PICO•TAG AMINO ACID ANALYSIS COLUMN

I. INTRODUCTION

Waters exclusive bonding and packing processes, developed for the Pico•Tag® Column (3.9 mm x 15 cm), P/N 88131, produce a durable, high efficiency stationary phase ideally suited for the reverse-phase separation of phenylthiocarbamyl amino acids. Our stringent quality control procedures result in a column which, when used in conjunction with Waters Pico•Tag reagents, eluents, and HPLC system is guaranteed to provide the speed, resolution, and sensitivity needed for amino acid analysis.

This information provides the means to ensure that you derive the full benefit of our research, manufacturing, and quality control. For additional information on the complete method, refer to Section 4.4 of the Amino Acid Analysis Manual, P/N 07124, or the Pico•Tag Amino Acid Analysis System Operator’s Manual, P/N 88140.

WARNING: THE AMPULES OF PHENYLISOTHIOCYANATE WHICH ARE CONTAINED IN THE PICO•TAG CHEMICAL PACKAGE REACT ON CONTACT WITH STRONG ACIDS, EMITTING HIGHLY TOXIC CYANIDE FUMES AND/OR OXIDES OF SULPHUR. CARE MUST BE TAKEN TO ASSURE THAT THESE AMPULES AND CONTENTS ARE DISPOSED OF PROPERLY AND THAT THEY DO NOT COME INTO CONTACT WITH ACIDS.

Figure 1. Pico•Tag Method Amino Acid Analysis 250 pmol of Pierce H Standard

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II. PREPARATION FOR OPERATION

a. Column Installation

Remove the end plugs from your steel column with a 5/16" wrench and save them for storage after the column has been removed from the system. The column outlet is indicated by an arrow on the label (showing the direction solvent should flow). Tighten the fittings 1/4-to-1/2 turn beyond finger tight. DO NOT OVERTIGHTEN - THIS WILL DAMAGE THE FITTING SEAT. A properly prepared and assembled compression fitting in good condition is all that is required.

Follow the next four steps of this procedure if tubing cutting is required to connect a new column or to improve the end connections on your existing fittings.

1. Using a file with a cutting edge (such as the file included in the Startup Tool Kit, P/N 96146, supplied with each Waters Liquid Chromatography System) scribe the circumference of the tubing at the desired break.

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. File the ends smooth and assemble as shown.

4. Slide the compression fitting, followed by the ferrule (large end of the taper first) over the tube. Be certain to bottom the tube in the fitting seat for which its use is intended to assure a leak-free connection.

Note: Attach a union in place of the column and flush the lines free of microparticulates before attaching the column.

Figure 5: Ferrule and Compression Assembly

b. Solvent Requirements

Your Pico•Tag column is shipped containing an aqueous/organic mixture compatible with the Pico•Tag eluents. It is recommended that only Waters Pico•Tag eluents be used with this column.

Waters Pico•Tag Eluent A (P/N 88108)
Waters Pico•Tag Eluent B (P/N 88112)
Waters Pico•Tag Diluent (P/N 88119)

c. Equilibration

A necessary step to successful use of your column is the initial solvation (or wetting) of the packing. Install the column in a column heater and bring temperature to 38 °C. Purge the column at 1 ml/min with Eluent B, then equilibrate with Eluent A for fifteen minutes. Equilibration between the mobile phase and packing is established when a stable baseline can be produced. If your result is unsatisfactory repeat the equilibration process.

III. CARE AND USE

Liquid chromatography columns have a finite life influenced by their care and use, number of injections, sample and solvent cleanliness, frequency of solvent changeover, and handling and storage procedures among other factors. If a change is observed in the:

Retention of a particular compound resolution between two compounds peak shape.

Take immediate steps to determine the reason for the changes, and until the determination is made, the results of any separations using the column must not be relied upon. Follow generally accepted procedures for quality control and methods development when using these columns.

Important Note: Before running the first analysis on your new column perform the Test Sample Separation given in the Test Conditions Section.
a. Sample and Eluent Preparation and Filtration

Use HPLC grade solvents, filtered to remove microparticulate matter above 0.45 µm. (Waters Pico-Tag prefiltered Eluents A [P/N 88108] and B [P/N 88112] are recommended.) This reduces the problem of plugged filters and preserves column life. Vacuum filtration or sonification may be used to remove dissolved gases which could affect your solvent delivery system.

Always filter prepared samples to prevent excessive pressure buildup due to particulate matter

- Adequate sample cleanup (using Sep-Pak® cartridges designed for this purpose) prevents alteration of the column chemistry by strongly adsorbing or precipitating sample components.
- Use of an in-line pre-column filter (P/N 84560), is recommended.

b. Precautions

Normal recommended pressure should not exceed 3500 psi.

- For all silica-based packing materials, stay within a pH range of 2-8 (i.e., avoid using concentrated acids or bases). Maximum column life (pH): 3.5 - 6.5.
- Filter all aqueous buffers (Waters Pico-Tag eluents are prefiltered). Avoid using turbid or cloudy buffers. Be sure that any solutions containing buffers, salts, etc. are compatible with the wetted surfaces of the column and equipment.
- Protect column from vibration, mechanical shock, and rapid changes in pressure. Column packings are based on a highly porous and delicate silica gel alignment. Any thermal, physical or chemical shock (such as changing solvents rapidly or at high flow rates) can cause the particles to shift and may result in a loss of efficiency.
- When using water, distill or treat with a Milli-Q® or equivalent system. De-ionized water is not acceptable because it contains organic compounds which alter column selectivity.
- Protect the column from rapid changes in solvent composition. DO NOT change the flow rate faster than 0.5 ml/min increments.

c. Storage Considerations (more than 72 hours without use)

Leaving the column unused for less than 72 hours does not generally require storage procedures.

- DO NOT store the column in water alone; this practice will promote microbial contamination. Store the column in Pico-Tag Eluent B or in 10% organic in water mixture.
- DO NOT allow buffers or other potentially harmful materials to remain in the system when not being used. Flush and replace with Eluent B or 10% organic in water mixture.
- Return the column to its box with the end plugs firmly in place for storage. Allowing steel columns to dry out can result in poor chromatographic performance.

d. Troubleshooting

Table 2: Typical Column Problems and Solutions

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excess pressure buildup</td>
<td>Filters plugged with Particulates</td>
<td>Clean in an ultrasonic bath or replace.</td>
</tr>
<tr>
<td></td>
<td>Sample precipitates on column</td>
<td>Always alter mobile phases and samples.</td>
</tr>
<tr>
<td></td>
<td>(sample not soluble in mobile phase)</td>
<td>Slowly purge with a strong mobile phase that is both appropriate to dissolve the contaminate and compatible with the column.</td>
</tr>
<tr>
<td>Loss of resolution, broad peaks, low plate counts</td>
<td>Contaminated column, insufficient equilibration</td>
<td>Slowly purge with very strong solvent (refer to Equilibration section)</td>
</tr>
<tr>
<td></td>
<td>Column collapse and void formation</td>
<td>Since this may occur the 1st 2 weeks of use, Waters will replace any column with this defect. Refer to warranty for details.</td>
</tr>
<tr>
<td></td>
<td>Filters partially plugged</td>
<td>Replace or clean inlet and outlet filters.</td>
</tr>
</tbody>
</table>

e. Service and Applications Information

Waters staff of trained and experienced Service Specialists provides maintenance for Waters instruments on preventative and/or corrective levels. Contact Waters at 1-800-252-HPLC or your local Waters Representative for answers to specific chromatography questions in areas such as methods development, applications, quality control, and service related matters.
IV. COLUMN EFFICIENCY

Waters measures column efficiency by using the 5-sigma method. Plate count, as an expression of efficiency, is determined by measuring the peak width at 4.4% of the peak height. Unlike half-peak height and tangent methods used by other manufacturers, this stringent method takes into consideration naturally occurring peak asymmetry.

Figure 3. Column Efficiency

\[
N = \frac{V_p}{W_{4.4\%}}
\]

V. TEST CONDITIONS

Columns are thoroughly tested in our quality control laboratories for adherence to our specifications. Since slight variations in your results will occur depending on the equipment used, test sample makeup and equipment settings and condition, perform the test sample run given here for your new column and record the results (retention time and the settings used) before attempting the first analysis. Use these results for comparison throughout the life of your column.

NOTE: BE SURE TO RECORD RESULTS AND INSTRUMENT SETTINGS (AND CONFIGURATIONS) TO ALLOW EXACT REPRODUCTION AND COMPARISON IN THE FUTURE.

Test Sample: Acenaphthene (0.05% in acetonitrile)
Solvent: Pico•Tag Eluent B
Flow Rate: 1.0 ml/min
Chart Speed: 10 cm/min
Injection Volume: 15 µl
Detection: 254 nm @ 0.5 AUFS

VI. RECOMMENDED SPARE PARTS (STEEL COLUMNS)

<table>
<thead>
<tr>
<th>Item</th>
<th>P/N</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pico•Tag Reagent Kit</td>
<td>88123</td>
<td>1</td>
</tr>
<tr>
<td>Phenylisothiocyanate (PITC)</td>
<td>88120</td>
<td>1</td>
</tr>
<tr>
<td>Triethylamine (TEA)</td>
<td>88121</td>
<td>1</td>
</tr>
<tr>
<td>Protein Hydrolyzate Standards</td>
<td>88122</td>
<td>1</td>
</tr>
<tr>
<td>3.9 mm Filter Replacement Kit</td>
<td>84095</td>
<td>1</td>
</tr>
<tr>
<td>Filter Insert Assembly (only)*</td>
<td>84056</td>
<td>2</td>
</tr>
<tr>
<td>Retainers (only)*</td>
<td>84057</td>
<td>2</td>
</tr>
</tbody>
</table>

* Included as components of 3.9 mm Filter Replacement Kit

VII. WARRANTY

Waters warrants its quality manufactured columns in accordance with the following terms and conditions. Waters will repack or replace the column (at our discretion) at no cost if it fails to perform satisfactorily and Waters is notified within 90 days of receipt. Columns being returned must have prior Return Authorization granted by Waters Customer Service Department. Approval is subject to the following exclusions:

- Physical damage to the column due to misuse or abuse.
- Chemical damage to the packing material due to operating with incompatible solvents, buffers, or pH.
- Physical damage to the packing material due to operation at incorrect temperatures or pressures.
- High internal pressure in the column due to improper solvent or sample filtration practices causing particulate buildup or precipitation in the column or end fittings.