I. INTRODUCTION

The Waters Carbamate Analysis Column (3.9 x 150 mm) is packed with a durable, high efficiency, 4μ spherical silica-based stationary phase ideally suited for the reversed-phase separation of carbamate pesticides and related compounds.

Waters exclusive sequential bonding and packing processes coupled with stringent quality control procedures ensure precise surface chemistry, reproducibility, and stability. When used as a component of Waters Carbamate Analysis System with the Waters Carbamate Analysis Method, this column is guaranteed to provide the resolution and sensitivity needed for successful analysis of the analytes listed in Figure 1.

Figure 1: A Typical Separation of Carbamate Pesticides & Related Compounds using Waters Carbamate Analysis System*

Please take a few moments to read this manual carefully. Use the information it contains to ensure that you obtain quality results and take full advantage of the features your Waters column offers.

Note: Liquid chromatography columns have a finite life, which is directly related to the care and use they receive. Column life is affected by contamination from samples and solvents, frequent solvent changeovers, and improper handling and storage.

* For additional information on the complete method, please refer to Waters Carbamate Analysis System Manual.
Follow generally accepted procedures for quality control and methods development when using this column.

If you observe a change in peak shape, retention of a particular compound, or resolution between two compounds, take immediate steps to determine the reason for the changes. Until the cause of the change is determined, do not rely on the results of the analyses.

II. INSTALLATION

a. Attaching the Column

1. If this column is to be used with an LC system previously used for a purpose other than carbamate analysis, then before installing the column in the flow path connect the column inlet and outlet lines to each other with a union and flush the lines, as well as the sample injector and injector loops, free of previous solvents. Make certain that the final flushing solvent is miscible with water. Remove the union.

2. Remove the end plugs from your column with a 5/16-inch wrench.

   NOTE: Be sure to replace and tighten the end plugs when the column is removed from the system for storage.

3. The column outlet is indicated by an arrow on the label showing the direction solvent should flow. Thread the inlet and outlet tubing fittings into the column until finger tight and then tighten the fittings with a wrench turning each - turn.

   CAUTION: Do not over-tighten. Over-tightening will damage the connection. Follow the next three steps of this procedure if you must remove a damaged compression screw or worn ferrule.

4. Using a miniature tubing cutter, scribe deeply the circumference of the tubing at the desired break point. Or, alternatively, using a three-cornered file with a cutting edge, cut 1/3 of the way through the tubing at the desired break point.

5. Grasp the tubing on both sides of the scribe mark with cloth-covered pliers (to prevent marring the tube surface) and gently work the tub back and forth until it breaks cleanly. Check that the end is straight and smooth with no burrs. Flush the tube from the opposite end with mobile phase to remove any metal particles that may have lodged in the interior of the tube.

6. Slide the compression screw head first followed by the ferrule (large end of the taper first) over the tube. Insert the end of the tube into the fitting seat to which it will be connected. Tighten the compression screw in the fitting seat as in Step 3. Assembly details are shown in Figure 2.

   CAUTION: Properly bottom the tubing in the fitting seat while tightening the compression screw to the column. Tubing not completely seated will result in dead volume which could cause excessive sample band spreading.

b. Equilibration

The Waters Carbamate Analysis Column is shipped in the mobile phase used for column storage: 50/50 v/v methanol/acetonitrile.

For equilibration, flush the column with the initial mobile phase used for the gradient analysis. This can be conveniently done while the post-column reaction system, column oven, and detector are warming up to stable operating conditions (about 30 minutes at 1.5 mL/min). Refer to the Waters Carbamate Analysis System Manual for details.
III. MOBILE PHASE AND SAMPLE GUIDELINE

a. Solvent Preparation and Filtration

1. Use only HPLC Grade or better solvents suitable for high sensitivity fluorescence analysis, filtered to remove micro-particulate matter about 0.45 µm.

   NOTE: All glassware used for solvent and sample preparation must be scrupulously clean. Detergent, fingerprint, cigarette smoke, breath residues, etc., may contain amines which can cause interference with the analysis.

   Acetonitrile and methanol, even HPLC grade, may contain traces of amines or ammonia, which will react with OPA/mercaptoethanol to form highly fluorescent impurities. These derivatives may cause baseline shifts or increased baseline noise. If this becomes a problem, clean the reservoirs and use fresh solvent. If necessary, switch to a different lot of solvent or to a different solvent vendor until a suitable grade is found.

   Distill or HPLC grade water. De-ionized water is not acceptable because it contains organic compounds which may alter column selectivity.

2. Use vacuum filtration, sonication, and/or helium sparging to remove dissolved gases, which could affect your solvent delivery system.

   Waters Solvent Clarification Kit is designed to assist in the degassing and preparation of mobile phases. Waters Carbamate Analysis System has provision for continuous sparging of each mobile phase component.

3. Use a Waters In-Line Pre column Filter to capture system particulates and extend column life. Note: a Waters In-Line Pre column Filter is supplied with the Waters Carbamate Analysis System.

b. Sample Preparation and Filtration

1. Use a Waters Sample Clarification Kit or 0.45 µ Membrane Filter Units to filter samples and prevent excessive pressure buildup.

2. Do not inject a sample that is dissolved in a solvent, which is not miscible with the mobile phase.

3. If samples contain contaminants which become irreversibly bound to the column packing under normal operating conditions, it may be desirable to use Waters Sep-Pak® Cartridges or Waters Guard-Pak™ Pre column module and Guard-Pak Cartridges to remove the contaminants off-line or on-line, respectively.

IV. OPERATION

a. Chromatography Guidelines

Liquid chromatography columns have a finite life which, is directly related to the care and use they receive. Column life is affected by contamination from; samples and solvents, frequent solvent changeovers, and improper handling and storage.

If you observe a change in peak shape, retention of a particular compound, or resolution between two compounds, take immediate steps to determine the reason for the changes. Until the cause of the change is determined, do not rely on the results of the analyses.

Follow generally accepted procedures for quality control and methods development when using this column.

NOTE: Before running the first analysis on your new column, perform the test sample separation given in Section IV. b., Efficiency Testing

PRECAUTIONS

a. Pressure

Maximum pressure should not exceed: 28 Mpa (4000 psi or 275 bars). Typical operating pressure in the Waters Carbamate Analysis System: 10-20 Mpa (1500-3000 psi or 100-200 bars).

b. Temperature

Recommended column operating temperature range: 20 °C - 40 °C. Typical operating pressure in the Waters Carbamate Analysis System: 30 °C.

c. Flow Rate

There are not flow rate restrictions as long as the recommended pressure limits are not exceeded. Typical operating flow rate is 1.5mL/min. Flow rate should be increased gradually (in 0.5 mL/min increments) to reach operating flow rate and decreased gradually to zero upon system shutdown.

d. pH Range

Maintain pH of mobile phase and samples between 3 and 8. Avoid using concentrated acids or bases.
e. Particular Contamination

Filter all mobile phases. Never use turbid or cloudy solvents or solutions.

f. Shock

Protect column from vibration, mechanical shock, and rapid changes in operating pressure. Any thermal, physical, or chemical shock (such as changing solvent composition rapidly) may cause the particles to shift and may result in void and a loss of efficiency.

Protect the column from rapid changes in solvent composition which may alter the mobile phase viscosity, and thereby, the system back pressure drastically.

g. Efficiency Testing

Waters columns are tested in our quality control laboratories for adherence to our specifications. Slight variations in your results will occur depending on:

- Equipment Used
- Test System makeup
- Equipment settings and experimental conditions

Each new column’s performance should be checked on your system to provide an initial efficiency standard for future comparison. After the column has been installed and equilibrated, run the test sample as described in the Waters Carbamate Analysis System Manual.

Choose a peak for one of the following analytes: aldicarb sulfoxide, aldicarb sulfone, oxamyl, or methomyl. Measure the column efficiency as shown in Figure 3.

**NOTE:** For convenience, VR and W can be expressed in units of length rather than volume as measure with a scale from the chromatogram.

The “5-Sigma” method shown in Figure 3 is a more stringent way to calculate plate count, N, than “half-peak height” and “tangent” methods. It takes into account naturally occurring peak asymmetry which can significantly reduce the resolution between adjacent peaks.

Save the chromatogram from this test. With the calculated column efficiency, record the retention times, system settings, and all experimental conditions so that they can be reproduced exactly in the future. If problems occur during normal operation of the column, repeat the initial efficiency test under the original conditions and compare the results.

Differences in the results may indicate the source of the problem. Refer to Table 1 and also the Waters Carbamate Analysis System Manual for troubleshooting guidelines.

![Figure 3: 5-Sigma Method for Measuring Column Efficiency](image_url)

**V. CARE AND MAINTENANCE**

a. Troubleshooting

Table 1 provides the corrective action for some typical column problems that may occur with the Carbamate Analysis Column. Refer also to the Waters Carbamate Analysis System Manual for more detailed information on troubleshooting Waters Carbamate Analysis Method.

**NOTE:** Eventually, column performance will degrade over time below an acceptable level as determined by periodic efficiency testing. When this happens, replace the old column with a new Waters Carbamate Analysis Column. See Section VI. for reorder information.
b. Shutdown and Storage

Between Analyses

During the course of a working day, between analyses, continue to pump the initial mobile phase mixture through the column. This will maintain the equilibrium in the column necessary for good retention time reproducibility. If a few hours will pass before the next injection, the flow rate may be slowed down in the interim to a few tenths of a mL/min to conserve solvent.

Overnight

When shutting down overnight or over a weekend, first flush the column with 15-30 mL of 50/50 v/v acetonitrile/methanol. Then turn the flow rate to zero mL/min and leave the column connected in the system. The oven temperature may be maintained at 30 °C, if desired.

Long-term Storage (more than 72 hours)

Flush the column with 15-30 mL of 50/50 v/v acetonitrile/methanol. Then turn the pump off. Allow the column to cool to ambient temperature. Disconnect the inlet and outlet tubes from the column and join them with a union. Install the end plugs in the column inlet and outlet fittings. Tighten the end plugs firmly in place with 5/16” open end wrench.

**CAUTION: Do not overtighten – Overtightening will damage the column fittings. Allowing columns to dry out may result in poor chromatographic performance. Return the column to its box for storage.**

### VI. COLUMN AND SUPPLIES ORDERING INFORMATION

<table>
<thead>
<tr>
<th>Item</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamate Analysis Column, 3.9x150mm</td>
<td>WAT035577</td>
</tr>
<tr>
<td>Filter Retainer</td>
<td>WAT088084</td>
</tr>
<tr>
<td>Filter Retainer Disc</td>
<td>WAT089567</td>
</tr>
<tr>
<td>In-Line Pre column Filter</td>
<td>WAT084560</td>
</tr>
<tr>
<td>Solvent Clarification Kit with pump</td>
<td>WAT085113</td>
</tr>
<tr>
<td>Solvent Clarification Kit without pump</td>
<td>WAT085124</td>
</tr>
<tr>
<td>Aqueous Replacement Filters (HATF 04700), pkg. of 100</td>
<td>WAT085147</td>
</tr>
<tr>
<td>Organic Replacement Filters (FHUP 04700), pkg. of 100</td>
<td>WAT085118</td>
</tr>
<tr>
<td>Aqueous Sample Clarification Kit</td>
<td>WAT026865</td>
</tr>
<tr>
<td>Guard-Pak Pre column Module: Kit</td>
<td>WAT080040</td>
</tr>
<tr>
<td>Nova-Pak® C18 Guard-Pak Cartridges (10/Pkg.)</td>
<td>WAT015220</td>
</tr>
<tr>
<td>Sep-Pak C18 Cartridges (50/Box)</td>
<td>WAT051910</td>
</tr>
<tr>
<td>Sep-Pak C18 Plus Cartridges (24/Box)</td>
<td>WAT011191</td>
</tr>
<tr>
<td>Sep-Pak C18 Plus Cartridges (96/Box)</td>
<td>WAT015402</td>
</tr>
</tbody>
</table>

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**Table 1: Troubleshooting the Carbamate Analysis Column**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Conditions</th>
<th>Corrective Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excess pressure buildup</td>
<td>Filters plugged with particulates.</td>
<td>Replace filter element or clean in an ultrasonic bath. Always filter solvents and samples.</td>
</tr>
<tr>
<td></td>
<td>Sample precipitates on column (sample not soluble in mobile phase).</td>
<td>Slowly purge with a solvent appropriate to dissolve the precipitate.</td>
</tr>
<tr>
<td></td>
<td>Clogged tubing.</td>
<td>Replace tubing.</td>
</tr>
<tr>
<td>Loss of resolution, broad peaks, or low plate counts</td>
<td>Mass overload</td>
<td>Dilute sample and run it again.</td>
</tr>
<tr>
<td></td>
<td>Incorrect tubing size (0.040” or 0.020” i.d.)</td>
<td>Install correct 0.009” ID stainless steel tubing to column inlet &amp; outlet.</td>
</tr>
<tr>
<td></td>
<td>Contaminated column.</td>
<td>Slowly flush with a 50-100 mL of 50/50 v/v acetonitrile/MeOH; then equilibrate with initial mobile phase and run sample again.</td>
</tr>
<tr>
<td></td>
<td>Insufficient equilibration</td>
<td>Continue equilibration</td>
</tr>
<tr>
<td></td>
<td>Filters partially plugged</td>
<td>Replace or clean (inlet and outlet) the Filter Retainer Disc and the Filter.</td>
</tr>
<tr>
<td></td>
<td>Failing Injector</td>
<td>Repair Injector.</td>
</tr>
</tbody>
</table>
VII. WARRANTY/SERVICE INFORMATION

Waters Corporation staff of experienced specialists provides maintenance assistance on both preventative and/or corrective levels. For complete information and assistance, please call Waters Service Department at 1-800-252-HPLC. For solutions to particular applications questions, Waters team of technical support personnel are available to help you with specialized support. They may be contacted at 1-800-252-HPLC in Milford, MA, USA.

WARRANTY

Waters Corporation warrants its high performance liquid chromatography columns in accordance with the following terms and conditions:

Waters will repack or replace (at our discretion) without cost any steel column that fails to perform satisfactorily if notice within 90 days from your receipt. Any column returned must have a Return Authorization Number granted by the 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 because of use with incompatible solvents or buffers, or 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 which has occurred due to improper solvent or sample filtration practices.