

AccQ-Tag Amino Acid Analysis Column

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I. INTRODUCTION

This manual describes procedures for using the Waters® AccQ-Tag™ Amino Acid Analysis Column for the separation of hydrolysate amino acids derivatized according to the AccQ-Tag Method.

The AccQ-Tag Amino Acid Analysis Column is manufactured in Waters ISO 9002-certified facilities under strict guidelines to assure the highest quality products. Please take a few moments to read this manual carefully. Follow its recommendations to ensure reproducible chromatography and to maximize column lifetime.

a. Column Description

The AccQ-Tag Column separates the hydrolysate amino acid derivatives produced in the reaction with Waters AccQ-Fluor™ reagent. The AccQ-Tag Column is a high-efficiency 4 µm Nova-Pak® C₁₈ Column specifically certified for use with the AccQ-Tag Method.

When you use the AccQ-Tag Column as part of a Waters AccQ-Tag System, sub-picomole detection limits can be achieved.

b. Column Specifications

AccQ-Tag Amino Acid Analysis Column	
Dimension	3.9 x 150 mm
Particle size	4 µm
Packing material	Silica base bonded with C ₁₈
Shipped in	Acetonitrile/water (50%/50%)

II. INSTALLING THE COLUMN

a. System Preparation

This procedure requires a zero dead-volume union. Before installing the column in the flow path:

1. Directly connect the injector to the detector by replacing the old column with a zero dead-volume union.
2. Flush the HPLC system with acetonitrile/water (60%/40%) at a flow rate of 1.0 mL/min.
3. Remove the union.
4. Install the column in the column heater (see Section II.b.).

b. Column Installation

To install the column:

1. Remove the end plugs from the column and save them for use when you store the column.
2. Screw the inlet and outlet fittings into the column until finger-tight. An arrow on the label indicates the direction of flow.

If you are using a stainless steel system, use two 5/16 inch wrenches to tighten the fittings 1/4 to 1/2 turn. Do not over tighten. Over tightening damages the connection.

Preparing a new PEEK connection

Prepare a new tubing-ferrule connection (Figure 2-1) if the existing connection is damaged or worn.

To prepare a new PEEK™ tubing-ferrule connection:

1. Use a single-edge razor to make a straight, square cut in front of the compression screw on the worn fitting.
2. Slide a compression screw and washer over the end of the cut tubing.

Properly bottom the tubing in the fitting seat. Dead volume results in sample bandspreading.

To prepare a new stainless steel tubing-ferrule connection:

1. Use a file with a cutting edge or a tube cutter to scribe the circumference of the steel tubing at the desired break point.
2. Grasp the tubing on both sides of the scribe mark with cloth-covered pliers (to prevent marring the tube surface), and gently work the tube back and forth until it separates.
3. Ensure that the tubing end is straight, open, and free of burrs.

4. Slide the compression fitting, followed by the ferrule (large end of the toper first) over the tube.
5. Properly bottom the tubing in the fitting seat. Dead volume results in sample bandspreading.

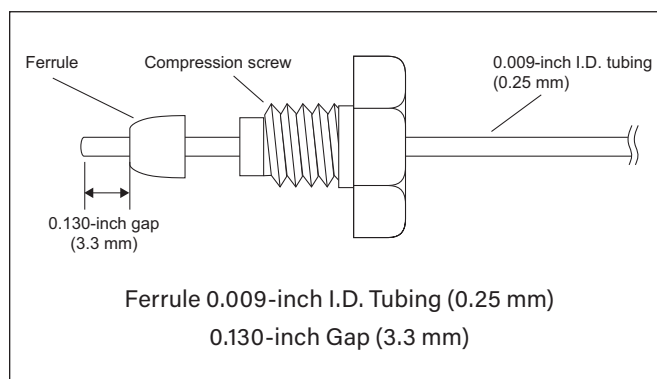


Figure 1. Ferrule and compression screw assembly.

III. USING THE COLUMN

a. Chromatography Guidelines

Before running the first amino acid analysis on the new column, perform the efficiency test in Section IV. b. Efficiency Testing.

General Considerations

The following operating guidelines will help you obtain the best performance from your AccQ•Tag Column:

- Do not exceed an operating pressure of 26 MPa/260 atm or 4000 psi).
- Filter all eluents with a 0.22 µm membrane filter. Never use turbid or cloudy eluents.
- Protect the column from vibration and mechanical shock.
- Protect the column from rapid changes in pressure, which can result from rapid changes in eluent composition or flow rate.
- Use water that has been purified with a Millipore® Water System capable of delivering 18 megohm water. De-ionized water may contain organic compounds that alter column selectivity.

b. Efficiency Testing

Perform an efficiency test before attempting the first analysis. Run the test sample using the conditions detailed in the following pages. Monitor column performance by measuring efficiency regularly over the life of the column.

There are four parts to performing the efficiency test:

- 1) Conditioning the column.
- 2) Preparing the acenaphthene standard.
- 3) Running the acenaphthene standard.
- 4) Calculating column efficiency.

If you experience problems during operation, repeat the efficiency test and compare the results. Differences may indicate a column problem.

1) Conditioning the Column

Condition the column with acetonitrile/water (60%/40%) when you install it. To condition the column:

1. Set the column heater temperature to 37 °C.
2. Set the pump flow rate to 0.0 mL/min.
3. Increase the flow rate gradually in 0.1 mL/min increments to 1.0 mL/min.
4. Equilibrate at 37 °C for 20 minutes.

2) Preparing the Acenaphthene Standard

The test standard is 0.05% acenaphthene in acetonitrile. Prepare an acenaphthene sample by dissolving 0.05 gram acenaphthene in 100 mL acetonitrile.

3) Running the Acenaphthene Standard

1. Set the gain on the detector to obtain a peak between 50 and 70% of full scale.
2. Inject 10 µl test standard and run the analysis for 10 minutes.
3. Record results, instrument settings, and instrument configuration for future comparison.

Table 1. AccQ-Tag Efficiency Test Conditions

Test Sample	Acenaphthene (0.05% in acetonitrile)
Mobile phase	Acetonitrile/water (60%/40%)
Flow rate	1.0 mL/min
Injection volume	10 µL
Detection (Waters 470 Scanning Fluorescence Detector)	Excitation wavelength – 250 nm Emission wavelength – 395 nm Gain – 10 Filter – 0.5
Temperature	37 °C
Run time	10 minutes

4) Calculating Column Efficiency

Record the retention time of the acenaphthene peak. Measure the width of the peak at 4.4% of peak height and perform the calculation shown in Figure 2. The column efficiency (N) should yield >9000 theoretical plates.

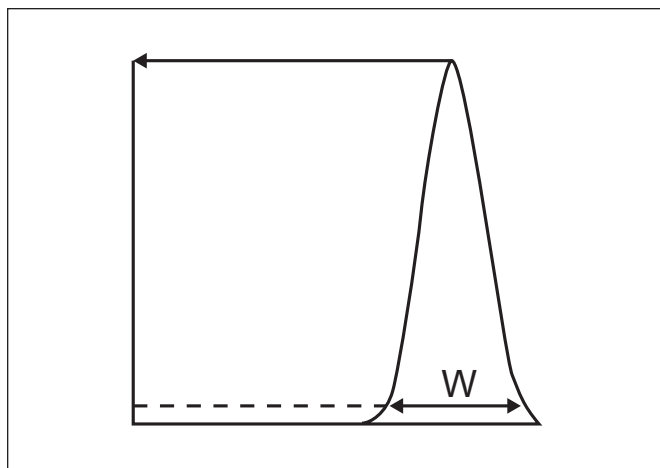


Figure 2. 5-Sigma method test calculations.

c. Equilibrating the Column for the AccQ-Tag Method

1. Equilibrate the column in 100% AccQ-Tag Eluent A for 10 minutes at 1 mL/min.
2. If the column has been stored in 100% acetonitrile, condition the column with acetonitrile/water (60%/40%) at 1 mL/min for 5 minutes before equilibrating in Eluent A.
3. After equilibrating the column, run a blank gradient.

V. CARE AND MAINTENANCE

a. Column Storage

Short Term (i.e., <2 days):

20/80 (acetonitrile/water)

Long Term (i.e., >2 days):

Flush column with water for 45 minutes at 1.0 mL/min.

Then follow with 100% acetonitrile for 45 minutes at 1.0 mL/min and store.

Note: Do not allow the column to dry out or freeze.

Table 2. Column Problems and Solutions

Symptom	Conditions	Corrective action
Excess pressure buildup	In-line precolumn filter plugged. Check for injector or pump seal shedding.	Replace filter.
	Column frit plugged	Replace frit.
	Clogged tubing	Unclog or replace tubing.
	Failing injector	Repair the injector.
	Column contaminated	Flush column with 100% acetonitrile at 0.5 mL/min for 30 minutes.
Loss of resolution, broad peaks, low plate counts	Insufficient equilibration	Continue equilibration.
	Column contaminated	Flush column with 100% acetonitrile at 0.5 mL/min for 30 minutes.
	Incorrect diameter tubing	Install 0.005 inch PEEK tubing.

APPENDIX A. SPARE PARTS

To order the parts below, contact the nearest [Waters subsidiary office](#).

Table A. Spare Parts and Accessories

Product Description	Quantity	P/N
AccQ•Fluor Reagent Kit	—	WAT052880
AccQ•Tag Chemistry Package	—	WAT052875
AccQ•Tag Column 3.9 x 150 mm	—	WAT052885
Solvent Charication Kit with pump and filters (110V, 60 Hz)		WAT085113
Compression Screw, PEEK	—	WAT021812
Compression Screws and Ferrules, Steel	5/pkg	WAT025604
Ferrule, PEEK	—	WAT021817
Replacement frit for 3.9 mm column	—	WAT015931
In-line Precolumn Filter Kit, steel	—	WAT084560
Replacement filters for In-line Precolumn Filter	5/pkg	WAT005139
Non-Metallic Filter Assembly	—	WAT077152
Tubing, PEEK 0.005-inch I.D., 24 inches	—	WAT033390
Tubing, PEEK 0.010-inch I.D., 24 inches	—	WAT033391
Tubing, Steel, 0.009-inch I.D., 10 feet	—	WAT026973
Zero Dead-Volume Union, PEEK	1	WAT026.04
Zero Dead-Volume Union, Steel	—	WAT097332

APPENDIX B. WARRANTY/SERVICE INFORMATION

Waters Corporation warrants its columns in accordance with the following terms and conditions:

Waters repacks or replaces without cost any column that fails to perform satisfactorily if notified within 90 days of your receipt of the column. A returned column must have a return merchandise authorization number (RMA) granted by Waters Technical Support. Approval is subject to the following exclusions:

- Physical damage to the column because of misuse or abuse.
- Chemical damage to the packing material because of use with incompatible solvents or buffers, or because of use at an incorrect pH.
- Physical damage to the packing material because of operation at incorrect temperatures or pressures.
- Particulate buildup or precipitation in the column or end fittings (causing high internal pressure) resulting from improper mobile phase or sample filtration.

For technical service and assistance with applications questions, contact the nearest [Waters subsidiary office](#).

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