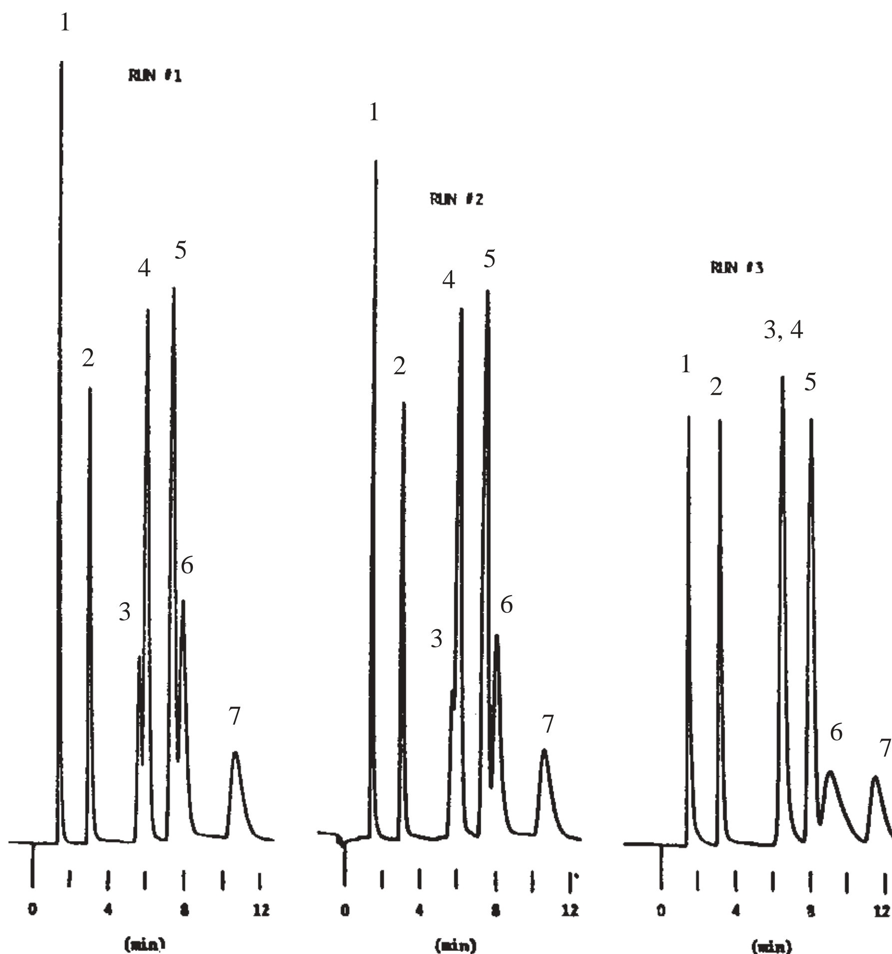


Figure 6
Effect of Metal Contamination

AminoPac® PA1
Analytical (4 X 250 mm)
Product No. 037022

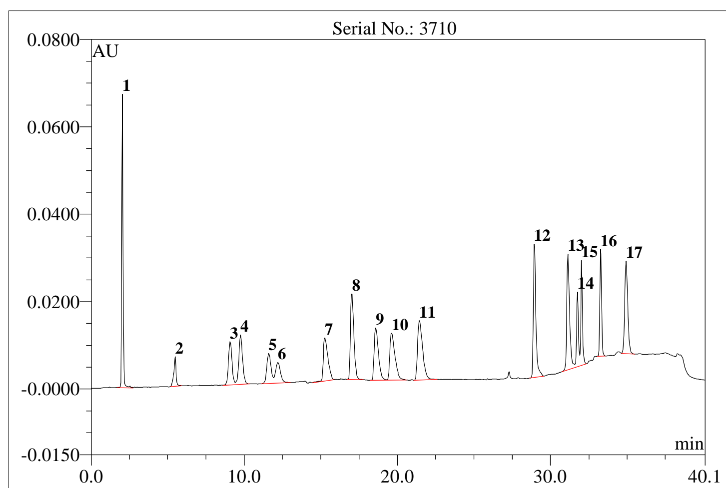
Date: 30-Jan-04 10:11
Serial No. : 003710
Lot No. : 014-98-131

Flow Rate: 1.0 mL/min
Temperature: (Reaction Coil) 130 °C
Detection: Absorbance Detection using AD25 VIS at 500 nm
Standard: Pierce hydrolyzate H = 2.5µM/mL Cystine, 1.25 µM/mL in 0.1 M HCl
Diluted Standard: (in Deionized water) 200 nmol/mL Each analyte is 4 nmoles per injection
Injection Volume: 20 µL
PCR Flow Rate: 0.66 mL/min
Storage Solution: 0.023 M NaOH/ 0.007 M Na2B4O7

Eluent:
%A: 0.56 M NaOH/0.64 M H3BO3
%B: 0.023 M NaOH/ 0.007 M Na2B4O7
%C: 0.080 M NaOH/0.018 M Na2B4O7/10% Methanol
%D: 0.40 M NaOAc/0.001 M NaOH/2% Methanol

Eluent Profile:

Time	%A	%B	%C	%D	V5	V6
0.0	0	100	0	0	Inject	Off
4.0	0	100	0	0	Inject	Off
4.1	100	0	0	0	Inject	Off
13.9	100	0	0	0	Inject	Off
14.0	0	100	0	0	Inject	Off
25.8	0	100	0	0	Load	On
26.0	0	100	0	0	Inject	Off
36.0	0	100	0	0	Inject	Off
40.0	0	0	100	0	Inject	Off
46.0	0	0	100	0	Inject	Off
46.1	0	0	90	10	Inject	Off
56.0	0	0	0	100	Inject	Off
62.0	0	0	0	100	Inject	Off
62.1	0	100	0	0	Inject	Off
66.1	0	100	0	0	Inject	Off



No.	Peak Name	Ret.Time (min)	Asymmetry (EP @ 10%)	Resolution (EP)	Efficiency (EP)
1	Arginine	2.03	1.10	17.27	2881
2	Lysine	5.50	0.79	10.67	7685
3	Threonine	9.07	n.a.	1.62	7475
4	Alanine	9.77	0.97	3.91	7679
5	Glycine	11.60	n.a.	1.12	8773
6	Serine	12.20	n.a.	5.47	7077
7	Valine	15.27	1.62	3.75	12557
8	Proline	17.03	1.37	3.49	29023
9	Isoleucine	18.60	1.52	1.87	22099
10	Leucine	19.63	1.61	3.02	16866
11	Methionine	21.43	1.69	17.62	21218
12	Histidine	28.93	1.83	7.67	190571
13	Phenylalanine	31.13	1.53	2.50	161226
14	Glutamate	31.77	n.a.	1.39	410733
15	Aspartate	32.03	n.a.	6.43	475564
16	Cystine	33.27	1.25	6.13	447875
17	Tyrosine	34.93	1.23	n.a.	162454

QA Results:

Analyte	Parameter	Specification	Results
Glycine	Efficiency	>=5850	Passed
Glycine	Retention Time	10.05-13.35	Passed
	Pressure	<=1650	1370

Production Reference:

APPENDIX B - COLUMN CARE

Recommended Operation Pressures

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for the columns covered by this manual are listed in Table 2, "Standard Flow Rates and Back Pressures".

Column Storage

For short-term storage, the strongest eluent in use can be used as the storage solution. For long-term storage, use the storage solution listed in Table 1, "Storage Solutions, Eluents and Regenerants". Flush the column for 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column.

Column Cleanup

The following column cleanup protocols have been divided into three general isocratic protocols to remove acidsoluble, base-soluble or organic contaminants. They can be combined into one gradient protocol if desired but the following precautions should be observed.

Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface bands in the column. High pressure bands can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column. High pressure bands in the column can be created by pumping successive eluents through the column that are not miscible or that have eluent components that will precipitate out in the other eluent. The precipitation of the salts in solvents during column rinses can result in very high pressure bands.

High viscosity mixing bands can be created between two eluents having solvents with a very high energy of mixing.

CAUTION

Eluents used on columns containing low cross-linked resins must contain less than 5% organic solvents.

Base-soluble Contaminants

This procedure is used to elute anionic contaminants from anion exchange columns. Typical eluents are formulated from carbonate/bicarbonate mixtures, borate and hydroxide.

A. **Prepare a 500 mL solution of 10X eluent concentrate.** See Table 1, "Storage Solutions, Eluents and Regenerants."

B. **Disconnect the suppressor from the analytical column.** If your system is configured with both a guard column and an analytical column, place the guard column after the analytical column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels. Direct the effluent from the outlet line of the guard column to a separate waste container.

CAUTION

When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the analytical column and irreversibly damage it. If in doubt, clean each column separately.

C. Set the pump flow rate to that listed in Table 2, "Standard Flow Rates and Back Pressures."

D. If your eluent contains a solvent that is not compatible with the 10X eluent concentrate, rinse the column for 15 minutes with deionized water before pumping the 10X eluent concentrate over the column.

E. Pump the 10X eluent concentrate solution through the column for 30–60 minutes.

F. Reconnect the suppressor to the analytical column and place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

G. Equilibrate the column(s) with eluent before resuming normal operation.

Organic Contaminants

CAUTION

Eluents used on columns containing low cross-linked resins must contain less than 10% organic solvents.

A. Prepare a 500 mL solution of 5% acetonitrile in deionized water having a specific resistance of 18.2 megohm-cm.

B. If your system is configured with both a guard column and an analytical column, reverse the order of the guard and analytical column in the eluent flow path. Double check that the eluent flows in the direction designated on each of the column labels. Direct the effluent from the outlet line of the guard column to a separate waste container.

CAUTION

When cleaning an analytical column and a guard column in series, ensure that the guard column is placed after the analytical column in the eluent flow path. Contaminants that have accumulated on the guard column can be eluted onto the analytical column and irreversibly damage it. If in doubt, clean each column separately.

C. Set the pump flow rate to that listed in Table 2, “Standard Flow Rates and Back Pressures”.

D. If your eluent contains a solvent that is not compatible with 5% acetonitrile, rinse the column for 15 minutes with deionized water before pumping the 5% acetonitrile over the column.

E. Pump a 5% acetonitrile solution through the column for 30–60 minutes.

F. If your eluent contains a solvent that is not compatible with 5% acetonitrile, rinse the column for 15 minutes with deionized water before pumping eluent over the column.

G. Reconnect the suppressor column to the analytical column and place the guard column in line between the injection valve and the analytical column if your system was originally configured with a guard column.

H. Equilibrate the column(s) with eluent before resuming normal operation.