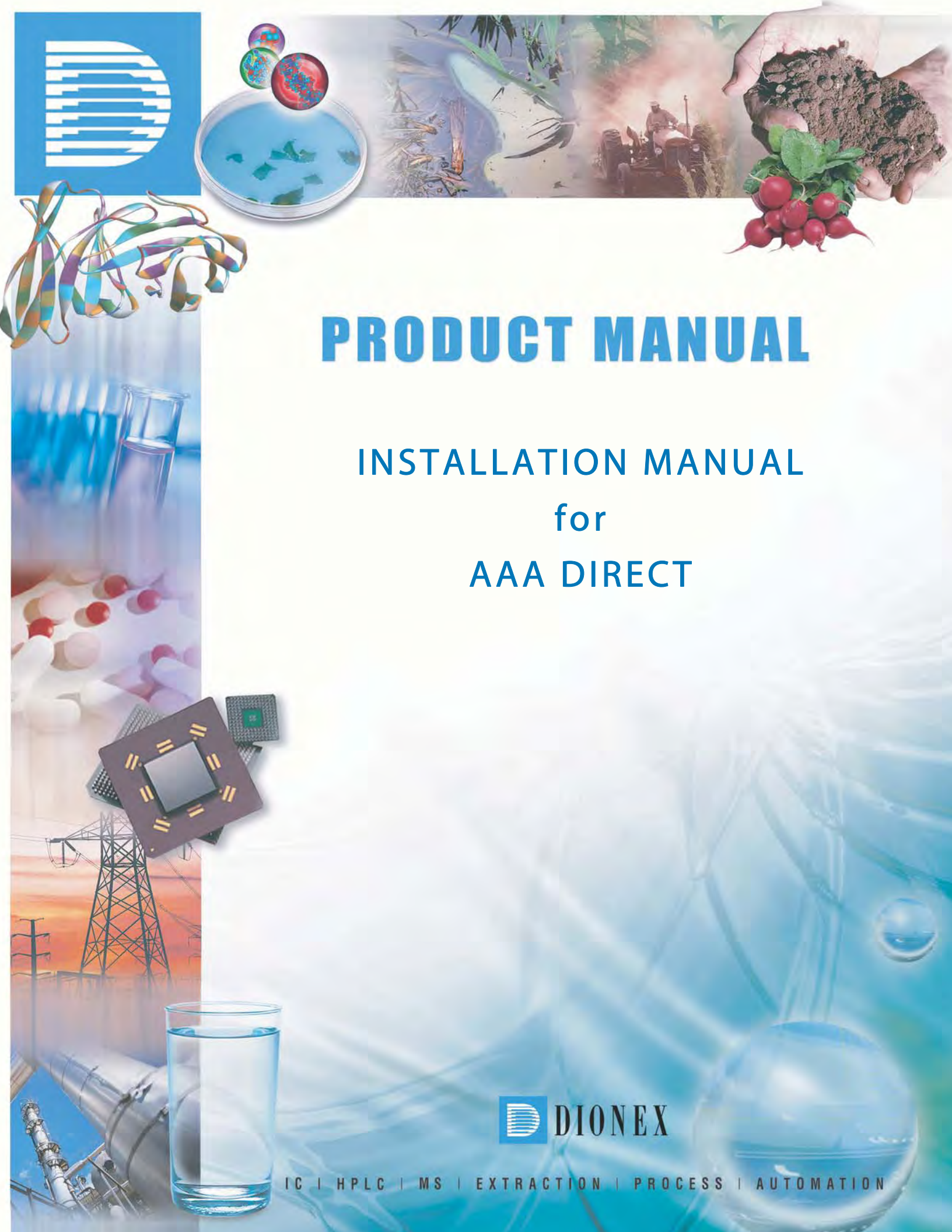

PRODUCT MANUAL

INSTALLATION MANUAL
for
AAA DIRECT

DIONEX

IC | HPLC | MS | EXTRACTION | PROCESS | AUTOMATION

INSTALLATION MANUAL
for
AAA DIRECT

Document No. 031481
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Standard Operating Procedure: Installation of a Dionex AAA Direct System

SECTION 1 - INTRODUCTION

Amino acid analyses require attention to detail, regardless of the analytical approach. The Dionex AAA-Direct system simplifies that task because it eliminates the need for additional high purity reagents that are required by other techniques.

This procedure is written specifically for Dionex service representatives working closely with the nearest Dionex Applications Lab to ensure a successful installation experience. Please read the entire document carefully before arranging an installation.

SECTION 2 - THE MOST IMPORTANT RULES

2.1 IMPORTANT PREPARATIONS: (Always do the following)

SAFETY: Wear appropriate gloves (non-powder) when handling eluent bottles, samples, or electrode cell parts. Don't touch these with your bare hands. Gloves will protect you from the chemicals and protect the parts from fingerprint damage.

- Use 50% NaOH solution rather than NaOH pellets to make eluent.
- Use dedicated glassware and disposable glass or plastic ware for volume adjustments.
- Keep your NaOH eluent blanketed by inert gas. Prepare new NaOH eluent if left unblanketed for more than 30 minutes.
- Pull at least 40 mL of new eluent through the lines when changing eluent or adding fresh eluent. This will ensure that your fresh eluent is primed through the lines up to the pump heads.
- Use pre-slit septa with the injection vials.
- Use 25 µL loop size; larger loops will cause loss of resolution.
- Install and use the piston wash option.

2.1 IMPORTANT REMINDERS: (Never do the following)

- Do not continue to the next step of the procedure if the previous has failed.
- Do not start an installation with any of the check list items missing (see Section 3 for Check List).
- Never use bottled HPLC water. Do not store deionized water (DI water), always use freshly drawn DI water for any preparation of eluents.
- Never use 'communal' filtration units or filters made of unknown or unsuitable (cellulose derivatives, polysulfone) materials.
- Never use eluent inlet filters; cover the ends of the eluent lines with parafilm when changing bottles.
- Never use MeOH or other organic solvent as rinse fluid in the autosampler. Use only 20 ppm sodium azide, or water if replaced daily.
- Do not run above 50 °C or 3,500 psi.

SECTION 3 - INITIAL CHECK LIST

These items ***MUST*** be available at the customer site. Check with the customer when you call to schedule the installation.

_ Laboratory water unit delivering 18.2 megohm-cm water at the installation site.

_ Vacuum pump available for use with the vacuum filtration units.

_ Three sterile-packed Nylon Nalgene Filtration Units, Funnel Size 1.0 L (VWR P/N 28198-514).

_ Inert gas cylinder (helium or nitrogen) with a regulator valve (about 0–200 psi at the low pressure side) and the appropriate size adaptors plus tubing.

_ Dionex AAA-Direct Installation Kit (P/N 059539) containing: This Standard Operating Procedure, Histidine quality solution, two bottles of sodium acetate, one bottle of 50% sodium hydroxide, restriction tubing and a disk with pgm, qnt, qnd rpt methods for the startup.

_ Product Manual for the AAA-Direct (Document. No. 031481) available on the CD enclosed with the AminoPac PA10 column.

_ NIST Amino Acid standards (SRM 2389, 2.5 mM solution).

_ One spare AAA certified Au electrode P/N 055832 (separate from the Au electrode ordered inside the cell kit (AS50, LC25 or LC30 style)).

_ One spare pH-Ag/AgCl reference electrode P/N 044198 (in addition to the reference electrode ordered inside the cell kit (AS50, LC25 or LC30 style)).

_ Sterile-packed, 10 mL and 25 mL disposable pipets and suitable pipeting bulbs or pumps.

_ Sodium azide solid, reagent grade for preparation of diluent solution.

_ Powder-free, disposable gloves (at least 1 box).

_ Disposable, plastic (PE) large-size (at least 20 mL) syringe for priming the pump.

_ Nitric acid, 6 N, concentrated nitric acid, diluted 1:1 (v/v) for system cleanup.

SECTION 4 - INSTALLATION

Make sure that each of the following sections are completed successfully before moving onto the next section. If you are having problems, check the troubleshooting guide at the end of this procedure. If you are still having problems, call Dionex.

NOTE: ***Refer to the AAA-Direct manual for assistance and precautions.***

NOTE: ***The instructions in this manual are for use with non-disposable gold working electrodes only (P/N 055832). Please refer to the AAA-Direct Product Manual (Document No. 031481) when working with disposable electrodes (P/N 060082 - 6 electrode and P/N 060140 - 24 electrode).***

4.1 System Configuration and Start-up

4.1.1 System Configuration (Time Requirement: 120 min)

CAUTION: Never polish a disposable gold electrode.

a) Configure the system as follows:
   1. The AS50 autosampler on the left
   2. The injection module in the middle
   3. The pump on the right
   4. The detector on top of the pump.
b) Ensure that nitrogen or helium is delivered to the eluent organizer with about 5–6 psi at each bottle.
c) Check that the AS50 is plumbed with red tubing (0.005 i.d).
d) Check that the red tubing is minimized to reduce dead volume.

NOTE: ***Do not connect the column as that will be completed in a future step.***

e) Make all fluid and electrical connections.
f) Install the yellow tubing from the Installation Kit between the injector and detector cell inlet.
g) Assemble the electrochemical cell with the gold (Au) AAA-Direct-Certified working electrode (P/N 055832).
h) Verify that the modules are communicating.

4.1.2 Software Installation (Time Requirement: 5 min)

a) Restore the sequence “HisNIST” from the Installation Disk into the “Data” directory of Chromeleon, using the same sequence name “HisNIST.”
b) Using the "Save As" function, create a copy of this sequence under a different name, e.g. “Installation.” This installation sequence contains raw data and will be used during the installation process.
c) Remove any signal “offset” from the program file so that actual detector response measurements can be recorded.
d) Verify that the sequence was copied successfully, see Figure 1 for an example of the "HisNIST" file. This sequence contains raw data for comparison with chromatograms you are going to generate during installation.

Sequence: HisNIST Page 1 of 1
Operator jcheng Printed 7/2/01 5:15:43 PM

Title: eds sequence
Datasource: PJANDIK_SYS2
Location: sequence\Jul2001\CareKit
Timebase: PJANDIK_SYS2_1 Created: 6/28/01 5:48:10 PM by jcheng
#Samples: 4 Last Update: 7/2/01 1:37:53 PM by jcheng

No	Name	Type	Pos	Inj.	Program	Method	Status	Inj. Date/Time	Comment
1	WaterBlankIsocratic	Unknown	1	25.0	36A24B40C_10min	His	Finished	6/28/01 6:52:25 PM	Response test
2	8uMHis	Standard	2	25.0	36A24B40C_10min	His	Finished	6/28/01 9:58:44 PM	Response test
3	WaterBlankGradient	Unknown	1	25.0	GP50Comp_010PN6	NIST	Finished	6/29/01 3:48:29 PM	System test
4	8uMNISTsrm2389	Standard	3	25.0	GP50Comp_010PN6	NIST	Finished	6/29/01 5:04:36 PM	System test

Figure 1
HisNIST Sequence as it should appear in the Data Directory following Restore

4.1.3 System Rinse (Time Requirement: 75–90 min)

a) Prepare a solution of 2 M NaOH to rinse each bottle:
   1. Dilute 104 mL of 50% sodium hydroxide to 1 L of Deionized Water (DI water)
b) Place the 2 M NaOH in a pre-rinsed bottle.
c) Place all 4 eluent lines in the pre-rinsed bottle.
d) Withdraw at least 40 mL of sodium hydroxide from each line, using a syringe.
e) Close the solvent "draw-off" valve.
f) Leave the pump proportioning at 25/25/25/25 for 15 minutes.
g) Ensure all surfaces come into contact with the sodium hydroxide (rotate the injection valve).
h) Repeat the process with DI Water (18.2 megohm-cm water).
i) If everything completed successfully then continue to the next section.

4.2 Verification of System Cleanliness

4.2.1 Eluent Preparation (Time Requirement: 45 min)

a) Prepare eluents as described in the AAA-Direct Product Manual (Document No. 031481) and transfer them into their respective eluent reservoirs making sure to follow all of the precautions.
b) Set the eluent component to 100% for each eluent
c) Draw out at least 40 mL of eluent from each reservoir after filling the eluent bottles.

4.2.2 System Background Check (Time Requirement: 30 min)

a) Verify system background using the initial conditions of the program “GP50Comp_010PN6” from the installation floppy disk. This file uses Waveform Table 1 and Gradient Table 4 for protein hydrolysates in the AAA-Direct Manual.
b) Ensure the following:
   1. The detector is set to pH mode (not Ag mode)
   2. The cell is not on
   3. The pump is pumping
      - 76% DI water at 0.25 mL/min (Channel A)
      - 24% 0.25 M NaOH at 0.25 mL/min (Channel B)
c) Confirm the pH reading in the Detail Screen of the detector is between 12.1 and 13.0.
d) Turn on the cell and begin monitoring the background signal from the control panel for at least 30 min.
e) Confirm the baseline is < 80 nC. If the background exceeds 80 nC or the pH is out of range, see the Troubleshooting Section.

4.3 Verification of System Response

4.3.1 Adjusting Eluent Composition (Time Requirement: 10 min)

a) Change eluent composition to:
   1. 36% DI water at 0.25 mL/min (Channel A)
   2. 24% 0.25 M NaOH at 0.25 mL/min (Channel B)
   3. 40% 1.0 M NaOAc at 0.25 mL/min (Channel C)

b) Wait 10 minutes until the background is stable and <130 nC.
   - If it is drifting down, wait as long as it takes to stabilize below 130 nC
   - If the background exceeds 130 nC, see the troubleshooting section
   - If the background is stable and <130 nC, proceed to the next section

4.3.2 Column Installation (Time Requirement: 15 min)

a) Stop the flow.
b) Turn off the cell voltage.
c) Remove the yellow restrictor tubing.
d) Install the AminoPac PA10 guard and analytical columns, but **DO NOT** connect the column outlet to the cell inlet yet.
e) Turn the pump back on.
f) Pump the following through the column and into a waste container for 10 min.
   1. 36% DI water at 0.25 mL/min (Channel A)
   2. 24% 0.25 M NaOH at 0.25 mL/min (Channel B)
   3. 40% 1.0 M NaOAc at 0.25 mL/min (Channel C)

f) Connect the column tubing to the cell.
g) Verify the background is still <130 nC.
   - If it is not, see the troubleshooting section
   - If the background is stable and <130 nC, proceed to the next section

4.3.3 Histidine Injection (Time Requirement: 30 min)

a) Make an 8 µM solution of histidine by adding 1 mL of water to the dry residue in the micro vial shipped with installation kit.
b) Place a vial with DI water in position 1 of the autosampler.
c) Place the histidine quality solution in position 2.
d) Run lines 1 and 2 as shown in the Installation sequence created as a copy of the HisNIST sequence from the Installation floppy disk.
e) Confirm the peak height for histidine is >200 nC (see Figure 2).
f) Ensure the % RSD for "His" peak height is <5%.
   - If this is not the case, see the troubleshooting section

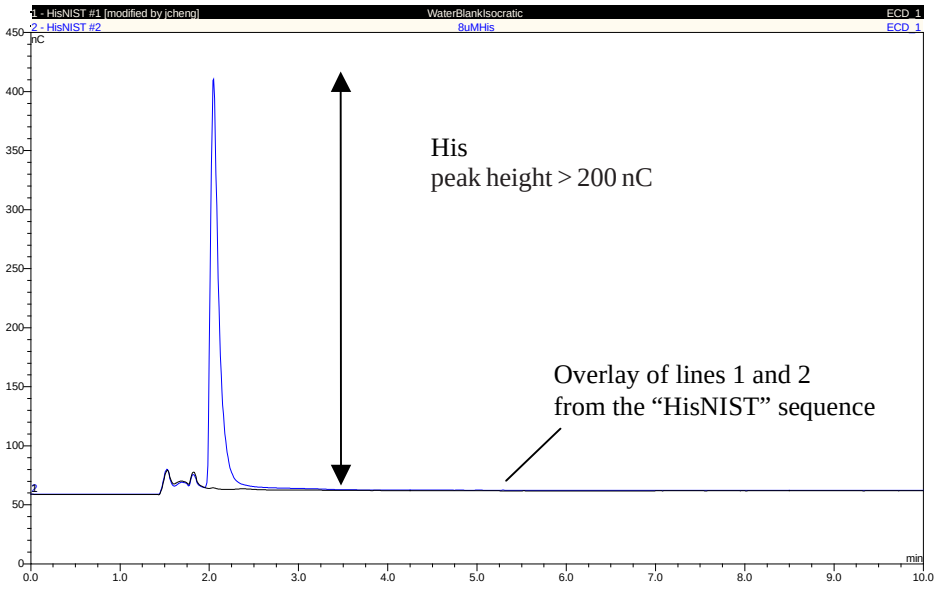

Figure 2
Testing the Detection Response

4.4 Verification of System Functionality

4.4.1 Injection of NIST SRM 2389 Standard (Time Requirement: 100 min)

a) Program the pump to deliver:
   - 76% DI water at 0.25 mL/min (Channel A)
   - 24% 0.25 M NaOH at 0.25 mL/min (Channel B)

b) Let the system equilibrate.
c) Set the column oven to 30 °C.
d) Verify that the background level is < 80 nC.
e) Prepare 1 L of 20 mg/L of sodium azide in DI water.
f) Prepare 100.00 mL of 8 µM NIST standard by doing the following:
   1. Pipeting exactly 320.0 µL of NIST SRM 2389 concentrate into a clean 100 mL volumetric flask
   2. Filling up the flask to 100 mL with the 20 mg/L azide solution.

g) Ensure that there is still a water blank (vial of DI water) in position 1 of the autosampler.
h) Place the 8 µM NIST standard into position 3.
i) Execute lines 3 and 4 of the Installation sequence.
j) Confirm that the baseline rise from the start of the run to the top of the acetate gradient does not exceed 50 nC.
   - If this is not the case, see the troubleshooting section

k) Confirm that the Arginine peak is >120 nC/235 pmol (see Figure 3).
   - If this is not the case, see the troubleshooting section

l) Overlay your separation with the information from line 4 of the HisNIST sequence.
m) Confirm that the resolution between Ala and Thr is comparable.
   - If this is not the case, see the troubleshooting section

NOTE: ***If everything is successful to this point then installation is complete.***

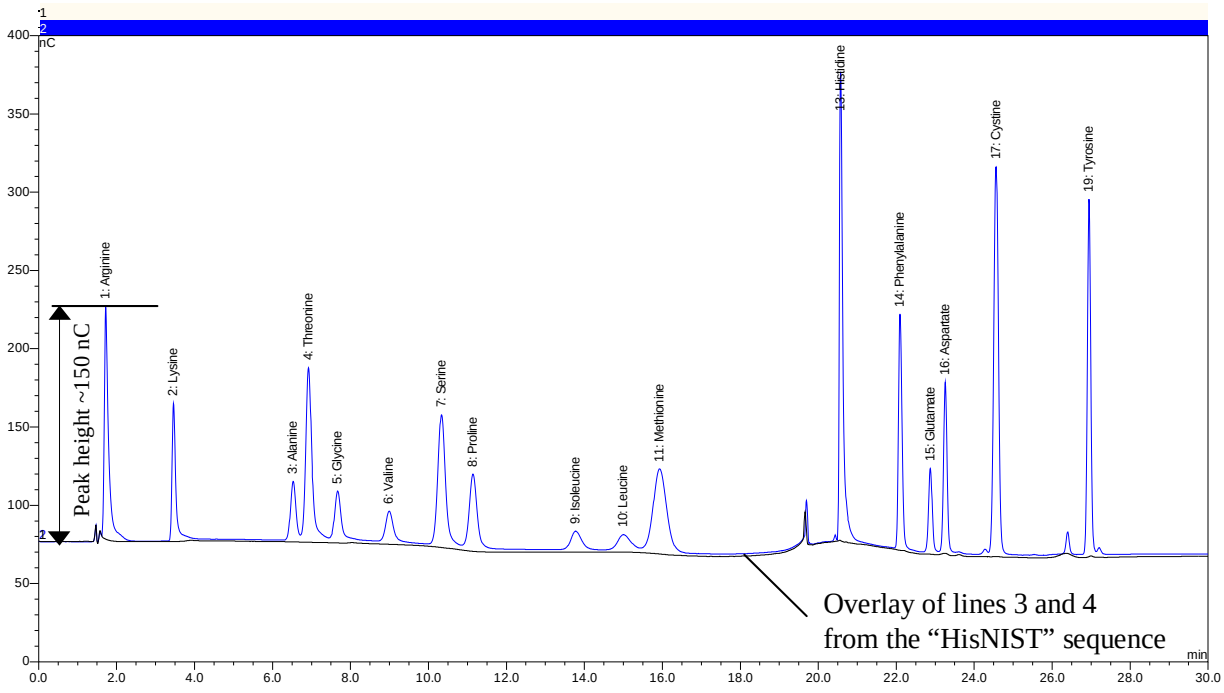

Figure 3
System Test

SECTION 5 - INSTALLATION TROUBLESHOOTING

5.1 Verification of System Cleanliness

- If pH is not within range:
  a) Confirm that the eluent composition is set to 76/24% (A/B).
  b) If eluent is correct, recalibrate Reference Electrode (see instructions in the ED50 Manual).
     - If the reference electrode cannot be calibrated, check the cable from the detector to the cell.
     - Alternatively, replace the reference electrode.

- If background >80 nC and not decreasing:
  a) Repeat the 2 M sodium hydroxide rinse through all lines.
     - If the baseline remains at >80 nC after the second sodium hydroxide rinse, rinse the entire system, including all the bottles and eluent lines with 6 N nitric acid, overnight.
     - Replace the AAA-Direct gold working electrode with the spare if the baseline continues to exceed 80 nC.

- If either the baseline or the pH remain out of range, call Dionex Support.

5.2 Verification of System Background

- If background remains steady but exceeds 130 nC:
  a) Re-rinse the acetate channel eluent lines and eluent bottle with 2 N NaOH.
  b) In addition, rinse the acetate channel eluent lines and eluent bottle 3-times with purified filtered water.
  c) Make up fresh acetate and try again.

- If after the column is connected to the cell, the background exceeds 130 nC and is not dropping:
  a) Remove the column and replace the restrictor tubing, pumping 36:24:40 (A:B:C).
  b) If the background is < 130 nC, install a new AminoPac PA10.
  c) If the background exceeds 130 nC with the restriction tubing and is not dropping:
     1. Discard the acetate eluent
     2. Rinse line C and the rest of the system thoroughly with DI water from line A.
     3. Prepare new acetate and re-check the background.
        - If it is high again, you either have a contamination source in your labware or a bad lot of acetate. Call Dionex for help.

- If the histidine peak height is out of range:
  a) Check the injector and confirm that the 25 µL injection loop is installed.
  b) Check if the autosampler is set properly and functioning correctly.
     - If the autosampler is set properly and functioning correctly:
       1. Remove the column
       2. Install the restriction tubing
       3. Inject the histidine.
  c) The height of the histidine peak near the void should exceed 180 nC:
     - If the peak height is > 180 nC, install a new column.
     - If the peak height is < 180 nC, replace the AAA-Direct gold working electrode with the spare and repeat the installation section.

5.3 Verification of System Functionality

- If the acetate gradient causes a baseline rise exceeding 50 nC but the second injection shows less rise than the first:
  a) Run 6:24:70 (A:B:C) through the column for 1 hour
  b) Re-equilibrate for 30 minutes at 76:24 (A:B)
  c) Repeat the gradient test.

- If the first and second injections show similar and excessive baseline rise, the eluent lines should be cleaned with 1 M NaOH as described in the AAA-Direct Manual.

- If the arginine peak is <140 nC/200 pmol replace the AAA-Direct gold working electrode with the spare.
  a) Call Dionex should the arginine peak height be lower than 140 nC after installation of the spare Au electrode.

- If you observe a resolution loss between Ala and Thr:
  a) Check the supply of inert gas to channel B (250 mM NaOH).
     - Carbonated NaOH will cause resolution loss and retention time fluctuation in the Ala to Met region of the chromatogram.
  b) If poor resolution persists, remove guard column and re-inject the same standard using the main column only.
  c) Replace the guard if resolution is restored.
  d) Replace the analytical column if poor separation persists without the guard.

- Another possible source could be the temperature accuracy of the AS50 TC. Call Dionex should poor separations persist between Ala and Thr after removing the guard.

WARNING: AVOID PRESSURE FLUCTUATIONS. SEVERE PRESSURE PULSES CAN DAMAGE THE GUARD COLUMN CAUSING POOR PEAK SHAPES (SPLIT PEAKS, PEAK FRONTING, TAILING) AND RESOLUTION.

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