# Waters Alliance System for Carbamate Analysis

## Method Guide

71500017102 / Revision E

Waters

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| Telephone and fax | From the USA or Canada, phone 800 252-4752, or fax 508 872 1990.<br>For other locations worldwide, phone and fax numbers appear in the Waters Web site. |
| Conventional mail | Waters Corporation<br>34 Maple Street<br>Milford, MA 01757<br>USA |

## Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow Good Laboratory Practice, and consult your organization's standard operating procedures.

### FCC radiation emissions notice

Changes or modifications not expressly approved by the party responsible for compliance, could void the users authority to operate the equipment. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference,

and (2) this device must accept any interference received, including interference that may cause undesired operation.

## Canada spectrum management emissions notice

This class A digital product apparatus complies with Canadian ICES-001.

Cet appareil numérique de la classe A est conforme à la norme NMB-001.

## Electrical power safety notice

Do not position the instrument so that it is difficult to operate the disconnecting device.

## Safety hazard symbol notice

Documentation needs to be consulted in all cases where the  symbol is used to find out the nature of the potential hazard and any actions which have to be taken.

## Equipment misuse notice

If the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

## Safety advisories

Consult Appendix A for a comprehensive list of warning and caution advisories.

# Operating this instrument

When operating this instrument, follow standard quality-control (QC) procedures and the guidelines presented in this section.

## Applicable symbols

| Symbol | Definition |
|---|---|
|  | Manufacturer |
|   | Authorized representative of the European Community |
|  | Confirms that a manufactured product complies with all applicable European Community directives |
|   | Australia C-Tick EMC compliant |
|   | Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements |
|  | Consult instructions for use |
|  | Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste.<br>For compliance with the Waste Electrical and Electronic Equipment Directive (WEEE) 2012/19/EU, contact Waters Corporation for the correct disposal and recycling instructions. |

## Audience and purpose

This guide is intended for personnel who install, operate, and maintain the Waters Alliance System for Carbamate Analysis.

## Intended use of the Waters Alliance System for Carbamate Analysis

The Waters Alliance System for Carbamate Analysis is for research use only and is not intended for use in diagnostic applications.

## Calibrating

To calibrate LC systems, follow acceptable calibration methods using at least five standards to generate a standard curve. The concentration range for standards must include the entire range of QC samples, typical specimens, and atypical specimens.

## Quality control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. If sample trays are the same or very similar, vary the location of the QC samples in the trays. Ensure that QC sample results fall within an acceptable range, and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report these data until you are certain that the instrument performs satisfactorily.

# ISM classification

## ISM Classification: ISM Group 1 Class A

This classification has been assigned in accordance with IEC CISPR 11 Industrial Scientific and Medical (ISM) instruments requirements. Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment. Class A products are suitable for use in commercial (that is, nonresidential) locations and can be directly connected to a low voltage, power-supply network.

# EC authorized representative


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# Table of Contents

# 1 Introduction

The Waters® Alliance® System for Carbamate Analysis is a complete method and instrumentation package for the high-sensitivity analysis of N-methylcarbamate and N-methyl-carbamoyloxime pesticides and related compounds in a variety of environmental and food samples, including drinking and raw source waters, crop residues, and processed foods.

## Contents



Chapter 3 introduces you to Waters Carbamate Analysis Method. This method is an integral part of the Waters Alliance System for Carbamate Analysis. It is a complete protocol for the baseline separation of 11 analytes (12, if the internal standard BDMS is included) by a complex reversed-phase gradient sequence followed by post-column reaction and fluorescence detection.

The Alliance System for Carbamate Analysis provides high-sensitivity operation and the lowest possible limits of detection. The system can separate subpart-per-billion levels of carbamates in drinking water or trace residues in processed foods.

The system includes the following features to deliver optimum sensitivity as well as operational convenience:

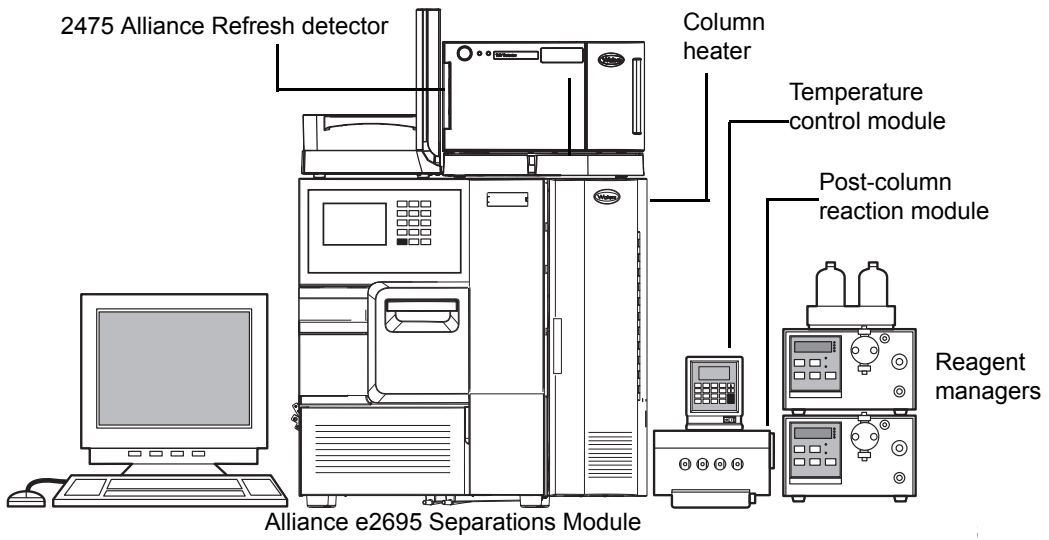
- A short, small-particle, high-efficiency column is used to keep peak volumes as small as possible for the highest response and best resolution.
- The column packing has been chemically optimized for maximum selectivity.

- A mobile phase gradient has been developed for ruggedness, baseline resolution of all analytes, maximum fluorescence response, and minimum separation time.
- Flow rates, column and reactor temperatures, and all fluidics component dimensions are matched for optimum sample throughput, reaction yields, product integrity, and sensitivity.
- Pumping systems for mobile phases and reagents have been chosen for highest reliability and reproducibility.
- Detection systems are selected for optimal lamp spectral output, excitation, and emission wavelength capabilities suited to the properties of the isoindole derivative, and quiet, stable performance over extended periods of time.
- Typical bandspreading for the entire system under normal operating conditions is measured in terms of volume standard deviation(s) to be under 50 µL.

## System components

The figure below shows the principal hardware components of the Waters Alliance System for Carbamate Analysis.

### System components:



The components include the following items:

**Alliance e2695 Separations Module** – An HPLC system platform that combines solvent and sample management. Options include sample heating/cooling and a two-column switching valve.

**Column Heater** – Provides accurate microprocessor-based temperature control that enhances chromatographic results by creating a consistent separation environment for method reproducibility.

**Carbamate Analysis Column** – A high-efficiency, octadecylsilyl-bonded, 4-micron, spherical silica column that possesses unique selectivity for this analysis.

**Post-Column Reaction Module (PCRM)** – Consists of an oven containing an RXN™ 1000 Reaction Coil and a CHEX™ Countercurrent Heat Exchanger. Three bulkhead fittings on the PCRM panel provide fluid path entry to the Post-Column Reaction Module. A fourth bulkhead fitting provides a fluid path to the detector.

**Temperature Control Module** – Controls the temperature of the PCRM.

**Reagent Manager (RM)** – A single-piston, eccentrically driven, pulse-dampened pumping system. This system has two reagent managers, one each for the NaOH and OPA/ME post-column reagent solutions. Each reagent manager has controls for optimum performance, operational convenience, and safety.

**Waters Model 2475 Multi-Channel Fluorescence Detector** – The fluorescence detector illuminates a sample with a narrow band of high-intensity light. The detector then measures the low levels of fluorescence emitted from the sample. The emitted light is filtered, amplified, and converted to electrical signals that can be recorded and analyzed.

## System options

In addition to the standard system components listed above, you can add the following options to your system:

- Waters Empower™ software automates system control, data collection, analysis, and storage.
- A Waters Model 746 Data Module is a lower-cost alternative for peak area integration and recording capability.

- A two-column switching valve can be helpful if you intend to do a lot of online scanning of excitation or emission wavelength spectra using the 2475 Detector.
- Waters Solvent Clarification Kit complete with glassware, vacuum pump, and aqueous filters (part number WAT200538) for aqueous solvents can be used to filter and degas both mobile phase solvents and reagent solutions.
- A Millipore Milli-Q® Water System or equivalent is strongly recommended as the source of clean water for all mobile phase, reagent, and sample preparation needs.
- Other columns and detectors such as the Waters 2489 Dual λ Absorbance Detector or Waters 2998 Photodiode Array Detector can be added to the system to accommodate a variety of other applications. For more information on all these and other system options, consult your local Waters representative.

# 2 Installation

The Waters Alliance System for Carbamate Analysis consists of a number of multiple purpose components. This chapter provides an overview of the system installation. See the accompanying operator's manuals for detailed information on installation requirements and setup procedures for each respective component.

**Contents**



**Tip:** The Alliance System for Carbamate Analysis will be installed and started by a trained Waters service engineer.

You will need to supply the following items:

- Solvents for mobile phase preparation
- Carbamate pesticide standards
- Reservoirs for mobile-phase components and post-column reagent solutions
- A balance, glassware, micropipets, and other supplies for preparing solutions

**Tip:** Chapter 3, contains information about the requisite quality of reagents and includes other precautions and suggestions that you should observe before you perform high-sensitivity analysis with the system.

## Unpack the components

Each component of the system is packed separately in shock-absorbing packing material to protect it during shipment. Retain the packing material and shipping cartons for future use (e.g., storage, repacking, or shipment).

Open the shipping cartons and remove the protective packing material. Carefully lift out the components. Inspect all items. If you find any damage or discrepancy in the contents of the order, and you are a U.S. or Canadian customer, contact the shipping agent and Waters Technical Service at 800 252-4752. Other customers should call their local Waters subsidiary, their local Waters Technical Service Representative, or Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance. For more information about the converter warranty, see *Waters Licenses, Warranties, and Support*.

After inspecting each unit for damage, check the contents of each carton against the packing list. Where a startup kit is included with a component, check the contents of each kit against the items listed on the corresponding startup kit list. Report discrepancies or missing items to Waters.

# Installation requirements

## System layout

**Tip:** Fluorescence detector sensitivity is inversely proportional to temperature. Changes in temperature affect detector response.

The Waters Alliance System for Carbamate Analysis should be located in a clean area free from extremes of temperature, humidity, appreciable shock, and vibration. Make sure the site is also free of ammonia or amine vapors.

- You need at least 91.44 cm (36 inches) of linear bench space.
- Depending upon the system options purchased and the layout you choose to employ, you may need as much as 60.96 to 121.92 cm (24 to 48 inches) of additional bench space.
- Your bench should be at least 60.96 cm (24 inches) deep to permit clearance at the rear for access to cable connections, power cords, and fluid lines.
- An additional 7.62 cm (3 inches) of clearance should be allowed on each side of the system for unrestricted air circulation.
- Vertical space required may range from 60.96 to 91.44 cm (24 to 36 inches).

The figure “System components:” on page 16 shows a suggested layout for the principal components of the system. Three mobile phase reservoirs (not

shown) in the solvent tray are located on top of the Alliance e2695 Separations Module. To conserve space, you can locate two post-column reagent solution reservoirs on top of the Reagent Managers (only if the reservoirs are placed in solvent-resistant pans or trays sufficiently large to contain the entire contents of both reservoirs in case of a spill or breakage).

**Caution:** The measured, preassembled tubing pieces included in the system startup kit are designed to be installed only with the relative locations of the components shown in the figure “System components:” on page 16.

# System component installation

This section summarizes information about installing the individual components which comprise the system and highlight important installation considerations, where applicable. The figure “System communication connections:” on page 24 and the figure “Connect to an Alliance e2695 Separations Module:” on page 26 show suggested locations for each component and reservoir.

## Alliance e2695 Separations Module

Install the Alliance e2695 Separations Module as described in the *Waters Alliance e2695 Separations Module Operator's Guide*.

**Tip:** Make certain that the 2-mL volume sample loop and a 2500-µL syringe are installed in the Alliance e2695 Separations Module. See the *Waters Alliance e2695 Separations Module Operator's Guide* for more details.

## Carbamate Analysis Column and guard column in the column heater

Install the column heater module and column as described in the *Waters Alliance e2695 Separations Module Operator's Guide*.

The Carbamate Analysis Column is placed in the column heater with its outlet end (indicated by a directional arrow on the column label) pointed upward as shown in the figure “Fluid path schematic of the Alliance System for Carbamate Analysis:” on page 27.

A Waters Sentry™ Guard column is attached to the column inlet with a 0.009-in. ID steel jumper tube supplied in the startup kit. Routing a portion of the inlet tube from the injector through the column heater blocks helps hold the tubing and filter assembly in place.

**Tip:** Replace the guard column with a new one whenever you observe a sustained, significant increase in system operating pressure on the system controller display. Order additional guard columns so you always have replacements when needed.

The column outlet is connected to the long inlet tube of the PCRM unit shown in the figure “Fluid line connections to the PCRM:” on page 29.

## Post-Column Reaction Module

Install the PCRM as described in the *Waters Post-Column Reaction Module Installation Guide*.

## Reagent Managers

Install the two Reagent Managers as described in the *Waters Reagent Manager Operator’s Manual*. Suggested locations for the Reagent Managers are shown in the figure “System communication connections:” on page 24 and the figure “Fluid path schematic of the Alliance System for Carbamate Analysis:” on page 27.

The electrical connections between the Reagent Manager and the Alliance e2695 Separations Module are shown in the figure “Connect to an Alliance e2695 Separations Module:” on page 26.

The fluid connections to each post-column reagent pump head are shown in the figure “Fluid delivery end of a Reagent Manager:” on page 28.

Setup for the post-column reagent reservoirs is shown in the figure “Fluid path schematic of the Alliance System for Carbamate Analysis:” on page 27 and the figure “Fluid line connections to the PCRM:” on page 29.

Connect the inlet fitting of one Reagent Manager to the sodium hydroxide post-column reagent reservoir. Connect the inlet fitting of the second Reagent Manager to the OPA/ME post-column reagent reservoir. See the figure “Fluid

path schematic of the Alliance System for Carbamate Analysis:" on page 27 for details.

**Caution:**

- Verify that you have connected the proper reservoirs to the corresponding pumps and that the tubing connections have been made according to the figure "Fluid path schematic of the Alliance System for Carbamate Analysis:" on page 27. If you add post-column reagents to the column effluent in the wrong sequence, you will not obtain acceptable results.
- To meet the regulatory requirements of immunity from external electrical disturbances that may affect performance of this instrument, do not use cables longer than 9.8 feet (3 meters) when you make connections to the screw-type barrier terminal strips. In addition, ensure you always connect the shield of the cable to the chassis ground at one instrument only.

**Tip:** The Installation Category (Overvoltage Category) for this instrument is Level II. The Level II category pertains to the equipment that receives the electrical power from a local level, such as an electrical wall outlet.

## Waters 2475 Scanning Fluorescence Detector

Install the 2475 Scanning Fluorescence Detector as described in the *Waters 2475 Multiwavelength Fluorescence Detector Operator's Guide*. Suggested locations for the detector are shown in the figure "System components:" on page 16 and the figure "System communication connections:" on page 24.

Connect the 1-V detector output to the optional integrator or data system you have purchased, following instructions in the corresponding manuals.

**Tip:** See the *Waters 2475 Multiwavelength Fluorescence Detector Operator's Guide* for information on connecting a 2475 Detector to your system.

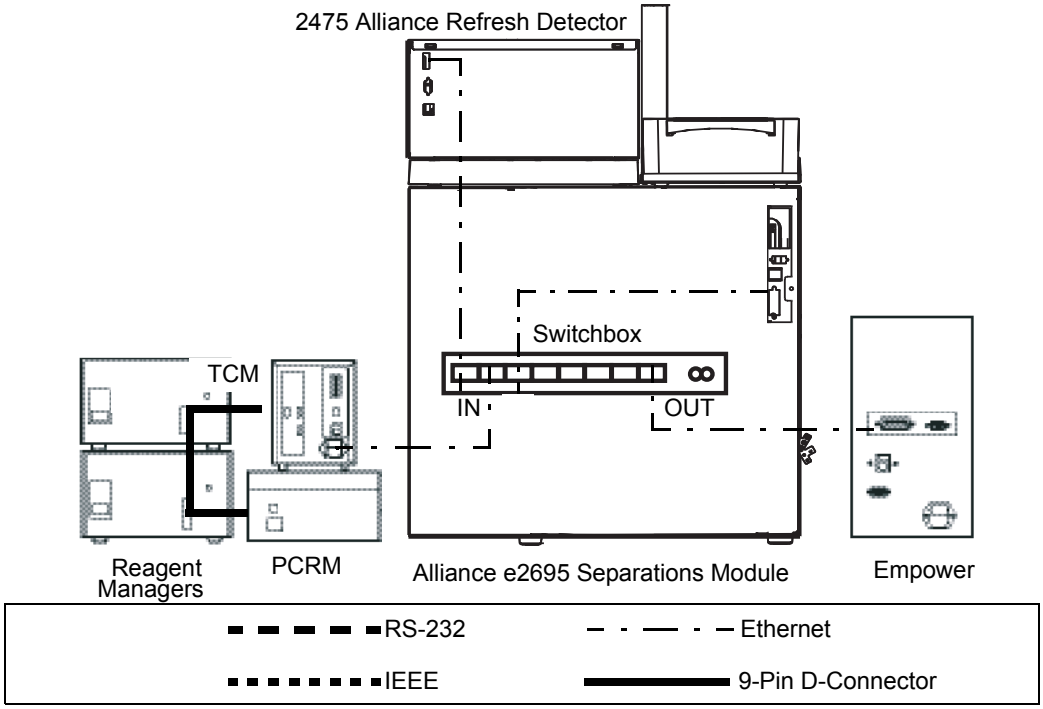
To provide a real-time output chromatogram and continuous, concurrent system diagnostic record, Waters recommends reporting the Alliance e2695 Separations Module pressure output channel in the Empower instrument method.

## System wiring

The following figure shows the electrical connections of the Alliance System for Carbamate Analysis.

**Caution:** When making communication and signal wiring connections between system components, use the same media type, such as Ethernet. Do not mix media types (for example, Ethernet and IEEE).

### System communication connections:




## Power wiring

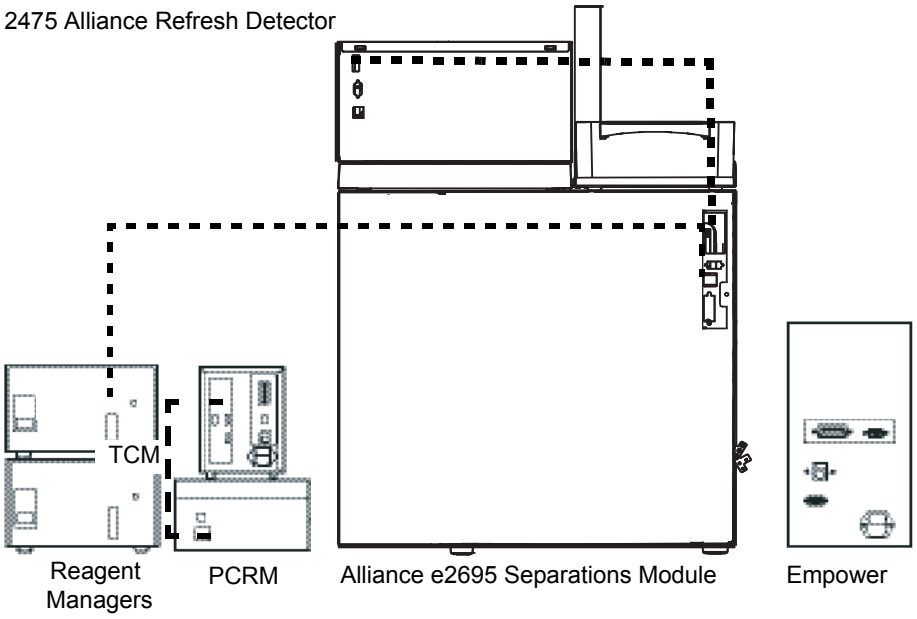
Each system component requires a grounded AC power outlet. Make sure the power line is free of transients and fluctuations. An isolated circuit is recommended. If a transient-free circuit is not available, use an isolation transformer for critical components, e.g., those containing microprocessor chips.

For information on voltage, current, fusing, and AC power wiring, see the operator's manuals for the individual instruments that come with your system.

## Signal wiring

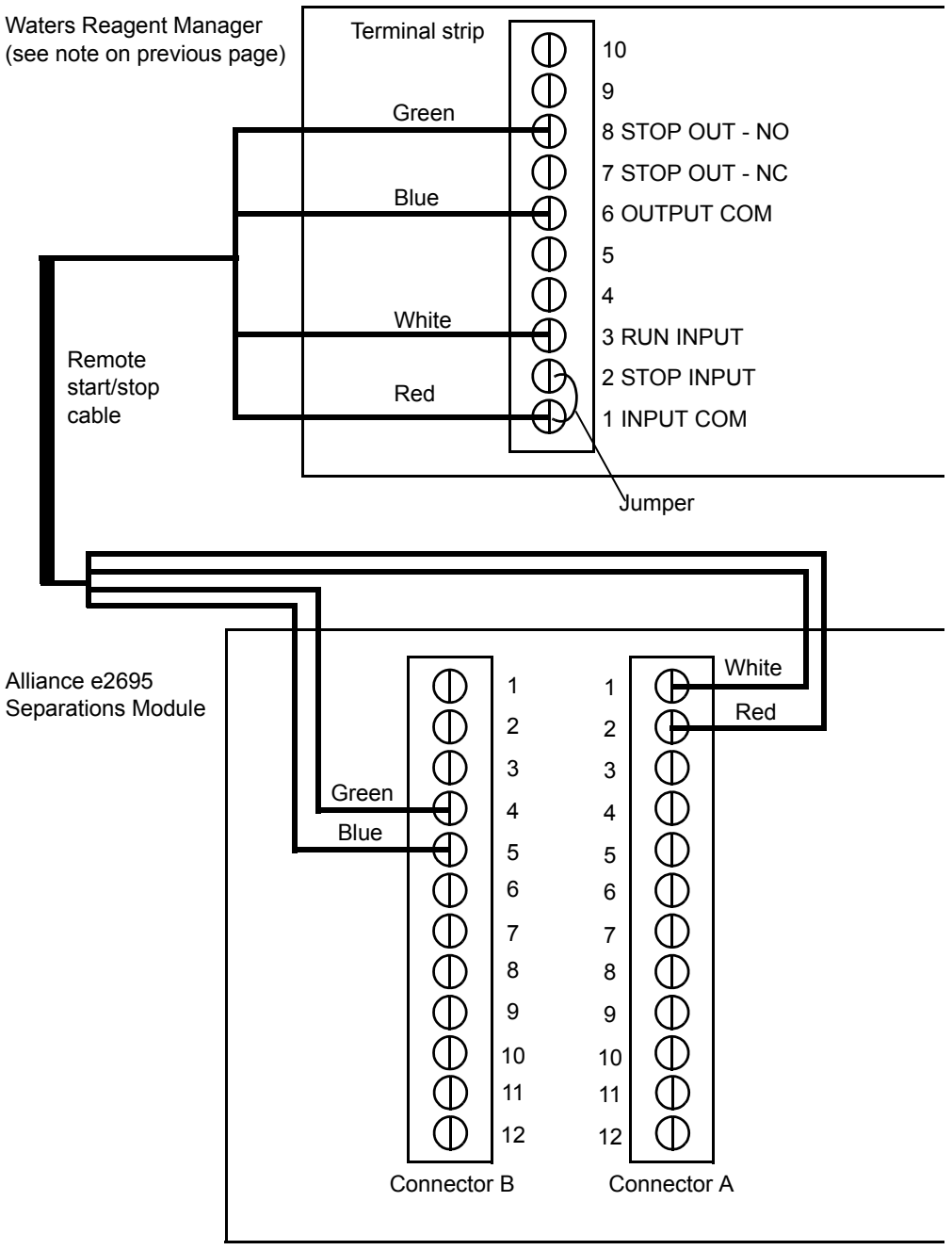
See the figure "Signal connections:" on page 25 for information on connecting signal wires between the Reagent Managers and the Alliance e2695 Separations Module. For additional information, see the operator's manuals for the individual instruments that are supplied with your system.

### Signal connections:




**Tip:** For clarity, the figure below shows only one Reagent Manager. The second Reagent Manager is wired to the Alliance e2695 Separations Module using a second Remote Start/Stop cable in parallel with the first.

## Connect to an Alliance e2695 Separations Module:

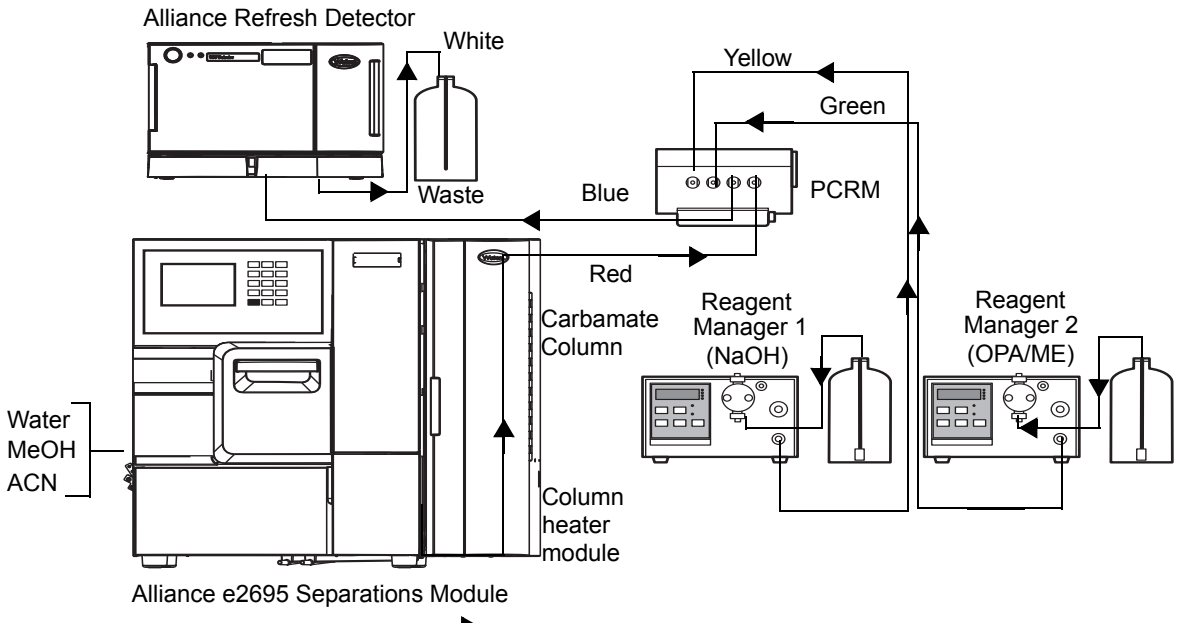


## Fluidic connections

The figure below shows the fluidic connections of the Alliance System for Carbamate Analysis.

The figure only shows the connections for the components. Do not use this schematic for component layout.

### Fluid path schematic of the Alliance System for Carbamate Analysis:

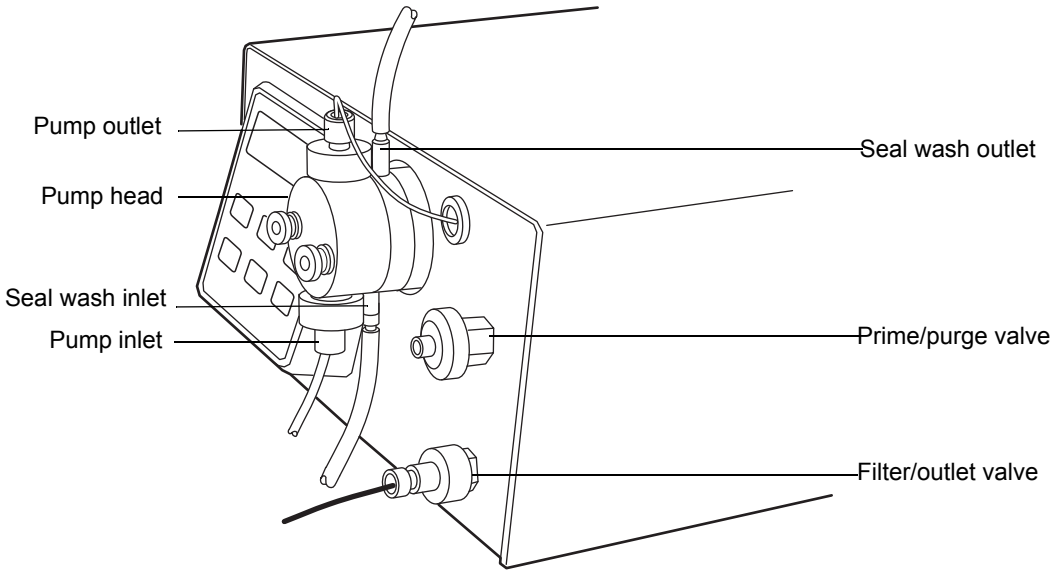



### Tips:

- The Waters Reagent Manager requires at least 1 L of seal wash solvent. You should check the seal wash solvent level regularly to ensure there is an adequate amount of solvent in the Reagent Manager.
- The 2475 Multi-Channel Fluorescence Detector must be the last detector in line because of the flow cell backpressure limit of 145 psi.

The fluid-handling components of the Waters Reagent Manager are shown in the figure “Fluid delivery end of a Reagent Manager:” on page 28.

### Fluid delivery end of a Reagent Manager:




## Mobile phase reservoirs

**Warning:** Always observe safety precautions and good laboratory practices when handling and disposing solvents. Keep solvent containers and bottles in approved protective carriers at all times. Protect system components from potential spills by using appropriate pans or trays.

Assemble three reservoirs, one each for water, methanol, and acetonitrile. Connect the reservoirs to the 2695 inlet lines A, B, and C, respectively, as described in the *Waters Alliance e2695 Separations Module Operator's Guide*.

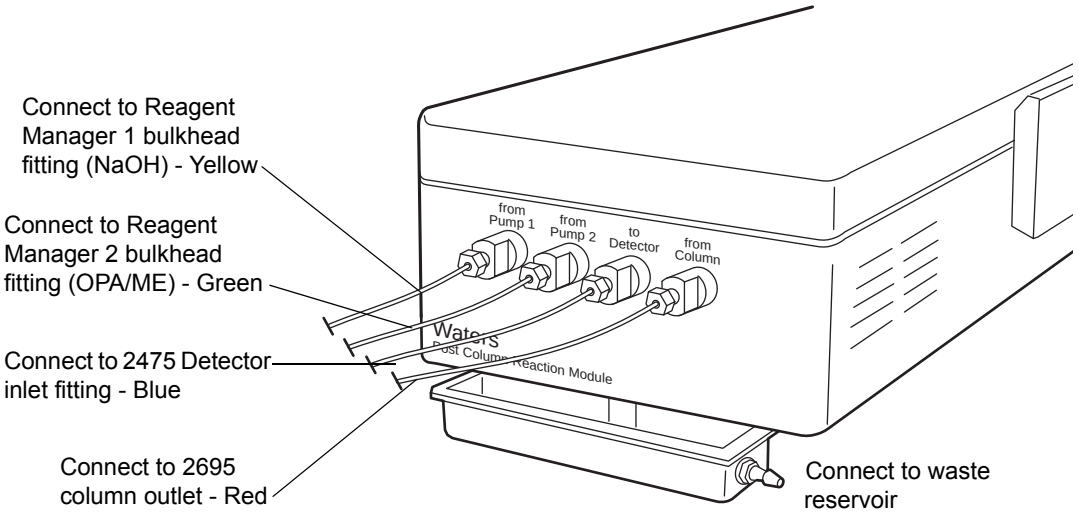
## Post-Column Reaction Module

Supplied in the PCRM startup kit is a Tubing Kit, part number 200000126, which contains five lengths of tubing. Four color-coded tubes have been cut to the proper length and flushed clean in the factory, have compression screws and ferrules attached at each end, and are ready to install (the fifth tube is to be connected to waste). The points at which the tubes are to be attached to the PCRM are shown in the figure “Fluid path schematic of the Alliance System for Carbamate Analysis:” on page 27 and the figure “Fluid line connections to

the PCRM:" on page 29. Take care not to kink these tubes or bend them into an excessively small radius.

**Tip:** See the *Waters Post-Column Reaction Module Installation Guide* for information on connecting a PCRM to your system.

### Fluid line connections to the PCRM:




## Waters 2475 Scanning Fluorescence Detector

Install the 2475 Scanning Fluorescence Detector as described in the *Waters 2475 Multiwavelength Fluorescence Detector Operator's Guide*. Suggested locations for the detector are shown in the figure "System components:" on page 16 and the figure "System communication connections:" on page 24.

Attach the waste line to the detector outlet bulkhead fitting in the following manner. The necessary parts are supplied in the PCRM startup kit.

Attach one end of the waste tubing (white) to the outlet of the 2475 Detector, and then insert the other end into a suitable waste container.

**Caution:**

- Keep the waste solvent reservoir on about the same level as the detector. If the waste container is placed below bench level, e.g., on the floor, a fluid siphon may be created.
- The flow cell backpressure limit is 145 psi. Avoid putting any connector behind the flow cell that could exceed this pressure rating.

# 3 Waters Carbamate Analysis Method

This chapter introduces the Waters Carbamate Analysis Method. This method is an integral part of the Waters Alliance System for Carbamate Analysis. By using this method with the other components of the Waters Alliance System for Carbamate Analysis, you will be able to meet or exceed the capability requirements of methods published by the U.S. Environmental Protection Agency and the Association of Official Analytical Chemists (see "References" on page 86, numbers 1 and 2).

**Contents:**



## About Carbamates

Carbamates are commercial pesticides derived from carbamic acid. Highly effective and having a broad spectrum of activity, carbamates are used worldwide to protect crops from insect pests.

Carbamates and their degradation products and metabolites are of great concern to members of the regulatory and scientific communities as more drinking water sources worldwide test positive for carbamates. Carbamates contaminate aquifers and surface water through agricultural runoff after being applied directly to food crops. Residues of carbamates and their by-products can remain on the produce when food crops are harvested too soon after application.

In an effort to protect drinking water resources, the U.S. Environmental Protection Agency and other international governing bodies regulate pesticide use and require routine monitoring of drinking and raw source water. Additionally, consumers are becoming increasingly vigilant for pesticide residues due to their toxic nature.

## Principle of the Carbamate Analysis method

The Waters Alliance System for Carbamate Analysis is a complete method and instrumentation package for the high-sensitivity analysis of N-methylcarbamate and N-methyl-carbamoyloxime pesticides and related compounds in a variety of environmental and food samples, including drinking and raw source waters, crop residues, and processed foods.

Waters Carbamate Analysis Columns have been tested and fully qualified for this method. When used as part of Waters Alliance System for Carbamate Analysis, the Carbamate Analysis Column will perform the reversed-phase nonlinear gradient separation as described in this Carbamate Analysis Method.

Inlet lines from three separate mobile phase reservoirs, containing water, methanol, and acetonitrile, respectively, meet at a proportioning valve in the solvent conditioning tray. A gradient table programmed into the Alliance e2695 Separations Module directs this valve to generate a mobile phase gradient consisting of linear, convex, and concave segments. The Alliance e2695 Separations Module independent pistons then deliver this gradient through a pair of pressure transducers and an inline filter, and then on to the sample management system. The gradient then passes through a second inline filter and onto the Carbamate Analysis Column.

Either small volume aliquots (10 to 20 µL) of sample extracts in water-miscible organic solvents or larger volume aqueous samples (250 to 1000 µL) are introduced by the sample management system into the mobile phase stream and carried into the column. Aqueous samples are trace-enriched on the column; i.e., the analytes are concentrated and retained

at the inlet end by the reversed-phase, hydrophobic column packing and then transported selectively through the column bed as the concentration of the organic solvent component(s) in the mobile phase (gradient strength) increases.

**Tip:** The Waters Alliance System for Carbamate Analysis can accommodate 1-mL injection volumes of water samples. This capability can be used to achieve lower detection limits or to increase reproducibility in the analysis of low-level samples.

Both the second inline filter and column are maintained in the column heater at 30° C, a few degrees above ambient temperature, for maximum retention time reproducibility. As each analyte is separated and leaves the column, it is prewarmed as it passes through the CHEX™ Countercurrent Heat Exchanger to the post-column reactor oven.

At the first tee inside the reactor oven, the column effluent is met by a stream of sodium hydroxide solution which has also been preheated by passage through the CHEX unit. These two streams mix and pass through the RXN 1000 Reaction Coil where 30 seconds of reaction time in the 1-mL volume coil maintained at 80° C are sufficient to hydrolyze the N-methylcarbamoyl moiety and release methylamine.

After exiting the coil, the effluent immediately enters a second tee where it is joined by the OPA/ME reagent solution, which has also been prewarmed by passage through the CHEX unit. An almost immediate reaction occurs at 80 °C to yield the isoindole derivative. In less than 1 second, the fluid stream leaves the oven, is cooled to ambient temperature by passing through the CHEX unit, and then passes out of the PCRM and into the detector cell.

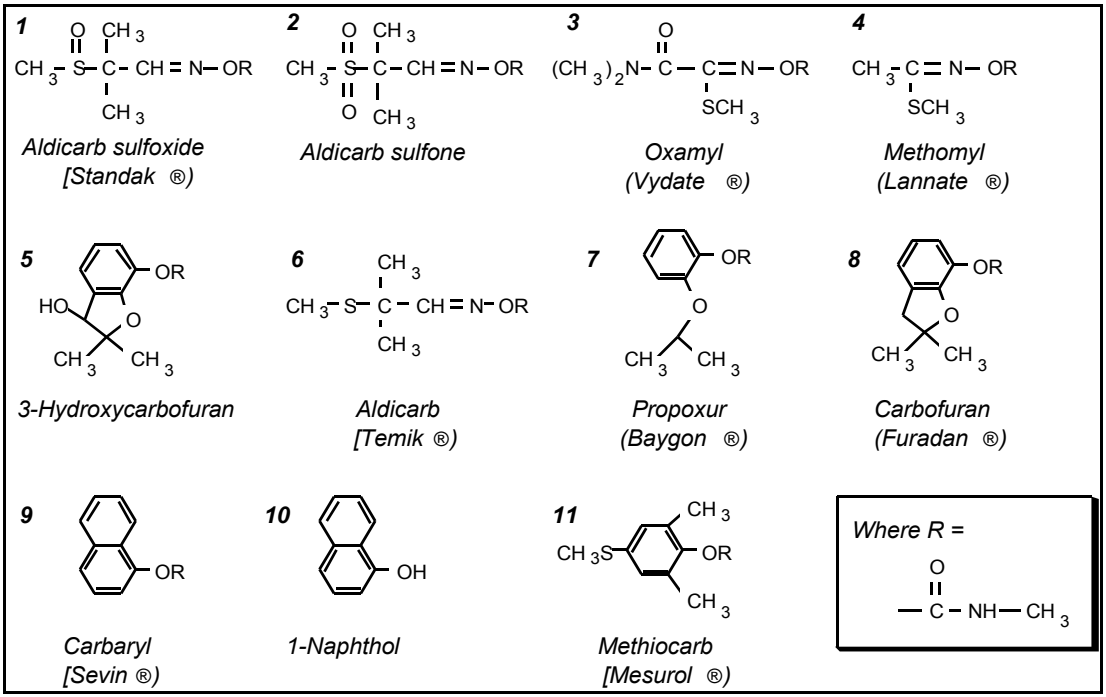
The detector effluent is collected in a suitable waste container and disposed of in an appropriate manner. The detector signal is monitored by one or more data collection or chromatography workstation devices.

This separation will enable the Alliance System for Carbamate Analysis to meet or exceed the performance requirements for single-operator precision and accuracy of EPA Methods 531.2 and 8318, as well as the performance standards of the AOAC (see “References” on page 86, numbers 1 and 2).

The structural formulas for the 11 analytes specified in EPA Method 531.2 are shown in the figure “Analytes in the Carbamate Analysis method:” on page 34. They are listed in the order in which they elute from an octadecylsilyl-bonded [$C_{18}$] silica HPLC column operated in a reversed-phase gradient separation mode. All but 1-naphthol [**10**], a hydrolysis product of carbaryl [**9**], contain the

N-methylcarbamoyl moiety [indicated by –OR]. Compounds **1** and **2** are toxic oxidation products of aldicarb [**6**] as is **5** of carbofuran [**8**].

### Analytes in the Carbamate Analysis method:




The N-methylcarbamoyloximes – oxamyl [**3**], methomyl [**4**], and aldicarb [**6**] – have UV extinction coefficients nearly 3 to 4 orders of magnitude lower than those of the aromatic ring-containing N-methylcarbamates – propoxur [**7**], carbofuran [**8**], carbaryl [**9**], and methiocarb [**11**]. Thus, it would be difficult to include all of these compounds in a multiresidue method using liquid chromatography with UV detection and achieve uniformly low detection limits for each analyte.

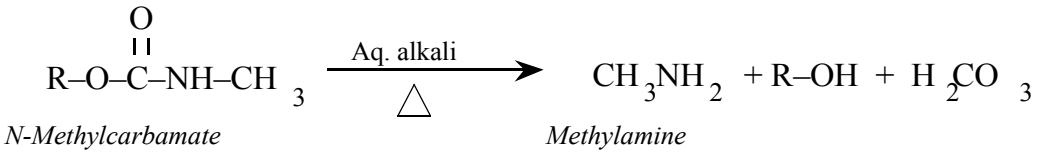
Professor Anson Moye and coworkers first applied an amino acid analysis post-column reaction scheme successfully to the analysis of carbamate pesticides (see "References" on page 86, number 3). This reaction sequence is shown in the figure "Post-Column Reaction sequence for Carbamate Analysis:" on page 35.

As each carbamate elutes from the reversed-phase liquid chromatographic column, it is hydrolyzed by a strong aqueous base (NaOH) at an elevated

temperature to release an alcohol, carbon dioxide (which forms carbonate), and methylamine. In the second step, the methylamine combines with *o*-phthaldialdehyde (OPA) and the nucleophilic 2-mercaptoethanol (ME) to form a highly fluorescent isoindole derivative (see "References" on page 86, number 4).

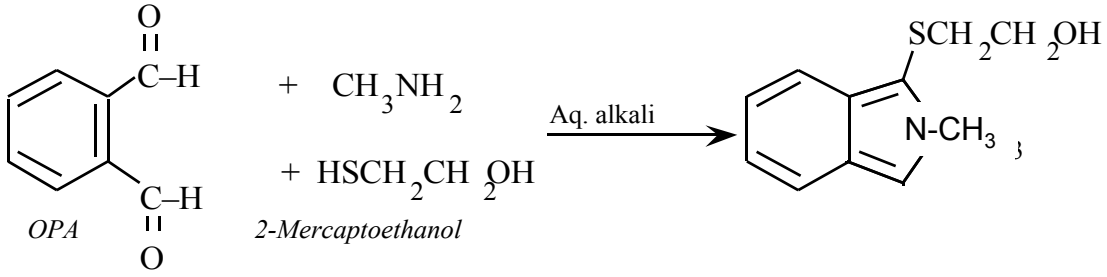
### Post-Column Reaction sequence for Carbamate Analysis:

*Step 1: Hydrolysis*




*N-Methylcarbamate* *Methylamine*

*Step 2: Derivatization of methylamine*




*OPA* *2-Mercaptoethanol*

1-Naphthol is unaffected by this reaction sequence, but exhibits an equally strong native fluorescence. Thus, this post-column derivatization method yields a single analyte from 10 different compounds (11, if the internal standard BDMS is included) and, when coupled with highly sensitive fluorescence detection, provides a means to measure part-per-billion levels of all 11 analytes (see the figure "Analytes in the Carbamate Analysis method:" on page 34) in a single liquid chromatographic analysis. All current standard multiresidue LC methods for carbamate analysis are based on this reversed-phase separation/post-column reaction/fluorescence detection scheme (see "References" on page 86, numbers 1 and 2).

The Alliance System for Carbamate Analysis described in this guide provides optimum sensitivity as well as operational convenience. The chromatographic method and system presented here have been redeveloped, refined, and ruggedized in Waters Application Laboratories and have been based, in part,

on work done by Neue, Engelhardt, and Lillig (see "References" on page 86, numbers 5 to 8) Carson and Roe (see number 9), and Wildman (see "References" on page 86, number 10).

**Caution:** Use the highest quality reagents and solvents and scrupulously clean glassware for all solutions, samples, and solvents. Detergent residues, fingerprints, cigarette smoke, breath, or anything which contains primary amines or ammonia can cause interference in this analysis.

The Alliance System for Carbamate Analysis provides high-sensitivity operation and the lowest possible limits of detection. Perhaps you need to look for sub-part-per-billion levels of carbamates in drinking water or trace residues in processed foods. If so, you will want to push the sensitivity of the system to low levels.

Our service representative will help you install the hardware correctly and guide you through its initial operation. This and the other manuals supplied will explain the method as well as startup, shutdown, maintenance, and troubleshooting procedures. For more help, U.S. and Canadian customers should contact Waters Technical Service at 800 252-4752. Other customers should call their local Waters subsidiary, their local Waters Technical Service Representative, or Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

Proper care in sample and reagent preparation and in routine operation of the system is very important. Some of the most difficult problems to diagnose relate to unforeseen sources of chemical contamination. At the nanogram level, it takes very little primary amine to react with the OPA/ME and be detected as a ghost peak or baseline noise. To be successful, you must use caution to prepare samples effectively (1,2,11 - see "References" on page 86). Carefully follow the method in this chapter, and follow good laboratory practice.

## Use the Carbamate Analysis method

Here are general steps to follow for successful system operation:

1. Familiarize yourself with the principles of the method and system operation as discussed in "Principle of the Carbamate Analysis method" on page 32.

2. Install the system components as shown in Chapter 2. See the operator's manuals supplied with each component of the system for detailed installation information.
3. Prepare mobile phases, analytical standards, and post-column reagent solutions.
4. Turn on the power switches for each component and allow sufficient time for circuits or lamps to warm up (5 to 60 minutes).
5. Prime the reagent manager pumps and check the system flow rate.
6. Set the column and post-column reactor oven temperatures and allow them to reach a stable operating temperature (40 to 60 minutes).
7. Program the gradient table into the Alliance e2695 Separations Module and check all the other instrument settings including excitation and emission wavelengths, detector attenuation, and data system/recorder parameters.
8. Prepare a sample, filter it if necessary, make an injection, and document all results.

In this chapter, you will find a more detailed discussion of reagent preparation, system setup parameters, and a recommended sequence for daily method operation as illustrated by running a test water sample.

**Tip:** Specific recommendations made in this method may differ from corresponding practice in standard methods. In such cases, be certain to follow the Waters Carbamate Analysis Method described here to obtain good results with your system. For supplemental information on sample preparation, system suitability requirements, calculation of recovery, precision, and detection limits, etc., please see the published standard methods and references contained therein (see "References" on page 86).

## Obtain standards, reagents, and solvents

**Warning:** Always observe safety precautions and good laboratory practice when handling reagents, solvents, and toxic chemicals. Use only approved procedures for discarding chemical waste. Carbamates are potent cholinesterase inhibitors.

This section provides information about the standards, reagents, and solvents that you must obtain for use with the Carbamate Analysis Method.

## Carbamate standards

### The carbamate standards listed in order of elution are:

1. Aldicarb sulfoxide
2. Aldicarb sulfone
3. Oxamyl
4. Methomyl
5. 3-Hydroxycarbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbaryl
10. 1-Naphthol
11. Methiocarb
12. BDMC (4-bromo-3, 5-dimethylphenyl N-methylcarbamate) – used as an internal standard

Compounds 2 to 4 and 6 to 11 are available as dry, crystalline powders from several reagent supply companies, including Riedel-de Haën (U.S. distributor: Crescent Chemical Co., 631-348-0333) and Chem Service, Inc, 610-692-3026.

All of these compounds are available in acetonitrile solution separately or as a mixed standard from AccuStandard, Inc., 800-442-5290, and Regis Technologies, Inc., 847-967-6000.

**Caution:** To obtain good quantitative results, you must use high-quality standards of known purity. Before doing any quantitative measurements, carefully check the concentration of any purchased stock solutions against reference solutions freshly prepared from solids of known purity. Check with the supplier of such solutions on lot freshness and on their methods of preparation and storage.

## Post-column reagent ingredients

The following post-column reagents are available from several reagent suppliers:

- Phthalic dicarboxaldehyde (*o*-phthaldialdehyde (OPA)), 97%, recrystallized grade preferred
- 2-Mercaptoethanol (ME), 98%
- Sodium borate, 10-hydrate (sodium tetraborate decahydrate), crystalline, ACS reagent grade
- Sodium hydroxide (NaOH) pellets, ACS reagent grade (low carbonate content)

**Tip:** NaOH must be low in carbonate content. If a suitable grade of fresh pellets is not available, then adapt the method in "Prepare Post-Column reagents" on page 42 to use 50% NaOH solution, which contains very little carbonate. Old pellets may have absorbed too much airborne $CO_2$. See "Pump shutdowns" on page 99 for more information.

## Solvents and reagents

The following solvents and reagents are available from several suppliers:

- Acetonitrile (ACN), HPLC grade or better
- Methanol (MeOH), HPLC grade or better

  **Tip:** Acetonitrile and methanol, even HPLC grade, can contain traces of amines or ammonia which will react with OPA/ME in this post-column system to form highly fluorescent impurities. These derivatives can cause baseline shifts which parallel the gradient changes in solvent composition, and/or they may add to baseline noise. Normally, these impurity levels are low enough so as not to pose a problem for this procedure. However, if the baseline shift or noise becomes objectionable

in time, clean the reservoirs and use fresh solvent. If there is a problem from the start, then use a different lot of solvent or try solvent from another vendor.

- Water, Milli-Q System or HPLC grade

  **Tip:** RO water might not be suitable for this procedure. The quality of water from any purification system must be monitored on a regular basis, and proper maintenance procedures must be followed strictly. If you plan to use bottled HPLC grade water, test it for suitability. If you find interfering peaks in your chromatograms, try water from another vendor.

- Potassium dihydrogen citrate, ACS reagent grade
- Sodium thiosulfate, ACS reagent grade

**Tips:**

- Use methanol and acetonitrile in 1-L bottles only. Use the solvents directly from the bottle; do not transfer to another reservoir. Do not top off or refill the bottles. When the solvent bottle is nearly empty, discard the remaining solvent (or use it for another purpose in the laboratory) and open a fresh bottle. Acetonitrile quality can deteriorate with age after a fresh bottle has been opened, so use it promptly and do not store open bottles for any length of time.
- Use a 2- to 4-liter reservoir for water, since much more water is consumed than either of the two organic mobile phase components under the gradient conditions in this procedure.

## Prepare standards, reagents, and eluents

**Caution:** Use scrupulously clean glassware, including vials, septa, and pipets, in every step of this procedure for all solutions and solvents. Detergent residues, fingerprints, cigarette smoke, breath, any amines, ammonia, etc., can cause interference, ghost peaks, baseline noise, etc.

### A standard solution used for injection is prepared in two stages:

1. Prepare more concentrated, separate stock solutions of each of the 11 analytes (1 to 11) and the internal standard BDMC solution.

2. Prepare a single standard mixture containing all 11 analytes and the internal standard BDMC solution by mixing and diluting aliquots of each of the stock solutions.

## Prepare stock solutions

Prepare from 10 to 100 mL of a solution of each analyte at a level of 0.1 mg/mL in acetonitrile; e.g., weigh 10 milligrams of an analyte and dissolve it in 100 milliliters of acetonitrile. Store it in an amber glass bottle or vial, under nitrogen, sealed tightly against moisture and air, in a freezer. Except for 1-naphthol [**10**], these solutions should be stable for up to 3 months under these storage conditions. Naphthol stock solution should be prepared fresh each time standards are to be chromatographed.

**Tip:** If standards are purchased from a vendor in the form of stock solutions, this step will not be necessary. Purchased solutions can be supplied at a concentration other than 0.1 mg/mL. If this is the case, make a suitable adjustment in the volume pipetted in step 2 below to achieve the desired final concentration.

## Prepare a standard mixture

### To prepare a 25 ppb (25 ng/mL or 25 µg/L) standard solution:

1. To prepare 1 liter of reserved water, add 9.35 ± 0.15 g potassium dihydrogen citrate and 200 ±120 mg sodium thiosulfate to 1 L of reagent water.
2. Pipet 25 µL of each of the 11 stock solutions and BDMC stock solution into a clean 100-mL volumetric flask.
3. Dilute the mixture to the mark with preserved water. Mix thoroughly. Store in an amber glass bottle, under nitrogen, sealed against air and moisture in a refrigerator. This solution is stable for up to 3 months (except for the concentration of 1-naphthol) at 4° C.
4. Use a fresh aliquot of this mixture for each daily set of analyses. Run a standard chromatogram at least every 8 hours to verify sample and reagent stability.

**Caution:** Do not store stock or standard solutions at room temperature for any length of time. Slow hydrolysis will occur and apparent response will diminish with time.

## Prepare Post-Column reagents

**Tip:** For quantitative work, make 500 mL of each solution fresh daily. For qualitative work only (e.g., system diagnostics or troubleshooting), if your lab conditions are not extreme, you may stretch the use of these solutions to a second day.

### 0.05 N. NaOH for Hydrolysis

Add 1 gram of NaOH pellets to a 500-mL volumetric flask containing about 250 mL of water. Dissolve the pellets by swirling, dilute to the mark with more water, and mix thoroughly. Degas by vacuum filtration using a Gelman Supor 0.45-µm membrane filter (part number WAT200538) and Waters Solvent Clarification Kit. Transfer filtered solution to a 1-liter amber glass solvent reservoir bottle for use. See "Pump shutdowns" on page 99, for additional recommendations on preparing NaOH solutions from pellets or from 50% w/w solutions.

### OPA/ME for derivatization

**Caution:** Do not use commercially prepared OPA/ME solutions designed for amino acid analysis. The higher OPA concentration in these solutions and the presence of surfactants, antioxidants, or other ingredients will interfere with carbamate analysis.

#### Prepare the OPA/ME reagent as follows:

1. Stock buffer (0.05 M sodium borate): Dissolve 19.1 grams of sodium tetraborate decahydrate in one liter of water with stirring; complete solution may take more than 1 hour. This stock can be stored in a sealed bottle indefinitely for future use.
2. OPA Reagent: Dissolve 50 milligrams of *o*-phthaldialdehyde in 5 mL of methanol; swirl slowly to dissolve. Transfer to a 500-mL volumetric flask. Dilute solution to mark with borate stock buffer from step 1 and mix thoroughly. Filter and degas as described for NaOH solution. Then,

add 500 µL of 2-mercaptoethanol and swirl gently to mix well. Once the 2-mercaptoethanol has been added, do *not* sparge or degas this solution. For use, transfer the solution to a sealed amber glass solvent reservoir, wrapped with aluminum foil to keep out all light.

**Tip:** If you use another brand or type of filter to degas and clarify the reagent solutions, test the filter first for suitability. For example, a nylon membrane may leach amine-containing impurities which can cause background fluorescence in the OPA/ME solution which will be manifested as increased baseline noise.

### Prepare eluents

Degas methanol and acetonitrile, if necessary, by ultrasonication under vacuum before use. Filter organic eluents with a Waters membrane filter (part number WAT200534). Degas water from sources other than a Milli-Q System by vacuum filtration with a Waters membrane filter (part number WAT200538) and Waters Solvent Clarification Kit before use.

**Recommendation:** On the Alliance e2695 Separations Module, use Solvent Line A for water, B for methanol, and C for acetonitrile.

## System setup: summary of parameters

This section describes setup procedures for various components in the Waters Alliance System for Carbamate Analysis. The section includes procedures for creating a separation method for the Alliance e2695 Separations Module, and for setting up the analysis column, the PCRM, the 2475 Detector (see the second Note), the recorder or integrator, the test sample, and the Reagent Managers.

**Tips:**

- If you are using Empower software to control instruments in your system, set Alliance e2695 Separations Module setup parameters in the Instrument Method Editor. See the Empower Help for more information.
- Empower control of the 2475 Fluorescence Detector requires specific hardware and software configurations. See the *Waters 2475 Fluorescence Detector Release Notes* and the *Empower System Installation and Configuration Guide* for details.

## Setup for a run method on an Alliance system

You enter separation method parameters in six different screens, which are arranged in the order shown in the table below. Each screen is described in the sections listed.

### Separation method parameter screens:

| Screen name | Reference |
|---|---|
| Mobile Phase | See “Set Mobile Phase screen parameter values” on page 44 |
| Gradient | See “Set gradient table parameter values” on page 49 |
| Sample | See “Set sample parameter values” on page 51 |
| Autosampler | See “Set Autosampler parameter values” on page 79 |
| Column | See “Set column parameter values” on page 54 |
| I/O | See “Set I/O parameter values” on page 55 |

## Set Mobile Phase screen parameter values

The Mobile Phase screen appears when you select the separation method to edit. Press the Next or Previous screen key to move among the six method parameter screens. The icon between the Next and Previous screen keys shows where you are in the sequence of the six screens. Press Exit to return to the Methods screen. A dialog box prompts you to save the changes to the separation method.

### To enter parameters in the Mobile Phase screen:

1. Press the Next or Prev screen key (if necessary) to open the Mobile Phase screen.
2. Enter values in the fields, as appropriate. The table below describes the fields and screen keys in the Mobile Phase screen.

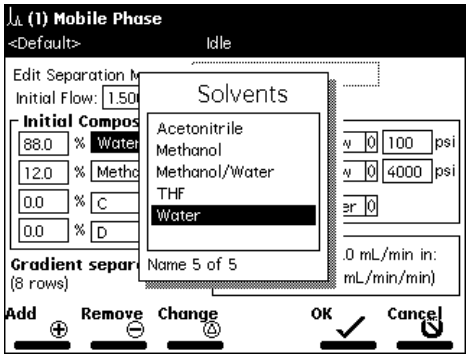
### Mobile phase screen parameters:

| Parameter | Function | Value range |
|---|---|---|
| Initial Flow | Specifies the initial flow rate of the method. For isocratic operation, this is the flow rate for the entire separation. | 0.000 and 0.010 to 10.000 in 1.5-mL/min increments. |
| Initial Composition | Specifies the initial composition of the eluents. The sum of the four fields must equal 100%. (You enter solvent names using the Labels screen key.) | 0 to 100.0 in 0.1% increments:<br>• 88% water<br>• 12% methanol |
| Alarms: Min | Specifies the system pressure (set in psia, bar, or kPa) below which the alarm condition (selected in the adjacent box) is executed. To enable access to the Pressure fields, set the alarm parameter to anything other than "Disable." | 0 to 4500 in 1-psi increments, 0 to 310 bar in 1-bar increments, or 0 to 31025 kPa in 1-kPa increments.<br><br>See "Alarms" on page 48. |
| Alarms: Max | Specifies the system pressure (set in psia or bar) above which the alarm condition (selected in the adjacent box) is executed. Use to detect problems with method conditions and to protect your column from overpressure. | 0 to 5000 in 1-psi increments, 0 to 344 bar in 1-bar increments, or 0 to 34473 kPa in 1-kPa increments.<br><br>See "Alarms" on page 48. |

### Mobile phase screen parameters: (Continued)

| Parameter | Function | Value range |
|---|---|---|
| Alarms: Bubble detect | Specifies the response that occurs when the solvent management system detects a bubble in the flow path. | See "Alarms" on page 48. |
| Flow Ramp | Specifies the time (in minutes) for the solvent delivery system to reach the maximum system flow rate. This limits the rate of change of the flow rate to protect the column from potentially damaging sudden changes in pressure.<br>This flow ramp is used for all applications of the separation method and overrides any flow rate time-based changes defined within a gradient table. | 0 to 30 min in 0.01-min increments. |
| Gradient (screen key) | Displays the Gradient Table screen, which allows you to build a gradient table. | See "Set gradient table parameter values" on page 49. |
| Degas (screen key, for units equipped with an inline vacuum degasser) | Displays the Degasser screen which allows you to set the Degasser Mode. See the *Waters Alliance e2695 Separations Module Operator's Guide*.<br><br>On Degasser Error: Specifies that an alarm response occurs when an inline vacuum degasser fault is detected. The inline degasser is disabled on any fault regardless of the alarm setting you select. Waters recommends that you enable either the "Stop Function" or "Stop Flow" alarm setting. | Off<br>On<br><br>See "Alarms" on page 48. |

## Mobile phase screen parameters: (Continued)

| Parameter | Function | Value range |
|---|---|---|
| Labels (screen key) | Displays the Solvents dialog box, which allows you to add, remove, or change the names of solvents used in methods.<br>Use the Add, Remove, and Change screen keys to edit the list of solvents.<br>A, B, C, and D are not valid user-entered solvent names. |  |

**Mobile phase screen parameters: (Continued)**

| Parameter | Function | Value range |
|---|---|---|
| Other Params (screen key) | The Preferred Stroke Volume field sets the volume of solvent delivered with each piston stroke.<br><br>You can override the default stroke volumes, but do not exceed the flow rate limits displayed on the screen for each of the stroke volume settings. See the "Preferred Plunger Stroke Volume" discussion in the *Waters Alliance e2695 Separations Module Operator's Guide*.<br><br>The Plunger Seal Wash period sets the time interval between successive plunger seal-wash pump cycles. | 130 µL – default<br>100 uL – stroke volume<br>50 µL<br>25 µL<br><br>Off, 0.50 to 10.00 in 0.01-min increments |

## Alarms

The Alliance e2695 Separations Module maintains a log of all enabled errors that occur during operation. Each error can produce a variety of user-defined responses, as shown in the table titled "Alarm responses:" on page 48.

**Alarm responses:**

| Alarm response | Function |
|---|---|
| Disable | All alarm response reporting is disabled. |
| Log Quietly | The error is entered into the error log without alerting the operator. |
| Alert User | The error is entered into the error log and the operator is alerted with a dialog box. |

### Alarm responses: (Continued)

| Alarm response | Function |
|---|---|
| Stop Funct | The error is entered into the error log, the operator is alerted with a dialog box, and operation is suspended at the end of the current function. You can abort or resume the operation of the sample set by pressing the appropriate screen key. |
| Stop Flow | The error is entered into the error log, the operator is alerted with a dialog box, the current function is suspended, and solvent flow is stopped. |

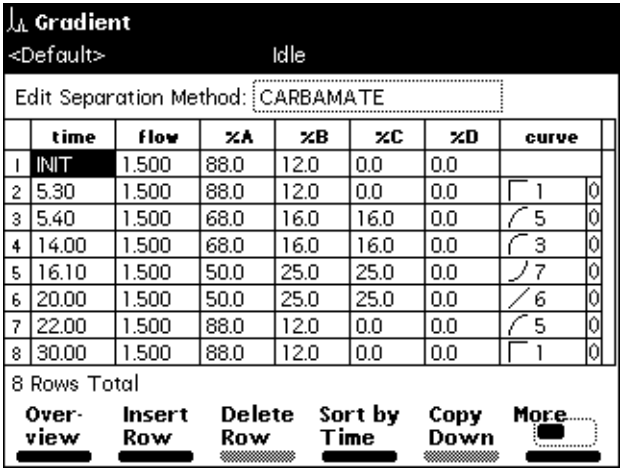
## Set gradient table parameter values

The gradient table allows you to make time-based changes to the composition of the mobile phase during a run. You can program up to 25 lines in the gradient table.

### To set the parameters in the gradient table:

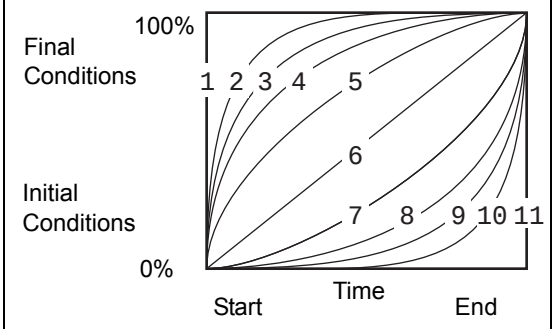
1. Press the Gradient screen key in the Mobile Phase screen to open the Gradient screen.

   #### Gradient screen:




2. Enter values in the gradient table as appropriate. The table below describes the parameters in the gradient table.

### Gradient table parameters:

| Parameter | Function | Value range |
|---|---|---|
| Time | Specifies the time after the start of the run at which the change is to occur. (INIT is allowed only in the first row of this table.) | INIT, 0.00 to 999.99 in 0.01-minute increments. |
| Flow | Sets the flow rate of the solvent delivery system. | 0.000 and 0.010 to 10.000 in 0.001-mL/min increments. |
| %A, %B, %C, %D | Sets the proportion of each solvent in the mobile phase. The sum of these four fields must equal 100%. | 0 to 100 in 0.1% increments. |
| Curve | Sets the rate at which the solvent is to change to the new proportions and/or flow rates. The figure at right shows the curve shape for each value.   | |
| | Select the desired gradient curve from the list of profiles, or select the curve number by pressing the appropriate numeric key. Press 0 for curve 10 and . for curve 11. | |
| Overview (screen key) | Displays a time-ordered summary of the events in the gradient, detector, and timed events tables. | N/A |
| Insert Row (screen key) | Inserts a row above the current row. | N/A |
| Delete Row (screen key) | Deletes the current row. | N/A |

### Gradient table parameters: (Continued)

| Parameter | Function | Value range |
|---|---|---|
| Sort by Time (screen key) | Sorts the rows based on time. | N/A |
| Copy Down (screen key) | Copies the contents of the current table cell into subsequent cells in the column. | N/A |
| Reset Table (screen key) | Clears the table. | N/A |
| Print (screen key) | Prints the gradient table. | N/A |

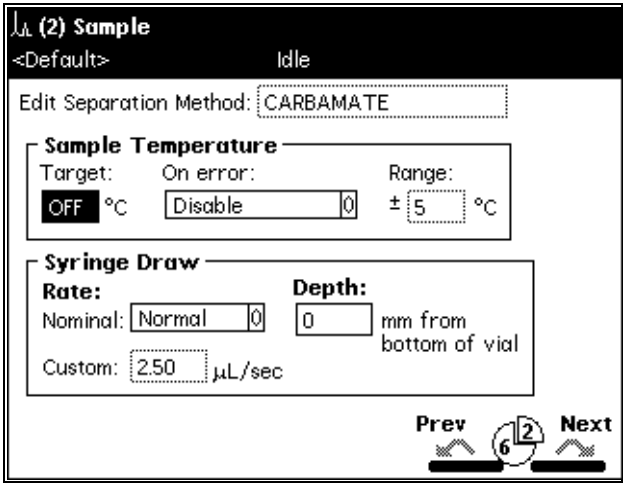
3. Press Exit to save the gradient table and return to the Mobile Phase screen.

## Set sample parameter values

### To enter parameters in the Sample screen:

1. Press the Next or Prev screen key (as appropriate) to open the Sample screen.

### Sample screen:



2. Enter or select values in the Sample screen parameter fields as appropriate. The table below describes the parameters in the Sample screen.

### Sample parameters:

| Parameter | Function | Value range |
|---|---|---|
| Syringe Draw Rate<br><br>Custom | Selects one of three preset syringe draw rates to accommodate viscous samples. The rate changes with the size of the installed syringe.<br>Specifies a user-entered custom draw rate value. | Fast (5.0 µL/sec)[1]<br>Normal (2.5 µL/sec)<br>Slow (1.0 µL/sec)<br>Custom (1.00 to 5.00 in 0.01-µL/sec increments). |
| Sample Draw Depth[2] | Adjusts the depth of the needle tip to accommodate for sedimented samples or nonstandard vials. A value of 0 corresponds to the bottom of the vial.[b] | 0 to 20 in 1-mm increments[b]. |

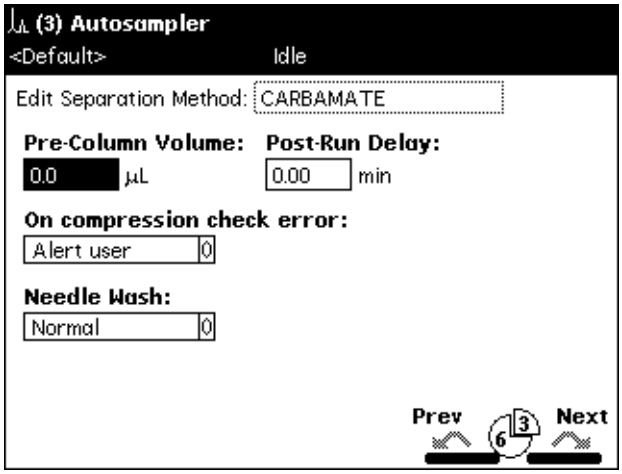
1. These draw rates are for a 250-µL syringe. The values automatically change to reflect the syringe size entered in the Configuration screen. Be sure that the 2500 ul syringe and 2 ml loop are present and are correctly configured.
2. See the *Waters Alliance e2695 Separations Module Operator's Guide* for a list of sample draw depths required for use with Alliance e2695 Separations Module vials and Low Volume Inserts.

## Set Autosampler parameter values

### To enter parameters in the Autosampler screen:

1. Press the Next or Prev screen key (as appropriate) to open the Autosampler screen.

### Autosampler screen:




2. Enter values in the Autosampler screen as appropriate. The table below describes the parameters in the Autosampler screen.

### Autosampler parameters:

| Parameter | Function | Value range |
|---|---|---|
| Pre-Column Volume | The sample management system starts the gradient and delivers this volume before making an injection. Use this parameter when transferring a method from a system that has a delay volume smaller than that of the Alliance e2695 Separations Module. This parameter can also be used to reduce the delay volume of the Alliance e2695 Separations Module for narrow or microbore columns, because the sample is held in the sample loop (if desired) until the gradient front reaches the sample loop. | 0.0 to 10000.0 in 0.1-µL increments. |

### Autosampler parameters: (Continued)

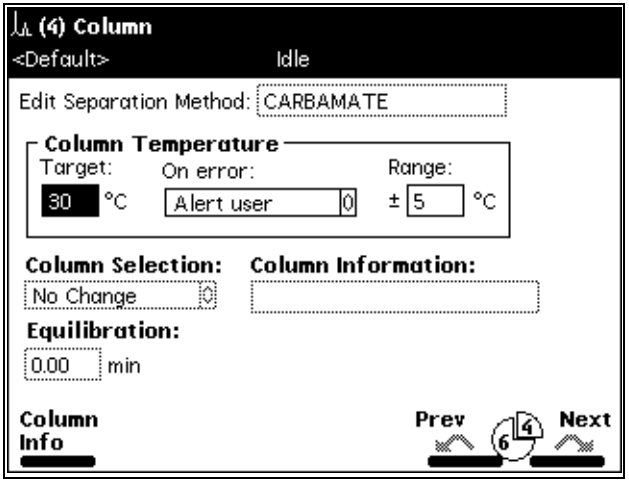
| Parameter | Function | Value range |
|---|---|---|
| Post-Run Delay | Provides time for a data system to process data from the run or to reequilibrate. During the delay, the Alliance e2695 Separations Module draws the next sample into the loop but does not perform an injection. | 0 to 999.99 in 0.01-min increments. |
| On compression check error | Response that occurs when the compression check fails while running a sample set or sample template that specifies an automatic compression check. | See the table titled "Alarm responses:" on page 48. |

## Set column parameter values

### To enter parameters in the Column screen:

1. Press the Next or Prev screen key (as appropriate) to open the Column screen.

### Column screen:




2. Enter values in the Column screen as appropriate. The table below describes the parameters in the Column screen.

### Column parameters:

| Parameter | Function | Value range |
|---|---|---|
| Column Temperature Target | Sets the temperature of the column heater (if installed). To turn off the column heater, press the Clear key. | 30 (ambient +5 °C) to 60 °C in 1 °C increments. |
| On error | Response that occurs when the column temperature is out of the specified range. | See the table titled "Alarm responses:" on page 48. |
| Column Temperature Range | Sets the maximum allowable variation in column temperature. If the temperature variation exceeds the range, the alarm condition selected in the adjacent box is triggered. | ±10 °C in 1 °C increments. |

## Set I/O parameter values

You set I/O parameter values when you want to use analog signals to notify other instruments of the status of the Alliance e2695 Separations Module. For example, you may want to:

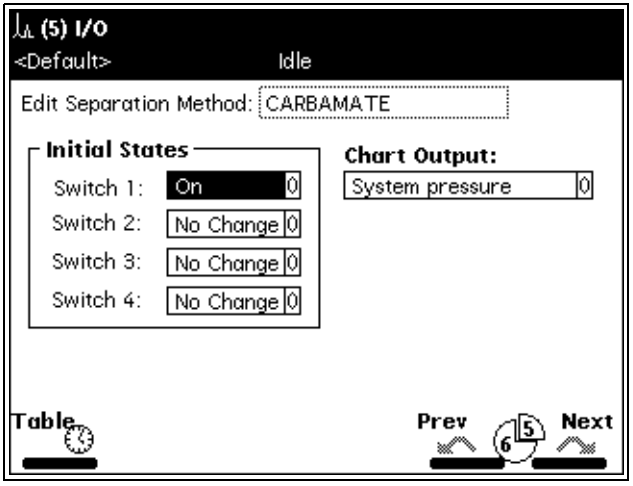
- Notify a detector that an injection has begun.
- Turn on a heating plate or a stirring bar in a solvent reservoir.
- Notify a third-party data system or integrator that an event has occurred.
- Activate a switching valve.
- Sound a buzzer.

You can also select the system parameter signal that is sent out through the Chart Out terminals.

### To enter parameters in the I/O screen:

1. Press the Next or Prev screen key to open the I/O screen.

### I/O screen:




2. Enter values in the I/O screen as appropriate. The table titled "I/O parameters:" on page 56 describes the parameters in the I/O screen.

### I/O parameters:

| Parameter | Function | Value range |
|---|---|---|
| Initial States | Defines the initial condition for each of the four event switches. At the beginning of each injection cycle, each switch returns to the state defined in this parameter. | On<br>Off<br>Toggle[1]<br>Pulse[2]<br>No Change |
| Chart Output | Defines the signal sent out on the Chart Out terminals on the rear panel of the Alliance e2695 Separations Module module. | low rate<br>System pressure<br>Sample Loop Pressure<br>%A, %B, %C, %D<br>Column Temp<br>Sample Temp<br>Degasser Vacuum<br>Primary Head Pressure |

### I/O parameters: (Continued)

| Parameter | Function | Value range |
|---|---|---|
| Timed Table (screen key) | Displays the I/O Events table. | See "Edit the I/O events table" on page 57. |

1. Toggle is a one-time change of state.
2. Pulse is a single pulse with a width defined in the Param column of the I/O Events table.

## Edit the I/O events table

The I/O Events table allows you to set the times for the following events to occur during a run:

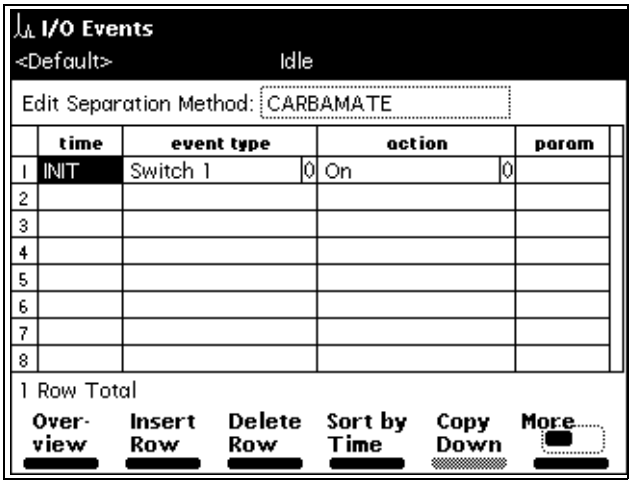
- Changing the state of event switches
- Changing the sparge rate
- Setting the column temperature
- Setting the sample compartment temperature
- Alerts

You can program up to 25 lines in the I/O Events table.

### To enter events in the I/O Events table:

1. Press the Table screen key in the I/O screen. The I/O Events table appears.

### I/O events table:




2. Enter values in the I/O Events table as appropriate. The table titled "I/O events table parameters:" on page 58 describes the parameters in the I/O Events table. The table titled "Action parameters:" on page 59 lists the parameters you can use in the action column in the I/O Events table.

### I/O events table parameters:

| Parameter | Function | Value range |
|---|---|---|
| time | Determines the time after the start of a run at which the change is to occur. Press the Clear key to select INIT. Conditions in the INIT line apply when the system is initialized to a method, while events at time 0.00 occur immediately upon an injection. | INIT, 0.00 to 999.99 in 0.01-min increments |
| event type | Sets the type of event to occur. | Switches 1 to 4<br>Set Sparge<br>Set Temperature<br>Alert |

### I/O events table parameters: (Continued)

| Parameter | Function | Value range |
|---|---|---|
| action | Selects the action to perform with the specified event. | See the table titled "Action parameters:" on page 59. |
| param | Selects the value for the action. | See the table titled "Action parameters:" on page 59. |

### Action parameters:

| Event type | Action | Value range (Param colum) |
|---|---|---|
| Switches 1 to 4 | On<br>Off<br>Toggle[1]<br>Pulse[2]<br><br>No Change | <br><br><br>0.01 to 10.00 in 0.01-min increments (Pulse only) |
| Set Temperature (if column heater or sample heater/cooler is installed) | Column | 30 (ambient +5 °C) to 60 °C in 1 °C increments |
| Alert | No action | N/A |

1. Changes the state of the switch (open to closed, or closed to open).
2. A single pulse with a width defined in the param column of the I/O Events table.

3. Press Exit to return to the I/O screen.

## Setup for a run method on the 2475 Fluorescence Detector

After you press HOME to return to the Home screen and select a channel mode (λ or λλ), you are ready to set up the detector for a run. See the *Waters*

*2475 Multiwavelength Fluorescence Detector Operator's Guide* for more information.

The table below contains the function descriptions, fields, screen number, display units, allowable ranges, and default settings for the Home screen and its secondary function screens.

### Primary and secondary function (method) parameters:

| Function | Screen | Type | Units | Range | Default |
|---|---|---|---|---|---|
| **xλ (excitation wavelength)** | Home | numeric | nm | integer 200 to 890 nm | 339 nm |
| **eλ (emission wavelength)** | Home | numeric | nm | integer 210 to 900 nm | 445 nm |
| **Tip:** The emission λ setting must always be at least 10 nm above the excitation λ setting. | | | | | |
| **Gain** | 1 | numeric | emission or energy units | 0 to 1,000 | 10 |
| **EUFS** | 1 | numeric | EUFS | 1 to 100,000 | 10,000 |
| **filter type** | 2 (of 4) | choice | none | Hamming<br>RC<br>None | Hamming |
| **analog out (single λ)** | 2 (of 4) | choice | none | emission A<br>energy A<br>sample energy<br>ref energy | sample energy |
| **time constant** | 2 (of 4) | numeric | sec | Hamming (λ): 0.1 to 5.0<br>Hamming (λλ): 1 to 50<br>RC (λ): 0.1 to 99<br>RC (λλ): 1 to 99<br>0 to disable filtering | 2.0 |

**Primary and secondary function (method) parameters: (Continued)**

| Function | Screen | Type | Units | Range | Default |
|---|---|---|---|---|---|
| **data offset** | 3 (of 4) | numeric | EU | –100 to +1000 | 0.000 |
| **voltage offset** | 3 (of 4) | numeric | mV | integer –1000 to +1000 | 0 |
| **chart polarity** | 3 (of 4) | choice | none | +<br>– | + |
| **Auto Zero on inject** | 4 (of 4) | check box | none | Checked<br>not checked | Checked |
| **Enable keypad & event-in Auto Zero** | 4 (of 4) | check box | none | Checked<br>not checked | Checked |
| **Enable keypad & event-in chart mark** | 4 (of 4) | check box | none | Checked<br>not checked | Checked |

## Store methods

You can store and retrieve up to 10 methods. The detector designates stored methods as the numerals 1 to 10. If you are using a stored method during operation, the method number appears on the Home screen. An asterisk in the method number icon indicates that conditions are not stored.

If you edit a parameter such as wavelength or EUFS, you are editing the current conditions (method *). You can store the method in one of the 10 available method storage slots, or you can replace the current method with one previously stored. When you retrieve a previously stored method, you replace the existing method conditions with those of the stored method.

The method number displayed on the Home screen is that of the retrieved method until you make a change. Any parameter change (for example, wavelength or EUFS) alters conditions so that the original recalled method is no longer in effect, causing the method number to change to an asterisk.

On startup, the operating parameters at the time the detector was last shut down are restored. However, any timed events or thresholds associated with the method are deactivated when power is restored. Thus, on startup, an asterisk always appears inside the method icon on the Home screen.

When the detector is operating under remote control by Empower software, the remote icon appears.

## Program timed events

You can program up to 48 timed events to the nearest 0.01 minute. As you enter timed events, each new event appears at the end of the timed event list. You can specify a time that is not in sequence with the events specified previously, and the timed event list is sorted automatically when you press Next. The table below lists the 12 timed events.

### Timed event parameters:

| Number | Event | Units | Desired setting | Specify channel |
|---|---|---|---|---|
| 1. | excitation wavelength | nm | 339 | Yes |
| 2. | emission wavelength | nm | 445 | Yes |
| **Tip:** The emission λ setting must always be at least 10 nm above the excitation λ setting. | | | | |
| 3. | time constant | seconds | Hamming: (λ) 2.0 sec | Yes |
| 4. | gain | | 10 | Yes |
| 5. | sensitivity | EUFS | 5000 | Yes |
| 6. | chart mark (10% of full scale) | N/A | N/A | Yes |
| 7. | polarity | 1. –<br>2. + | + | Yes |
| 8. | Auto Zero | N/A | N/A | Yes |
| 9. | lamp | 1. off<br>2. on | off | No |
| 10. | switch 1 | 1. high<br>2. low<br>3. pulse<br>4. rect wave | low | No |

### Timed event parameters: (Continued)

| Number | Event | Units | Desired setting | Specify channel |
|---|---|---|---|---|
| 11. | switch 2 | 1. high<br>2. low<br>3. pulse<br>4. rect wave | low | No |
| 12. | threshold | EU | –100.0 to 1100.0 EUs or variable, depending on output selection | Yes |

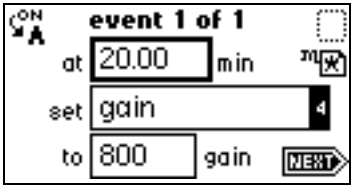
### To program a new timed event:

1. Select METHOD (Shift, A/B). The Method list appears.

   **Method list:**


2. Press 1 Timed events. An active field for specifying the time of the event appears.
3. Specify the time for the event. When you begin, additional fields appear.

   **Timed events screen:**




4. Press Enter. To advance to the Set field (Events list), press ▼.

5. Press Enter again to display the list. If you know the event number, simply press it (see the table titled "Timed event parameters:" on page 62).
6. Enter the appropriate selection in the To field if it appears.

   **Tip:** If you want the same event programmed on both channels, you must enter two events, one for Channel A and one for Channel B.
7. Press A/B to set the threshold on the other channel.

   ON A or ON B indicates the channel on which the event is programmed. You can program all or some events on Channel A and all or some events on Channel B. Event programming is time-based, not channel-specific.
8. Press Next to advance to a new timed event. To delete a timed event, press CE when the time field is active to change it to OFF.
9. Press HOME to return to the Home screen, and then press Run/Stop to start the method.
10. Select Reset (Shift, Run/Stop) to reset the run clock to 0.

    If the detector is configured with the 717plus Autosampler or another external device, the inject start signal programmed from that device starts the method.

**Caution:** If you are working in real time under current conditions (method *), a power failure or shutdown will cause loss of all timed or threshold events if you have not stored them as a method (see "Store methods" on page 61).

## Setup for the Carbamate analysis column

See the *Column Care and Use Manual* for information about installation and equilibration. A new column is preequilibrated with a 50:50 v/v mixture of methanol:acetonitrile.

After connecting the column inlet, but *before* connecting the column outlet fitting and fastening the column in the heater (see the figure "Fluid path schematic of the Alliance System for Carbamate Analysis:" on page 27), deliver 5 to 10 mL of initial mobile phase (88:12 water:methanol) through the column. Collect the column effluent directly from the column outlet in a waste vessel and discard in an appropriate manner. Stop the solvent manager, connect the column outlet fitting, install the column in the oven, and resume

flow of the initial mobile phase. Check all fittings for leaks; further tighten if necessary. When completed, shut the oven door and allow sufficient time for the column to reach thermal and chemical equilibrium at 30 °C.

## Setup for the Post-column reaction module

This section lists the Temperature Control Module parameter that you must set to use the PCRM in the Carbamate Analysis Method. See the *Waters Temperature Control System Operator's Guide* for more information on operating the Temperature Control Module. See the *Waters Post-Column Reaction Module Installation Guide* for information on operating the PCRM.

- Post-Column Reaction Module Temperature: 80 °C

## Setup for the model 2475 detector

This section lists the Model 2475 Scanning Fluorescence Detector parameters that you must set to use the Carbamate Analysis Method. See the *Waters 2475 Multiwavelength Fluorescence Detector Operator's Guide* for more information.

- Gain: 10
- Excitation: $\lambda$: 339 nm
- Emission: $\lambda$: 445 nm
- Hamming: 2.0 s

## Setup for the recorder, integrator, or data system

- Chart Speed: 0.5 cm/min
- Channel One: 10-mV detector signal (recorder) or 1-V signal (integrator or data system)
- Channel Two: 10-mV system pressure signal from the Alliance e2695 Separations Module

## Setup for the test sample

- Sample concentration: 25 ppb of each of the 11 analytes and BDMC (25 µg/L)
- Sample injection volume: 400 µL

## Setup for the Reagent Managers

The fluid delivery end of the Reagent Manager is shown in the figure "Fluid delivery end of a Reagent Manager:" on page 28, and a fluid path schematic is shown in the figure "Flow paths of solvents through pumps:" on page 67. These pumps have a flow rate range of 0 to 2.0 mL/min.

This section lists the Reagent Manager parameters that you must set to use the Carbamate Analysis Method. See the *Waters Reagent Manager Operator's Manual* for more information.

- Flow Rate: 0.50 mL/min
- Low Pressure Limit: 0 psi
- High Pressure Limit: 1200 psi

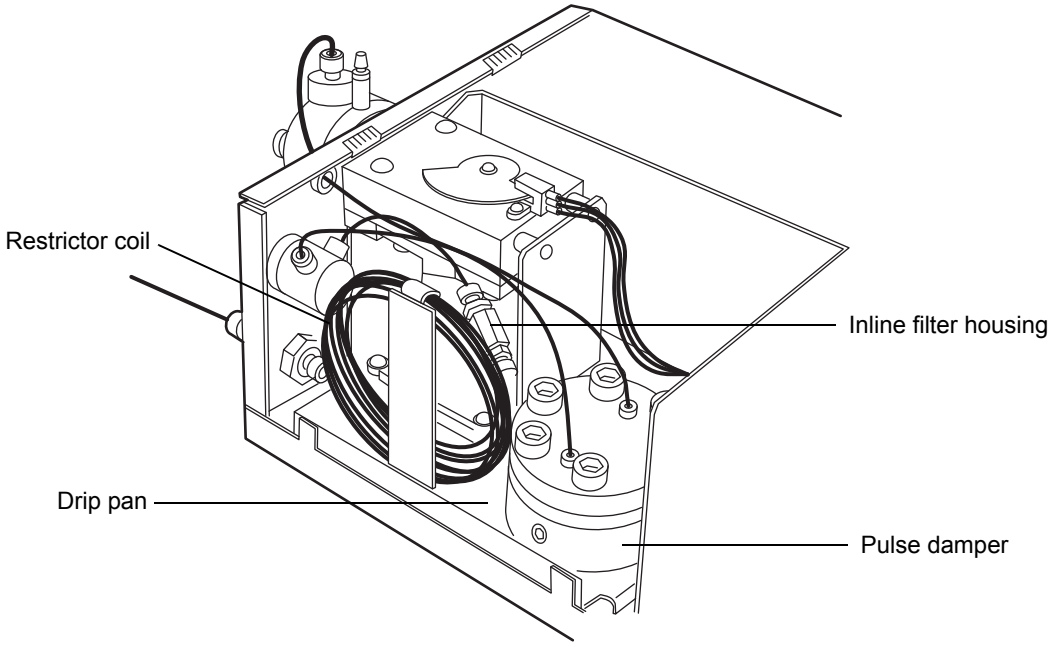
**Tip:** Both Reagent Managers are equipped with user-settable high-pressure cutoff limits that automatically shut off all pumps if the backpressure exceeds the value set by the user (1200 psi). This prevents damage to the RXN 1000 Reaction Coil in the event of blockage in the fluid lines.

**Caution:** The maximum operating pressure of the RXN 1000 Coil is 500 psi (3.4 MPa)

**Tips:**

- The Waters Reagent Manager requires at least 1 L of seal wash solvent. You should check the seal wash solvent level regularly to ensure that there is an adequate amount of solvent in the Reagent Manager.
- Waters recommends using a seal wash mixture of 80:20 water:methanol for both Reagent Managers. See the *Reagent Manager Operator's Manual* for more information.

### Flow paths of solvents through pumps:




Turn the power switch on the front panel of the Reagent Managers to On. This allows switch S1 in the Alliance e2695 Separations Module to control power to each pump.

**Tip:** There are two ways to turn the Reagent Manager pumps on and off: the Reagent Manager front panel switch; and the Alliance e2695 Separations Module switch S1. In normal operation, all switches are turned on.

**Recommendation:** Use the main power switch on the Reagent Managers for long-term system shutdown, for diagnostic purposes, or for quick on/off control of a particular pump. Use switch S1 on the Alliance e2695 Separations Module for automatic shutdown (see "System shutdown: summary of parameters" on page 76).

- NaOH Flow Rate: 0.5 mL/min
- OPA/ME Flow Rate: 0.5 mL/min

These flow rates are set after the initial calibration procedure outlined in "Reagent Manager initial pump flow rate calibration" on page 68, and should not have to be reset again unless a different flow rate is desired.

## Reagent Manager initial pump flow rate calibration

When the Reagent Manager pumps are used for the first time (or after the seals have been changed), the following general sequence needs to be carried out for each pump.

### To use Reagent Manager pumps for first time:

1. Prime each pump with methanol.
2. Pump methanol at 1.5 mL/min for 30 minutes to thoroughly wet the seals.
3. Pump water at 1.5 mL/min for 30 minutes to reach the normal motor operating temperature.
4. Check the flow rate.
5. Switch to the reagent solution and prime the pump again. Check the flow before running samples.

# Test procedure

## Operational protocol

Before beginning, empty the waste vessel, discarding its contents in an appropriate manner. Follow these steps for daily system startup and operation:

### To perform daily system startup and operation:

1. Turn on the power switches for all components.

   **Tip:** If you have a 2475 Detector, read its diagnostic messages immediately after power-on to verify that the proper operating condition has been reached. See the Waters 2475 Multiwavelength Fluorescence Detector Operator's Guide for more information.
2. Verify that the column oven temperature is set at 30 °C on the Alliance e2695 Separations Module so that the column will begin to warm up to operating temperature at this time.
3. Prepare and fill the mobile phase reservoirs with properly degassed solvents.

4. Prepare fresh NaOH and OPA/ME reagent solutions. Clean and fill each reagent manager reservoir with the appropriate solution.
5. Prepare fresh analytical standard mixtures, as required, for system calibration.
6. Prime the Alliance e2695 Separations Module. More information on priming is in the *Waters Alliance e2695 Separations Module Operator's Guide*.

   **Recommendation:** Dry and wet prime the solvent manager with each of the three solvents individually, and then again with the initial mobile phase mixture (88:12 water:methanol).

   a. See the *Waters Alliance e2695 Separations Module Operator's Guide* for information about priming the system.

   b. When all priming operations have been completed, set the initial flow rate to 1.5 mL/min (incrementally).
7. Continue delivering the initial mobile phase mixture through the system while performing the next steps.

**Caution:** It is essential to have the Alliance e2695 Separations Module delivering mobile phase through the system before elevating the PCRM to operating temperature.

8. Set the Post-Column Reaction Module temperature to 80 °C.
9. Turn on and prime the Reagent Managers with their respective solutions at 0.5 mL/min.
10. Check that the gradient and event tables are properly programmed into the Alliance e2695 Separations Module.
11. Check that the detector wavelength and attenuation/gain settings are correct.

    Check the parameters on the data recording/integrating device(s) being used.
12. When the Post-Column Reaction Module has reached the proper operating temperature, check the system flow rate. The total flow should be 2.5 mL/min at the waste line outlet. If not, verify that each pump has been properly primed. Then, recheck the flow rate. If a problem still

persists, see the maintenance and troubleshooting procedures in Chapter 4.

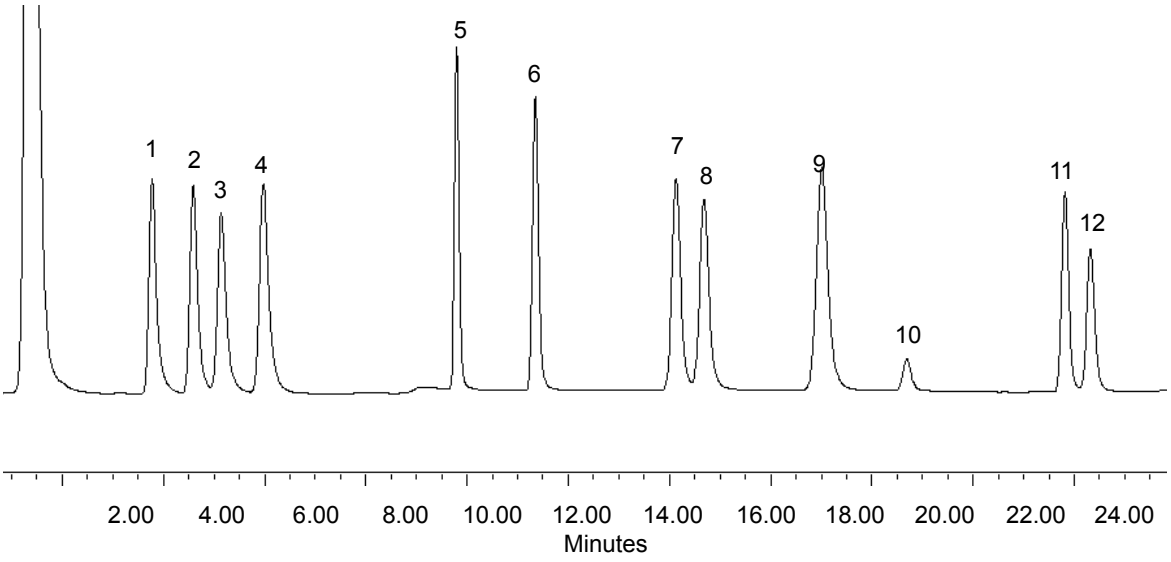
After the initial Alliance e2695 Separations Module and Reagent Manager flow rates have been stabilized and checked, the Post-Column Reaction Module and column heater have reached operating temperature, the detector baseline has stabilized, and the column has been equilibrated with the initial mobile phase for at least 30 minutes, run duplicate injections of the standard test solution.

Compare the chromatogram obtained with that in the figure "Separation of analytes – 400 µL injection volume, 25 ppb concentration, and 10 ng of each analyte on the column:" on page 71 or the figure "Separation of analytes – 400 µL injection volume, 1 ppb concentration, and 0.4 ng of each analyte on the column:" on page 71. Typical retention times for the 11 analytes and BDMC are listed in the table titled "Typical retention times:" on page 72. If any problem is observed, see the troubleshooting procedures in Chapter 4. Once the problem has been diagnosed and corrected, repeat the test and verify that satisfactory results have been obtained.

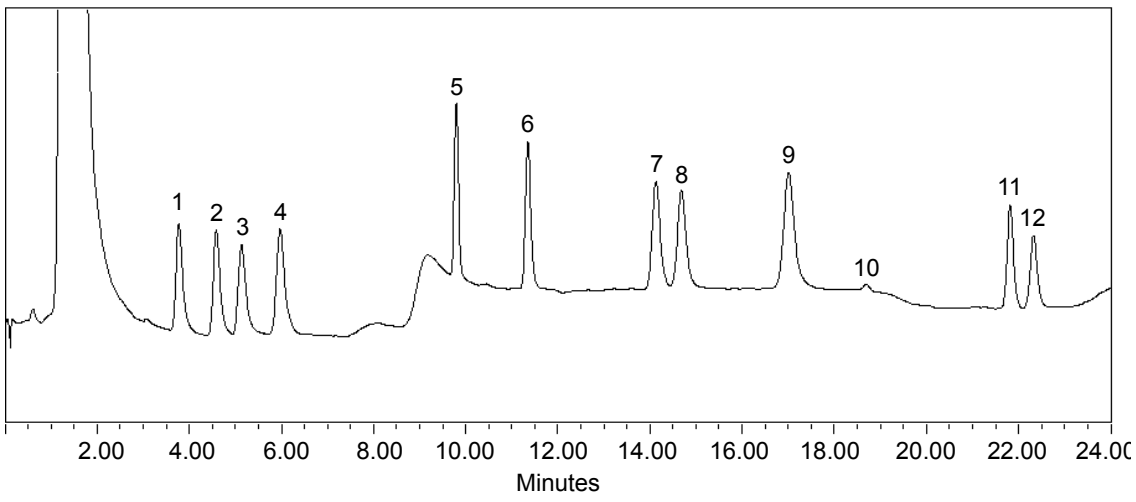
When your system is new, you may need to determine if there are any sources of contamination in the system, sample, or reagents that need to be eliminated. Making several injections of the test sample will familiarize you with operating the system as well as help to flush any contaminants from the system that may have been introduced during installation and startup.

Also see Appendix B, on how to test the efficiency of a new column. Use one of your initial runs to perform this test.

**Separation of analytes – 400 µL injection volume, 25 ppb concentration, and 10 ng of each analyte on the column:**




**Separation of analytes – 400 µL injection volume, 1 ppb concentration, and 0.4 ng of each analyte on the column:**



### Typical retention times:

| Peak | Analyte name | 400 µL | 1000 µL |
|---|---|---|---|
| 1 | Aldicarb sulfoxide | 3.77 | 4.18 |
| 2 | Aldicarb sulfone | 4.66 | 5.07 |
| 3 | Oxamyl | 5.17 | 5.57 |
| 4 | Methomyl | 6.03 | 6.42 |
| 5 | 3-Hydroxycarbofuran | 9.83 | 9.84 |
| 6 | Aldicarb | 11.46 | 11.48 |
| 7 | Propoxur | 14.35 | 14.35 |
| 8 | Carbofuran | 14.94 | 14.92 |
| 9 | Carbaryl | 17.37 | 17.35 |
| 10 | 1-Naphthol | 18.99 | 18.98 |
| 11 | Methiocarb | 22.02 | 22.01 |
| 12 | BDMC | 22.56 | 22.55 |

**Tip:** Peak 12 in the figure “Separation of analytes – 400 µL injection volume, 25 ppb concentration, and 10 ng of each analyte on the column:” on page 71 to the figure “Separation of analytes – 1000 µL injection volume, 0.1 ppb concentration, and 0.1 ng of each analyte on the column:” on page 73 represents BDMC, which is an internal standard.

**Tip:** In the table titled “Typical retention times:” on page 72, notice that the retention times, especially for the first four analytes which elute isocratically in 88:12 water:methanol, shift to longer values as the sample volume is increased in the trace enrichment mode. Remember to set the retention time/peak recognition parameters on your data system based upon results for the sample volume that you intend to use.

The figure “Separation of analytes – 1000 µL injection volume, 0.1 ppb concentration, and 0.1 ng of each analyte on the column:” on page 73 shows a chromatogram for a 1000-µL injection of a water sample containing the 11 analytes each at a concentration of 0.1 µg/L (0.1 ppb). This is one of eight replicate runs.

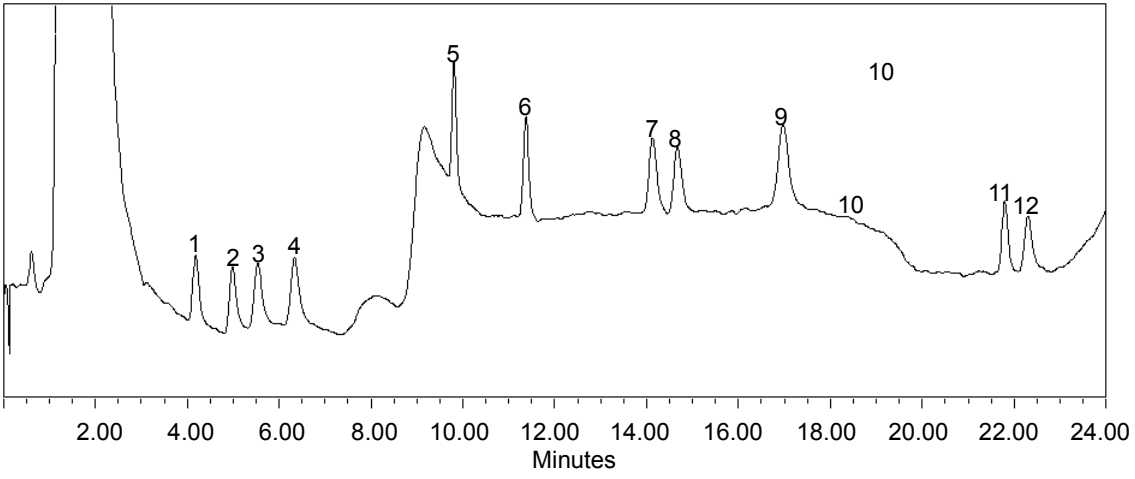
## Confirmation of peak identity

When doing trace level analysis, it is desirable to determine with certainty the chemical identity of an analyte detected after elution from a chromatographic column. One good tool for confirmation of identity is mass spectrometry.

Another means of raising your confidence level about the identity of a particular sample component is to use a second, *mechanistically different* type of separation system and compare the component's elution behavior (retention time) in this system with that of known standards. An example of this type of approach has been published for aldicarb and its metabolites (11). However, an effective means of confirming the identity of all 11 analytes by a second type of liquid chromatography column has not yet been developed.

**Tip:** A "confirmation" column has been identified in some published procedures (1), but the separation mechanism is still reversed-phase chromatography. The elution order on a C1, C8, or CN column is not sufficiently different from that on a $C_{18}$-type column to provide a reliable means of identity confirmation.

### Separation of analytes – 1000 µL injection volume, 0.1 ppb concentration, and 0.1 ng of each analyte on the column:



### Typical recovery, precision, and detection limits: comparing EPA and Waters methods:

| Peak | Analyte | % Recovery | | % Precision | | Detection limits µg/L (ppb) | |
|---|---|---|---|---|---|---|---|
| | | EPA | Waters | EPA | Waters | EPA | Waters |
| 1 | Aldicarb sulfoxide | 112 | 102 | 6.2 | 2.9 | 0.038 | 0.019 |
| 2 | Aldicarb sulfone | 92 | 96 | 9.5 | 6.9 | 0.033 | 0.041 |
| 3 | Oxamyl | 101 | 96 | 8.6 | 8.2 | 0.044 | 0.050 |
| 4 | Methomyl | 101 | 98 | 6.5 | 5.1 | 0.054 | 0.031 |
| 5 | 3-Hydroxycarbofuran | 105 | 100 | 6.8 | 3.0 | 0.038 | 0.022 |
| 6 | Aldicarb | 95 | 102 | 7.4 | 3.3 | 0.049 | 0.022 |
| 7 | Propoxur | 109 | 99 | 5.9 | 5.9 | 0.061 | 0.038 |
| 8 | Carbofuran | 112 | 94 | 6.7 | 4.6 | 0.050 | 0.028 |
| 9 | Carbaryl | 112 | 96 | 7.0 | 2.3 | 0.043 | 0.013 |
| 10 | 1-Naphthol | 113 | 105 | 12.6 | 8.2 | 0.115 | 0.053 |
| 11 | Methiocarb | 105 | 99 | 5.9 | 3.7 | 0.055 | 0.022 |
| 12 | BDMC | 108 | 100 | 4.3 | 5.2 | – | 0.031 |

## Sample

Preserved reagent water (spiked as described below)

## Conditions

EPA Method 531.2: Spike levels at 3× EDL for MDL, 7 replicates; Injection volume = 200 µL

Spike levels at 0.2 µg/L for all analytes (except BDMC surrogate = 2.0 µg/L) for Recovery and Precision, 7 replicates; Injection volume = 1000 µL

Waters Method: Spike levels at 0.2 µg/L for all analytes (including BDMC surrogate) for MDL, Recovery and Precision, 7 replicates; Injection volume = 1000 µL

Precision is defined as the standard deviation of the percent recovery.

**Tip:** Results shown are for a single operator/laboratory. Your results may vary, depending upon operating conditions. See the EPA method for acceptance criteria.

### Typical linearity and retention time reproducibility:

| Peak # | Analyte | Linearity ($R^2$)* | Retention Time (minutes)** | Retention Time Reproducibility (%RSD)** | RT Variability (SD in sec) |
|---|---|---|---|---|---|
| 1 | Aldicarb sulfoxide | 0.9999 | 3.77 | 0.20 | 0.48 |
| 2 | Aldicarb solfone | 0.9999 | 4.68 | 0.23 | 0.66 |
| 3 | Oxamyl | 0.9999 | 5.16 | 0.20 | 0.60 |
| 4 | Methomyl | 0.9999 | 6.04 | 0.16 | 0.54 |
| 5 | 3-Hydroxycarbofuran | 0.9999 | 9.83 | 0.07 | 0.42 |
| 6 | Aldicarb | 0.9999 | 11.45 | 0.06 | 0.42 |
| 7 | Propoxur | 0.9999 | 14.31 | 0.04 | 0.36 |
| 8 | Carbofuran | 0.9999 | 14.89 | 0.04 | 0.36 |
| 9 | Carbaryl | 0.9999 | 17.33 | 0.04 | 0.42 |
| 10 | 1-Naphthol | 0.9999 | 18.99 | 0.04 | 0.42 |
| 11 | Methiocarb | 0.9999 | 22.01 | 0.02 | 0.30 |
| 12 | BDMC | 0.9999 | 22.56 | 0.02 | 0.30 |

## Sample

Preserved reagent water

## Conditions

*Linearity: Peak area vs. ng on column. $R^2$ = correlation coefficient for least squares variance analysis. 400 µL injection volume, 2 to 20 ng of each analyte injected on column.

**Retention Time Study: Average of 3 replicates; 400 µL injection volume, 25 ppb analyte concentration.

**Tip:** Results shown are for a single operator/laboratory. Your results may vary, depending upon operating conditions.

# System shutdown: summary of parameters

This section describes shutdown procedures for the Alliance e2695 Separations Module, column heater, Reagent Managers, and degasser at the end of an automated run, so that they can be left unattended (e.g., overnight or over a weekend). The section includes a procedure for creating a Alliance e2695 Separations Module shutdown method.

### Tips

- If you are using Empower software to control instruments in your system, set Alliance e2695 Separations Module shutdown parameters in the Instrument Method Editor. See the Empower Help for more information.
- Empower control of the 2475 Fluorescence Detector requires specific hardware and software configurations. See the *Waters 2475 Fluorescence Detector Release Notes* and the *Empower System Installation and Configuration Guide* for details.

## Shutdown parameters for the Alliance e2695 Separations Module

This section describes how to create a new Alliance e2695 Separations Module shutdown method, and lists the parameters you need to program for the method. (You could also create a shutdown method by editing an existing method.) You enter shutdown parameters in six different screens, which are arranged in the order shown in the table titled "Separation method parameter screens:" on page 44. Each screen is described in the sections listed in the table titled "Separation method parameter screens:" on page 76.

### Separation method parameter screens:

| Screen name | Reference |
|---|---|
| Mobile Phase | "Set Mobile Phase screen parameter values" on page 44 |
| Gradient | "Set gradient table parameter values" on page 49 |
| Sample | "Set sample parameter values" on page 51 |
| Autosampler | "Set Autosampler parameter values" on page 52 |

**Separation method parameter screens: (Continued)**

| Screen name | Reference |
|---|---|
| Column | “Set Column parameter values” on page 80 |
| I/O | “Set I/O parameter values” on page 81 |

## Set Mobile Phase Screen parameter values

The Mobile Phase screen appears when you select the separation method to edit. Press the Next or Previous screen key to move among the six method parameter screens. The icon between the Next and Previous screen keys shows where you are in the sequence of the six screens. Press Exit to return to the Methods screen. A dialog box prompts you to save the changes to the separation method.

### To enter parameters in the Mobile Phase screen:

1. Press the Next or Prev screen key (if necessary) to open the Mobile Phase screen.
2. Enter values in the fields. The table titled “Mobile phase screen parameters:” on page 45 describes the fields and screen keys in the Mobile Phase screen.

### Alarms

The Alliance e2695 Separations Module maintains a log of all enabled errors that occur during operation. Each error can produce a variety of user-defined responses, as shown in the table titled “Alarm responses:” on page 48.

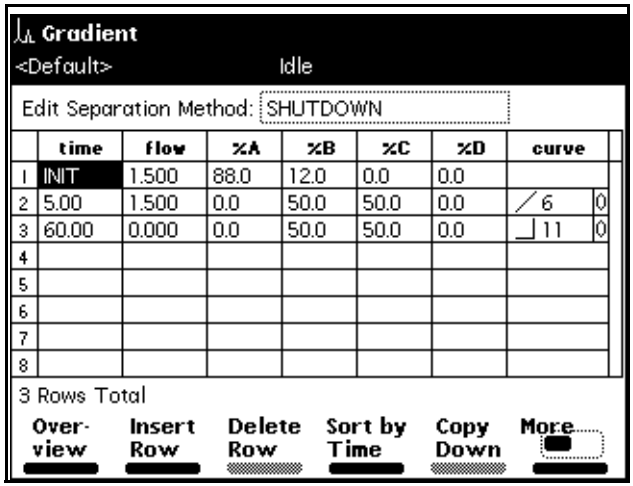
## Set Gradient table parameter values

The gradient table allows you to make time-based changes to the composition of the mobile phase during a run. You can program up to 25 lines in the gradient table.

### To set the parameters in the gradient table:

1. Press the Gradient screen key in the Mobile Phase screen to open the Gradient screen.

### Gradient screen:



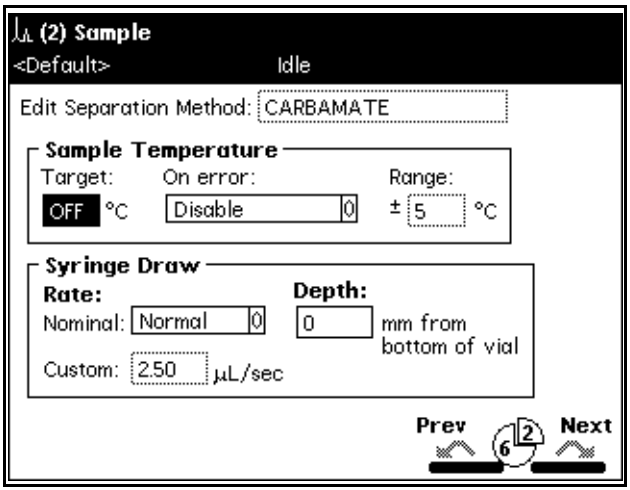

2. Enter values in the gradient table as shown in the figure above. The table titled “Gradient table parameters:” on page 50 describes the parameters in the gradient table.
3. Press Exit to save the gradient table and return to the Mobile Phase screen.

## Set Sample parameter values

### To enter parameters in the Sample screen:

1. Press the Next or Prev screen key (as appropriate) to open the Sample screen.

### Sample screen:



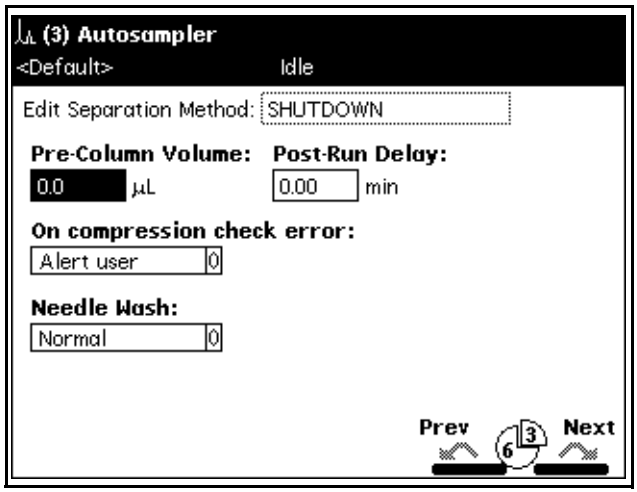

2. Enter or select values in the Sample screen parameter fields as shown in the figure above. The table titled “Sample parameters:” on page 52 describes the parameters in the Sample screen.

## Set Autosampler parameter values

### To enter parameters in the Autosampler screen:

1. Press the Next or Prev screen key (as appropriate) to open the Autosampler screen.

### Autosampler screen:



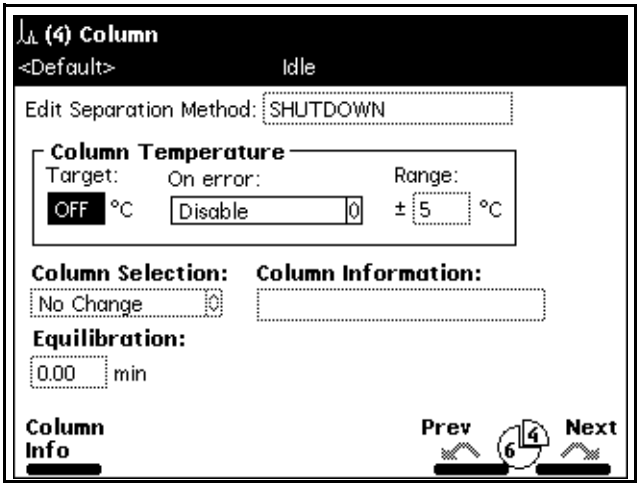

2. Enter values in the Autosampler screen as shown in the figure above. The table titled “Autosampler parameters:” on page 53 describes the parameters in the Autosampler screen.

## Set Column parameter values

### To enter parameters in the Column screen:

1. Press the Next or Prev screen key (as appropriate) to open the Column screen.

### Column screen:




2. Enter values in the Column screen as shown in the figure above. The table titled “Column parameters:” on page 55 describes the parameters in the Column screen.

## Set I/O parameter values

You set I/O parameter values when you want to use analog signals to notify other instruments of the status of the Alliance e2695 Separations Module. For example, you may want to:

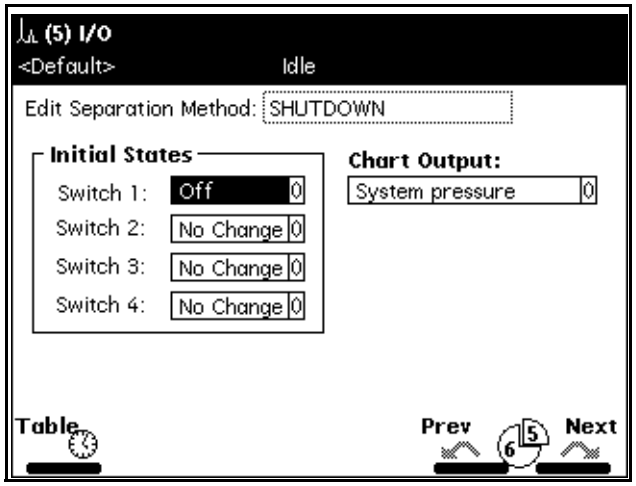
- Notify a detector that an injection has begun.
- Turn on a heating plate or a stirring bar in a solvent reservoir.
- Notify a third-party data system or integrator that an event has occurred.
- Activate a switching valve.
- Sound a buzzer.

You can also select the system parameter signal that is sent out through the Chart Out terminals.

### To enter parameters in the I/O screen:

1. Press the Next or Prev screen key to open the I/O screen.

### I/O screen:




2. Enter values in the I/O screen as shown in the figure above. The table titled "I/O parameters:" on page 56 describes the parameters in the I/O screen.

## Edit the I/O events table

The I/O Events table allows you to set the times for these events to occur during a run:

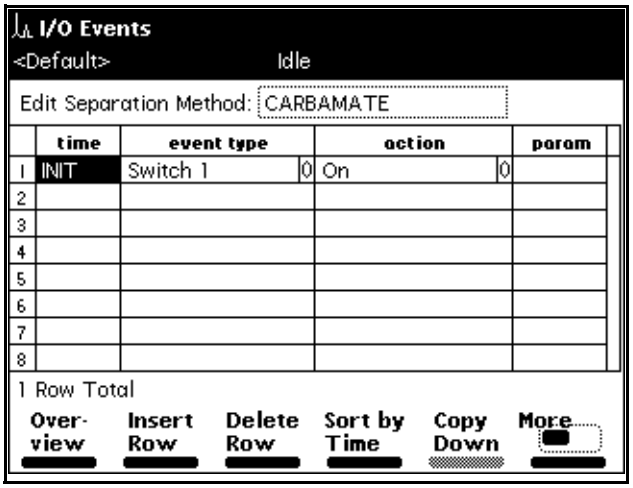
- Changing the state of event switches
- Changing the sparge rate
- Setting the column temperature
- Setting the sample compartment temperature
- Alerts

You can program up to 25 lines in the I/O Events table.

### To enter events in the I/O Events table:

1. Press the Table screen key in the I/O screen. The I/O Events table appears.

### I/O events table:




2. Enter values in the I/O Events table as shown in the figure above. The table titled "I/O parameters:" on page 56 describes the parameters in the I/O Events table. The table titled "I/O events table parameters:" on page 58 lists the parameters you can use in the action column in the I/O Events table.
3. Press Exit to return to the I/O screen.

## Detectors screen settings

There are no detector settings that apply to the carbamate shutdown method. Press Next on the Detectors screen to return to the Methods screen.

**Tip:** If you are using Empower software, the 2475 lamp can be turned off using the Lamp Off feature in the setup screen. The Temperature Control Module can be similarly disabled.

## Save the shutdown method

### To save the shutdown method:

1. From the Methods screen, press Exit. The Save Changes prompt appears.

2. Press the Yes screen key to save the method and return to the Methods screen.

## Activate the shutdown method

### To activate the shutdown method:

1. Press the Config screen key to open the Configuration screen.
2. Press the More screen key to display an additional set of screen keys.
3. Press the Auto-Shutdn screen key to open the Auto-Shutdown dialog box.
4. Select the shutdown method.
5. In the Shutdown After field, enter a time period (in minutes) after the last injection which you want the Alliance e2695 Separations Module to shut down. For the 30-minute carbamate analysis run time, Waters recommends entering a Shutdown After time of 60 minutes.
6. Press the OK screen key.

## Shutdown for the Post-Column reaction module

This section lists the Temperature Control Module parameter that you must set to use the PCRM shutdown method. See the *Waters Temperature Control System Operator's Guide* for more information.

- Post-Column Reaction Module Temperature: Off

**Tip:** Automated shutdown of the PCRM/TCM can be performed via the Ethernet by using Empower control. The PCRM/TCM cannot be shut down using the Alliance e2695 Separations Module.

## Shutdown for the model 2475 Detector

See the *Waters 2475 Multiwavelength Fluorescence Detector Operator's Guide* for information about shutting down the detector.

## Shutdown for the recorder, integrator, or data system

See the documentation supplied with the recorder, integrator, or data system for information about shutting down that instrument.

## Shutdown for the Reagent managers

See "Shutdown parameters for the Alliance e2695 Separations Module" on page 76, for information about shutting down the Reagent Managers.

# Shut down and store the system

## Between analyses

### Observe the following guidelines for shutting down the system between analyses:

1. During the course of a working day, between analyses, continue to deliver the initial mobile phase mixture through the column. This will maintain the equilibrium in the column necessary for good retention time reproducibility.
2. 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. Keep the inline vacuum degasser turned on. Make certain that Auto-Shutdown for your shutdown method is deactivated.
3. Allow the Reagent Manager pumps to continue to pump at 0.5 mL/min. Keep the detectors on and the Post-Column Reaction Module and column heater at operating temperature.

## Overnight or weekends

### Observe the following guidelines for shutting down the system overnight or for a weekend:

1. Use the shutdown procedures in "System shutdown: summary of parameters" on page 76. This will turn off the Post-Column Reaction Module, maintain flow to hasten the cool down of the reactor system, while also flushing the column with 50:50 v/v mixture of methanol:acetonitrile necessary to maintain the column bed in an active wetted state. Then turn off the two Reagent Manager pumps, the Alliance e2695 Separations Module flow, and the degasser.
2. If possible, turn off the detector lamp to lengthen lamp life, especially for the 2475 Detector.

3. The column heater can be left on overnight, but should be shut down over the weekend.

## Long-term (more than 72 hours)

### Observe the following guidelines for long-term shutdown or storage:

1. Follow the above steps for overnight or weekend shutdown.
2. After flushing the column and cooling it down to ambient temperature, disconnect the inlet and outlet tubes and join them together with a union (see Appendix B, for more information). Install end plugs in the column inlet and outlet fittings, and return the column carefully to its box for storage.

   **Tip:** Flushing the column will also leave methanol:acetonitrile in the fluid lines of the Alliance e2695 Separations Module, post-column reactor, and detector.

   **Tip:** It is desirable to flush high ionic buffers from the system and minimize solute precipitation.
3. Empty and clean both reagent reservoirs. Pump water through the Reagent Manager modules for 10 to 20 minutes at 1.5 mL/min, followed by isopropyl alcohol for another 10 to 20 minutes. Then turn the pumps off, leaving isopropyl alcohol in the fluid lines.

**Caution:**

- If any components of the system are to be used for another type of analysis, make certain that any liquids pumped initially through the system are miscible with methanol, water, methanol:acetonitrile, or isopropyl alcohol. Likewise, before starting up the system for carbamate analysis again, make certain that any residual material not miscible with the initial methanol:water mobile phase has been flushed thoroughly from the system with an appropriate intermediate solvent.
- Avoid pumping any compounds that contain amines.

# References

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*Injection HPLC with Post Column Derivatization*, EPA/600/4-85/054, USEPA, EMSL, Cincinnati, Ohio 45268 (1985).

(b) National Pesticide Survey, *Method 5. Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Ground Water by Direct Aqueous Injection HPLC with Post Column Derivatization*, USEPA (1987).

(c) *Method 531.1.Revision 3.1 Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization,* USEPA (1995).

(d) *Method 531.2.Revision 1.0 Measurement of N-Methylcarbamoyloximes and N-Methylcarbamates in Water by Direct Aqueous Injection HPLC with Post Column Derivatization,* USEPA (2001).

(e) Foerst, DL, and Moye, HA, *Aldicarb and Related Compounds in Drinking Water via Direct Aqueous Injection HPLC with Post Column Derivatization,* EPA/600/D-85/051, USEPA, EMSL, Cincinnati, Ohio 45268 (1985).

2. (a) Krause, RT, *Liquid Chromatographic Determination of N-Methylcarbamate Insecticides and Metabolites in Crops. I. Collaborative Study,* J. Assoc. Off. Anal. Chem., 68(4), 726-733 (1985).

   (b) Krause, RT, *Liquid Chromatographic Determination of N-Methylcarbamate Insecticides and Metabolites in Crops. II. Analytical Characteristics and Residue Findings,* J. Assoc. Off. Anal. Chem., 68(4), 734-741 (1985).

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   (b) Roth, M, *Fluorescence Reaction for Amino Acids,* Anal. Chem., 43(7), 880-882 (1971).

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   (b) Simons, SS, Jr, and Johnson, DF, *The Structure of the Fluorescent Adduct Formed in the Reaction of o-Phthalaldehyde and Thiols with Amines,* J. Am. Chem. Soc., 98(22), 7098-7099 (1976).

(c) Simons, SS, Jr, and Johnson, DF, *Reaction of o-Phthalaldehyde and Thiols with Primary Amines: Formation of 1-Alkyl (and aryl) thio-2-alkylisoindoles,* J. Org. Chem., 43(14), 2886-2891 (1978).

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6. Engelhardt, H, and Neue, U, *Reaction Detector with Three Dimensional Coiled Open Tubes in HPLC,* Chromatographia, 15(7), 403-408 (1982).
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9. Carson, WW, and Roe, JS, *Heat Exchanger for Refractometer,* U.S. Patent No. 4,284,352 (1981).
10. Wildman, B.J., *Investigation of the Analysis of Carbamate Pesticides in Drinking Water*, 30th Rocky Mountain Conference, August 1988, Denver CO.
11. (a) Ting, KC, Kho, PK, Musselman, AS, Root, GA, and Tichelaar, GR, *High Performance Liquid Chromatographic Method for Determination of Six N-Methylcarbamates in Vegetables and Fruits,* Bull. Environ. Contam. Toxicol., 33, 538-547 (1984).

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# 4 Maintenance and Troubleshooting

See the other manuals included with the system for more detailed information on maintaining and troubleshooting each system component.

**Contents:**



## Contact Waters technical service

Waters service specialists provide preventive and/or corrective maintenance. U.S. and Canadian customers should contact Waters Technical Service at 800 252-4752. Other customers should call their local Waters subsidiary, their local Waters Technical Service Representative, or Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

**Caution:**

- Before performing maintenance or service on any system component, make certain that all power switches are turned off, power cords are unplugged from outlets, and fluid lines have been removed from all reservoirs so that siphoning cannot occur.
- When you need to perform maintenance on an instrument in the Alliance System for Carbamate Analysis, see the cautions and safety notices in the instrument operator's guide. Be aware of high voltages, high temperatures, or any potentially harmful situation.

# Troubleshoot the system

## General considerations

There are troubleshooting guidelines and repair procedures in each manual supplied with the system. Refer to them for more information. This chapter considers primarily symptoms of the system as a whole and suggests corrective actions. Before attempting any repairs suggested below, U.S. and Canadian customers should contact Waters Technical Service at 800 252-4752. Other customers should call their local Waters subsidiary, their local Waters Technical Service Representative, or Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

- Check for the most obvious cause of a problem first. Consider the age of the system when making a diagnosis. A new seal or detector lamp is less likely to have failed than an old seal or lamp, respectively. Consider esoteric options only after all the easy explanations have been systematically eliminated.
- Check flow rate and system pressure.
- Check for the following symptoms which are discussed in the table titled "Chromatographic troubleshooting:" on page 93.
  - No peaks
  - Poor response or missing peaks, but good separation
  - Poor separation
- You can track and pinpoint problems if you record system pressure alongside the detector output with the data system.

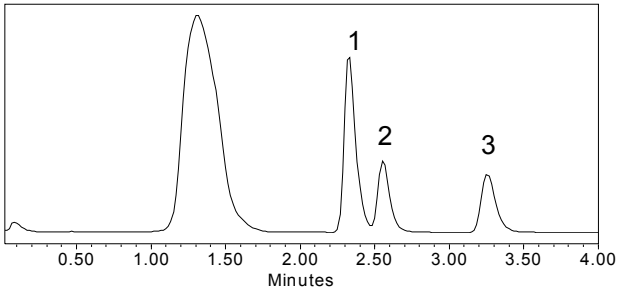
- Record and measure all results when the equipment is new or working properly so that you have a standard for comparison if something goes wrong. Measure column efficiency, record a typical pressure trace, learn to recognize normal baseline noise for a given detector attenuation and gain setting, etc. Then use this information when diagnosing a problem.
- A knowledge of the chemistry of the analysis is greatly beneficial when attempting to troubleshoot the system.

If you observe unsatisfactory analysis, check the system for obvious problems such as leaks or Reagent Managers not pumping. After this, you can perform the following check on the system and chemistry.

Inject 400 µL of a 25 ppb (25 µg/L) standard solution of carbaryl, 1-naphthol, and methiocarb prepared by appropriate dilutions of stock standard solutions using preserved water. Using an isocratic eluent of 40:30:30 water, methanol, acetonitrile, and standard operating conditions with a run time of 5 minutes, observe the resulting chromatogram. Compare the chromatogram with the chromatograms shown in the figure “Normal operation:” on page 92 to the figure “No OPA/ME reaction in the PCRM:” on page 92.

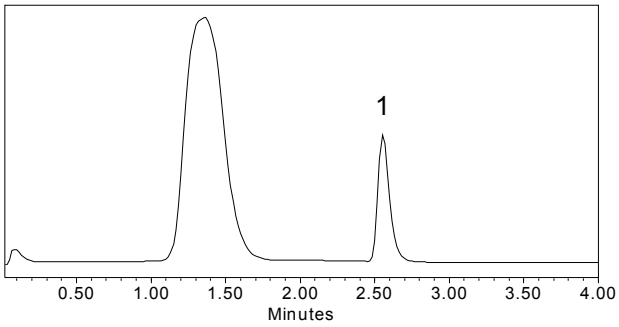
- If three peaks (see the figure “Normal operation:” on page 92) appear at the appropriate retention times with good peak height, the system is operating normally.
- If only the 1-naphthol peak appears (see the figure “No hydrolysis in the PCRM:” on page 92), then hydrolysis is not occurring. This indicates that the NaOH reagent has deteriorated, the Reagent Manager is not pumping the reagent, or the PCRM is not heating properly.
- If only the carbaryl and 1-naphthol peaks appear (see the figure “No OPA/ME reaction in the PCRM:” on page 92), then the OPA/ME reagent is not reacting with the effluent. This indicates that the OPA/ME reagent has deteriorated or the Reagent Manager is not pumping the reagent.
- If no peaks appear, troubleshoot the system using the suggestions in the table titled “Chromatographic troubleshooting:” on page 93.

### Normal operation:



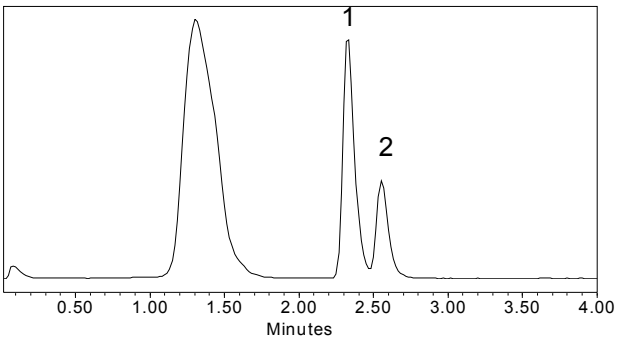

Sample: 400 µL of standard mixture in preserved water (25 µg/L each compound)
1. Carbaryl
2. 1-Naphthol
3. Methiocarb

### No hydrolysis in the PCRM:




Sample: 400 µL of standard mixture in preserved water (25 µg/L each compound)
1. 1-Naphthol

### No OPA/ME reaction in the PCRM:




Sample: 400 µL of standard mixture in preserved water (25 µg/L each compound)
1. Carbaryl
2. 1-Naphthol

### Chromatographic troubleshooting:

| Symptom | Possible cause | Corrective action |
|---|---|---|
| No peaks | Mobile phase not being delivered | Check system backpressure on the Alliance e2695 Separations Module screen to determine if the solvent manager is delivering mobile phase; reprime the Alliance e2695 Separations Module as necessary and remeasure flow rate.<br>Make certain solvents have been adequately degassed. Degas solvents for at least 5 minutes before starting. Then, keep the vacuum degasser turned on during use. |
| | One or both Reagent Managers not pumping | Visually inspect the LED display for flow rate and pressure for each Reagent Manager. Verify that all switches are on.<br>If the pump is running but not delivering reagent:<br>• Reprime the pump to clear possible air bubble by drawing reagent solution through priming valve with 10-mL gas-tight syringe, and then press the Prime key.<br>• Replace piston seals and seal backup washers, if necessary.<br>• Replace check valves, if necessary. |

### Chromatographic troubleshooting: (Continued)

| Symptom | Possible cause | Corrective action |
|---|---|---|
| No peaks (continued) | Detector not operating | Check the 2475 lamp for proper operation using built-in diagnostics (see the *2475 Multiwavelength Fluorescence Detector Operator's Guide*).<br>**Warning:** Do not remove cover from the detector cabinet. For lamp replacement or any other interior service procedures, contact your nearest authorized Waters field service engineer for help. |
| | Analytes not eluting from column | Remove the column from the system and replace it with a coupler union. Make an injection under initial conditions. If a large peak elutes, the problem is in the column/mobile phase chemistry and the rest of the system is functioning properly. If a large peak fails to elute, the problem is elsewhere in the system.<br>Injector not working or injection not made. Diagnose the problem and fix it. |
| | Leak in plumbing | Check all tubing, fittings and unions, especially to and from tees, hydrolysis coil, PCRM bulkhead unions, etc. Tighten if necessary. Replace any defective parts. |

### Chromatographic troubleshooting: (Continued)

| Symptom | Possible cause | Corrective action |
|---|---|---|
| Poor response (Chromatography is correct, but peak response is less than expected or peaks are missing.) | Insufficient temperature in RXN 1000 Coil for hydrolysis | Turn on the PCRM and check temperature. It should be 80 °C; a lower temperatures may not enable sufficient hydrolysis to occur. Fix heating element, if necessary. |
| | Improper addition order of post-column reagents | Verify that all the fluid connections from the reagent reservoirs to each RM and from each RM to the PCRM match those shown in the figure "Fluid line connections to the PCRM:" on page 29. Make certain that NaOH solution is being pumped by Reagent Manager #1 and that OPA/ME solution is being pumped by Reagent Manager #2. |
| | Deteriorated post-column reagents or standards | Prepare fresh reagent solutions daily according to protocol. OPA solution is good for about 24 hours at best, even if kept in the dark. After this, response falls off. NaOH deteriorates due to conversion to sodium carbonate by reaction with absorbed carbon dioxide.<br>Recalibrate system response every 8 hours to check for degradation of standards or reagents. See the Attentions in Chapter 3 for standard preparation and storage. |

### Chromatographic troubleshooting: (Continued)

| Symptom | Possible cause | Corrective action |
|---|---|---|
| Poor response (continued) | One post-column reagent is not pumping | If NaOH is not flowing, only the 1-naphthol peak is in the chromatogram (no hydrolysis of the carbamates occurred). If OPA/ME is not flowing, only carbaryl and 1-naphthol peaks appear (the carbaryl response appears reduced, since only the 1-naphthol produced during the hydrolysis of carbaryl is responding to the detector; no isoindole derivative is present under this peak envelope). Based on which peaks are missing, check the proper pump and reprime or fix as necessary.<br>If peak(s) are missing from the middle of a chromatogram accompanied by a downward baseline shift and reduced baseline noise, then the OPA/ME pump stopped briefly. Reprime the pump.<br>If peak(s) other than carbaryl or 1-naphthol are missing from the middle of a chromatogram, but there is no baseline shift and noise remains basically normal, then the NaOH pump stopped briefly. Reprime the pump. |

### Chromatographic troubleshooting: (Continued)

| Symptom | Possible cause | Corrective action |
|---|---|---|
| Poor response (continued) | Detector lamp may need replacement | Check the 2475 lamp for proper operation using built-in diagnostics (see the *2475 Multiwavelength Fluorescence Detector Operator's Guide*).<br><br>**Warning:** Do not remove cover from the detector cabinet. For lamp replacement or any other interior service procedures, contact your nearest authorized Waters field service engineer for help. |
| | Detector PMT may be wearing out or be receiving insufficient voltage | Check the power board for proper voltages - contact your nearest field service representative. Replace defective or worn components. |
| Poor separation<br>**Tip:** Run through at least one or more complete gradient cycles before making an injection to troubleshoot. | Column improperly equilibrated | Verify that the column was conditioned in 50:50 methanol:acetonitrile overnight before use and stored in this solvent between work periods. If not, flush column with this mixture and leave overnight. Then flush with initial mobile phase (88:12 water:methanol) until stable baseline and pressure traces are obtained; then cycle through gradient, return to initial conditions, and make another injection. |

### Chromatographic troubleshooting: (Continued)

| Symptom | Possible cause | Corrective action |
|---|---|---|
| Poor separation (continued) | Reduced column effluency<br>**Tip:** This will be apparent by broadly tailed peaks or double peaks though peak maxima positions are at the correct retention times. | Check the column efficiency. Replace with new column, if necessary.<br>Check the sample preparation procedure. Ensure the column is not contaminated by extraneous, strongly retained sample components. Use a Sentry Guard Cartridge and holder if necessary to prolong column life, or refine the sample preparation protocol to produce a cleaner extract or water sample, as necessary or if practical. |
| | Gradient not being formed properly<br>**Tip:** This is indicated by a change in relative peak positions, especially overlapping peaks among the first four test analytes. | Check proportioning valve in Alliance e2695 Separations Module for proper delivery by alternating valve pairs and measuring volume delivered per unit time (see the *Waters Alliance e2695 Separations Module Operator's Guide*). |
| | Wrong solvent in reservoirs | Check that each reservoir contains the correct solvent and is matched by letter to the proper column on the gradient table. |

**Chromatographic troubleshooting: (Continued)**

| Symptom | Possible cause | Corrective action |
|---|---|---|
| Poor separation (continued) | Unstable ambient temperature: peak positions shift during the course of day or night | Make certain column has been placed in column heater and that the heater temperature is set at 30 °C. If ambient temperature nears 30 °C, the heater may not maintain a uniform temperature. In this case, raise the heater temperature to at least 5 °C above ambient, or replace the heater with a heating/cooling combination bath to maintain the column at a constant 30 °C temperature.<br>**Tip:** Keep in mind that if the separation is run at a different temperature, peak retention times shift to different values and resolution of close peaks can change significantly. |

## Pump shutdowns

The table titled “Troubleshooting pump shutdowns:” on page 100 describes the likely cause and corrective action to take if you experience high-pressure pump shutdowns in your Reagent Managers. While infrequent, these shutdowns may occur within a few weeks to a few months of using your Carbamate System, and are typically a result of sodium carbonate precipitating in the reaction coil and stainless steel tubing in the heat exchanger and reactor outlet line. This precipitation can usually be avoided by preparing your NaOH solution using fresh reagents on a daily basis and flushing post column reagents from your system during extended shutdowns.

### Troubleshooting pump shutdowns:

| Symptom | Possible cause | Corrective action |
|---|---|---|
| High-pressure shutdown of the Reagent Manager pumps or the Alliance e2695 Separations Module.<br>System runs fine at ambient temperatures, but high-pressure shutdown occurs around 80 °C. | Sodium carbonate is precipitating in the reaction coil and stainless steel tubing in the heat exchanger and reactor outlet line.<br>**Tip:** Carbonate may be residual in the type of NaOH or in the water used to prepare the solution, or it may be formed over time from carbon dioxide absorbed from the atmosphere. | Isolate and test each fluid path element for abnormally high backpressure. The RXN-1000 coil is the element most likely to be affected. In addition, the heat exchanger and tubing may also be clogged.<br>Once you have isolated the clogged elements, do the following:<br>Replace all blocked elements.[1]<br>Prepare a fresh NaOH solution daily.[2] |

1. Do not attempt to dissolve the precipitate. Much like boiler scale, the sodium carbonate precipitate is very hard to dissolve. Wash procedures run the risk of contaminating the post-column reaction system.
2. Use caution when using sodium hydroxide pellets for the preparation of NaOH solutions. Waters recommends using ACS reagent grade pellets (with sodium carbonate levels below 1.0%). Other grades may contain a higher percentage of sodium carbonate. Be aware that when opened, atmospheric moisture leaks into the container and coats the pellets. This surface solution then readily absorbs $CO_2$, resulting in excessively high carbonate formation over time. When using commercially available NaOH solutions, look for 50% w/w concentrated solutions. Waters does *not* recommend purchasing NaOH in ampoule form or in previously prepared bottles.

## Maintain the reagent managers

Supplied with the reagent manager is a Piston Seal Kit. As a preventive maintenance procedure, it is important to change these parts in each pump every year of operation (or whenever a leak occurs). Reorder parts as necessary to continue this maintenance schedule.

If a pump, after reaching operating temperature and being set to the calibrated flow rate, has been properly primed and, and yet does not deliver

the correct flow rate, then suspect that it may be necessary to change the piston seal and backup washer.

**Caution:** It is important to replace the piston seal and backup washer immediately if a leak occurs, since reagent can pass into the mechanical housing of the pump and cause corrosion damage. A leak may not become visible outside the pump, especially at low flow rates, until after a lot of corrosive fluid has damaged the inner housing. This makes regular bimonthly seal replacement an important preventive maintenance operation. See the *Waters Reagent Manager Operator's Manual* for more information.

## Replacement parts required

Required Reagent Manager replacement parts include one Piston Seal Kit (part number 700000152) containing a piston seal, backup washer, diaphragm, O-ring, seal installation/removal tool, scouring pad, and instructions.

# A Safety Advisories

Waters® instruments and devices display hazard symbols that alert you to the hidden dangers associated with a product's operation and maintenance. The symbols also appear in product manuals where they accompany statements describing the hazards and advising how to avoid them. This appendix presents the safety symbols and statements that apply to all of the products that Waters offers.

**Contents:**

# Warning symbols

Warning symbols alert you to the risk of death, injury or seriously adverse physiological reactions associated with an instrument's use or misuse. Heed all warnings when you install, repair, or operate any Waters instrument or device. Waters accepts no liability in cases of injury or property damage resulting from the failure of individuals to comply with any safety precaution when installing, repairing, or operating any of its instruments or devices.

The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device, or a component of an instrument or device. When one of these symbols appear in a manual's narrative sections or procedures, an accompanying statement identifies the applicable risk and explains how to avoid it.

**Warning:** (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)

**Warning:** (Risk of burn injury from contacting hot surfaces.)

**Warning:** (Risk of electric shock.)

**Warning:** (Risk of fire.)

**Warning:** (Risk of sharp-point puncture injury.)

**Warning:** (Risk of hand crush injury.)

**Warning:** (Risk of injury caused by moving machinery.)

**Warning:** (Risk of exposure to ultraviolet radiation.)

**Warning:** (Risk of contacting corrosive substances.)

**Warning:** (Risk of exposure to a toxic substance.)

**Warning:** (Risk of personal exposure to laser radiation.)


**Warning:** (Risk of exposure to biological agents that can pose a serious health threat.)


**Warning:** (Risk of tipping.)


**Warning:** (Risk of explosion.)


**Warning:** (Risk of eye injury.)

## Specific warnings

The following warnings (both symbols and text) can appear in the user manuals of particular instruments and devices and on labels affixed to them or their component parts.

### Burst warning

This warning applies to Waters instruments and devices fitted with nonmetallic tubing.


**Warning:** To avoid injury from bursting, nonmetallic tubing, heed these precautions when working in the vicinity of such tubing when it is pressurized:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to incompatible compounds like tetrahydrofuran (THF) and nitric or sulfuric acids.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, significantly reducing the pressure at which the tubing can rupture.

## Biohazard warning

The following warning applies to Waters instruments and devices that can process material containing biohazards, which are substances that contain biological agents capable of producing harmful effects in humans.

**Warning:** To avoid infection with potentially infectious, human-sourced products, inactivated microorganisms, and other biological materials, assume that all biological fluids that you handle are infectious.

Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication, *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the biohazard safety representative for your organization regarding the proper use and handling of infectious substances.

## Biohazard and chemical hazard warning

This warning applies to Waters instruments and devices that can process biohazards, corrosive materials, or toxic materials.

**Warning:** To avoid personal contamination with biohazards, toxic materials, or corrosive materials, you must understand the hazards associated with their handling.

Guidelines prescribing the proper use and handling of such materials appear in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Disposal of Chemicals.*

Observe Good Laboratory Practice (GLP) at all times, particularly when working with hazardous materials, and consult the safety representative for your organization regarding its protocols for handling such materials.

# Caution advisory

Caution advisories appear where an instrument or device can be subject to use or misuse that can damage it or compromise a sample's integrity. The exclamation point symbol and its associated statement alert you to such risk.

**Caution:** To avoid damaging the instrument's case, do not clean it with abrasives or solvents.

# Warnings that apply to all Waters instruments and devices

When operating this device, follow standard quality-control procedures and the equipment guidelines in this section.

**Attention:** Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

**Important:** Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.

**Achtung:** Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.

**Avvertenza:** qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

**Atencion:** cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.

**注意：**未經有關法規認證部門允許對本設備進行的改變或修改,可能會使使用者喪失操作該設備的權利。

**注意：**未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

**주의:** 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

**注意：**規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザーとしての承認が無効になる可能性があります。

**Warning:** Use caution when working with any polymer tubing under pressure:

- Always wear eye protection when near pressurized polymer tubing.
- Extinguish all nearby flames.
- Do not use tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

**Attention:** Manipulez les tubes en polymère sous pression avec precaution:

- Portez systématiquement des lunettes de protection lorsque vous vous trouvez à proximité de tubes en polymère pressurisés.
- Eteignez toute flamme se trouvant à proximité de l'instrument.
- Evitez d'utiliser des tubes sévèrement déformés ou endommagés.
- Evitez d'utiliser des tubes non métalliques avec du tétrahydrofurane (THF) ou de l'acide sulfurique ou nitrique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

**Vorsicht:** Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:

- In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.
- Alle offenen Flammen in der Nähe löschen.
- Keine Schläuche verwenden, die stark geknickt oder überbeansprucht sind.
- Nichtmetallische Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.
- Durch Methylenchlorid und Dimethylsulfoxid können nichtmetallische Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.

**Attenzione:** fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamenti nei tubi non metallici, riducendo notevolmente la pressione di rottura dei tubi stessi.

**Advertencia:** se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Si hubiera alguna llama las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el cloruro de metileno y el sulfóxido de dimetilo dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

**警告：**當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致非金屬管線膨脹，大大降低管線的耐壓能力。

**警告：**当有压力的情况下使用管线时，小心注意以下几点：

- 当接近有压力的聚合物管线时一定要戴防护眼镜。
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚枫会导致非金属管线膨胀，大大降低管线的耐压能力。

**경고:** 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

**警告：**圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン(THF)や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

**Warning:** The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.

**Attention:** L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.

**Vorsicht:** Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwenddung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

**Attenzione:** si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

**Advertencia:** el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.

**警告：**使用者必須非常清楚如果設備不是按照製造廠商指定的方式使用，那麼該設備所提供的保護將被消弱。

**警告：使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用，那么该设备所提供的保护将被削弱。**

**경고:** 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

**警告：**ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

# Warnings that address the replacing of fuses

The following warnings pertain to instruments equipped with user-replaceable fuses.

### If the fuse types and ratings appear on the instrument:

**Warning:** To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.

**Attention:** pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boite à fusible de l'instrument.

**Vorsicht:** Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.

**Attenzione:** per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.

**Advertencia:** Para evitar incendios, sustituir los fusibles por aquellos del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.

**警告：**為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。

**警告：**为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。

**경고:** 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

**警告：**火災予防のために、ヒューズ交換では機器ヒューズカバー脇のパネルに記載されているタイプおよび定格のヒューズをご使用ください。

### If the fuse types and ratings do not appear on the instrument:

**Warning:** To protect against fire, replace fuses with those of the type and rating indicated in the “Replacing fuses” section of the Maintenance Procedures chapter.

**Attention:** pour éviter tout risque d'incendie, remplacez toujours les fusibles par d'autres du type et de la puissance indiqués dans la rubrique "Remplacement des fusibles" du chapitre traitant des procédures de maintenance.

**Vorsicht:** Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert im Abschnitt "Sicherungen ersetzen" des Kapitels "Wartungsverfahren" angegeben sind.

**Attenzione:** per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate nel paragrafo "Sostituzione dei fusibili" del capitolo "Procedure di manutenzione".

**Advertencia:** Para evitar incendios, sustituir los fusibles por aquellos del tipo y características indicados en la sección "Sustituir fusibles".

**警告：**為了避免火災，更換保險絲時，應使用「維護步驟」章節中「更換保險絲」所指定之相同類型與規格的保險絲。

**警告：**为了避免火灾，应更换“维护步骤”一章的“更换保险丝”一节中介绍的相同类型和规格的保险丝。

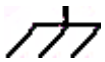
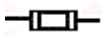
**경고:** 화재의 위험을 막으려면 유지관리 절차 단원의 “퓨즈 교체” 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

**警告：**火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

# Electrical and handling symbols

## Electrical symbols

The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument's front or rear panels.

| | |
|---|---|
|  | Electrical power on |
|  | Electrical power off |
|  | Standby |
|  | Direct current |
|  | Alternating current |
|  | Protective conductor terminal |
|  | Frame, or chassis, terminal |
|  | Fuse |

## Handling symbols

The following handling symbols and their associated statements can appear on labels affixed to the packaging in which instruments, devices, and component parts are shipped.

| | |
|---|---|
|  | Keep upright! |
|  | Keep dry! |
|  | Fragile! |
|  | Use no hooks! |

# B Column Care and Use

This appendix provides information on caring for and using the Waters® Carbamate Analysis Column.

**Contents**

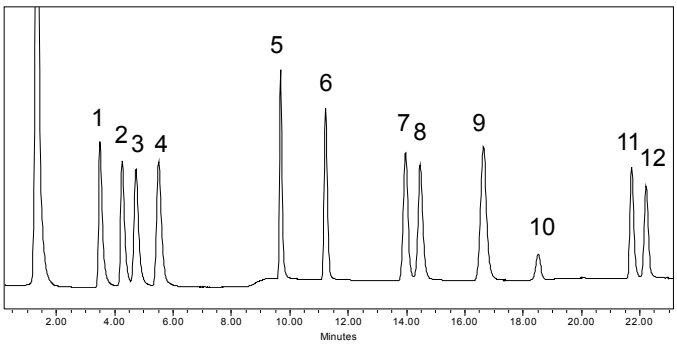


## Introduction

The Waters Carbamate Analysis Column (3.9 mm x 15 cm) is packed with a durable, high-efficiency, 4-micron, 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 the Waters Alliance® System for Carbamate Analysis 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 the figure "Typical separation of carbamate pesticides and related compounds using the Waters Alliance System for Carbamate Analysis:" on page 118.

### Typical separation of carbamate pesticides and related compounds using the Waters Alliance System for Carbamate Analysis:




Sample: 400 µL of standard mixture in preserved water (10 ng each compound)

1. Aldicarb Sulfoxide
2. Aldicarb Sulfone
3. Oxamyl
4. Methomyl
5. 3-Hydroxycarbofuran
6. Aldicarb
7. Propoxur
8. Carbofuran
9. Carbary
10. 1-Naphthol
11. Methiocarb
12. BDMC

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

**Tip:** 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.

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.

## Installation

### To attach 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.

   **Tip**: 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 1/4 to 1/2 turn.

   **Caution:** To avoid damaging the connection, do not overtighten the fittings.

   **Requirement:** Follow the next three steps if you must remove a damaged compression screw or worn ferrule.

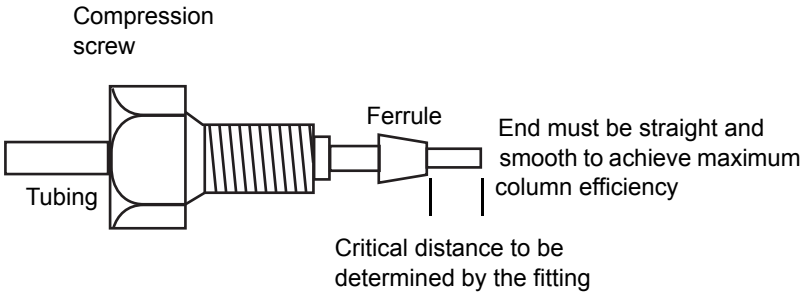
4. Using a miniature tubing cutter, scribe deeply the circumference of the tubing at the desired break point. Or, alternatively, using a three-cornered file with a cutting edge, cut 1/3 of the way through the tubing at the desired break point.

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 tube 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 the figure “Ferrule and compression screw assembly:” on page 120.

**Caution:** Make sure you fully seat the tubing in the fitting while tightening. Tubing not fully seated may result in dead volume that could cause excessive band spreading.

### Ferrule and compression screw assembly:




## Equilibrate the column

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

For equilibration, flush the column with the initial mobile phase used for the gradient analysis. This is 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). See "System setup: summary of parameters" on page 43, for details.

# Mobile phase and sample guidelines

## Solvent preparation and filtration

Observe the following guidelines for solvent preparation and filtration:

- Use only HPLC grade or better solvents suitable for high-sensitivity fluorescence analysis, filtered to remove microparticulate matter above 0.45 microns.

  **Tips:**

  - 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 that 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 treat water with a Milli-Q® or equivalent water purification system or use HPLC grade water. Deionized water is not acceptable because it contains organic compounds which may alter column selectivity.

- Use vacuum filtration, sonication, and/or helium sparging to remove dissolved gases which could affect your solvent delivery system. The Waters Solvent Clarification Kit is designed to assist in the degassing and preparation of mobile phases. The Waters Alliance System for Carbamate Analysis provides for continuous degassing of each mobile phase component.
- Use a Waters Inline Precolumn Filter to capture system particulates and extend column life.

## Sample preparation and filtration

Observe the following guidelines for sample preparation and filtration:

- Use a Waters Sample Clarification Kit or appropriate filters to filter samples and prevent excessive pressure buildup.
- Do not inject a sample that is dissolved in a solvent which is not miscible with the mobile phase.

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™ Precolumn module and Guard-Pak Cartridges or Waters Sentry™ Guard columns to remove the contaminants offline or online, respectively.

# Operation

## Chromatography guidelines

Observe the following guidelines for chromatography:

- 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, immediately 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.

  **Tip:** Before running the first analysis on your new column, perform the test sample separation given in "Efficiency testing" on page 123.

## Precautions

- **Pressure:** Maximum pressure should not exceed 28 MPa (4000 psi or 275 bars). Typical operating pressure in the Waters Alliance System for Carbamate Analysis is 10 to 20 MPa (1500 to 3000 psi or 100 to 200 bars).
- **Temperature:** Recommended column operating temperature range is 20 to 40 °C. The typical column operating temperature in the Waters Alliance System for Carbamate Analysis is 30 °C.
- **Flow rate:** There are no flow rate restrictions as long as the recommended pressure limits are not exceeded. Typical operating flow rate is 1.5 mL/min. Flow rate should be increased gradually (in 0.5-mL/min increments) to reach operating flow rate and decreased gradually to 0 upon system shutdown.
- **pH Range:** Maintain pH of mobile phase and samples between 3 and 8. Avoid using concentrated acids or bases.
- **Particulate contamination:** Filter all mobile phases. Never use turbid or cloudy solvents or solutions.

- **Shock:** Protect the 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 voids 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 backpressure, drastically.

## 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 sample 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 "System setup: summary of parameters" on page 43.

Choose a peak for one of the following analytes: aldicarb sulfoxide, aldicarb sulfone, oxamyl, or methomyl. Measure the column efficiency as shown in the figure "5-Sigma method for measuring column efficiency:" on page 124.

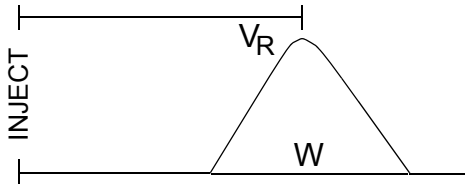
**Tips:**

- For convenience, VR and W can be expressed in units of length rather than volume, as measured with a scale directly from the chromatogram.
- The 5-Sigma method shown in the figure "5-Sigma method for measuring column efficiency:" on page 124 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. See "Troubleshooting the Carbamate Analysis column:" on page 124 and "Troubleshoot the system" on page 90.

### 5-Sigma method for measuring column efficiency:

N = Column efficiency (plates)
VR = Volume to peak apex (mL)
W = Volume at 4.4% of peak height (mL)




$$N = 25 \left( \frac{V_R}{W} \right)^2$$

# Care and maintenance

## Troubleshoot

The table titled "Troubleshooting the Carbamate Analysis column:" on page 124 provides the corrective action for some typical column problems that may occur with the Carbamate Analysis Column. Also see "Troubleshoot the system" on page 90.

**Tip:** 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 "Ordering information for columns and supplies" on page 127, for information.

### Troubleshooting the Carbamate Analysis column:

| Symptom | Conditions | Corrective action |
|---|---|---|
| Excessive pressure buildup | Filters plugged with particulate | Replace filter element or clean in an ultrasonic bath. Always filter solvents and samples. |
| | Sample precipitates on column (sample not soluble in mobile phase) | Slowly purge with a solvent appropriate to dissolve the precipitate. |
| | Clogged tubing | Replace tubing. |

### Troubleshooting the Carbamate Analysis column: (Continued)

| Symptom | Conditions | Corrective action |
|---|---|---|
| Loss of resolution, broad peaks, low plate counts | Mass overload | Dilute sample and run it again. |
| | Incorrect tubing size | Install 0.009-inch stainless steel tubing to column inlet and outlet. |
| | Contaminated column | Slowly flush with 50 to 100 mL of 50:50 v/v mixture of acetonitrile:MeOH, and then equilibrate with initial mobile phase and run sample again. |
| | Insufficient equilibration | Continue equilibration. |
| | Filters partially plugged | Replace or clean the Filter Retainer Disk and the Filter (both inlet and outlet). |
| | Sample injected in a solvent incompatible with or stronger than the mobile phase | Change sample solvent. |
| | Failing injector | Repair injector. |

## Shut down and store

### Between analyses

#### Observe the following guidelines for shutting down and storing carbamate analysis columns between analyses:

1. During the course of a working day, between analyses, continue to deliver the initial mobile phase mixture through the column. This will maintain the equilibrium in the column necessary for good retention time reproducibility.

2. 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 or weekends

### Observe the following guidelines for shutting down and storing carbamate analysis columns overnight or for a weekend:

1. Flush the column with 15 to 30 mL of 50:50 v/v mixture of acetonitrile:methanol.
2. Turn the flow rate to 0 mL/min and leave the column connected in the system.
3. Maintain the oven temperature at 30 °C, if desired.

## Long-term (more than 72 hours)

### Observe the following guidelines for long-term shutdown or storage of carbamate analysis columns:

1. Follow the steps for overnight or weekend shutdown above.
2. Flush the column with 15-30 mL of 50:50 v/v mixture of acetonitrile:methanol.
3. Turn the flow off and allow the column to cool to ambient temperature.
4. Disconnect the inlet and outlet tubes from the column and join them with a union.
5. Install the end plugs in the column inlet and outlet fittings.
6. Tighten the end plugs firmly in place with a 5/16-inch open end wrench.

**Caution:**

- To avoid damaging the connection, do not overtighten the fittings. To maintain chromatographic performance, do not allow the columns to dry out.
- To avoid sodium carbamate precipitating in the reaction coil and stainless steel tubing in the heat exchanger and reactor outlet line, flush post column reagents from your system during extended shutdowns.

7. Return the column to its box for storage.

# Ordering information for columns and supplies

### Ordering information for columns and column supplies:

| Item | Part number |
|---|---|
| Carbamate Analysis Column, 3.9 mm x 15 cm | WAT035577 |
| Filter Retainer | WAT088084 |
| Filter Retainer Disk | WAT089567 |
| Inline Precolumn Filter | WAT084560 |
| Solvent Clarification Kit with pump (110 V) | WAT085113 |
| Aqueous Sample Clarification Kit | WAT098697 |
| Organic Sample Clarification Kit | WAT026870 |
| Aqueous Replacement Filters, 0.45 µ, 47 mm, 100/pkg | WAT200538 |
| Aqueous Replacement Filters, 0.2 µ, 47 mm, 100/pkg | WAT200539 |
| Organic Replacement Filters, 0.2 µ, 47 mm, 100/pkg | WAT200535 |
| Guard-Pak Precolumn Module:<br>• Kit<br>• Nova-Pak® $C_{18}$ Guard-Pak Cartridges (10/pkg) | <br>WAT084550<br>WAT015220 |
| Sentry Guard Columns:<br>• Universal Guard Holder<br>• Nova-Pak $C_{18}$ Cartridges (2/pkg) | <br>WAT046910<br>WAT046830 |
| Sep-Pak $C_{18}$ Cartridges (50/box) | WAT051910 |

### Ordering information for columns and column supplies:

| Item | Part number |
|---|---|
| Sep-Pak $C_{18}$ Plus Cartridges (50/box) | WAT020515 |
| Oasis® HLB Extraction Cartridge (30/box) | WAT106202 |

## Order information

To order by phone, call 800 252-4752, press 1, and then press 1. To order by fax, call 508 482-4820 (U.S.A.) or 905 678-2350 (Canada).

Mail orders should be sent to:

Waters Corporation
Mail Stop CM
34 Maple St.
Milford, MA 01757

Online orders can be placed at:

c.shop.waters.com

## Service and applications assistance

Waters experienced service specialists provide maintenance assistance on both preventive and/or corrective levels. For complete information and assistance, call Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.).

### Warranty

For information about the liquid chromatography column warranty, see *Waters Licenses, Warranties, and Support*.