

APPLICATION MANUAL

Amino Acids

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High performance liquid chromatography (HPLC) with post-column derivatization is a technique for rendering analytes more detectable than they would otherwise be in their native forms. Post-column derivatization gives improved sensitivity or better selectivity (reduction of interference) leading to lower detection limits. The Pickering Laboratories PCX5200 was developed to facilitate the determination of amino acids in protein hydrolysates using sodium ion-exchange or in native samples using lithium ion-exchange. There are two options for post-column detection of amino acids. The first is the use of Pickering's patented TRIONE[®] ninhydrin reagent, which will react with both primary and secondary amino acids. The second is the use of o-phthalaldehyde (OPA), a fluorescent reagent that gives greater sensitivity but will detect only primary amino acids.

Post-column Analysis

A complete post-column analysis system for amino acids consists of the following components:

- HPLC ternary or greater gradient pump
- Manual injector or autosampler equipped with high pH compatible Tefzel[®] or PEEK[™] seals
- Pickering Laboratories ion-exchange columns
- Pickering PCX5200 post-column derivatization instrument
- Eluants, reagents, and standards
- Visible or fluorescence detector
- Chart recorder, integrator, or data system

Ion-exchange Chromatography of Amino Acids

Ion-exchange chromatography followed by post-column derivatization has been the method of choice for amino acid analysis since S. Moore, D.H. Spackman and W.H. Stein published it in 1958—work which merited a Nobel prize.

The separation is a multi-modal process wherein ion-exchange, ion-exclusion, and partition all take place. The primary process is cation-exchange where a pH gradient mobilizes amino acids in order of their isoelectric points; acidic amino acids such as glutamic acid elute early and basic amino acids such as lysine elute late. Partitioning is affected by ionic strength and organic modifiers; for example threonine and serine are resolved by partition effects. Ion-exclusion only occurs for highly acidic amino acids such as taurine.

Sodium ion-exchange is used for fast analysis of the 22 amino acids found in hydrolyzed protein or in simple formulated products.

Lithium ion-exchange is a slower technique with higher resolution to separate as many as 46 amino acids and compounds found in the complex mixtures of biological fluids or tissue extracts.

Post-column Derivatization

TRIONE® (Ninhydrin)

The most popular reagent for post-column detection is ninhydrin. Ninhydrin reacts with primary amines and hydrindantin to form Ruhemann's Purple (Figure 1-1) which is detectable at 570nm. Ninhydrin reacts with secondary amines to form a yellow complex detectable at 440nm.

The ninhydrin reaction is carried out at 130°C with a reactor volume of 500 µL. The elevated temperature is required to because at room temperature, the ninhydrin reaction is very slow and takes hours to go to completion.

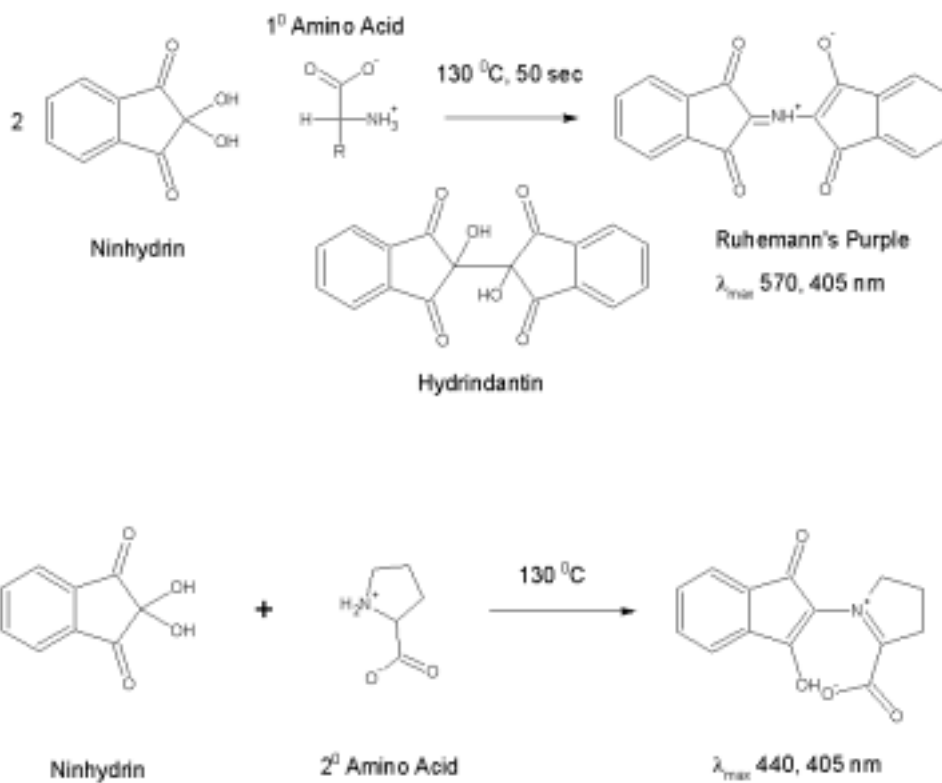


Figure 1-1

**OPA
(Fluorescence)**

An alternative reagent system based on *o*-phthalaldehyde can be used for high-sensitivity detection of primary amino acids. OPA reacts rapidly with primary amines and Thiofluor™ (N,N-dimethyl-2-mercaptoethylamine) under mild basic conditions to produce a strongly fluorescent isoindole derivative (Figure 1-2). OPA does not react with secondary amines or aryl amines, so fails to detect Proline and other secondary amino acids. However, it is possible to detect secondary amino acids by using a two-step reaction in which they are first oxidized and then reacted with OPA. This technique has some disadvantages, and is not often used. Contact Pickering for details.

The Pickering PCX5200 derivatization instrument for fluorescent detection of amino acids is similarly designed to the ninhydrin instrument, except that it contains a 150 μ L reactor and a higher pressure restrictor (in the pulse damper) for the less viscous OPA reagent. Also, the reaction is carried out at 40°C.

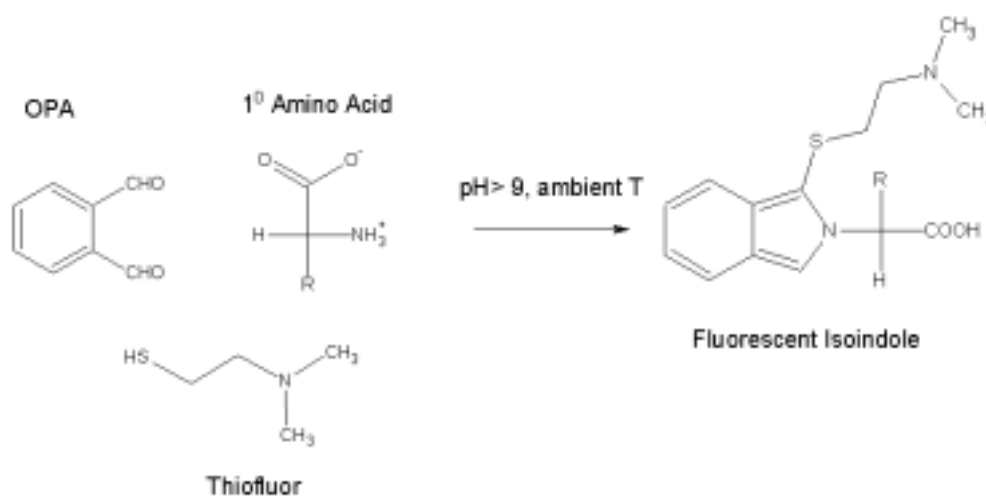


Figure 1-2

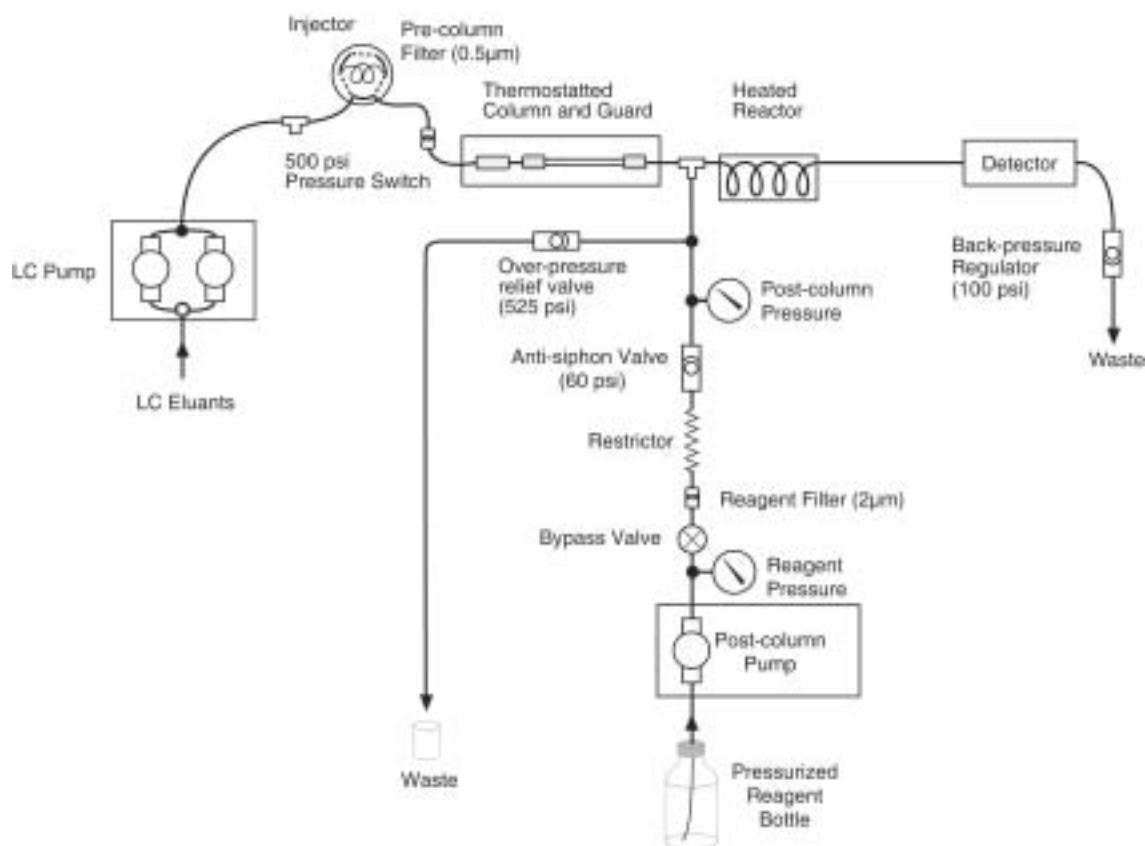


Figure 1-3

Post-column Reaction Hardware

The Pickering single-reagent design (Figure 1-3) uses a thermostatted column oven, single piston reagent pump, heated reactor coil. However, there are many refinements to the system which increase sensitivity and ease of use:

- Pump pulses are eliminated by the mechanical action of the Bourdon tube inside the reagent gauge, and then released through a packed-bed restrictor.
- Elevated reactor temperatures cause boiling inside the reactor and flow cell. This is eliminated by a back-pressure regulator placed after the flow cell.
- A post-column gauge monitors pressure at the first mixing tee, which is also the pressure at the first reactor to warn of potential blockage inside the system.
- Bypass valves are provided for priming or purging the reagent pumps.
- Pressurized reagent reservoirs allow the pump to operate more precisely at low flow rates, and also provides an inert atmosphere to protect air-sensitive reagents.

Safety Systems

Safety systems have also been incorporated into the design to protect against 1) rupture of the reactor because of the excessive pressure and 2) back-flow of caustic reagent onto the analytical column:

- An over-pressure relief valve opens at 525 ± 10 psi (36 bar) and diverts flow away from the reactor.
- A pressure switch ensures eluant flow through the column during operation by enabling the system only when there is a column pressure > 500 psi (34 bar).
- Anti-siphon valves in the reagent delivery system prevent reagents from siphoning when the pump is off.

NOTES:

Chapter 2

Installation & System Operation

Read all installation instructions in the PCX5200 Operation Manual before operating your post-column derivatization instrument and HPLC system. The following assumes that the PCX5200 has been installed according to the directions in the Operation Manual.

HPLC system requirements

Pump :

- Minimum ternary gradient elution
- Piston wash capability is preferable

Injector:

- Tefzel® or PEEK® rotor seal for injector valve
- Tefzel® or PEEK® needle seat if it is an autosampler

The buffers and column regenerant used in the amino acid application require special preparation of the HPLC system:



- The column regenerant is ***strongly alkaline (pH 12+)***. Autosamplers typically contain seals made of Vespel®, a soft polymer that will dissolve at high pH. The rotor seal and needle seat must be Tefzel® or PEEK®.
- Lithium and Sodium salts are mildly corrosive to stainless steel when *exposed to air*. Remove steel eluant filters and gas dispersers.
- Thoroughly clean the HPLC system before using it with the PCX5200. The post-column reaction is extremely sensitive, and contamination will show in the chromatogram.
- Thoroughly purge all organic solvents from the HPLC before starting the installation. Organic solvents will swell the resin inside the column and cause it to over-pressure.
- The pulse dampener on the HPLC and the pressure transducer on the PCX5200 must be dead-headed prior to running amino acid analysis. See page 2-2 for more details.

On most HPLC pumps, the pressure transducer and/or pulse damper must be “Deadheaded” to prevent corrosion of the internal steel and to ensure reproducible gradient formation (see below).

1. The diaphragm of the pressure transducer on the PCX 5200 is type 316 stainless steel, and will corrode rapidly if it comes into contact with lithium or sodium eluants. This is also true for some HPLC pulse dampeners.
2. Correct gradient formation is critical in amino acid analysis. Even slight changes in pH can drastically affect the separation. The large internal volume of diaphragm-type pulse dampeners is not adequately flushed at the low flow rates associated with amino acid analysis. That is, from run to run, a small amount of eluant remains in the pulse dampener, and will alter the gradient composition.

The solution to both of these problems is to plumb the HPLC pump so that the eluant does not flow directly through the pulse dampener or the pressure transducer. This is done by installing a tee between the pump head and the pulse damper and/or pressure transducer (Figure 2-1). In this configuration, the eluant flows from the tee to the column, and the pulse damper or pressure transducer exit line is plugged.

Procedure Following figure 2-1:

1. Use 1/16 inch x 0.020 inch ID capillary tubings for this section. Connect a tee-fitting between the outlet of the HPLC pump and the inlet of the injector.
2. Connect the third port of the tee to the inlet of the pulse damper.
3. Connect the outlet of the pulse dampener to the lower inlet of the pressure sensor on the post-column pressure panel, labelled “From Pump”.
4. Flush the system without the guard or column attached with 20% methanol / 80% water.
5. Install a high-pressure plug in the upper outlet of the pressure sensor, labelled “To Injector”.

Optional Restrictor Installation

There are certain situations that require the installation of an eluant restrictor to increase the column pressure. For example, some older model autosamplers cause a large drop in pressure on injection that will cause the column pressure to drop below 35 bar, and the PCX 5200 to shutdown. Should this be the case, it is first very important to rule out another source of the pressure drop (i.e. worn seals). If you are certain that the system is in good working order, then you can install a restrictor between the pump and the injector (See Figure 2-2).

Your HPLC pump may require the installation of an eluant restrictor if:

- The pressure-feedback control becomes unstable because of compliance in the pressure switch (in the PCX5200).
- The normal operating pressure of the column is too low for the pulse dampening in the HPLC to function properly.
- The HPLC pump uses a single high-pressure piston with a rapid refill stroke.

Pickering manufactures eluant restrictors in several pressure ratings. Please contact Pickering Laboratories' Technical Support Department for available sizes, or if you are unsure if you require a restrictor.

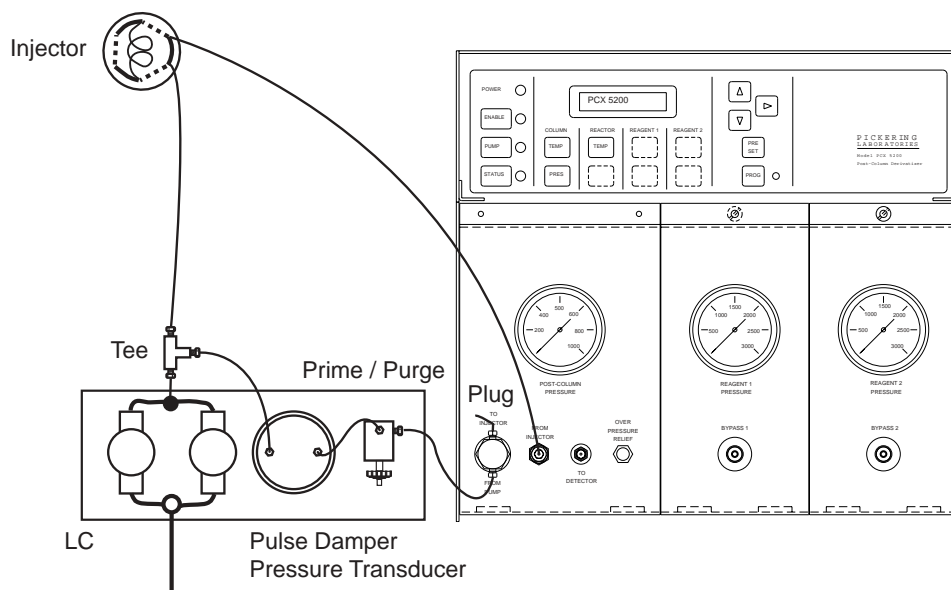


Figure 2-1. Typical example of an HPLC pump with the bypass tee installed

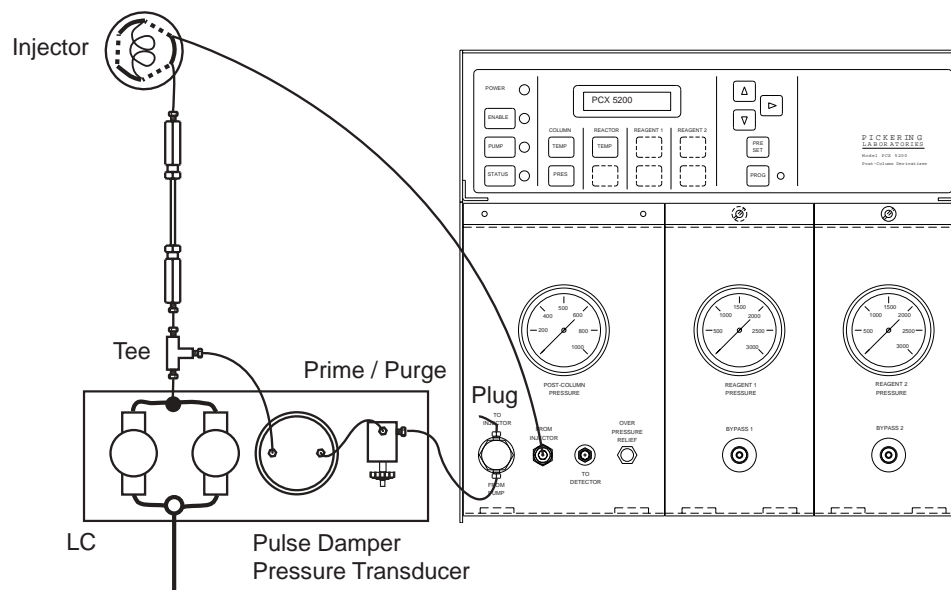


Figure 2-2. Typical example of an HPLC pump with the bypass tee and restrictor installed

Getting Started

Eluant Priming

The Pickering Laboratories amino acid analysis protocol requires three eluants: an acidic buffer, a neutral buffer, and column regenerant. Use the eluant system that is matched to your column and application. You may choose other conditions or eluants for your routine work, but during installation and checkout, we recommend you use Pickering's standard conditions. See pages 2-15 through 2-17 for recommended conditions.



Note: Pickering mobile phases are filtered before bottling, so it is unnecessary to filter the mobile phases before use. Filtering with marginally clean glassware has been known to introduce large amounts of contaminating compounds to the mobile phases.



Note: Degassing the eluants is not necessary. *Do not* continuously sparge the mobile phases, as this will alter the composition over time.



Caution! Always wear gloves for this operation. Avoid touching the inside of reservoirs or handling the solvent with bare fingers since amino acid contamination present on hands causes high background or baseline irregularities.

1. Fill eluant reservoir "A" with the acidic buffer
2. Fill eluant reservoir "B" with the neutral buffer
3. Fill eluant reservoir "C" with column regenerant
4. Flush at least 30 mL of each solvent through the HPLC system, including the "From Injector" tubing.
5. Flush the lines thoroughly with the three eluants *before* installing the column. Use pH paper to measure the pH of the solution at the outlet of the "From Injector" tubing to ensure that the lines are thoroughly flushed. It should correspond to the pH of the eluant in the reservoir. Start flushing with 100% regenerant, then 100% near-neutral eluant, and finally 100% low-pH eluant.

Column and Guard Installation

1. Be certain that ALL organic has been removed from the system and that the lines contain only buffer. Use pH paper to check the pH of the liquid at the outlet of the "From Injector" tubing.

A set of preformed capillary tubing has been provided for installing the guard column and the column.

2. Loosely connect the outlet of the guard column to the inlet of the analytical column
3. Loosely fit the inlet of the guard column to the outlet of the reducing union

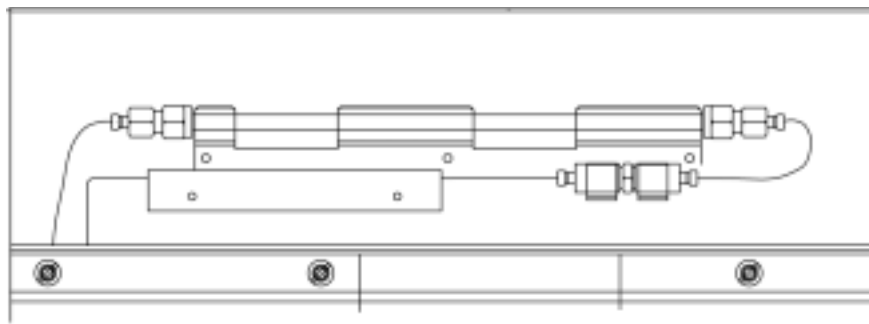


Figure 2-3 Front view of column oven with column and guard in place

(Figure 2-3). Carefully lay the analytical column into its slot in the heating block.

4. Start the HPLC pump at 100% Regenerant, 0.2ml/min. Wait for liquid to drip, and then successively tighten the connections, using caution not to over-tighten.
5. A loose end of tubing in the column oven leads to the first mixing tee. Attach the loose end to the outlet of the analytical column.
6. Set the flow rate of the HPLC pump to the appropriate flow rate for your column and with the correct Eluant A. ***Do not exceed the maximum flow rate for the ion-exchange column.***
7. Inspect the column and all connections for leaks. Even the slightest leak can have severe consequences. Stop all leaks before proceeding.

TRIONE Preparation

1. Thoroughly wash the reagent reservoir and then rinse with methanol. Wipe down the dip tubes with methanol and a clean cellulose tissue.
2. TRIONE® reagent requires little to no preparation, depending on what type you use.

T100: The one-part TRIONE® (Cat. No. T100C) requires no preparation - simply pour the TRIONE® directly into the reagent reservoir and put the cap on the reservoir.

T200: To prepare two-part TRIONE® (Cat. No. T200), pour Bottle 1 into the reservoir, add Bottle 2 to the reservoir, and cap tightly under Nitrogen. Swirl until homogeneous.

Note: TRIONE® is air sensitive, and must be kept under Nitrogen. The useful lifetime of T100 is three months* unopened, and one month in the reservoir. The shelf-life of the two-part TRIONE® is one year* unmixed, and one month in the reservoir.

*From date of manufacture

OPA Preparation

1. Pour 945mL of the OPA Diluent (Cat.No. OD104) into the reagent reservoir.
2. Put the cap on the bottle, open the vent valve, and turn on the gas supply. Thoroughly deaerate the contents by sparging with inert gas. Continue bubbling for at least 10 minutes.
3. Dissolve 300mg of OPA (Cat. No. O120) in 10mL of HPLC-grade methanol in a clean, dry container.
4. Turn off the gas supply and remove the cap from the bottle. Add the OPA solution to the deoxygenated Diluent in the reservoir. Wash any residual mixture into the reservoir with an additional 1–2 mL of methanol.
5. Dissolve 2g of Thiofluor™ (Cat. No. 3700-2000) in the remaining 5 mL of OPA Diluent and add to the reservoir.
6. Add 3mL of 35% Brij-35® solution.
7. Replace the cap and close the vent valve. Gently swirl the reagent to complete the mixing. Turn on the gas.



Note: the OPA reagent is sensitive to air oxidation and will degrade over time. The PCX5200 system is designed to minimize this oxidation. When the OPA reagent reservoir is maintained under inert gas pressure, the OPA reagent can maintain its activity for up to one week without significant loss of activity.

Priming of Reagent Pump

1. Connect a 20 mL disposable syringe to the Luer fitting in the center of the prime/purge valve.
2. Open the prime/purge valve 1 full turn (CCW) and let the flow exit into the syringe.
3. To purge air bubbles from the reservoir line, pump head, or reagent gauge, syringe suction may be applied.
4. Close the valve, remove the syringe, and wash the Luer fitting with a water.

Piston Wash*

5. Push 3 mL of 80/20 Water/Methanol through the piston wash on the reagent pump to wet the seals. Perform this step three times each day the system is in operation.

* Refer to the PCX5200 Operation Manual for more details on the Piston Wash

Starting the PCX5200

1. Turn on the main power at the rear of the PCX5200. The temperature displays should activate. The column oven starts warming immediately but the heated reactor remains off (the temperature display is on but there is no heat). Wait until the column oven is near its operating temperature.
2. Turn on the pump, start with Eluant A, and wait until at least 500 psi (35 bar) of pressure develops. The ENABLE LED will glow amber when the column pressure is sufficient.
3. Press the ENABLE key. Now the heated reactor controller should activate and the ENABLE LED should change to green.
4. Press and hold the PRESET key; the LCD shows: "Load preset..."
5. While holding down the PRESET key, press the ▼ key until the LCD shows: "1 Na AA" or "1 Li AA" (or others, if applicable).
6. Check that the column temperature setting is correct (lithium columns operate at 40–45°C and the sodium columns operate at 50–55°C) and the reactor temperature setting is 130°C for TRIONE® and 45°C for OPA. Press the COLUMN TEMP key or REACTOR TEMP key on the keypad to view the setpoint and release it to show the actual temperature.
7. Once the temperatures of the heated reactor and column oven reach their setpoints, press the PUMP key.

The POWER LED remains green.

The ENABLE LED remains green.

The PUMP LED turns green.

The STATUS LED turns green.

The reagent gauge should begin pulsing with a maximum of about 1,300–2,000 psig. Note! The pulsating pressure reading of the reagent pump (approximately 700 psig swing) is normal.

Inspect all tubing connections in the post-column reaction instrument to ensure there are no leaks.

HPLC Detector Set Up

UV/Visible

- Refer to your HPLC instruction manual for setup details.
- For TRIONE® ninhydrin with UV/VIS detection, primary amino acids have an optimum response at 570nm. Secondary amino acids are optimum at 440nm. Detection at 405nm will detect both primary and secondary amino acids with about the same sensitivity, but may show increased baseline noise. Since most peaks are relatively broad in ion exchange, you can use a response time of 2–5 seconds in most cases.
- If your detector is capable of acquiring two or more channels, use a baseline subtraction of 700 nm to increase sensitivity.

Fluorescence

- For OPA with fluorescence detection, optimum conditions for most detectors are excitation at 330nm and emission at 465nm. If your detector has a selectable time-constant, use about 2–5 seconds.

Integration Set Up

Prepare the HPLC data station or integrator and set up a data handling method to accept data from the detector. Initially, an area % method without naming peaks is adequate. This method should have a peak width of about 10 seconds; the data end time is about 60 minutes for the sodium columns, 130 minutes for the 15cm lithium or 200 minutes for the 25cm lithium column.

HPLC Pump Method Set Up

Pickering Laboratories recommends seven different gradient conditions depending on the column and type of sample. Use the program recommended on the column data sheet for the initial testing. Do not change this program until you are sure that the other aspects of the system are functioning properly.

Set the maximum pressure limit on the HPLC to 220 bar to protect the column.

Chromatograms

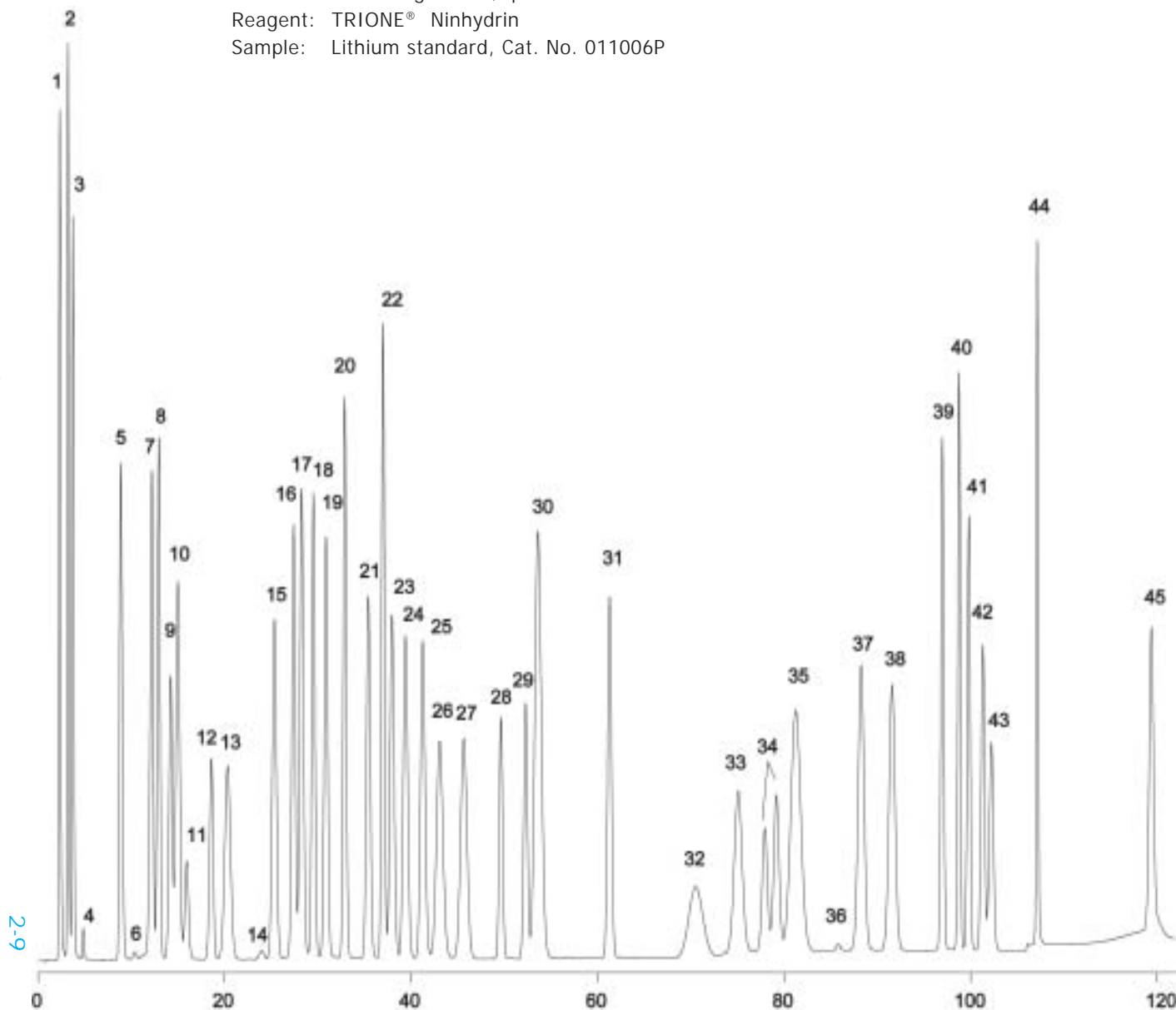
Allow the column to equilibrate for about 30 minutes under initial conditions. Inject 10µL of the 3-Component Test Mixture, and collect three chromatograms. The first chromatogram will not be representative of the performance the system, so use the second two to evaluate the performance.

METHOD 1: HIGH EFFICIENCY LITHIUM

PEAK IDENTIFICATION

1. Phosphoserine
2. Taurine
3. Phosphoethanolamine
4. Urea
5. Aspartic acid
6. Hydroxyproline
7. Threonine
8. Serine
9. Asparagine
10. Glutamic acid
11. Glutamine
12. Sarcosine
13. α -Aminoadipic acid
14. Proline
15. Glycine
16. Alanine
17. Citrulline
18. α -Amino-*n*-butyric acid
19. Valine
20. Cystine
21. Methionine
22. Cystathionine
23. Isoleucine
24. Leucine
25. Norleucine
26. Tyrosine
27. Phenylalanine
28. β -Alanine
29. β -Amino-*i*-butyric acid
30. Homocystine
31. γ -Amino butyric acid
32. Tryptophan
33. Ethanolamine
34. Hydroxylysines
35. Ammonia
36. Creatinine
37. Ornithine
38. Lysine
39. Histidine
40. 3-Methylhistidine
41. 1-Methylhistidine
42. Carnosine
43. Anserine
44. α -Amino- β -guanidino-propionic acid
45. Arginine

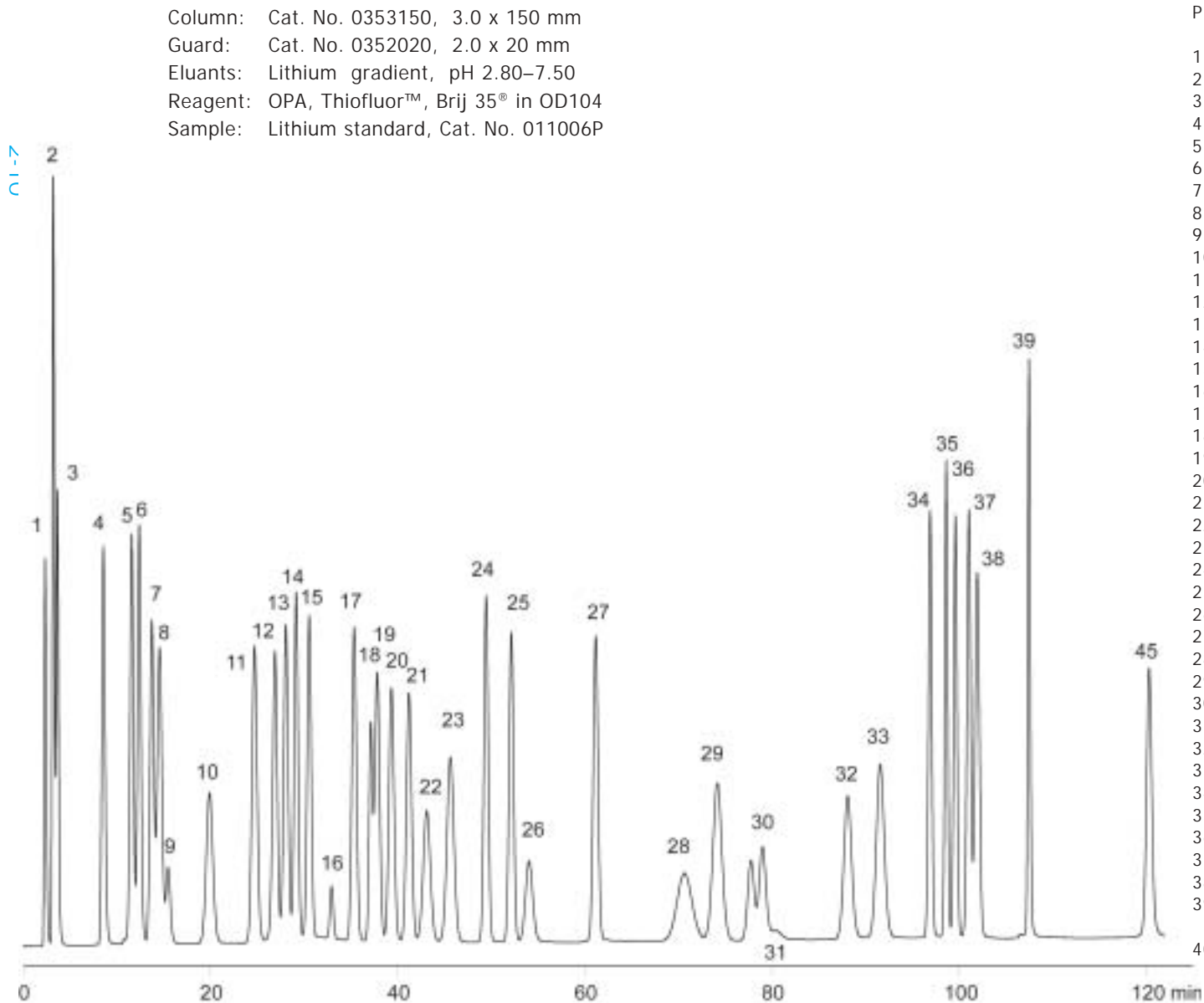
Column: Cat. No. 0353150, 3.0 x 150 mm
 Guard: Cat. No. 0352020, 2.0 x 20 mm
 Eluants: Lithium gradient, pH 2.80–7.50
 Reagent: TRIONE® Ninhydrin
 Sample: Lithium standard, Cat. No. 011006P



**METHOD 1b: HIGH EFFICIENCY LITHIUM, FLUORESCENCE
DETECTION**

PEAK IDENTIFICATION

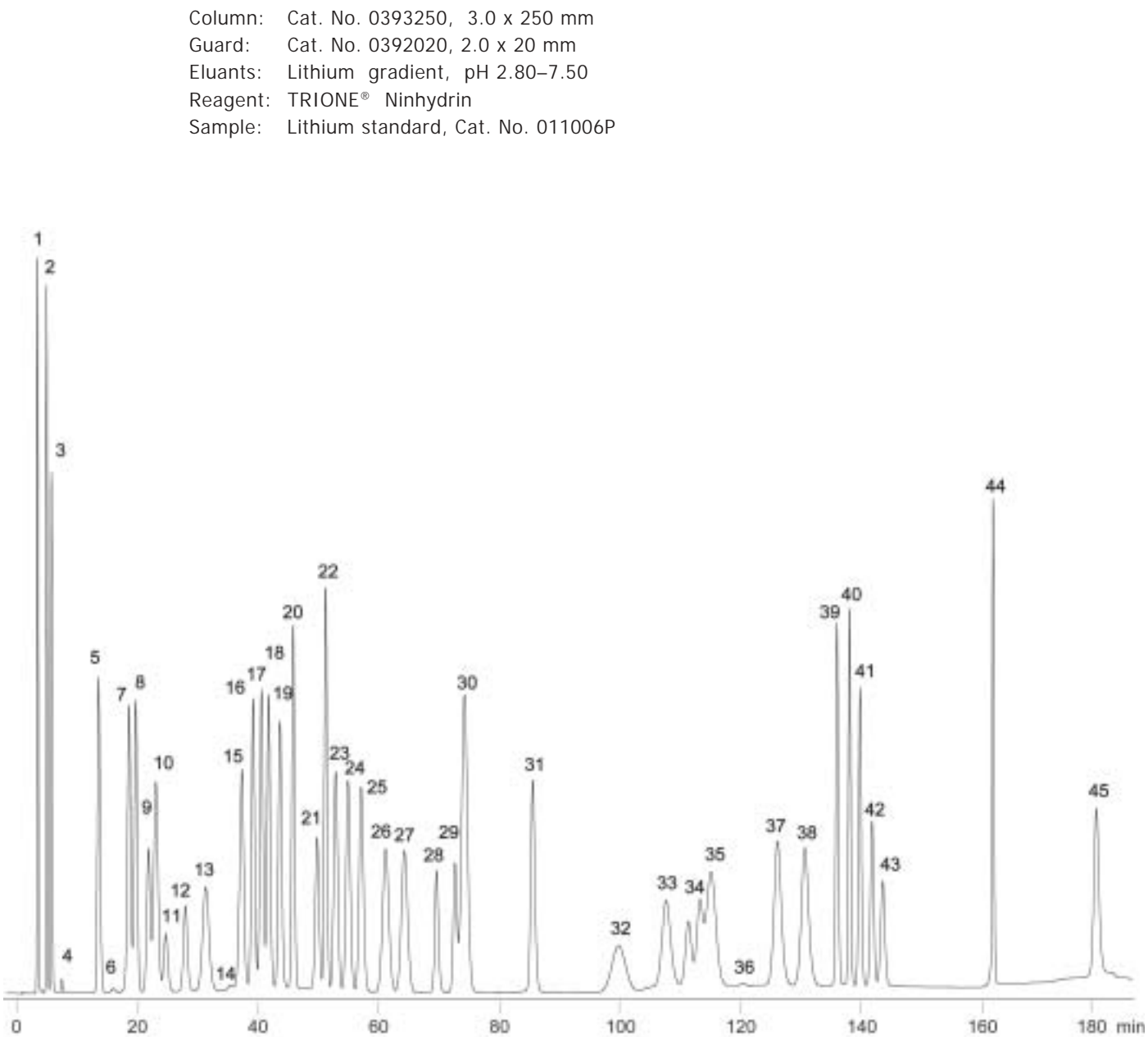
1. Phosphoserine
2. Taurine
3. Phosphoethanolamine
4. Aspartic acid
5. Threonine
6. Serine
7. Asparagine
8. Glutamic acid
9. Glutamine
10. α -Amino adipic acid
11. Glycine
12. Alanine
13. Citrulline
14. α -Amino-*n*-butyric acid
15. Valine
16. Cystine
17. Methionine
18. Cystathionine
19. Isoleucine
20. Leucine
21. Norleucine
22. Tyrosine
23. Phenylalanine
24. β -Alanine
25. β -Amino-*i*-butyric acid
26. Homocystine
27. γ -Amino butyric acid
28. Tryptophan
29. Ethanolamine
30. Hydroxylysines
31. Ammonia
32. Ornithine
33. Lysine
34. Histidine
35. 3-Methylhistidine
36. 1-Methylhistidine
37. Carnosine
38. Anserine
39. α -Amino- β -guanidino-propionic acid
40. Arginine



METHOD 2: STANDARD LITHIUM

PEAK IDENTIFICATION

1. Phosphoserine
2. Taurine
3. Phosphoethanolamine
4. Urea
5. Aspartic acid
6. Hydroxyproline
7. Threonine
8. Serine
9. Asparagine
10. Glutamic acid
11. Glutamine
12. Sarcosine
13. α -Aminoadipic acid
14. Proline
15. Glycine
16. Alanine
17. Citrulline
18. α -Amino-*n*-butyric acid
19. Valine
20. Cystine
21. Methionine
22. Cystathionine
23. Isoleucine
24. Leucine
25. Norleucine
26. Tyrosine
27. Phenylalanine
28. β -Alanine
29. β -Amino-*i*-butyric acid
30. Homocystine
31. γ -Amino butyric acid
32. Tryptophan
33. Ethanolamine
34. Hydroxylysines
35. Ammonia
36. Creatinine
37. Ornithine
38. Lysine
39. Histidine
40. 3-Methylhistidine
41. 1-Methylhistidine
42. Carnosine
43. Anserine
44. α -Amino- β -guanidino-propionic acid
45. Arginine

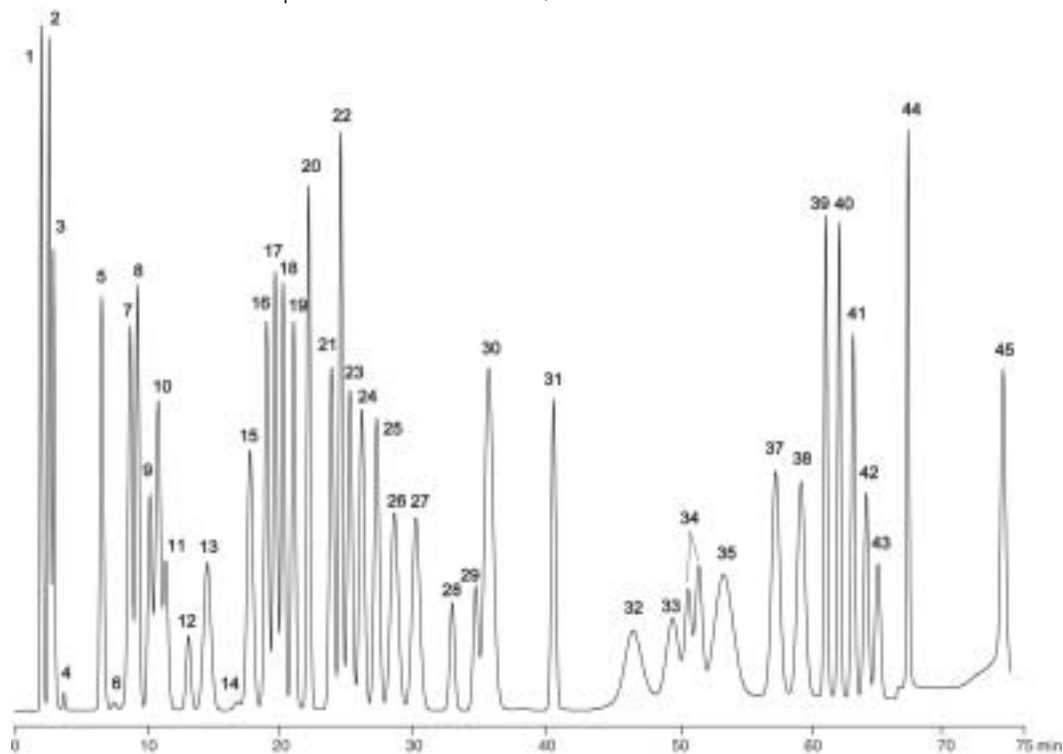


PEAK IDENTIFICATION

1. Phosphoserine
2. Taurine
3. Phosphoethanolamine
4. Urea
5. Aspartic acid
6. Hydroxyproline
7. Threonine
8. Serine
9. Asparagine
10. Glutamic acid
11. Glutamine
12. Sarcosine
13. α -Aminoadipic acid
14. Proline
15. Glycine
16. Alanine
17. Citrulline
18. α -Amino-*n*-butyric acid
19. Valine
20. Cystine
21. Methionine
22. Cystathionine
23. Isoleucine
24. Leucine
25. Norleucine
26. Tyrosine
27. Phenylalanine
28. β -Alanine
29. β -Amino-*i*-butyric acid
30. Homocystine
31. γ -Amino butyric acid
32. Tryptophan
33. Ethanolamine
34. Hydroxylysines
35. Ammonia
37. Ornithine
38. Lysine
39. Histidine
40. 3-Methylhistidine
41. 1-Methylhistidine
42. Carnosine
43. Anserine
44. α -Amino- β -guanidino-propionic acid
45. Arginine

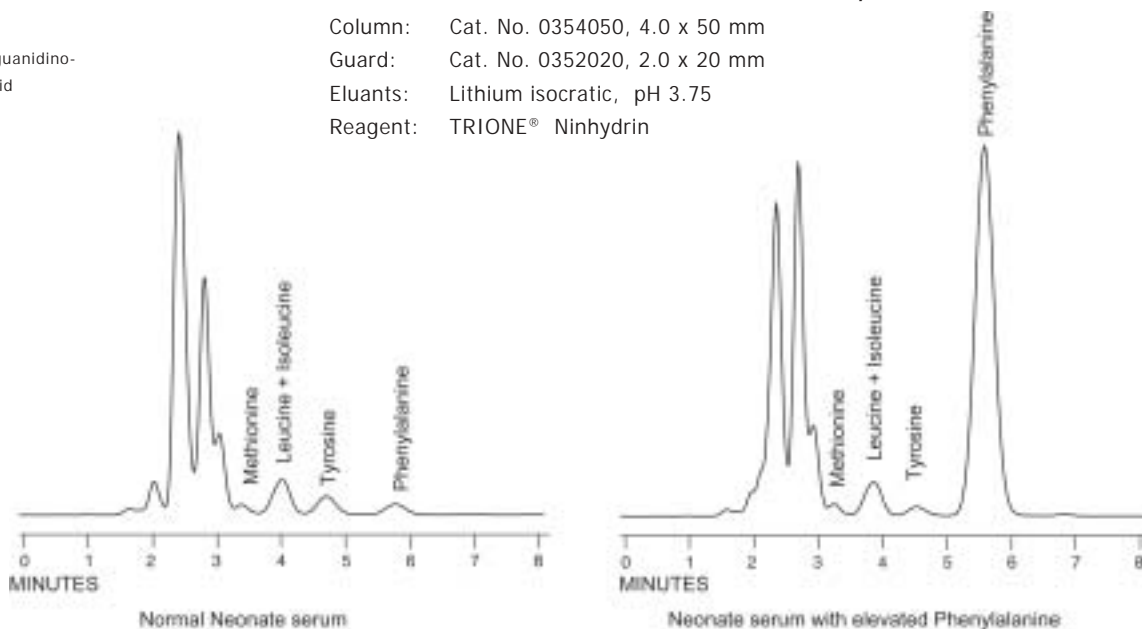
METHOD 3: 75 MINUTE ACCELERATED LITHIUM

Column: Cat. No. 0355080, 5.0 mm x 80 mm
 Guard: Cat. No. 1700-0207, Lithium Guard Column Kit
 Eluants: Lithium gradient, pH 2.80–7.50
 Reagent: TRIONE® Ninhydrin
 Sample: Lithium standard, Cat. No. 011006P



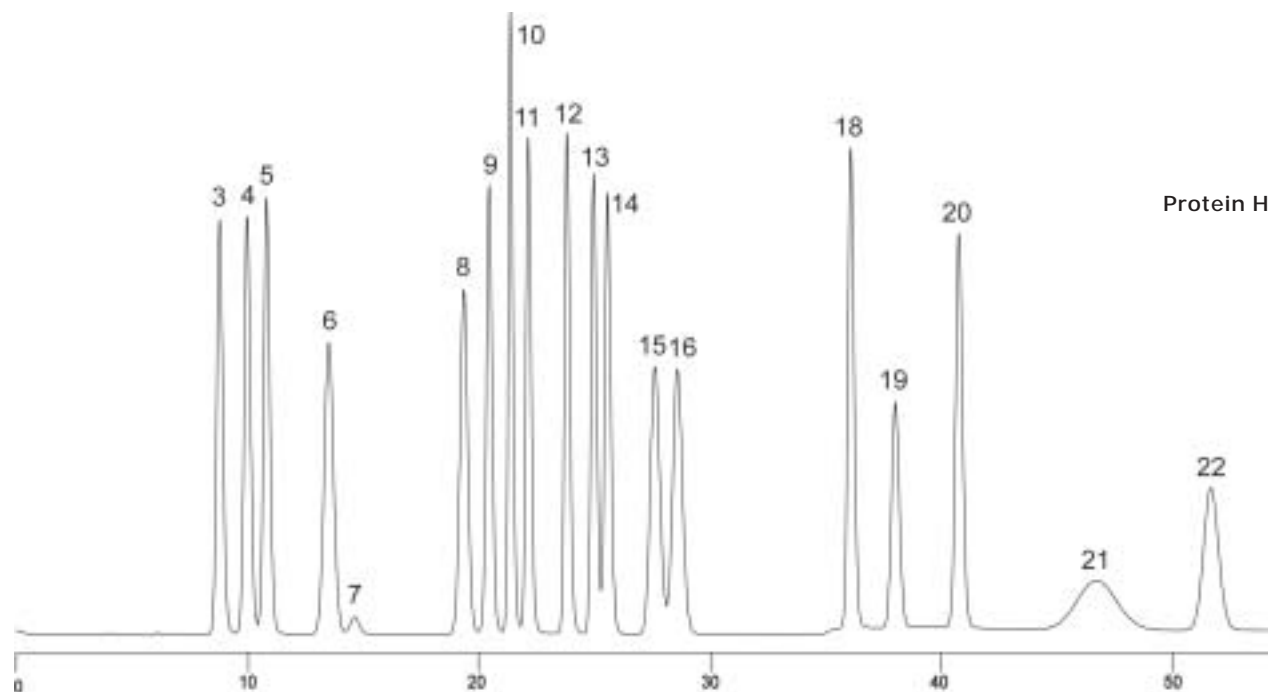
METHOD 4: PKU AND MSUD SCREENING, LITHIUM

Column: Cat. No. 0354050, 4.0 x 50 mm
 Guard: Cat. No. 0352020, 2.0 x 20 mm
 Eluants: Lithium isocratic, pH 3.75
 Reagent: TRIONE® Ninhydrin



METHOD 5: HIGH EFFICIENCY SODIUM, TRIONE DETECTION

Protein Hydrolysate Standard, Cat. No. 012506H

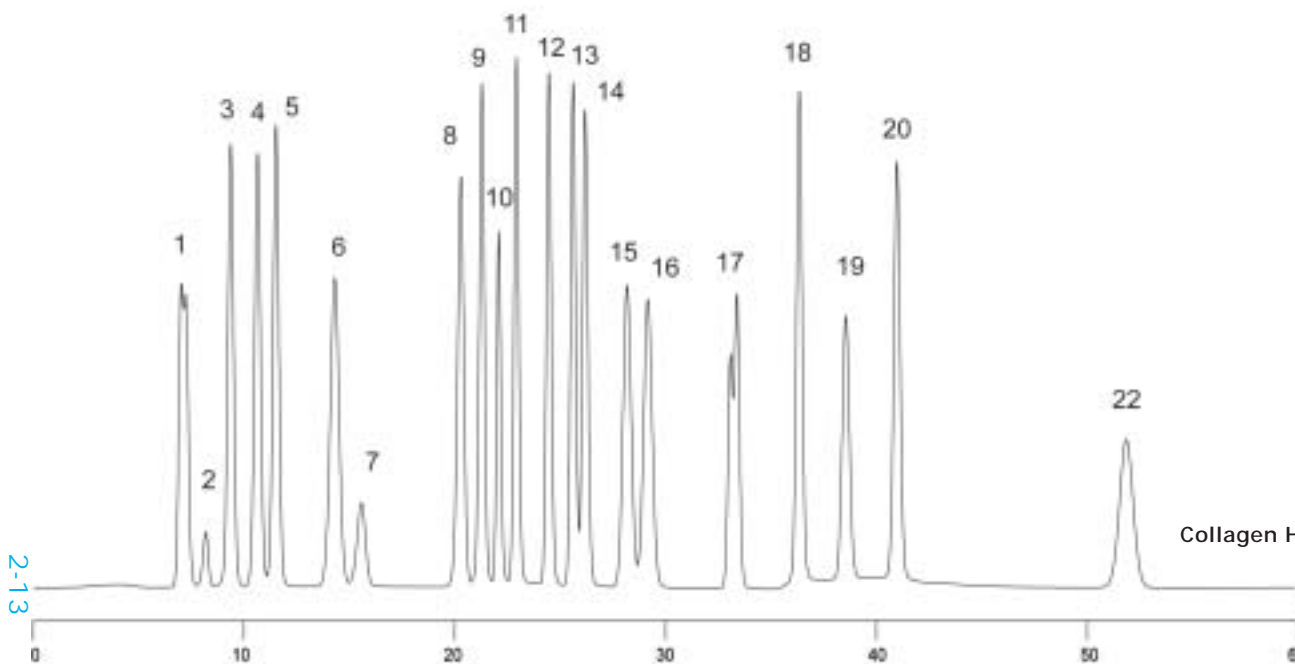


PEAK IDENTIFICATION

1. Methionine sulfoxide
2. Hydroxyproline
3. Aspartic acid
4. Threonine
5. Serine
6. Glutamic acid
7. Proline
8. Glycine
9. Alanine
10. Cystine
11. Valine
12. Methionine
13. Isoleucine
14. Leucine
15. Tyrosine
16. Phenylalanine
17. D,L & allo-Hydroxylysine
18. Lysine
19. Ammonia
20. Histidine
21. Tryptophan
22. Arginine

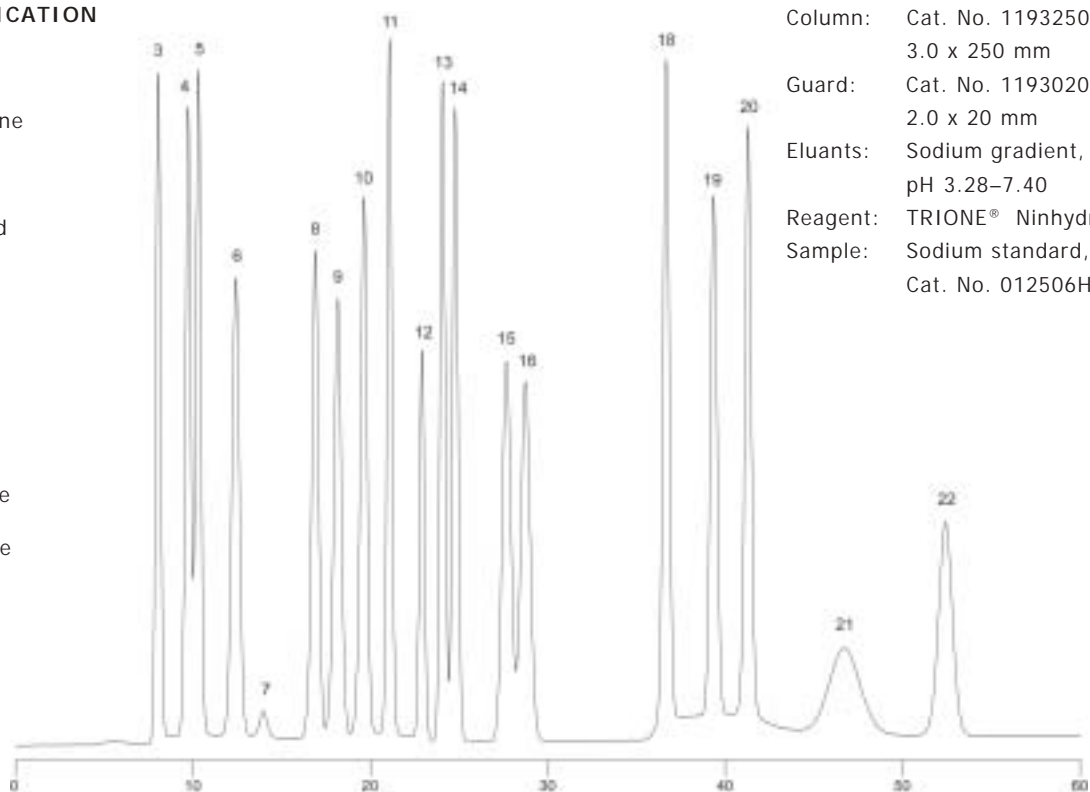
Column: Cat. No. 1154150, 3.0 x 150 mm
 Guard: Cat. No. 1192020, 2.0 x 20 mm
 Eluants: Sodium gradient, pH 3.15–7.40
 Reagent: TRIONE® Ninhydrin

Collagen Hydrolysate Standard, Cat. No. 012506C

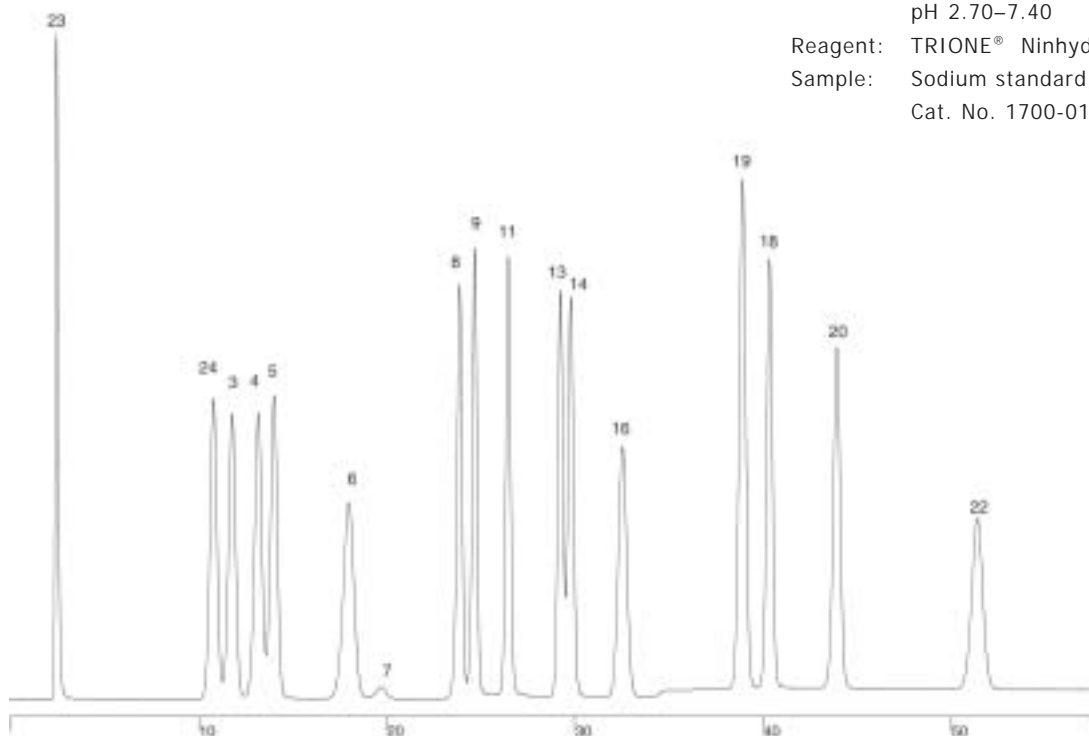


METHOD 6: STANDARD PROTEIN HYDROLYSATE, SODIUM**PEAK IDENTIFICATION**

1. Methionine sulfoxide
2. Hydroxyproline
3. Aspartic acid
4. Threonine
5. Serine
6. Glutamic acid
7. Proline
8. Glycine
9. Alanine
10. Cystine
11. Valine
12. Methionine
13. Isoleucine
14. Leucine
15. Tyrosine
16. Phenylalanine
17. D,L & allo-Hydroxylysine
18. Lysine
19. Ammonia
20. Histidine
21. Tryptophan
22. Arginine
23. Cysteic Acid
24. Methionine Sulfone



Column: Cat. No. 1193250,
3.0 x 250 mm
Guard: Cat. No. 1193020,
2.0 x 20 mm
Eluants: Sodium gradient,
pH 3.28–7.40
Reagent: TRIONE® Ninhydrin
Sample: Sodium standard,
Cat. No. 012506H

METHOD 7: OXIDIZED FEEDS, SODIUM

Column: Cat. No. 1193250
Guard: Cat. No. 1193020
Eluants: Sodium gradient,
pH 2.70–7.40
Reagent: TRIONE® Ninhydrin
Sample: Sodium standard,
Cat. No. 1700-0155

Gradient Methods

There are seven recommended gradients methods for four columns. These conditions are typical; the optimum conditions may be slightly different for your HPLC system.

METHOD 1: HIGH EFFICIENCY LITHIUM

0353150 Lithium column for physiologic fluids

Guard column: 0352020 Temperature: 40°C Flow rate: 0.30 mL/min

Step	Time(min)	Interval	%Li280	%Li750	%RG003	Comment
0	0	0	100	0	0	inject
1	12	12	100	0	0	isocratic
2	48	38	65	35	0	linear gradient
3	90	40	0	100	0	linear gradient
4	95	5	0	100	0	isocratic
5	120	25	0	94	6	linear gradient
6	122	2	0	94	6	isocratic
7	122.1	0.1	100	0	0	step change
7	140	17.9	100	0	0	re-equilibration

METHOD 2: STANDARD LITHIUM

0393250 Lithium column for physiologic fluids

Guard column: 0392020 Temperature: 40°C Flow rate: 0.30 mL/min

Step	Time(min)	Interval	%Li275	%Li750	%RG003	Comment
0	0	0	100	0	0	inject
1	17	17	100	0	0	isocratic
2	65	48	65	35	0	linear gradient
3	128	63	0	100	0	linear gradient
4	145	17	0	100	0	isocratic
5	185	40	0	94	6	linear gradient
6	185.1	0.1	100	0	0	step change
6	210	24.9	100	0	0	re-equilibration

METHOD 3: ACCELERATED LITHIUM

0355080 Lithium column for physiologic fluids

Guard column: Kit 1700-0207 Temperature: 38°C Flow rate: 0.40 mL/min

Step	Time(min)	Interval	%Li280	%Li750	%RG003	Comment
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0	0	0	100	0	0	inject
1	8	8	100	0	0	isocratic
2	22	14	77	23	0	linear gradient
3	32	10	65	35	0	linear gradient
4	38	6	40	60	0	linear gradient
5	55	17	0	100	0	linear gradient
6	59	4	0	100	0	isocratic
6	68	9	0	94	6	linear gradient
6	75	7	0	94	6	isocratic
6	75.1	0.1	100	0	0	step change
6	85	9.9	100	0	0	re-equilibration

METHOD 4: HIGH-EFFICIENCY LITHIUM FOR PKU AND MSUD SCREENING

0354050 Lithium column for physiologic fluid screening

Guard column: 0352020 Temperature: 65°C Flow rate: 0.40 mL/min

Step	Time(min)	Interval	%Li375	%RG003	Comment
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0	0	0	100	0	inject
1	8	8	100	0	isocratic
2	8.1	0.1	0	100	step change
3	10	1.9	0	100	isocratic
4	10.1	0.1	100	0	step change
5	16	5.9	100	0	re-equilibration

METHOD 5: HIGH EFFICIENCY SODIUM

1154150 Sodium column for collagen and protein hydrolysates

Guard column: 1193020 Temperature: 48°C Flow rate: 0.40 mL/min

Step	Time(min)	Interval	%1700-0112*	%Na740	%RG011	Comment
------	-----------	----------	-------------	--------	--------	---------

0	0	0	100	0	0	inject
1	12	12	100	0	0	isocratic
2	34	22	0	100	0	linear gradient
3	53	19	0	100	0	isocratic
4	53.1	0.1	0	0	100	step gradient
5	55	1.9	0	0	100	isocratic
5	55.1	0.1	100	0	0	isocratic
6	67	11.9	100	0	0	re-equilibration

* For use with columns with serial numbers above 1314

METHOD 6: STANDARD SODIUM FOR HYDROLYSATES

1193250 Sodium column for protein hydrolysates

Guard column: 1192020 Temperature: 48°C Flow Rate: 0.30 mL/min

Step	Time(min)	Interval	%Na328	%Na740	%RG011	Comment
0	0	0	100	0	0	inject
1	10	10	100	0	0	isocratic
2	32	22	0	100	0	linear gradient
3	56	24	0	100	0	isocratic
4	56.1	0.1	0	0	100	step gradient
5	58	1.9	0	0	100	isocratic
6	58.1	0.1	100	0	0	step change
5	70	0.1	100	0	0	re-equilibration

METHOD 7: STANDARD SODIUM FOR OXIDIZED FEED HYDROLYSATES

1193250 Sodium column for sulfur amino acids in oxidized feed hydrolysates

Guard column: 1193020 Temperature: 55°C Flow Rate: 0.40 mL/min

Step	Time(min)	Interval	%Na270	%Na740	%RG011	Comment
0	0	0	100	0	0	inject
1	14	14	100	0	0	isocratic
2	42	28	0	100	0	linear gradient
3	56	14	0	100	0	isocratic
4	56.1	0.1	0	0	100	step gradient
5	58	1.9	0	0	100	isocratic
5	58.1	0.1	100	0	0	step change
5	70	11.9	100	0	0	re-equilibration

Post-column Conditions

For all the above programs the same post-column conditions apply

PCX5200 Cat. No. 1152-1221 or 1152-1222

Reagent: TRIONE Ninhydrin (Cat. No. T100 or T200)

Pump: 0.30 mL/min

Reactor: 500µL at 130°C

PCX5200 Cat. No. 1152-1111 or 1152-1112

Reagent: *o*-Phthalaldehyde (Cat. No. O120) and Thiofluor™ (Cat. No. 3700-2000) in pH 10.4 borate buffer (Cat. No. OD104) + 30 mL Brij 35®

Pump: 0.30 mL/min

Reactor: 150µL at 45°C

Shutdown Procedures

Upon completion of the analyses, use one of the following procedures to shut down the PCX5200 system properly. These procedures can prevent potential column damage, reaction coil blockage, high background fluorescence signal, reagent precipitation, or other problems.

Short Term (Up to 7 days)

- Turn off the PCX5200 either manually by pressing the ENABLE key or via the "Slowdown" program (see below).
- Continue to pump Regenerant at the analytical flow rate for at least 20 minutes.
- Set the HPLC pump to < 0.1 mL/min Regenerant so that the column pressure is below 500 psi (35 bar).
- Turn off the detector lamp.

Shutdown Method

- You may also program a slowdown method to accomplish all the above steps.

Step	Time (min)	% RG	Flow (mL/min)
0	0	100	0.02
1	5	100	0.02
2	8	100	0.30
3	30	100	0.30
4	31	100	0.02



Note! The automatic valves prevent reagents from back-flowing onto the column. The inert gas should be left on to preserve the reagent.

Long Term (7 days or more)

- Set the HPLC to pump 100% Regenerant at 0.3 mL/min.
- Turn off the reagent pump by pressing the PUMP key.
- Stop the HPLC pump.
- Depressurize the PCX5200 by disconnecting the "To Detector" fitting.
- Remove the analytical column. **Disconnect the outlet first !!**
- Replace the column with a union or a restrictor, if available to close the lines.
- Turn off the gas at the toggle valve and vent the reagent reservoir.
- Replace the reagent with water/isopropanol (80/20) and draw 10 mL through the prime/purge valve.
- Replace the eluants with water only and pump 25 mL through each line to flush thoroughly.



- Replace the water in the eluant bottles with water/ isopropanol (80/20) and start the HPLC pump at 1 mL/min. If this flow rate is not enough to create 500 psi of pressure, it is acceptable to increase the flow until this is achieved. However, do not exceed 2 mL/min.
- Turn on the reagent pump and flush for 30 minutes
- Turn off the PCX5200 and the HPLC pump.
- Relieve the pressure in the reagent gauge by briefly opening the prime/purge valve.
- Turn off the inert gas source.
- Loosen the fitting at the inlet of the 100 psi external back-pressure regulator, relieving pressure on the post-column system. Place paper towels under the back-pressure regulator to absorb any escaping liquid.
- Turn off the HPLC system.

Initial System Testing

This testing is part of the installation process. In the event of later problems, you can refer to these qualification tests to help with diagnosis. Part of this initial testing is to establish standard conditions so that you can return to them for later diagnostics.

Set up the HPLC and the PCX5200 as recommended in Chapter 2. Inject 10 μ L of the Calibration mixture you received with your instrument.

Test Chromatogram

Collect two or more chromatograms to be sure that the system is stable and repeatable. Do not make any comparisons until you have obtained the same chromatogram twice in a row. Compare your chromatograms to the test chromatogram supplied with the Pickering column. Your chromatograms should not be significantly different. If there is a problem, see the later portion of this section for troubleshooting. Keep copies of your test chromatograms and the Pickering test chromatogram on file.

Make sure that the cysteic acid elutes at the same retention time and that the threonine/serine resolution is comparable to that on the QC chromatogram included with the column.

If the chromatograms do not meet these requirements, the system may need to be adjusted. Please contact Pickering Laboratories' Technical Support Department for assistance (800-654-3330 or 650-694-6700).

Parameter Log

Complete the parameter log in Appendix A of the PCX5200 Operation Manual. Your system should have come with a similar log from factory testing. Use the same conditions as for the test chromatogram above. Report the pressures for the system equilibrated under initial conditions. The pressure reported for the Reagent should be the maximum swing of the needle. Although the parameters will not be identical to the factory, they should be similar.

There is also a sheet for you to record the HPLC system parameters. Include all the settings for the pump, injector, detector, and integrator. Keep copies of this document, as it will be very helpful for troubleshooting.

Your conditions for routine analysis may be different than the ones for testing. You may be using a different sample, sample volume, standard solution, or gradient. Set up the system for injection of your calibration solution, and collect two or more chromatograms. The only standard for comparison is your expectations.

Make copies of the blank forms in Appendix A. Complete the parameter log for your initial conditions if they are different than the Pickering standard conditions. Record all the HPLC settings for your method. Keep copies of these chromatograms and logs for future use. We suggest posting this information near your instrument.

Chapter 3

Precautions and Troubleshooting

Technical Support

If you have any questions, contact Pickering Laboratories' Technical Support Department Monday thru Friday, 8 AM to 5 PM, Pacific Standard Time:

**800-654-3330 or 650-694-6700 or
by email at support@pickeringlabs.com**

Precautions General

The PCX5200 has two safety systems to prevent accidental backflow of reagent onto the column. The pressure interlock requires that the HPLC pump deliver at least 500 psi before the reagent pump can be engaged. The second is an automatic valve that prevents gas pressure from pumping reagents back through the column during extended shutdowns.

However there are ways that the safety systems can be **accidentally** bypassed. There is some residual pressure in the gauges immediately after shutdown, and it takes some time to leak down to zero. Follow these procedures to avoid such accidents:



- **NEVER disconnect** any fitting between the HPLC pump and the column until the post-column system has been shut down and **de-pressurized** by loosening the "To Detector" fitting on the right side of the post-column system.
- Any leaking fitting between the HPLC pump and the column can permit backflow in the event of an un-attended shutdown. Even a "negligible" leak can cause trouble. Leaky check-valves or seals in the HPLC pump also can cause this problem.
- When removing the column, always disconnect the **outlet** fitting first.
- Always follow the proper shutdown procedures.
- Do not allow reagent to flow into the column by depressurizing the post-column system. When ninhydrin diffuses into the column, it binds irreversibly to the polymer. This destroys the column, or at the minimum, results in unacceptable separation of the amino acids.

Mobile Phase

- Use Pickering Laboratories reagents and eluants. The quality of the chemicals is excellent, and the cost is low relative to the worth of your analytical results.
- Use the proper start-up and shutdown procedures consistently (see Chapter 2).
- Avoid touching the interior of the mobile phase reservoirs and the dip tubes with your skin. Amino acids in fingerprints will cause contamination. Gloves are suggested.

Chapter 3

Precautions and Troubleshooting

Column Precautions

- When switching a system between ion-exchange and reversed-phase applications, be sure to flush the HPLC and injector with water before connecting the column. Eluants for one analysis may damage the column for the other.
- Always protect the analytical column by use of the pre-column filter and guard column.
- Daily check for leaks at the column fittings; the eluants can be corrosive.
- Do not operate with a column pressure above 2800 psi (193 bars for an extended period of time. Isolate the source of the high pressure—guard column, analytical column, or the 0.5 μ m in-line filter— and replace items causing the increased back pressure. **Note:** Back-pressure from filter and guard column should be < 36 bars.
- During shutdown, flush the column with regenerant for 15–20 min. Store the column in regenerant.
- When removing the column, rinse the end-fittings with water then plug the column to prevent corrosion.
- Contamination usually occurs on the guard column first. Wash it separately from the analytical column. This will save much time in the washing and re-equilibration.
- Contaminants to be especially wary of: iron and other polyvalent cations, organic dyes, lipids, surfactants, and detergents. These may cause irreversible damage.
- Organic solvents will cause the resin in the column to swell leading to high back-pressure and broadened peaks. The column sometimes can be regenerated.
- Use the Pickering column and eluants. They are designed to work together.
- Filter all samples through a 0.45 μ m membrane filter. Some samples may require even more stringent filtration, especially if colloids are present.

Sample Precautions

- Samples must always be properly buffered. The ideal pH for sample injection is pH 2.2 \pm 0.2.
- For native samples, be sure that all proteins have been removed before analysis.
- Always wear gloves during the preparation of the reagents. The OPA and Thiofluor™ can cause skin irritation. Also fingerprints can cause contamination of the reagent. TRIONE® will stain skin.

Chapter 3

Precautions and Troubleshooting

Reagent Precautions

- The OPA reagent is sensitive to air oxidation, degrades over time, and should be prepared fresh for optimum sensitivity. OPA reagent is stable for at least one week when pressurized with inert gas.
- Thiofluor™ is extremely hygroscopic. Always keep in a tightly closed container.
- The preparation of the OPA Diluent by the user is not recommended because sodium borate (any grades) contains excessive amounts of heavy metal contaminants and insoluble matter. These impurities will eventually precipitate in the reactor and flowcell. ***The one year warranty does not cover damage caused by these contaminants.***
- The pre-mixed TRIONE® has a shelf life of 3 months*. As it ages, the risk of precipitate formation increases. Using outdated TRIONE® is a major cause of clogging in post-column systems.
- As TRIONE® ages, the color intensity for primary amines increases by up to 20%. A small drop in sensitivity when changing to a new lot of TRIONE® is not unusual.
- Air oxidation of TRIONE® causes the intensity for primary amines to decrease, but does not affect the intensity for secondary amines. Also the reagent becomes more yellow when it is oxidized.

Instrument Precautions

- Frequently observe and record the pressures and check for leaks. You may find a problem before it becomes serious.
- Do not operate the heated reactor above the boiling point of the eluant unless the back-pressure regulator is connected to the waste line of the detector. Boiling inside the reactor can cause precipitates to block the reactor. Operating above the boiling point without a back pressure regulator will void your warranty.
- Do not operate the heated reactor above 130°C. This can weaken and deform the PTFE capillary.
- Do not operate with a post-column pressure above 500 psi.

Electrical Precautions

- Always use the correct fuse. For the 120 volt system, use a fast-acting 3A, 250V, 5 x 20mm fuse, type GMA3 (Cat. No. 3543-0045). For the 240 volt system, use a fast-acting 1.6A, 250V, 5 x 20mm fuse, meeting IEC127 specifications (Cat. No. 3543-0044).
- Disconnect the power cord before removing the case of the PCX5200.

* From date of manufacture

Troubleshooting Guide

General Advice *Rules of Dolan and Snyder (see references)*

- Rule of One: Make one change at a time.
- Rule of Two: Confirm the problem before fixing it.
- Substitution Rule: Swap in a good part for a questionable one.
- Put it Back: If swapping doesn't fix it, put the original back.
- Write it Down: Changes or modifications, incidents.
- Crystal Ball: Preventive maintenance saves more time in the long run.
- Buffer Rule: Remove buffers from HPLC when not in use.

General Procedure for Troubleshooting

- Examine the system front to back.
- Verify that all settings, eluants, reagents, valves, etc. are according to specifications.
- Have there been any changes in the system?
- Compare against reference conditions: standard sample, column, and parameter log as appropriate.
- Gather information: observations, manuals, books, and technical assistance.
- Test your conclusions about the nature of the problem.
- Start working
- When in doubt, contact Technical Support

Chromatography Optimization

Note: Before making any change in the gradient, temperature, or other operating conditions, get at least two chromatograms in a row with the same problem. After you make a change, get at least two chromatograms showing the same effect of the change. This is especially true when you are trying to optimize gradient conditions.

- Make only one small change at a time.
- Make a change only after you have collected at least two chromatograms showing the same separation. This usually means three injections, as the first injection of a series rarely is representative of the rest of the series.
- Optimize the separations in the early part of the gradient before optimizing the late part.
- Every model of HPLC forms gradients differently. The programs suggested in this manual or in the information sheets are typical of the more popular HPLC pumps. Consult Pickering Laboratories if you need advice.
- If you need only the early part of the chromatogram, you can save time by truncating the gradient. Go to the final concentration of regenerant and hold it until the most basic component (arginine) elutes, then re-equilibrate with the initial buffer.
- The separation is temperature sensitive. Adjusting the temperature may improve it. For example, the resolution of threonine and serine improves when the column temperature is cooler, however the resolution of tyrosine and phenylalanine is best when the column temperature is warmer.

Interpretation of Instrument Pressures

The **most useful** diagnostic tool is a pressure log. Note that it is important to record all three pressures at initial conditions. Each permutation indicates a specific problem.

Condition	Column	Post-column Reagent	
Normal example (approximate in psi)	2000	250	1800
Pre-column filter blocked	▲	—	—
Reactor obstructed	▲	▲	▲
Reagent not pumping	—	▼	▼
Restrictor or reagent filter blocked	—	▼	▲

Common Problems

High post-column pressure—caused by:

- obstruction of flow path by deposits
- over-tightened fittings pinching a Teflon tubing closed
- obstruction of detector flowcell
- heat-exchanger in detector is too restrictive
- defective or obstructed back-pressure regulator

High background signal—caused by:

- Contaminated eluant
- bacterial growth
- fingerprints
- Contaminated reagents
- defective chemicals

Reagent backflow into column—caused by:

- Not following proper shutdown procedure
- Not shutting down and depressurizing post-column before working on HPLC
- Leaking fittings between column and HPLC pump, or leaking check valves in HPLC pump
- Defective reagent control valves

Air in reagent pump or flow conditioners—look for:

- Reagent pressure is low or inconsistent
- Some peaks disappear or change relative intensity
- Noisy baseline with 2 second period
- Pump takes too long to come up to pressure

Deposits in reactor or detector—caused by:

- Out-of-date TRIONE®
- Contaminated reagents
- Preparing your own reagents with poor quality chemicals

High column pressure—caused by:

- Filter is clogged—replace the frit
- Guard column is contaminated—replace it
- Worn HPLC pump seal or worn injector rotor seal
- Unfiltered samples—replace the frit and/or guard
- Particulate matter in eluant reservoirs
- Post-column pressure is high
- Column is damaged—replace it
- Organic solvent in column—wash column
- An episode of excessive eluant flow through column—replace column

Poor peak shape—caused by:

- Column worn out
- Guard column dirty
- Bad column
- Deposits in post-column reactor
- Partial obstruction of flowcell
- Too large a sample injected
- Wrong pH or buffer for sample
- Bad tubing connection: wrong style nut, too large tubing, wrong type union
- Reagent flow rate too high
- Injector problems

What to do if...

Noisy baseline—look for:

- Is there a pattern or rhythm in the noise?
- Match the frequency of the noise to one of the pumps. The Pickering pump has a 2 second period. Most HPLC pumps have a period of 5–30 sec. The problem is related to the pump with the matching frequency
- If the noise is random, check your detector
- Particles in flowcell
- If the background signal is also elevated, check for chemical contamination, or an error in formulation
- Reagent is too old or oxidized
- Improper electrical connection between detector and integrator/data system

Peaks disappear or diminish

- Oxidized TRIONE® or OPA
- Reactor at wrong temperature
- Metal contamination of column due to poor sample preparation or corrosion in system
- Reagent pump mis-adjusted
- Dirty flowcell

What to do if... *Threonine/Serine Resolution decreases*

- Change pre-column filter
- Reverse guard column and flush with 100% regenerant
- Repack or replace guard column / analytical column

Retention times not stable, especially in early part of chromatogram

- Column temperature not stable
- Re-equilibration time is too short. Increase it by two minutes increments
- pH of the samples varying. Use Li220 or Na220 to dilute samples. Use Seraprep or Uriprep for physiologic samples
- Too much internal volume in HPLC pump, or poorly swept pulse dampener. Re-plumb the HPLC pump as described in Chapter 2.
- Leaking proportioning valve in HPLC pump
- Autosampler problems

Late eluting broad peaks

- Add (or increase) regeneration step at end of run
- Make sure that all protein has been removed from samples

Artifacts in baseline

- Contamination in eluant, especially in first buffer. Replace eluants.
- Contamination in eluant reservoir. Carefully clean reservoir with soap and water, and replace eluant.
- Corrosion of eluant spargers or filters. Replace or remove them.
- Corrosion of pressure transducer diaphragm. Repair transducer, and re-plumb the HPLC pump as described in Chapter 2.
- Volatile amines used and worn in laboratory (reagents used in amino acid synthesizer, cigarette smoke laden clothing, etc.) Remove them from room.

Retention times drift over a long time

- Buildup of contaminants, especially on guard column. Relief the post-column pressure, disconnect the column, and reverse the guard column, then connect the outlet of the guard column to waste. Flush with regenerant for 30 min. Reconnect the system in the normal direction and test them. Replace the guard column if there is no improvement.
- Room temperature changes greatly with the seasons. Air condition the room.

Reagent pump stops pumping or delivers wrong flow rate

- Prime all the air bubbles from the lines
- Check pump setting and on/off switch
- Check pump seal for leakage
- Check reagent pressurization
- Check inlet and outlet check valves

Reactors or mixing tees have deposits

- Mineral deposits from water or reagents can usually be dissolved by pumping 20% nitric acid through the reactor. The Pickering pumps and most (but not all) HPLC pumps will tolerate this. Columns and autosamplers probably will not tolerate this.
 - a. Start HPLC pump at < 0.3 mL/min (Eluant A).
 - b. Replace the post-column reagent with water. Run the post-column pump for 5 min.
 - c. Stop the post-column pump. Replace deionized water with 20% nitric acid and run the post-column pump for 15 min.
 - d. Reverse the order of washing with water and then replace with the reagent.

Note: The washing solution can be stored in Erlenmeyer flasks or spare bottles. Pressurizing the washing solution is not necessary.

- Hydrindantin deposits (from out-of-date TRIONE®) are best removed by washing with ethanol. Once hydrindantin crystals have nucleated in a system, you must clean everything contacted by TRIONE®. Replace the reagent filter.
 - a. Start HPLC pump at < 0.3 mL/min (Regenerant).
 - b. Replace ninhydrin with deionized water. Run the post-column pump for 5 min.
 - c. Stop the post-column pump. Replace deionized water with ethanol and run the post-column pump for 30 min.
 - d. Reverse the order of washing with water and then replace with TRIONE® ninhydrin.

TRIONE® ninhydrin backflows onto a column

- Find out why it happened. In most cases, the backflow of post-column reagent is caused by releasing a fitting on the high pressure side of the column or opening the prime/purge valve of the HPLC without relieving the post-column pressure.
- Follow the steps below (organic solvent in column). However, if the column back-pressure remains high or the test chromatogram shows poor resolution, replace the column.

Organic solvent in a column

This procedure usually works, but it may not work every time.

- a. Shut down the PCX 5200 and remove the analytical and guard columns.
- b. Flush out all organic solvents from the HPLC and injector.
- c. Backflush both columns with regenerant. Use a very slow flow rate so that the back pressure does not exceed 2000 psi.
- d. Keep flushing until the pressure drops. Keep raising the flow rate until the pressure is normal at the operating flow rate and temperature for that column.

Contamination of a column

- First eliminate the source of contamination and clean the HPLC system.
- Usually only the guard column is contaminated. We suggest you buy a spare guard column to minimize down-time.
- Proteins and peptides can be stripped from a column by rinsing with regenerant at an elevated temperature (65–80°C).
- Metal ions usually can be removed by extended washing with pH 2.2 citrate buffer (Li220 or Na220).
- Surfactants, dyes, ninhydrin, and lipids usually cannot be removed. Prevention is the only cure.

NOTES

RECOMMENDED CONSUMABLES AND SPARE PARTS

For routine maintenance and minimal interruptions to your operation, always keep the necessary consumables and spare parts available.

Post-Column Reagents

Catalog Number	Description
O120	<i>o</i> -Phthalaldehyde (OPA), Chromatographic Grade™ crystals, 5g
OD104	OPA Diluent for Amino Acid Analysis, case of 4 (950mL per bottle)
3700-2000	Thiofluor™, Chromatographic Grade™ crystals, 10g
T100	TRIONE® Ninhydrin Reagent, 3-month * shelf life, 950 mL
T100C	TRIONE® Ninhydrin Reagent, 3-month* shelf life, case of 4 (950 mL per bottle)
T200	TRIONE® Two-part Ninhydrin Reagent (12 month* shelf life before mixing), prepares 4 x 950 mL

* From date of manufacture

Sodium Columns and Eluants

Catalog Number	Description
1154150	High-efficiency sodium ion-exchange column, 4.0 x 150 mm
1193020	Guard column for 1154150, 3.0 x 20 mm
1193250	Sodium ion-exchange column, 3.0 x 250 mm
1192020	Guard column for 1193250, 2.0 x 20 mm
Na270	Sodium eluant, pH 2.80, case of 4 (950mL per bottle)
1700-0112*	Sodium eluant, pH 3.15, 5% sulfolane, case of 4 (950mL per bottle)
Na328	Sodium eluant, pH 3.28, case of 4 (950mL per bottle)
Na740	Sodium eluant, pH 7.50, case of 4 (950mL per bottle)
RG011	Sodium column regenerant (950mL)
Na220	Sodium sample diluent, pH 2.20 case of 4 (250mL per bottle)
012506H	Calibration standard, protein hydrolysate, 0.25 µmole/mL, (5mL)
012506C	Calibration standard, collagen hydrolysate, 0.25 µmole/mL, (5mL)
1700-0070	Amino acid test mixture, 3-component, 0.25 mmol/mL (1.5mL)

* For 1154150 columns with serial numbers above 1314

Lithium Columns and Eluants

Catalog Number	Description
0353150	High-efficiency lithium ion-exchange column, 3.0 x 150 mm
0352020	Guard column for 0353150, 2.0 x 20 mm
0393250	Lithium ion-exchange column, 3.0 x 250 mm
0392020	Guard column for 0393250, 2.0 x 20 mm
0355080	Accelerated lithium ion-exchange column, 5.0 x 80 mm
1700-0207	Accelerated lithium guard column kit for 0355080
0354050	High-efficiency lithium column for rapid screening, 4.0 x 50 mm
Li275	Lithium eluent pH 2.75 case of 4 (950mL per bottle)
Li280	Lithium eluent pH 2.75 case of 4 (950mL per bottle)
Li750	Lithium eluent pH 7.50 case of 4 (950mL per bottle)
RG003	Lithium column regenerant (950mL)
Li220	Lithium sample diluent pH 2.36 (950mL)
011006P	Calibration standard, physiologic fluid, 0.25 µmole/mL (5mL)
012006P	Calibration standard without norleucine, 0.25 µmole/mL (5mL)
1700-0070	Amino acid test mixture, 3-component, 0.25 µmole/mL (1.5mL)
SP 100	SERAPREP™, for sample preparation of serum, (250mL)
UP 100	URIPREP™, for sample preparation of urine (250mL)

Spare Parts for PCX 5200

Part Number	Description
3102-9042	Replacement frit, 0.5 µm (for pre-column filter)
3102-9040	Replacement frit, 2 µm (for reagent filters)
1100-2927	OPA Reactor, 0.011" ID TFE tubing
1100-0281	0.5mL Coil Assembly only, no heater
1100-2660	Heated Reactor, 0.5mL, 120 V (other volumes on request)
1100-2661	Heated Reactor, 0.5mL, 240 V
1100-0200	Restrictor, for OPA, NaOCl, & NaOH reagent, with nuts & ferrules
1100-0141	Restrictor, Trione Ninhydrin, with nuts & ferrules
3106-1330	Seal (1) for reagent pump
3106-1310	Seal Kit for reagent pump, includes 2 seals and seal installation tool
3106-1314	Inlet Check Valve for reagent pump
3106-1316	Outlet Check Valve for reagent pump
3106-1332	Piston, sapphire, for reagent pump with piston-washing system
3106-1322	Piston Guide / Retainer
3106-1324	Liquid End Assembly
3107-0137	Reagent bottle, coated, 1 liter borosilicate, with cap, for storage
3107-0300	Reagent bottle assembly, includes 1L bottle and cap with integrated 2-way valve
2103-0463	Tubing, Saran, 1/8" OD x 0.063" ID, per 3 ft (90 cm)
3104-0081	Seal Kit for bypass valve
3101-0060	Nut, Fingertight for 1/16" plastic tubing
3102-1202	Nut, male, Upchurch type, 10-32, 1/16"
3102-2102	Ferrule, Upchurch type, 1/16"
3102-1402	Nut, male, Valco type, 10-32, 1/16"
3102-2402	Ferrule, Valco type, 1/16"
3103-1030	Tubing, stainless steel, 1/16" OD x 0.010" ID x 30 cm
2101-0212	Tubing, TFE, 1/16" OD x 0.011" ID, per 3 ft (90 cm)
2101-0225	Tubing, TFE, 1/16" OD x 0.025" ID, per 3 ft (90 cm) (waste line)
3101-0007	Nut, 1/4-28 x 1/16"
3101-0008	Ferrule, for 1/4-28 x 1/16"
3101-0005	Nut, 1/4-28 x 1/8"
3101-0006	Ferrule, for 1/4-28 x 1/8"
3102-1518	Nut, Lite-Touch, for 10-32, 1/16"
3102-2507	Ferrule, Lite-Touch, for 10-32, 1/16"
3543-0045	Fuse for PCX5200, 120 V
3543-0044	Fuse for PCX5200, 240 V

Amino Acid Analysis

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- M. V. Pickering, *LC•GC*, **7** (1988) 484.*
- J. A. Grunau and J.M. Swiader, *J. Chromatogr.*, **594** (1992) 165.*
- A. A. Boulton, G. B. Baker and J.D. Wood (Eds.), "Neuromethods 3, Amino Acids," Humana Press, Clifton, NJ (1985), Chapter 1.

Instrumentation

- M.V. Pickering, "Assembling an HPLC post-column system: practical considerations," *LC•GC*, **6**, 11 (1988) 994–997.*
- M.V. Pickering, "Modifying HPLC equipment to tolerate corrosive solutions," *LC•GC*, **6**, 9 (1988) 800–809.*
- J.W. Dolan and L.R. Snyder, "Troubleshooting LC Systems," Humana Press, Clifton, NJ (1989).

* Reprints available from Pickering Laboratories

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Pickering Laboratories, Inc., (Pickering) Instruments are warranted to be free of defects in material and workmanship under normal installation, use, and maintenance, for a period of one year from the date of delivery to the Customer. Pickering will replace or repair, without cost, any defective items. Expendable items such as check valves, pistons, piston seals, and filters are excluded from this warranty. In addition, physical damage, poor-quality reagent- and sample-induced damage, and instrument damage due to Customer's misuse are not covered by this warranty.

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Pickering Laboratories, Inc.
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