# Waters 600E Multisolvent Delivery System

Quick Start Guide





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## **Quick Summary**

The information below summarizes the information contained in this guide. Use the summary as a reference *after* you have read this entire document.

To make a run:

Step	Procedure
1.	Prepare the sample.
2.	<ul> <li>Set up the hardware:</li> <li>Add eluents to the system</li> <li>Purge the pump and reservoir lines</li> <li>Select the flow path</li> <li>Equilibrate the system</li> </ul>
	• Set pump (and detector) operating parameters
3.	<ul><li>Program a run:</li><li>Create a gradient table</li><li>Create a detector table</li><li>Create a method table</li></ul>
4.	<ul> <li>Make the run:</li> <li>Load the sample</li> <li>Select initial operating parameters</li> <li>Start the run</li> </ul>

#### **Documentation Conventions**

This guide uses the following conventions:

- When you are instructed to "press the X key", press the indicated keypad key.
- When you are instructed to "press the X screen key," press the keypad key directly below the key name displayed on the screen.

#### Notes, Attentions, and Cautions

Notes call out information that is important to the operator. For example:

Note: Record your result before you proceed to the next step.

• Attentions provide information about preventing possible damage to the system or equipment. For example:



**Attention:** To avoid damaging the detector flow cell, do not touch the flow cell window.

• Cautions provide information essential to the safety of the operator. For example:



*Caution:* To avoid chemical or electrical hazards, always observe safe laboratory practices when you operate your system.



**Caution:** To avoid electrical shock and possible injury, remove the power cord from the rear panel of the instrument before you perform the procedures in this section.

# Chapter 1 Introduction

This guide introduces you to the basic features of the Waters<sup>®</sup> 600E Multisolvent Delivery System and proceeds through the steps necessary for making a run.

This guide is intended for both novice and experienced chromatographers who are unfamiliar with the Waters 600E system.

The *Waters 600E Quick Start Guide* contains basic procedural information to help you set up the Waters 600E system and make a run.

**Note:** This guide is not designed to teach you chemistry and does not contain background or reference information. For information, see the Waters 600E Multisolvent Delivery System Installation and Maintenance Guide.

For additional information, or if you want to modify any of the steps in this guide, see the *Waters 600E Multisolvent Delivery System User's Guide*.

### **Before You Begin**

This guide assumes that:

- The system is properly installed (see the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*)
- You are using the Rheodyne 7725i manual injector to make injections
- You are using the 600 Controller in its default PowerLine<sup>™</sup> configuration

## **Documentation Conventions Used in this Guide**

This guide uses the following conventions:

• Special screen display type represents information or instructions displayed on the liquid crystal display (LCD).

For example, Enter Table Number (1-15)

- When instructed to "move the cursor," use the arrow keys or press the Enter key until the cursor is in the indicated field.
- When instructed to "press the X function key," press the key labeled "X" in the bottom row of keys on the front panel of the controller (Figure 1-1).
- When instructed to "press the X screen key," press the unlabeled key directly below the key name displayed on the screen.



Figure 1-1 Waters 600 Controller Front Panel

### **System Description**

The Waters 600E system is a high-performance liquid chromatography (HPLC) multisolvent delivery system. The 600E system is available in two configurations:

- 225-µL pump heads
- 100-µL pump heads

Consult Figure 1-2 as necessary to identify the components of the system when following the procedures in this guide.



Figure 1-2 Waters 600E Multisolvent Delivery System

# Chapter 2 Powering Up the System

To power up the system as a PowerLine controller:

- 1. If you are using any IEEE-488 PowerLine devices (detectors or integrators), power up these modules first.
- 2. Power up the 600E system by pressing the On/Off switch to the 1 (On) position. The On/Off switch is located in the corner of the front panel of the controller (see Figure 1-1).

Electrical connections for powering up are in the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*.

During powerup, the system automatically runs self-diagnostic tests and then displays the Powerup screen (Figure 2-1).

```
Waters
Multisolvent Delivery System
REVISION X.XX DATE: XX/XX/XX
POWER DIAGNOSTIC STATUS: OK
TYPE: W600E POWERLINE CONTROLLER
TO CONTINUE, PRESS ANY FUNCTION KEY
TO START EXTENDED DIAGNOSTICS,
PRESS DECIMAL POINT
Software Copyright 1993-1995
Waters Corporation
```

Figure 2-1 Powerup Screen

If all tests run successfully, the Powerup screen indicates that the diagnostic status is "OK."

If any tests fail, the Powerup screen displays an error message. See the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide* for information on error messages. See Chapter 2, Basic Operation, in the *Waters 600E Multisolvent Delivery System User's Guide* for additional information on powering up the system.

# Chapter 3 Preparing the System

Preparing the system involves:

- Verifying that PowerLine devices are active
- Adding eluents
- Flushing the system
- Equilibrating the system
- Setting operating parameters

## 3.1 Verifying That PowerLine Devices Are Active

Before you begin operating the Waters 600E system, take a few moments to check the system configuration. This involves verifying that the system recognizes PowerLine devices as follows:

- 1. Press the Setup function key to display the Pump Setup screen.
- 2. Press the System Config screen key to display the System Configure screen (Figure 3-1).

SYSTEM CONFIGURE		
UV #1	UV #2	DET #3
486 13	490E 12	410 21
	AUTOINJECTOR	
	717 3	
RETURN		RESCAN SYSTEM

Figure 3-1 System Configure Screen

- 3. Verify that the PowerLine modules installed in your system appear on the System Configure screen. See the *Waters 600E Multisolvent Delivery System User's Guide*, Section 4.2, Verifying that PowerLine Devices Are Active, for more information.
- 4. Press the Return screen key to exit the System Configure screen.

# 3.2 Adding Eluents

Use the procedures in the following sections when no eluent resides currently in the fluid path of the system.

If there is eluent in the system, see the *Waters 600E Multisolvent Delivery System User's Guide*, Section 3.6, Changing Eluents, for the procedure for changing eluents. Note that you must take special care when changing between buffered solutions and organic eluents, or between immiscible eluents.



**Caution:** Always observe safe laboratory practices when handling eluents. Know the chemical and physical properties of eluents. See the Materials Safety Data Sheets for the eluents in use.

Adding fresh eluent to the system involves:

- Sparging the reservoirs
- Priming the pump

## 3.2.1 Sparging the Reservoirs

**Caution:** Sparging certain eluents into the open air of a workspace may be harmful. Use eluent reservoir caps as described in the 600E Multisolvent Delivery System Installation and Maintenance Guide, Section 2.2.1, Setting Up the Eluent Reservoirs. Use an exhaust hood to remove vapor emitted from the reservoirs.

Use an initial sparge rate of 100 mL/min for a minimum of 15 minutes to degas the eluent(s) followed by a reduced rate (for example, 30 to 50 mL/min) to maintain the degassed condition. For additional information on sparging, see Appendix B, Eluent Considerations, and Chapter 3, Preparing Your 600E System for Operation, in the *Waters 600E Multisolvent Delivery System User's Guide*.

## **Preparing to Sparge**

- 1. Make sure sparge and eluent tubing are properly assembled and connected (see the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Chapter 2, Making Fluidic Connections to the Pump).
- 2. Ensure that the pump inlet manifold valve handle located on the front of the pump is in the Run position (see Figure 3-4). See the *Waters 600E Multisolvent Delivery System User's Guide*, Section 3.2, Priming the Pump.
- 3. Adjust the helium tank pressure to a setting between 345 KPa and 1035 KPa (50 to 150 psi).

### **Enabling Sparging**

To enable sparging for the reservoirs containing eluent:

1. Press the Setup function key. The Pump Setup screen appears (Figure 3-2).

```
      PUMP SETUP

      RESERVOIRS TO SPARGE:

      A: DISABLE B: DISABLE C: DISABLE D: DISABLE

      PUMP COL. TEMP. HEATER LIMIT: 0 C

      PRESS LIMITS: LOW: 0

      HIGH: 6000 PSI

      CHART OUT: %A

      FLOW FACTOR: 1.000

      AUTOSTART:IN
      HRN STEP VIAL

      NEXT
      SYSTEM
      PUMP
      LOCK

      SETUP
      CONFIG
      KEYBOARD
      HELP
```



- 2. Move the cursor to the Reservoir to Sparge field for the first reservoir that contains eluent (A, B, C, or D).
- 3. Type 1 and press Enter. This enables the reservoir for sparging. Gas does *not* flow when you enable the reservoir.
- 4. Repeat steps 2 and 3 for the remaining active reservoirs.

### Setting the Sparge Rate

1. Press the Direct function key. The Direct Control screen appears (Figure 3-3).

```
DIRECT CONTROL RUN TIME = 1.0 MIN

AUTOINJECTOR FLOW: 0.00 ML/MN

VIAL: MANL CURRENT NEW

INJ VOL: 0 %A: 100

DETECTOR #1 %B: 0

AUFS: 2.0 %C: 0

\lambda: 254 %D: 0

DETECTOR #2

AUFS: 0.1 SPARGE: OFF ML/MN

\lambda: 280 TEMP: C SET: OFF C

DETECTOR #3 PRESS: 0 PSI

SENS 1024 SWITCHES: (0=0FF 1=0N)

S1: 0 S2: 0 S3: 0 S4: 0

STOP

FLOW MORE HELP
```

Figure 3-3 Direct Control Screen

- 2. Move the cursor to the Sparge field.
- 3. Type 100 and press Enter to set an initial flow rate of 100 mL/min for the sparge gas. Gas flow begins in all enabled reservoirs.

Flow helium at 100 mL/min for a minimum of 15 minutes. Then repeat steps 1 to 3, but this time reduce flow to:

- 10 to 15 mL/min for aqueous mobile phases
- 20 to 30 mL/min for aqueous-acetonitrile mobile phases
- 50 mL/min for aqueous-methanol mobile phases

If helium is not available or is undesirable for sparging, use another degassing method. See the *Waters 600E Multisolvent Delivery System User's Guide*, Section B.6.2, Eluent Degassing Methods, for more information.

## 3.2.2 Priming the Pump

Priming is necessary to ensure proper pump operation. Priming involves:

- Venting the eluent
- Initiating flow
- Purging pumps and lines



**Caution:** Always observe safe laboratory practices when handling eluents. Know the chemical and physical properties of eluents. See the Materials Safety Data Sheets for the eluents in use.

#### Considerations

Consider the following when priming the 600E pump:

- The optional 225-µL heads usually do not require priming once eluent has been introduced to the draw-off valve. With some eluents, the standard 100-µL heads may require additional priming after you introduce eluent into the valve.
- You must prime with a suitable syringe, such as the one available from Waters. During purging, do not apply excessive force to the syringe by injecting eluent more quickly than the pumps can accept.

#### **Initiating Flow**

- 1. Rotate the inlet manifold valve handle located on the front of the pump to the Run position (Figure 3-4).
- 2. Place a waste beaker under the vent tube of the reference valve to collect the diverted eluent.
- 3. Open the reference valve (Figure 3-4) by rotating the handle from left to right to isolate the column from the flow path.



Figure 3-4 Pump Inlet Manifold Valve Positions

4. Press the Direct function key and the Direct Control screen appears. At the Flow field, type a flow rate of 1.0 mL/min (the gradient proportioning valve is not actuated at 0 mL/min).

#### **Drawing Off Eluent**

Once you have initiated flow, draw off eluent as follows:

- 1. Move the cursor to the %A field for the first eluent and type 100 in the New column. Press Enter.
- 2. Type 0 and press Enter for the remaining eluent composition fields.
- 3. Attach the priming syringe to the Luer<sup>™</sup> fitting on the inlet manifold valve (Figure 3-5).
- 4. Rotate the inlet manifold valve handle fully counterclockwise to the Draw position (see Figure 3-4). Draw off 10 mL of eluent, rotate the handle to the Run position, remove the syringe and discard the eluent properly.



Figure 3-5 Drawing Off Eluent

5. Repeat steps 1 through 4 for each of the remaining reservoirs that you intend to use.

#### Priming

1. Attach the priming syringe to the Luer fitting on the inlet manifold valve (Figure 3-5).

- 2. Move the cursor to the %A field for the first eluent and type 100. Press Enter. Type 0 and press Enter for the remaining % composition fields.
- 3. Move the cursor to the Flow field and press 10. Press Enter. This sets the flow rate to 10 mL/min for eluent reservoir A.



**Attention:** Confirm that the reference valve is open (i.e., injector and column bypassed) when selecting high flow rates or when making rapid changes in flow rate. Sudden flow and backpressure variations or high flow rates may damage installed columns.

- 4. Rotate the inlet manifold valve handle fully counterclockwise to the Draw position (see Figure 3-4). Draw off some eluent, rotate the valve handle to the Inj position and apply slight pressure to force eluent through the heads. Rotate the valve handle to the Run position and observe the flow. If flow is not constant repeat this step. When flow is constant repeat this procedure for other reservoirs that you intend to use.
- 5. When the pump has been primed using all applicable reservoirs, press the Stop flow screen key to stop the pump.

# 3.3 Flushing the System

Flushing the system ensures that all eluent in the fluid path is replaced with new eluent before running samples.



**Caution:** Be careful when venting eluents from a pressurized system. Follow good laboratory practices including the wearing of eye protection.

Flushing involves:

- Flushing system fluid lines
- Flushing the manual injector
- Connecting the column after flushing

#### **Before You Begin**

Before flushing the system:

1. Filter and sparge the fresh eluent. See the *Waters 600E Multisolvent Delivery System User's Guide*, Appendix B, Eluent Considerations, and Section 3.1, Sparging Eluents.

- 2. Prime the pump. See Section 3.2.2, Priming the Pump.
- 3. Open the slide-out drawer and install a union in place of the column during system flush.

## 3.3.1 Flushing the Rheodyne 7725i Manual Injector



**Caution:** Always observe safe laboratory practices when handling eluents. Know the chemical and physical properties of eluents. See the Material Safety Data Sheets for the eluents in use.

To flush the Rheodyne 7725i manual injector:

- 1. Set the injector handle to the Inject position. Setting the handle in the Inject position inserts the sample loop in the flow path.
- 2. Start flow of the flushing eluent at 1.0 mL/min. Flow for 2 to 3 minutes.
- 3. Fill a syringe (fitted with a 22-gauge, 90° point needle) with the new or intermediate eluent.
- 4. Set the injector handle to the Load position (Figure 3-6).





- 5. Fully insert the syringe needle into the injector port.
- 6. *Gently* discharge the contents of the syringe to completely fill the sample loop and expel any old or intermediate eluent from the injector vent tube. This step replaces the eluent in the sample loop with fresh eluent.
- 7. Leave the syringe in position and turn the injector handle to the Inject position.

- 8. Remove the syringe.
- 9. Allow the eluent to flow for several minutes at 1.0 mL/min.

## 3.3.2 Reconnecting the Column After Flushing the System

Perform the following procedure if the column has been replaced with a union, and the eluent inlet tubing, gradient proportioning valve, pump, injector, and detector flow cells are filled with new eluent.

To reconnect the column:

- 1. Press the Stop Flow screen key to stop the pump.
- 2. Remove the union and reinstall the column. See the column *Care and Use Manual* for column operating guidelines.
- 3. Continue with Section 3.4, Equilibrating the System, to prepare the system for samples.

## 3.4 Equilibrating the System

To equilibrate the system, set the following initial parameters on the Direct Control screen (see Figure 3-3):

- Sparge rate
- Eluent composition
- Flow rate
- AUFS
- Sensitivity

#### **Good Operating Practices**

When you equilibrate your system:

- Maintain sufficient eluent in the eluent reservoirs to ensure that the reservoirs do not run dry while operating. Running the pump without eluent adversely affects seal life.
- Equilibrate both the eluent and the system at ambient temperature before pump operation. Cold eluents that run through a system at room temperature outgas as they warm up.
- If you are operating in a cold room, equilibrate the system at the reduced temperature.

To equilibrate the system:

1. Press the Direct function key to access the Direct Control screen (see Figure 3-3).

#### **Setting Sparge Conditions**

- 2. If desired, set the sparge conditions as follows:
  - Move the cursor to the Sparge field.
  - Type in the maintenance flow rate for your eluents. Press Enter.

#### **Setting Eluent Composition**

- 3. Set the initial eluent composition as follows:
  - Move the cursor to the New %A field.
  - Type the desired % composition for the mobile phase (0 to 100, no decimals, in 1% increments) from reservoir A. Press Enter.
  - Repeat the previous two actions for each of the remaining three reservoirs. The sum of the four % composition values must equal 100.
  - After you type the value for eluent D, press Enter. The Current column displays the new composition.

#### Setting Initial Flow Rate

4. Set the initial flow rate as follows:



Attention: Be sure that the values for the maximum and minimum operating pressures programmed on the Pump Setup screen are appropriate for your column. See the Waters 600E Multisolvent Delivery System User's Guide, Section 4.3.2, Setting Pump Setup Parameters. Also, see the Care and Use Manual for your column.

**Note:** Some columns require a flow ramp-up. See the column Care and Use Manual for guidelines.

- Move the cursor to the Flow field.
- Type in the desired flow-rate value. Press Enter.

The pump immediately begins operating at the new flow and eluent composition conditions.

#### **Setting Detector Parameters**

- 5. Set the PowerLine detector parameters as necessary:
  - Move the cursor to the appropriate detector column.

- For a PowerLine UV/Vis absorbance detector:
- At the l field, type the required detector operating wavelength. Press Enter. Valid entries are 190 to 600 nm.
- At the AUFS field, type the required absorbance units full-scale (AUFS) value. Press Enter. Valid entries are 0.001 to 2.00 AU.
- For a PowerLine refractive index (RI) detector (Waters 410), at the Sens field, type the required sensitivity. Press Enter. Valid entries are 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024.
- For a PowerLine conductivity detector (Waters 431/432), at the Sens field, type the required sensitivity in microSiemens ( $\mu$ S). Press Enter. Valid values are 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, and 0.0005.

#### **Equilibration Period**

- 6. Allow a minimum of five to six column volumes of eluent to pass through the system before checking the stability of the baseline.
- 7. Observe the Press (pressure) value on the Direct Control screen. The pressure fluctuations should be within 5 to 10 percent of the average pressure value.

If the pressure fluctuates greatly, or every other pump stroke produces a significant pressure drop, purge the pump of air by repeating the procedures in Section 3.2.2, Priming the Pump, and Section 3.3, Flushing the System. If this does not correct the problem, see the *Waters 600E Multisolvent Delivery System Installation and Maintenance Guide*, Chapter 5, Troubleshooting, for additional help.

## 3.5 Setting Operating Parameters

Set operating parameters for the pump and any PowerLine Detectors present in your system. These parameters include:

- High and low operating-pressure limits
- Autozero
- Polarity

#### **Setting Pressure Limits**

To set high- and low-pressure limits:

- 1. Press the Set Up function key to display the Pump Setup screen (see Figure 3-2).
- 2. Enter the operating pressure limits for the system.

- Move the cursor to the Low field.
- Type a low-pressure limit value between 0 and 5950 psi (0 and 416.5 kg/cm2). Press Enter.
- Type a high-pressure limit value between 51 and 6000 psi (3.6 and 420 kg/cm2). Press Enter.

#### **Enabling and Disabling Autozero**

To enable and disable the Autozero feature:

- 1. From the Pump Setup screen, press the Next Setup screen key to display the AutoInjector Setup screen.
- 2. Press the Next Setup screen key to display the Detector Setup screen (Figure 3-7).

DETECTOR SETUP				
DET#1 DATA CHAN: FILTER CONST: AUTOZERO: POLARITY:	486 6 1.0 ON POS	DET #3 DATA CHAN: FILTER: POLARITY: BALANCE: 0	431 3 STD POS	
DET#2 DATA CHAN: FILTER CONST: AUTOZERO: POLARITY:	490E 8 1.0 ON POS			
NEXT SETUP				HELP

Figure 3-7 Detector Setup Screen

- 3. Move the cursor to the Autozero field of the appropriate detector.
- 4. Type 1 to enable or 0 to disable the Autozero feature. Press Enter.

#### **Selecting Output Polarity**

To select the polarity of the detector 10-mV output:

- 1. Move the cursor to the Polarity field of the appropriate detector.
- 2. Type 1 for positive or 0 for negative polarity. Press Enter.

# Chapter 4 Programming a Run

Programming a run involves setting up:

- Gradient table
- Event table
- Detector table (if you are using a PowerLine detector)
- Method table

For simplicity, an Event table will not be programmed in this section. For more information on programming tables, see the *Waters 600E Multisolvent Delivery System User's Guide*, Chapter 6, Creating PowerLine Methods.

# 4.1 Setting Up the Gradient Table

#### Accessing the Program Gradient Screen

Press the Program Table function key. The Program Gradient screen appears (Figure 4-1).

P

Figure 4-1 Program Gradient Screen

#### **Setting Parameters**

To set parameters on the Program Gradient screen:

- 1. Move the cursor to the Table # field. Type the desired gradient table number (1 to 15). Press Enter.
- 2. If a table is already stored under this number, the stored table now appears on the screen. You can overwrite or edit this table, or enter another table number.
- 3. Move the cursor to the first line of the gradient table. See Figure 4-3 for an example of a gradient table.
- 4. Move the cursor to the Flow field. Type the initial flow rate. Press Enter.
- 5. At the %A field, type the % composition for eluent A. Press Enter.
- 6. At the remaining % composition fields, type the % composition for each eluent. Press Enter after each value.
- 7. At the next line in the Time field, type the time after injection at which the gradient segment is to start. Press Enter.
- 8. Repeat steps 4 through 6 to enter the eluent conditions for that segment.
- 9. At the Curve field, type the number of the curve profile (Figure 4-2) for the gradient segment and press Enter.



Figure 4-2 Gradient Curve Profiles

10. For each additional line in the table, type in the appropriate values for each column.

#### Example

Figure 4-3 is an example of an Auto•Blend<sup>™</sup> gradient table for an analysis with a run time of 30 minutes. This gradient table includes a five-minute segment at the end of the run that returns the eluent composition to initial conditions. The table also contains a segment that

reduces the flow rate to 0.0 mL/min if an injection is not made within 50 minutes of the previous injection.

Time (min)	Flow (mL/min)	%A	%В	%C	%D	Curve
Initial	1.00	10	10	0	80	*
30.00	1.00	10	10	80	0	6
35.00	1.00	10	10	0	80	6
50.00	0.00	10	10	0	80	11

#### Saving the Table

To save the Gradient table:

1. Press the Save screen key. The screen displays:

Enter Table Number (1-15):

2. Type the number under which you want to save the table. Press Enter. The screen displays:

```
Replace with New Table? 1 = Yes 0 = No
```

3. If you entered a table number that is already stored in memory, that preexisting table appears on the screen. To overwrite the preexisting table, type 1. This erases the contents of the preexisting table and displays the new table with the assigned table number.

If you do not want to overwrite the preexisting table, type 0. The screen displays the newly created table again.

- 4. Repeat steps 1 through 3, assigning a different table number to the table until you locate a table that can be replaced with the new table.
- 5. If you entered a table number that is not already stored in memory, press 1 to save the new table under that number.

## 4.2 Setting Up the Detector Table

Skip this section if you are not using a PowerLine Detector in your 600E system.

#### Accessing the Detector Tables Screen

- 1. Press the Program Table function key to display the Program Gradient screen (see Figure 4-1).
- 2. Press the Next Table screen key to display the Program Event screen.
- 3. Press the Next Table screen key to display the Detector Tables screen (Figure 4-4).

DETECTOR	TABLES			Г	ABLE	<b>#:</b> 1	
410	SENS:	1024	431	SENS:	002		
TIME INITIAL INITIAL 3.10 4.62		DET/CHAN 1 2 1 1	λ 254 254 240 245			AUFS 1 .05 .005 .01	
NEXT TABLE	CLEAF LINF	R 5	CLEAR TABLE		SAVE		HELP

Figure 4-4 Detector Tables Screen

#### **Setting Parameters**

To set parameters on the Detector Tables screen:

- 1. Move the cursor to the Table # field. Type the desired gradient table number (1 to 15). Press Enter.
- 2. If a table is already stored under this number, the table now appears on the screen. Overwrite or edit this table, or enter another table number.
- 3. If you are using a RI detector:
  - a. Move the cursor to the 410 Sens field.
  - b. Type the required operating sensitivity for the RI detector and press Enter. Valid values are: 1, 2, 4, 8, 16, 32, 64, 128, 256, 512, and 1024.
- 4. If you are using a Waters 431 or 432 conductivity detector:
  - a. Move the cursor to the 431 Sens field.
  - b. Type the required conductivity sensitivity in microsiemens (μS) for the conductivity detector and press Enter. Valid values are 0.1, 0.05, 0.02, 0.01, 0.005, 0.002, 0.001, and 0.0005.

- 5. If you are using a UV/Vis detector:
  - a. Move the cursor to the Time field.
  - b. Type the time at which you wish the parameter values for l and/or AUFS to change. Press Enter.
  - c. At the Det/Chan field, type 1 for Det #1 or 2 for Det #2. Press Enter.
  - d. At the l field, type the wavelength in nm. Press Enter.
  - e. At the AUFS field, type the absorbance units full-scale (AUFS). Press Enter.
- 6. Repeat step 5 for each additional line that you need in the table.

#### Example

Figure 4-4 illustrates changing the wavelength for Det #1 12 minutes into the run.

Time	Det/Chan	Wavelength	AUFS
Initial	1	254	1
12.00	1	280	1

Figure 4-5 Changing the Detector 1 Wavelength

#### Saving the Table

To save the Detector table:

1. Press the Save screen key. The screen displays:

```
Enter Table Number (1-15)
```

2. Type the number under which you want to save the table and press Enter. The screen displays:

Replace with New Table? 1 = Yes 0 = No

- 3. If you entered a table number that is already stored in memory, that preexisting table appears on the screen. To overwrite that preexisting table, type 1. This erases the contents of the preexisting table and replaces them with the new table.
- 4. If you do not want to overwrite the existing table, type 0. The screen displays the newly created table again.
- 5. Repeat steps 1 and 2, assigning a different table number to the newly created table until you locate a table that can be replaced with the new table.

6. If you entered a table number that is not already stored in memory, press 1 to save the new table under that number.

## 4.3 Programming the Method Table

After you create the Gradient and Detector tables, link them together to create a method using the Method Table.

#### Accessing the Program Method Screen

Press the Program Method function key. The Program Methods screen appears (Figure 4-6).

PROGRA	M METHODS						
 STEP	FIRST	LAST	# TN T	INJ	RUN	TABLES	
1	1	1	1	15	1.0	# 1	
End of	Table						
	THEFT						
LINE	LINE		SAVE	М	IORE	HELP	
CLEAR							
TABLE	RECALL	SAV	/E	MORE	]	HELP	

Figure 4-6 Program Methods Screen

#### **Setting Parameters**

To set parameters on the Program methods screen:

- 1. Move the cursor to the First Vial field. Type 100 and press Enter. Vial code 100 indicates that the injection will be made using the manual injector or a non-PowerLine autoinjector.
- 2. At the # Inj field, type the number of injections (1 to 9). Press Enter.

- 3. At the Inj Vol field, type the injection volume in  $\mu$ L. Press Enter. (This field is for documentation purposes only.)
- 4. At the Run Time field, type the analysis run time in minutes. Press Enter.
- 5. At the Tables # field, type the table number for the set of tables (Gradient and Detector) for this step. Press Enter.
- 6. For each additional line (step) in the Method table, repeat steps 1 through 5.

#### Example

Figure 4-7 is an example of a Method table. The table illustrates a method that specifies five injections of a sample with a Rheodyne Injector, using Gradient and Detector tables #1.

Step	First Vial	Last Vial	# Inj	lnj Vol (μL)	Run Time (min)	Table #
1	100		5	500	30.00	1

Figure 4-7 Intelliou Table Example	Figure 4-7	Method	Table	Example
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#### Saving the Table

To save a Method table, press the Save screen key from the Program Methods screen.

# Chapter 5 Performing a Run

To run a sample, you must:

- Load the sample into the injector
- Set initial operating parameters
- Start the run

# 5.1 Loading the Sample



**Caution:** Always follow safe laboratory practices when handling eluents. Know the physical and chemical properties of the eluents. See the Material Safety Data Sheets for the eluents in use.



**Caution:** When using sample loops larger than  $100-\mu$ L, protect yourself from the rapid ejection of mobile phase from the needle port when you turn the valve from Inject to Load. A 1-mL loop expands by  $20-\mu$ L upon decompression from 2900 psi (200 bar).



**Attention:** Be sure to use the proper size of syringe needle to avoid damage to the valve. Proper needle dimensions are 0.028 inches x 2 inches (0.071 cm x 5.08 cm) OD, without electro taper. The point must be 90° (square).

To load the sample into the 7725i Rheodyne Manual Injector:

- 1. Turn the handle on the injector to the Load position.
- 2. Load a syringe with a volume of sample approximately five times the loop volume.
- 3. Fully insert the 22-gauge, 90°-point syringe needle into the filler port.
- 4. Gently discharge the contents of the syringe to completely fill the loop.

#### Accessing the Initial Operate Method Screen

Press the Operate Method function key. The initial Operate Method screen appears (Figure 5-1).

```
OPERATE METHOD
BEGIN METHOD AT
STEP #: 1
VIAL #: 1
INJ #: 1
KEYBOARD LOCK: YES
FLOW %A %B %C %D
CURRENT: 1.50 100 0 0 0
NEW: 2.00 90 10 0 0
Press OPERATE METHOD to go to the
New Conditions
```

Figure 5-1 Initial Operate Method Screen

#### **Setting Initial Operating Parameters**

Make the following changes only if you do not want to begin the run using the current settings.

- To select a different step in the method, move the cursor to the Step # field, type the new number, and press Enter.
- To change the number of the injection at which to start, move the cursor to the Inj # field, type the new value, and press Enter.

#### Accessing the Operate Methods Screen

Press the Operate Method function key again. The Operate Methods screen appears (Figure 5-2).

OPERATE	METHODS	RUI	I TIME	:	0.0	00	
AUTOINJE	CTOR	GRA	DIENT	TIME:	0.0	00	
STEP N	0.: 11						
VIAL:	MANL	GRA	DIENT	CONTH	ROL		
VOL:	15	I	'LOW:	0.00 1	4L/MN		
INJ VO	L: 1 of 2		%A:	100	%C:	0	
DETECTOR	#1		8B:	0	%D:	0	
AUFS:	1.000						
λ:	254	S	PARGE	: 15 MI	L/MN		
DETECTOR	#2	3	'EMP:	С	SET:	OFF C	2
AUFS:	0.500	I	RESS:	0 PSI			
λ:	280	S	SWITCH	ES:			
DETECTOR	#3		S1: 0	S2: (	) S3:	0 S4:	0
SENS	1024						
INI-	START	STO	)P				
TIAL	RUN	FLC	W	MORE	2	HOLD	
		STOP					
Al	BORT	FLOW	MC	DRE	HELP		

Figure 5-2 Operate Methods Screen

#### **Downloading the Operating Parameters**

When you press the Operate Method function key, the controller automatically downloads the operating parameters to the PowerLine devices (pump and detectors). The system status flag in the lower-left corner of the screen changes from Setup to Initial, and the Start Run screen key appears.

#### Starting the Run

- 1. When the system status is Initial, press the Start Run screen key to start the run. The system status flag changes to Inject Wait.
- 2. Turn the Injector handle to the Inject position. The system status flag changes to Running.

#### **Multiple Samples**

If you programmed the # Inj field in the Method table for more than one injection, the system status flag changes to Inject Wait when the run time expires. Load another sample into the Injector as described in Section 5.1, Loading the Sample. Turn the Injector handle to the Inject position to start the next run. When all programmed runs complete, the system status flag changes to Initial.

# Chapter 6 Powering Down the System

If you do not plan to use the 600E system for a long time (overnight or longer), power down the system.

#### **Before You Power Down**

Observe the following precautions when powering down your system:

- To prevent microbial growth, do not leave water or buffers in the fluidic lines while the system is not in use.
- Flush the lines with high-purity, filtered water or HPLC-grade water, followed by an aqueous 10% methanol solution.
- Remove the column if it is incompatible with this storage protocol.

### **Powering Down**

Turn off the Waters 600E by pressing the On/Off switch on the front panel of the controller to the 0 (Off) position.