Waters 2795 Separations Module

Quick Start Guide

NOTICE

Summary of This Guide

The following figure summarizes the information contained in this Quick Start Guide. Use it as a reference after reading the entire document.

```
            ┌─────────┐
            │  Start  │
            └────┬────┘
                 │
    ┌────────────────────────┐         ┌──────────────────┐         ┌──────────────────────┐
    │ Power on the           │         │ Configure the    │         │ Set Flow Rate and    │
    │ 2795 Separations       │         │ Plate Type(s)    │         │ Composition          │
    │ Module                 │         └────────┬─────────┘         └──────────┬───────────┘
    └───────────┬────────────┘                  │                              │
                │                      ┌─────────────────┐          ┌──────────────────────┐
    ┌────────────────────────┐        │ Load the Sample │          │ Select Direct        │
    │ Configure the          │        │ Plates          │          │ Function             │
    │ 2795 Separations       │        └─────────────────┘          └──────────┬───────────┘
    │ Module                 │                                                 │
    └───────────┬────────────┘                                        ┌────────────────┐
                │                                                      │   Function     │
    ┌────────────────────────┐                                        │   Begins       │
    │ Degas Solvents         │                                        └────────────────┘
    └───────────┬────────────┘        Perform an ─── No ────┘
                │                      Automatic Run?
    ┌────────────────────────┐              │
    │ Prime the Solvent      │             Yes
    │ Management System      │
    └────────────────────────┘
```
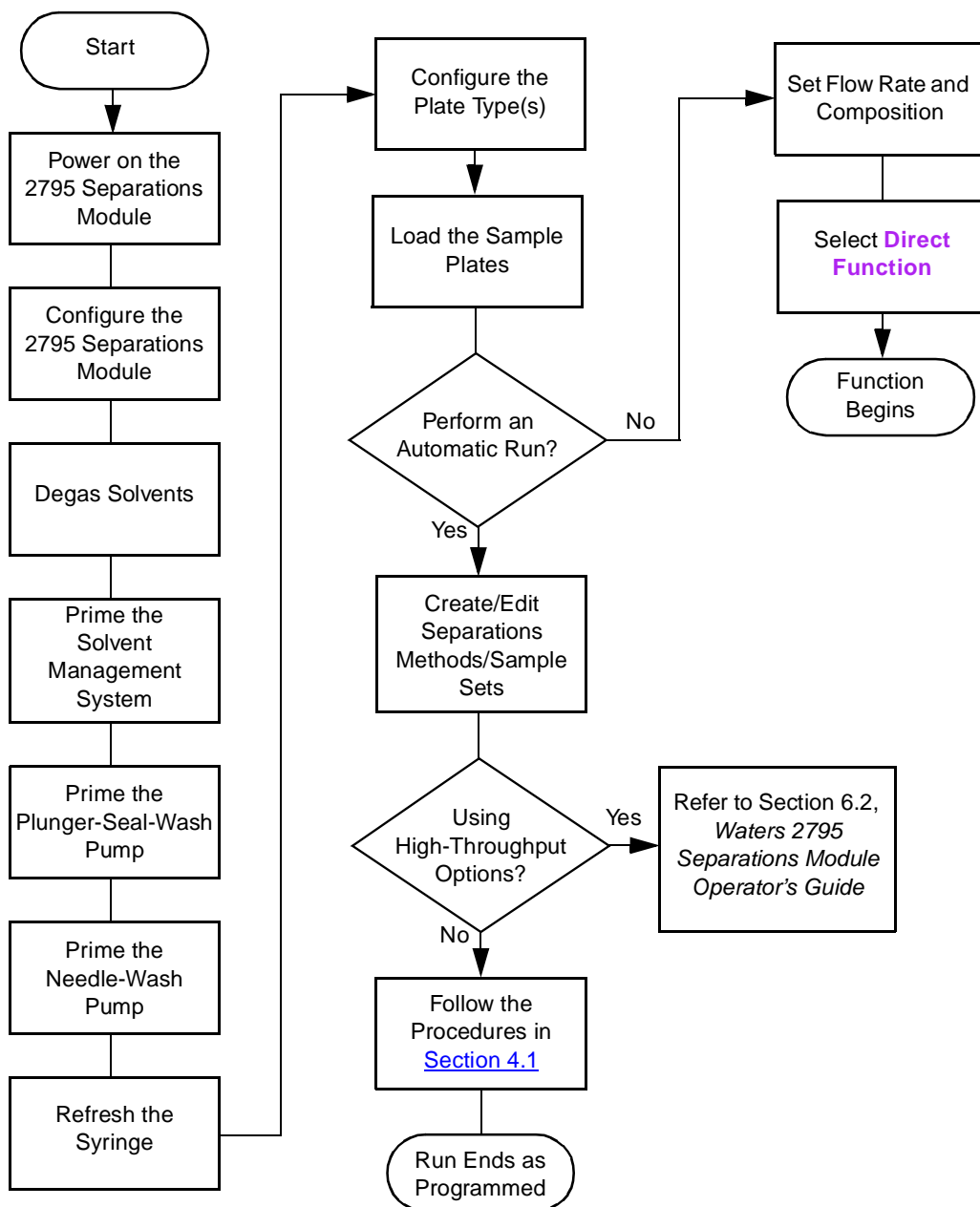
Conventions Used in This Guide

The *Waters 2795 Separations Module Quick Start Guide* uses the following conventions:

- Bold text indicates user input or action. For example, press **Enter**.

- 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 key directly below the key name displayed on the screen.

Notes, Attentions, and Cautions

This guide uses the following conventions for Notes, Attentions, and Cautions:

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

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

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

STOP *Atención: 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 the 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

The *Waters 2795 Separations Module Quick Start Guide* introduces you to the basic features of the Waters® 2795 Separations Module and describes how to make a run.

Who Should Use This Guide?

This guide is intended for both novice and experienced chromatographers who need to operate the 2795 Separations Module.

What Is in This Guide?

The *Waters 2795 Separations Module Quick Start Guide* contains basic procedural information to help you set up the 2795 Separations Module and make a run.

For additional information, or if you want to learn how to modify any procedures in this guide, refer to the *Waters 2795 Separations Module Operator's Guide*.

If you are using the 2795 Separations Module as part of an Alliance® HT LC/MS system (controlled by the MassLynx™ software), ensure you become familiar with the procedures in:

- *Waters Alliance LC/MS System Quick Start Guide*
- *MassLynx Data Acquisition Guide*
- *MassLynx User's Guide*
- *MassLynx BioLynx & ProteinLynx User's Guide*
- *MassLynx OpenLynx User's Guide*

Before You Begin

Ensure you have performed the following before you use the 2795 Separations Module:

- Your system is properly installed. Refer to the *Waters 2795 Separations Module Operator's Guide*, Chapter 2, Installing the 2795 Separations Module.

- Your separation methods and sample sets are created and stored. Refer to the *Waters 2795 Separations Module Operator's Guide*, Chapter 5, Creating and Editing Separation Methods and Sample Sets.

- Become familiar with the front panel and keypad operating procedures described in the *Waters 2795 Separations Module Operator's Guide*, Section 3.1, Using the 2795 Display and Keypad.

If you are using the 2795 Separations Module for high-throughput applications, refer to the *Waters 2795 Separations Module Operator's Guide,* Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation.

System Description

The Waters 2795 Separations Module (Figure 1-1) integrates a solvent management system and a three-axis sample management system to provide high-throughput analysis for HPLC, LC/MS, or flow injection-mass spectrometry applications.

When the 2795 Separations Module is installed with a sample heater/cooler and a column heater, it is known as the 2795 XE. Refer to the *Waters 2795 Separations Module Operator's Guide*, Section B.3, 2795 Separations Module Options, for information about the 2795 Separations Module options.

Solvent Bottle Tray

Detector Drip Tray

Front Panel Display and Keyboard

Syringe Access Door

Floppy Disk Drive

Column Heater Module

Sample Compartment Access Door

Solvent Delivery Tray Access Door

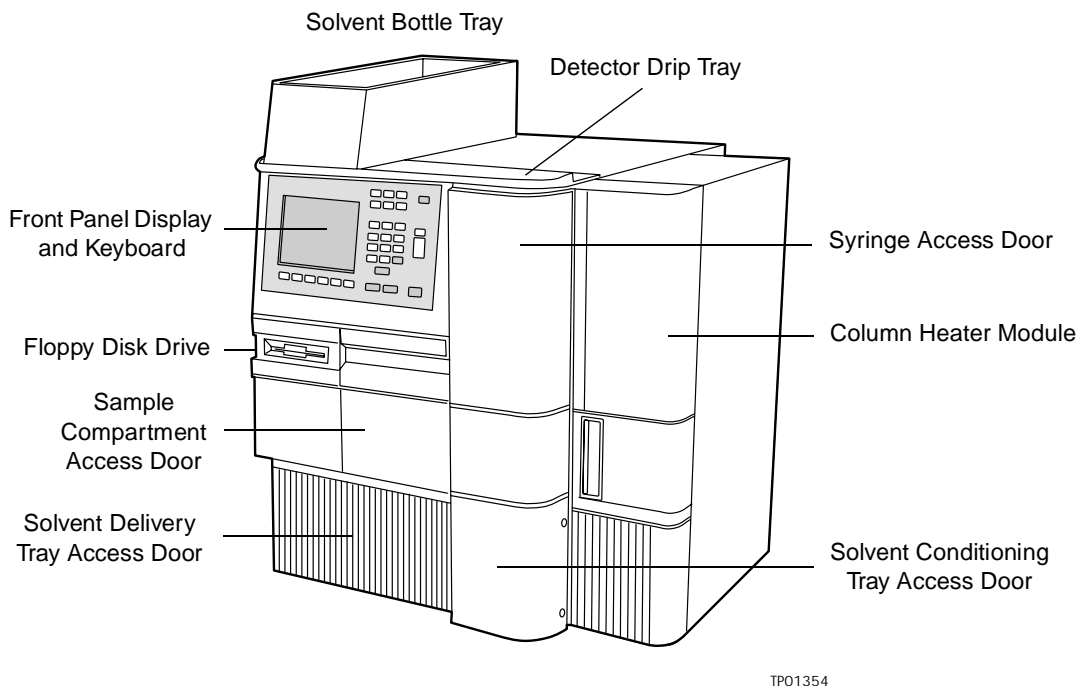Solvent Conditioning Tray Access Door

TPO1354

Figure 1-1 Waters 2795 XE Separations Module (Front View)

The sample management system in the 2795 Separations Module uses an XYZ needle assembly, up to four sample plates of up to 384 wells each, for a total capacity of 1536 samples. The 2795 Separations Module uses a variety of plate types including several that

are preconfigured (refer to the *Waters 2795 Separations Module Operator's Guide*, Table 3-6 and Table B-5).

Operating Modes

The 2795 Separations Module can operate in one of the following modes:

- Stand-alone HPLC system controller
- Component of an HPLC system controlled by Millennium®^{32} software
- Component of an LC/MS system controlled by MassLynx software

As a stand-alone HPLC system controller, the 2795 Separations Module manages both the solvent delivery and sample management systems as well as controlling one or more detectors. The 2795 Separations Module controls the sample plates using a rotating carrier. Optionally, the 2795 Separations Module can also control a three-column, six-column, or column regeneration selection valve.

An LC/MS system that includes the 2795 Separations Module and the Waters ZQ™ Mass Spectrometer is designated as a Waters Alliance HT LC/MS System. For further information on MassLynx control of the 2795 Separations Module, refer to the *Waters Alliance LC/MS System Quick Start Guide*.

1.1 Powering On the 2795 Separations Module

To power on the 2795 Separations Module, move the power switch (located at the top of the left side panel) to the **I** (ON) position. The startup diagnostics routine begins.

Startup Diagnostics

The startup diagnostics routine performs tests on the:

- CPU board
- Memory (ROM and RAM)
- Keypad
- Display
- External communication system
- Digital signal processor (DSP)
- Floppy disk drive

When initial startup diagnostics tests are complete, the front-panel screen displays the tests results, as shown in .

```
STARTUP DIAGNOSTICS
CPU      OK  DISPLAY OK  DSP      OK
ROM      OK  DMA     OK  FLOPPY OK
RAM      OK  GPIB    OK  CLOCK  OK
KEYPAD OK  FLASH   OK
```
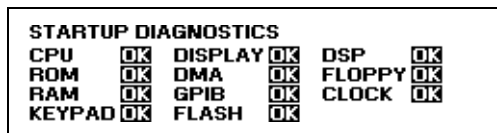
Figure 1-2 Diagnostic Test Results Screen

The Main Screen

If the initial tests of the startup diagnostics routine are successful, the Main screen (Figure 1-3) appears in the front panel display. As the startup diagnostics routine continues, the status of each of diagnostic test appears in the banner area of the Main screen. If the startup diagnostics routine fails, refer to the *Waters 2795 Separations Module Operator's Guide,* Section 8.5, Troubleshooting. When the startup diagnostics routine finishes, the 2795 Separations Module enters the Idle mode.

Banner Area

Data Area

Date & Time
Set By Clock

Screen Key Area

```
Main
<Default>              Idle

            Waters 2795
           Separations Module

  * Press Menu/ for monitoring and direct control *
         Status

           User: User

05/29/2001 09:33:43pm
© 2001 Waters Corporation        Revision 2.00

Develop Run
Methods Samples   Diag   Config   Log
```

Firmware Version
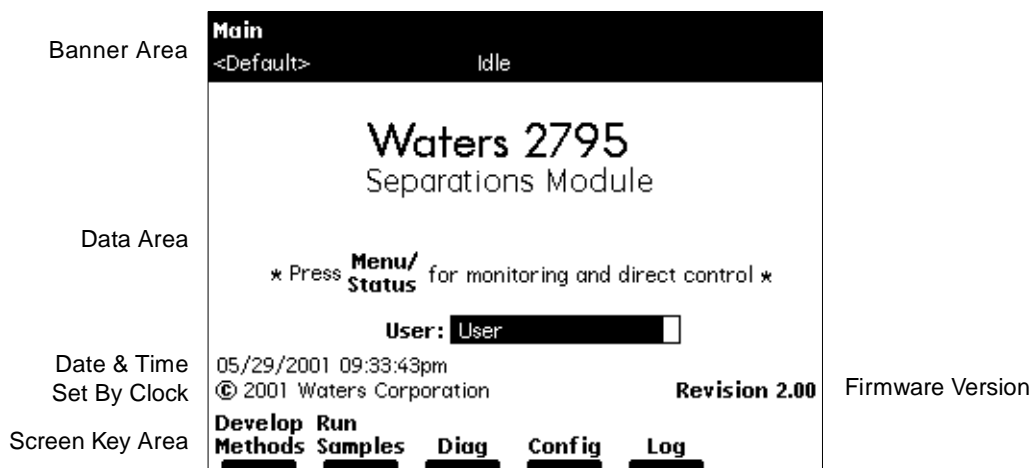
Figure 1-3 Main Screen

Note: Some 2795 Separations Module screens have additional screen keys that are not visible when you display the screen for the first time. Press the More screen key to display additional screen keys.

8 Introduction

1.2 Configuring the 2795 Separations Module

Before you can operate the 2795 Separations Module, you need to configure the 2795 Separations Module operating parameters. Press the **Config** screen key in the Main screen to display the Configuration screen (Figure 1-4).

Figure 1-4 Configuration Screen

1.2.1 Setting Configuration Parameters

Table 1-1 describes the parameters you can set for your system configuration.

Table 1-1 Configuration Parameters

Parameter	Description
Syringe Size and Loop Size	Specifies the sizes of the syringe and sample loop. These values are preset at the factory. Change these values when you install a different-sized syringe and/or sample loop (see the *Waters 2795 Separations Module Operator's Guide,* Table 7-5).
System	Specifies the operating mode and the IEEE-488 bus address (when required). See Section 1.2.2, Configuring the Operating Mode.
Printer	Specifies the type of printer and baud (data transfer) rate and flow control. *Note: The baud rate is automatically set to 1200 if you set the 746 Integrator as the printer type.*

Table 1-1 Configuration Parameters *(Continued)*

Parameter	Description
Date Format	Specifies MM/DD/YYYY (for example, 03/28/2001 for March 28, 2001) or DD.MM.YYYY (28.03.2001 for 28 March 2001).
Alarm Sound	Specifies the type of alarm (continuous or single beep).
Pressure Units	Specifies psi (pounds per square inch), kPa (kPascal), or bar.

1.2.2 Configuring the Operating Mode

On the Configuration screen, you configure the operating mode as follows.

No Interaction Mode

In this stand-alone mode, the 2795 Separations Module is not connected to the IEEE-488 interface bus, but can control non-IEEE-488 devices in the HPLC system using the I/O connections on the rear panel. Refer to the *Waters 2795 Separations Module Operator's Guide,* Section 2.5.2, Making Analog Signal Connections, for the procedure to make I/O connections.

To set the 2795 Separations Module to No Interaction mode:

1. Select **System** in the Configuration screen, then press **Enter** to list the operating modes.
2. Select **No Interaction**, then press **Enter**.
3. Press **Exit**.

System Controller Mode

In this stand-alone mode, the 2795 Separations Module can control up to three detector channels on the IEEE-488 bus. The channels can include up to two UV detector channels (a Waters 2487 Dual λ Detector and/or a Waters 486 Tunable Absorbance Detector) and one RI detector channel (Waters 2410 or 410 Differential Refractometer). For minimum firmware requirements of ancillary equipment, refer to the *Waters 2795 Separations Module Operator's Guide*.

Refer to the *Waters 2795 Separations Module Operator's Guide,* Section 2.5.3, Making Digital Signal Connections, for information on making IEEE-488 connections. You can also control other HPLC components using the I/O connections. Refer to the *Waters 2795 Separations Module Operator's Guide,* Section 2.5.2, Making Analog Signal Connections, for information on making I/O connections.

To set the 2795 Separations Module to System Controller mode:

1. Select **System** in the Configuration screen, then press **Enter** to list the operating modes.

2. Select **System Controller**, then press **Enter**.

3. Press the **Detectors** screen key. A list of active devices (with their IEEE-488 interface bus addresses) appears.

   a. Press **Scan** to update the list of devices. Up to three detectors (two absorbance channels and one RI detector) may appear in the list.

   b. Press the **OK** screen key to return to the Configuration screen.

4. Press **Exit**.

For information on setting detector parameters in a separation method, see the *Waters 2795 Separations Module Operator's Guide,* Section 5.2.6, Setting Detector Parameters.

Controlled by Millennium[32] Mode

Note: Millennium[32] workstations with software version 3.2 recognize a 2795 Separations Module with firmware version 2.00 as a 2790 Separations Module.

In this remote-control mode, a Millennium[32] Chromatography Manager workstation running Millennium[32] software controls the operation of your HPLC system using the IEEE-488 interface bus.

Note: This control capability will not function with Millennium software version 3.1 or earlier.

To set the 2795 Separations Module to Controlled by Millennium[32] mode:

1. Select **System** in the Configuration screen, then press **Enter** to list the choices.

2. Select **Controlled by Millennium32**, then press **Enter**. The IEEE 488 Address field is highlighted.

3. Press **Enter** to display a list the addresses.

4. Select an address that is unused by other chromatographic components connected to the Millennium[32] Chromatography Manager workstation, then press **Enter**.

5. Press **Exit**. The 2795 Separations Module is ready to be controlled from the Millennium[32] Chromatography Manager workstation.

Controlled by MassLynx Mode

Note: MassLynx software version 3.5 or earlier recognizes a 2795 Separations Module with firmware version 2.00 as a 2790 Separations Module.

Note: Refer to the software release notes for the Waters 2795 Separations Module that specify unique requirements and conditions for different MassLynx software versions.

In this remote-control mode, the Micromass® MassLynx software (version 3.5 or earlier) uses the IEEE-488 interface to control the 2795 Separations Module, and up to two detectors, including the:

- Micromass mass spectrometry (MS) detector
- Waters 996 or 2996 PDA Detector
- Other Waters detectors, such as the 2487 Dual λ Absorbance Detector

To set the 2795 Separations Module to Controlled by MassLynx mode:

1. Select **System** in the Configuration screen, then press **Enter** to list the operating modes.
2. Select **Controlled by MassLynx**, then press **Enter**. The IEEE-488 Address field is highlighted.
3. Press **Enter** to list the addresses.
4. Select an address that is unused by other chromatographic components connected to the Micromass computer, then press **Enter**.
5. Press **Exit**. The 2795 Separations Module is ready to be controlled from the MassLynx computer.

Operate Gradient by Event In Mode

In this mode, an external autosampler (a Waters 2700 Sample Manager, for example) initiates the start of the chromatographic run and performs the inject function (instead of the 2795 Separations Module). In this mode, the 2795 Separations Module has no control of IEEE-488 devices, but *can* control non-IEEE devices using the I/O connections on the rear panel. Refer to the *Waters 2795 Separations Module Operator's Guide,* Section 2.5.2, Making Analog Signal Connections, for information on I/O connections.

To set the 2795 Separations Module to Operate Gradient by Event In mode:

1. Select **System** in the Configuration screen, then press **Enter** to list the operating modes.
2. Select **Operate Gradient by Event In**, then press **Enter**.
3. Press **Events In** to display the Events In dialog box. For information on defining Events In conditions, refer to the *Waters 2795 Separations Module Operator's Guide*, Section 3.2.1, Setting Configuration Parameters, the "Specifying Signal Conditions for Events In" discussion.
4. Set the Stop Flow field to the appropriate condition, then press **Enter**.
   - Select **Ignore** if the Stop Flow I/O terminals of the 2795 Separations Module are not used.

- Select **On High** if the output signal from the external autosampler to the Stop Flow I/O terminals of the 2795 Separations Module is a high (more positive) TTL-level.

- Select **On Low** if the output signal from the external autosampler to the Stop Flow I/O terminals of the 2795 Separations Module is a low (more negative) TTL-level.

*Note: When you configure the 2795 Separations Module with a Waters 2700 Sample Manager, select **On Low**.*

5. Set the Hold 1 field to the appropriate condition, then press **Enter**.

- Select **On High** if the output signal from the external autosampler to the Hold Inject 1 terminals of the 2795 Separations Module is a high (more positive) TTL-level to start a chromatographic run and prevent an injection by the 2795 Separations Module.

- Select **On Low** if the output signal from the external autosampler to the Hold Inject 1 terminals of the 2795 Separations Module is a low (more negative) TTL-level to start a chromatographic run and prevent an injection by the 2795 Separations Module.

*Note: When configuring the 2795 Separations Module with a Waters 2700 Sample Manager, select **On Low**.*

6. Set the Logic field to **Or**, then press **Enter**.
7. Set the Hold 2 field to **Ignore**, then press **Enter**.
8. Press **OK** to save your selections and exit the Events In dialog box.

Controlled by RS-232 Mode

In this remote-control mode, a non-Waters data system (a mass spectrometry system, for example) controls the 2795 Separations Module using RS-232 communications. The RS-232 protocol is block/binary. The 2795 Separations Module disconnects from the IEEE-488 interface bus when this mode is selected. The 2795 Separations Module can control other non-IEEE devices in the HPLC system using the I/O connections on the rear panel.

To set the 2795 Separations Module to the Controlled via RS-232 mode:

1. Select **System** field in the Configuration screen, then press **Enter** to list the choices.
2. Select **Controlled via RS232**, then press **Enter**.
3. Press **Exit**. The 2795 Separations Module is ready to be controlled by a remote data system using RS-232 communications.

1.2.3 Specifying Report Options

The 2795 Separations Module can generate a report – a record of injections – during stand-alone or remote operation, if a printer or integrator is connected.

Note: Use Port A of the RS-232 connector to connect a cable from the 2795 Separations Module to a serial printer or to a Waters 746 Data Module.

To define the information to be sent to a printer, integrator, and/or floppy disk:

1. In the Configuration screen, press the **Reports** screen key to display the Report Options dialog box (Figure 1-5).
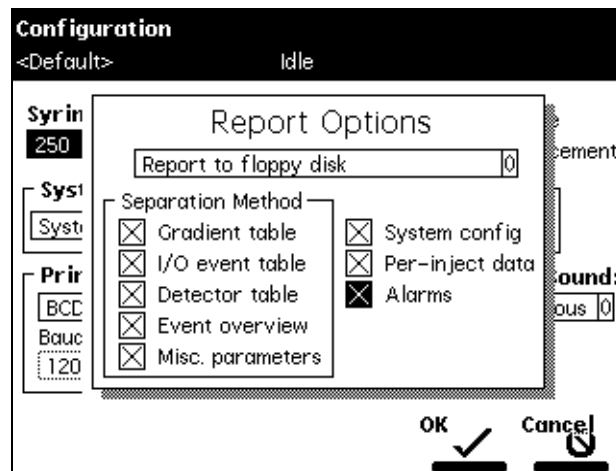
Figure 1-5 Report Options Dialog Box

2. From the drop-down list, select the destination for the report.
3. Select an option from the Separation Method list, then press any numeric key to enable or disable the option. An "X" in the check box indicates the option is enabled. The options are:
    *   **Gradient table** – Generates the gradient table
    *   **I/O event table** – Generates the I/O table
    *   **Detector table** – Generates the detector table
    *   **Event overview** – Generates an overview of all merged tables
    *   **Misc. parameters** – Generates a list of all parameters not included in tables
    *   **System config** – Generates a list of the instrument configuration parameters

- **Per-inject data** – Generates a list of the minimum, maximum, and average values for temperature and pressure, the time and date of each injection, and the bar code number
- **Alarms** – Generates a list of the error conditions that occur during each injection

4. Press the **OK** screen key to exit the Report Options dialog box (or press the **Cancel** screen key to exit the Report Options dialog box without saving your changes).

1.2.4 Specifying an Auto Shutdown

The Auto Shutdown feature allows you to set up the 2795 Separations Module to shut down automatically after a specified period of inactivity. Inactivity is defined as:

- No keyboard use
- No injections performed
- No changes sent to the 2795 Separations Module from a remotely connected MassLynx computer
- An error condition that suspends the operation of the 2795 Separations Module

You can use Auto Shutdown to:

- Minimize solvent flow when a run ends unattended
- Disable the vacuum degasser
- Disable temperature controls
- Turn off detector lamps

To enable an Auto Shutdown:

1. Press the **Config** screen key in the Main screen, then press the **More** screen key.
2. Press the **Auto Shutdown** screen key. The Auto Shutdown dialog box appears (Figure 1-6).
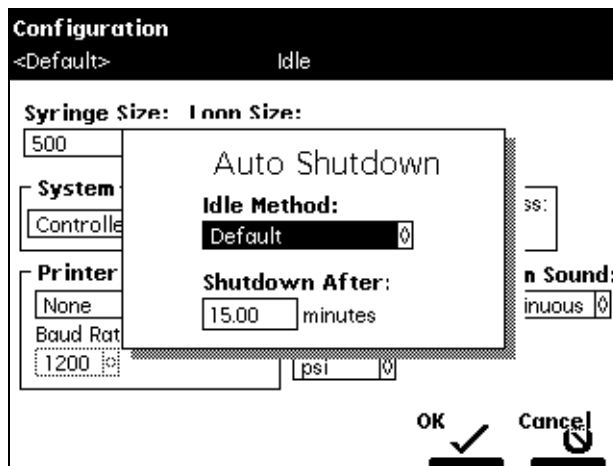
Figure 1-6 Auto Shutdown Dialog Box

3. In the Idle Method field, select the separation method to be applied when the 2795 Separations Module is shut down. Only the *initial* conditions in the method you select are applied during the shutdown period.

4. In the Shutdown After field, enter a time period of inactivity after which you want the 2795 Separations Module to shut down (or press **Clear** to disable Auto Shutdown).

5. Press the **OK** screen key. The 2795 Separations Module shuts down when there has been no activity for the specified period.

6. Press **Exit** to return to the previous screen.

Note: If you want to use Auto Shutdown to power off a 2487 or 486 detector lamp, specify a Lamp Off event and a time of INIT in the I/O Events Timed Table screen (see the Waters 2795 Separations Module Operator's Guide*, Section 5.2.5, Setting I/O Parameter Values).*

1.2.5 Configuring Plate Types

STOP *Attention: Waters recommends you consult the* Waters 2795 Separations Module Operator's Guide*, Table B-5, before configuring plates for the 2795 Separations Module. Read all the recommendations in the footnotes to Table B-5.*

There are 15 possible plate types you can configure for use with the 2795 Separations Module, some of which are preconfigured and not editable.

Note: When you select a preconfigured plate type, the parameter fields indicate the measured dimensions for the specific plate, which are not editable (indicated by a dotted line surrounding the field).

To customize a preconfigured plate type for the 2795 Separations Module:

1. In the Configuration screen, press the **Plate Types** screen key to display the Plate Type Configuration dialog box (Figure 1-7).

2. Select the plate type from the Name drop-down list. The remaining (noneditable) fields fill with values corresponding to the selected plate type. To configure a custom plate, see the "Configuring Custom Plates" discussion later.

3. In the Sequential reference field, select the processing order (see Table 1-6).

Table 1-2 describes the fields in the Plate Type Configuration dialog box. See the *Waters 2795 Separations Module Operator's Guide*, Table B-5, for recommended plate types (and their dimensions) for use with the 2795 Separations Module.

STOP *Attention: Using incorrect dimensions can cause needle damage.*
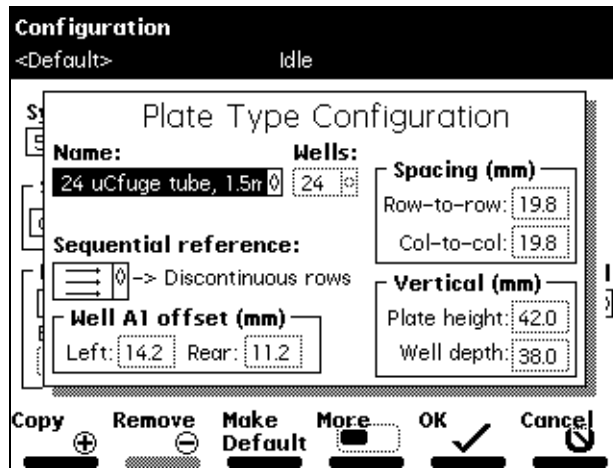
Figure 1-7 Plate Type Configuration Dialog Box

Table 1-2 Plate Type Configuration Parameters

Field	Description
Name	Name of the plate type. Use up to 30 characters to identify a plate type. Some of the 15 plate types are preconfigured.
	To move from one plate type to the adjacent plate type in the drop-down list, press the **Previous** or **Next** screen key. The default plate type appears with an asterisk in the Name field and the words "Default Plate Type" are displayed below the Name field.
Wells	Number of wells on the plate selected in the Name field. The parameters for each plate type are:

Number of Wells	Number of Rows	Number of Columns
24	4	6
48	6	8
48R	8	6
96	8	12
384	16	24

Field	Description
Sequential reference	Direction and sequence of well injections for the selected plate. See Table 1-6.

Table 1-2 Plate Type Configuration Parameters *(Continued)*

Field	Description
Well A1 offset (mm)	
Left	The distance from the center of the A:1 well to the left outer edge of the plate
Rear	The distance from the center of the A:1 well to the rear outer edge of the plate. See Figure 1-9.
Spacing (mm)	
Row-to-row	The distance from the centerline of the wells in one row to the centerline of the wells in an adjacent row
Col-to-col	The distance from the centerline of the wells in one column to the centerline of the wells in an adjacent column. See Figure 1-9.
Vertical (mm)[a]	
Plate height	Total plate height
Well depth	The well depth (distance from the top of the plate to the bottom of the well)

a. See the *Waters 2795 Separations Module Operator's Guide*, Table B-5, for dimensions of the plates recommended for use with the 2795 Separations Module.

Configuring Custom Plates

Note: You can use one of the preconfigured plate types to create a custom plate. Before following the next procedure, select the preconfigured plate type that most resembles the custom plate you want to create.

Required Material

To make plate measurements, you need a ruler or straight-edge scaled in millimeters.

Procedure

To configure a custom plate:

1. In the Plate Type Configuration dialog box, press the **Copy** screen key to display the Custom Plate Name field (Figure 1-8).
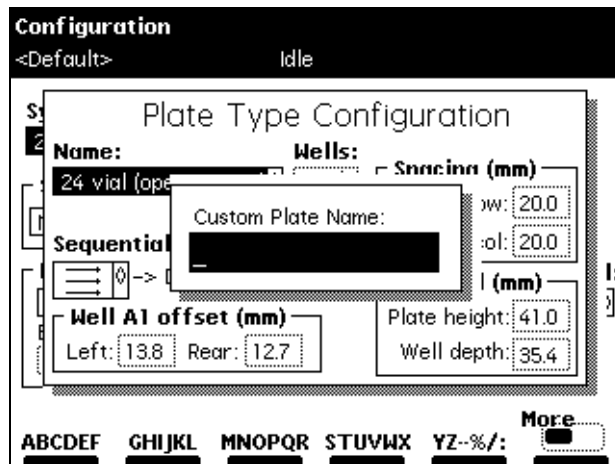
Figure 1-8 Custom Plate Name Field

2. Enter a custom plate name (see the *Waters 2795 Separations Module Operator's Guide*, Section 3.1.2, Using the Keypad, the "Entering an Alphanumeric String" discussion), then press **Enter**. The Plate Type Configuration parameter fields become editable (surrounded by a solid line).

3. Using a ruler or straight-edge scaled in millimeters, measure the dimensions for the custom plate as shown in <u>Figure 1-9</u> and <u>Figure 1-10</u>, then enter each parameter in the appropriate field in the Plate Type Configuration dialog box.

4. Press the **OK** screen key to store the custom plate (or press the **Cancel** screen key to discard the changes).

   To delete the selected custom plate type, press the **Remove** screen key. The Remove screen key is active only when a custom plate name is displayed in the Custom Plate Name field. You cannot remove a preconfigured plate type.

   To make the displayed plate type the default plate type, press the **Make Default** screen key. The factory-default plate type is 96 well, 300 µL. The words "Default Plate Type" appear below the default plate name in the Plate Type Configuration dialog box.

   Press the **Previous** or **Next** screen key to move from one plate type to another.

   Press the **Print** screen key to print a copy of the plate configuration list to a floppy disk or printer (if a printer is connected to your 2795 Separations Module).

Note: The Needle Depth Offset parameter (<u>Figure 1-10</u>) is used to specify an offset from the well bottom to accommodate sample vials or sedimentation. Enter the "Needle Depth Offset" in the Sample screen when you are creating or editing a separation method (see

the Waters 2795 Separations Module Operator's Guide*, Section 5.1, Creating and Editing Separation Methods).*

Top View Dimensions

Note: *Measure the A1 Left and A1 Rear Reference points (left and rear well offsets) from the outer edge of the plate.*
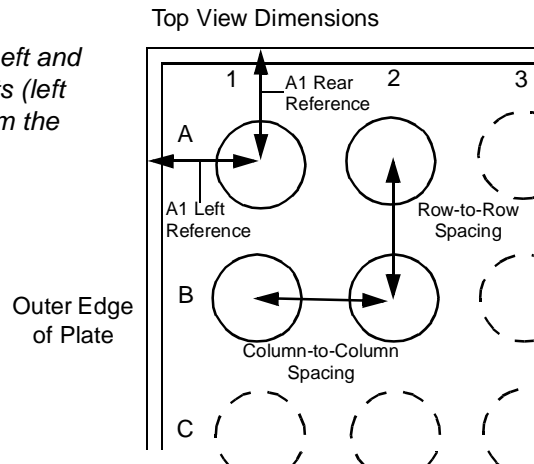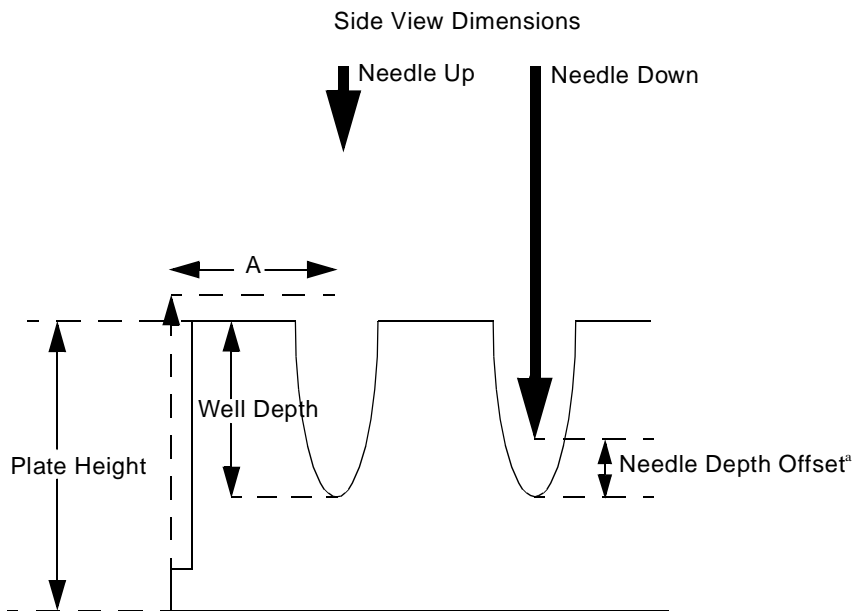
Outer Edge of Plate

Figure 1-9 Plate Parameters (Top View)

Note: *The minimum rear offset value is 8.3 mm. Refer to the* Waters 2795 Operator's Separations Guide*, Section 3.2.2, Configuring Plate Types, and Table B-5.*

Side View Dimensions

Needle Up   Needle Down

A

Well Depth

Plate Height   Needle Depth Offset[a]

a. The Needle Depth Offset parameter is part of the separation method. See Section 5.1, Creating and Editing Separation Methods in the Waters 2795 Separations Module Operator's Guide.

Figure 1-10 Plate Parameters (Side View)

Table 1-3 describes the preconfigured plate types for the 2795 Separations Module. The plate type names appear as displayed in the Name field of the Plate Type Configuration dialog box.

Table 1-3 2795 Separations Module Preconfigured Plate Types

Plate Type Name	Description	Part Number
24-uCfuge tube, 1.5 mL	24-well, 1.5 mL microcentrifuge tube holder	405000560
24-vial (open access), 2 mL	24-well, 2 mL vial, open access plate	200000114
48-vial holder, 2 mL	48-well, 2 mL vial holder	405000562
48-uCfuge tube, 0.5 mL	48-well, 0.5 mL microcentrifuge tube holder	405000561
96-well with 700 μL insert	96-well with 700 μL glass insert plate	186000349
96-well (tall), 350 μL	96-well tall, 350 μL polypropylene	WAT058943

Table 1-3 2795 Separations Module Preconfigured Plate Types *(Continued)*

Plate Type Name	Description	Part Number
96-well (tall), 1 mL	96-well tall, 1 mL, polypropylene	WAT058957
96-well (tall), 2 mL	96-well tall, 2 mL, polypropylene	WAT058958
96-well, 300 µL (Default)	96-well, 300 µL, polypropylene or polystyrene	Non-Waters[a]
384-well, 80 µL	384 well, 80 µL, polystyrene	Non-Waters[a]

a. Check the dimensions of all non-Waters plates. For more information on these and other 2795-compatible plates, see the *Waters 2795 Separations Module Operator's Guide*, Table B-5.

1.3 PerformancePLUS Degasser

Inline vacuum degassing reduces the total dissolved gas in the mobile phase. The 2795 Separations Module is outfitted with a factory-installed PerformancePLUS™ Degasser. This degasser is comprised of a variable-speed, continuously operating vacuum pump and low-volume degasser chambers that enable rapid instrument priming and equilibration. This allows you to become operational quickly from an idle state or following a solvent change. Refer to Figure 1-11.
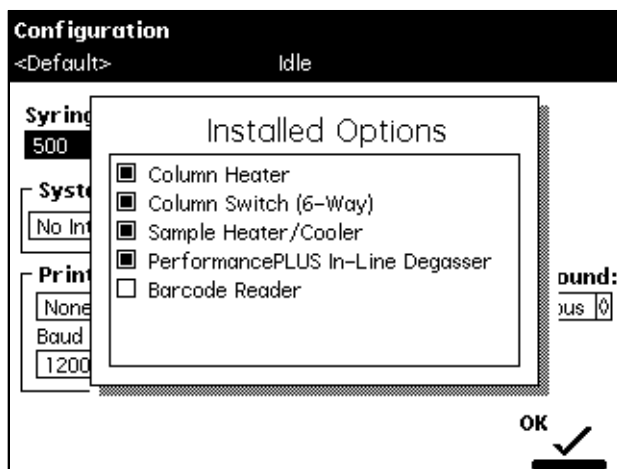
Figure 1-11 Installed Options Dialog Box Showing PerformancePLUS Degasser

The PerformancePLUS Degasser has five vacuum degasser chambers that enable you to degas eluents A, B, C, and D in addition to the purge solvent. The degasser chambers

have a low internal volume of less than 500 µL. This makes the 2795 Separations Module easy to prime. The Waters PerformancePLUS Degasser is fully menu supported (Figure 1-12).
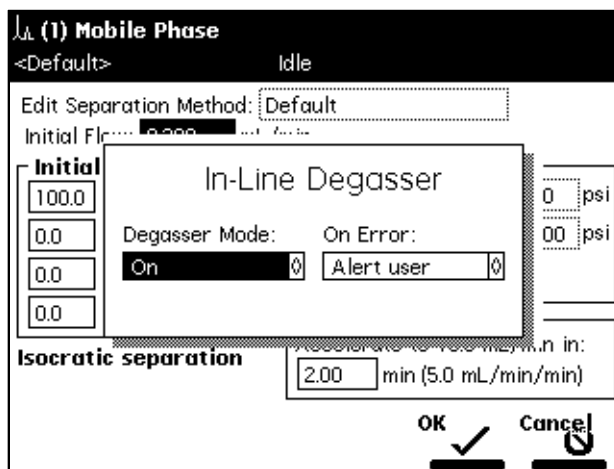
Figure 1-12 Degasser Mode On/Off Selection

1.4 System Preparation Function

The Waters 2795 Separations Module has an additional direct function for system preparation, labeled System Prep. This function prepares the 2795 Separations Module for operation.
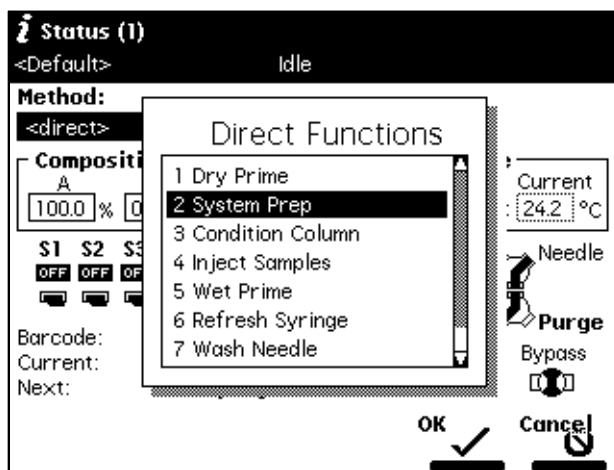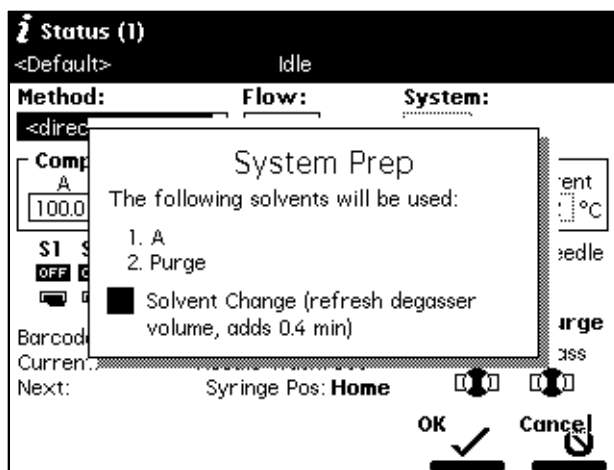

Figure 1-13 System Prep Selection from Direct Functions Dialog Box

Figure 1-14 System Prep Dialog Box

The System Prep function performs the following:

- After you have manually performed a dry prime to place eluent in the line from the reservoirs to the pump heads, System Prep fills the entire fluidic pathway of the 2795 Separations Module with fresh eluents, or wash and purge solvents, according to the selected separation method.

- System Prep performs a wet prime of 100% of each solvent used by the method, and concludes by setting the 2795 Separations Module at the initial composition. While priming, it washes the needle and purges the syringe.

- An optional extension of the solvent priming and the syringe purge time can be specified, that is useful when changing solvent or after extended periods of system inactivity.

- When you select the System Prep function immediately after the power-up of the 2795 Separations Module, a banner at the top of the Status screen reports that the System Prep function requires 5 minutes for the degasser to condition the eluents before initiating the System Prep function. The 5-minute period posted in the banner decrements until time zero, and the System Prep function begins automatically after time zero.

To accomplish this, System Prep does the following:

1. Performs an optional wet prime of 100% of each solvent specified by the given separation method using a flow rate of 7.0 mL/min. The length of each wet prime with the PerformancePLUS Degasser is approximately 0.4 minutes. The optional wet prime can be enabled or disabled using the Solvent Change option.

2. Performs a wet prime of 100% of each of the solvents specified by the given separation method using a flow rate of 1.0 mL/min (a flow rate that is optimum for degasser efficiency).

3. Performs a refresh syringe cycle that runs simultaneously with the wet primes until complete using the ratios in Table 1-4. In all cases, replacement volume is one stroke.

Table 1-4 Refresh Syringe Ratios

Solvent Status	PerformancePLUS Degasser
Solvent Change	10 strokes[a], 3.75 mL
No Solvent Change	3 strokes[a], 1.0 mL

a. Based on a standard configuration of a 500- μL syringe.

4. Performs a needle-wash cycle that runs simultaneously with the wet prime until complete, using the wash cycles, inject port wash time, and needle exterior wash time parameters from the selected separation method.

5. Leaves the system at an initial flow rate and composition of the selected separation method.

System Prep can be selected as a direct function, or it can be run as a sample set function in standalone mode, or sample list function controlled by MassLynx software version 3.5.

1.5 Preparing the Solvent Management System

For optimal performance of the 2795 Separations Module, you need to prepare the solvent and the sample management systems for operation. Preparing the solvent management system involves:

1. Preparing solvent reservoirs (Section 1.5.1)
2. Degassing solvents (Section 1.5.2)
3. Priming the plunger-seal-wash pump (Section 1.5.3)
4. Priming the solvent management system (Section 1.5.4)

For information on preparing the sample management system, see Section 1.6, Preparing the Sample Management System.

Note: To maintain the efficiency of the solvent management system, and to obtain accurate, reproducible chromatograms, use only HPLC-grade solvents.

⚠️ *Caution: Observe safe laboratory practices when you handle solvents. Refer to the Material Safety Data Sheets for the solvents you use.*

1.5.1 Preparing Solvent Reservoirs

Choose solvent reservoirs that provide a snug fit for the reservoir caps supplied in the Startup Kit. Waters recommends 1-L reservoirs.

🛑 STOP *Attention: To maintain adequate solvent head pressure and ensure proper solvent delivery, position the solvent reservoirs above the level of the solvent management system, at least 20 inches (50.8 cm) above the laboratory bench.*

STOP *Attention: To prevent solvent overflow, Waters recommends that pressurized solvent reservoir bottles not be used. Pressures above 5 psi can cause solvent to be forced through the internal components of the gradient proportioning valve (GPV), possibly causing a spillover into the solvent waste reservoir or an adverse mixture with other solvents in the GPV.*

Install the solvent and vent lines as described in the *Waters 2795 Separations Module Operator's Guide,* Section 2.4, Making Fluidic Connections.

1.5.2 Degassing Solvents

STOP *Attention: Do not run the degasser in the On mode for an extended period of time unless solvent is flowing through the supply tubes. Without solvent, excess vapors can condense in the degasser vacuum chamber walls, which could lead you to suspect a vacuum chamber leak.*

Note: For optimum efficiency when running the degasser in the On mode, ensure that all degasser solvent lines are filled with solvent.

To control the inline vacuum degasser:

1. Press **Menu/Status**. The Status screen is displayed.

2. Press the **Next Page** screen key (if necessary) to display the Degasser fields (Figure 1-15).

3. Select the **Degasser** mode field, then press **Enter** to display the following list of degasser modes:

   • **On** – The degasser is always enabled.

   • **Off** – The degasser is always disabled.
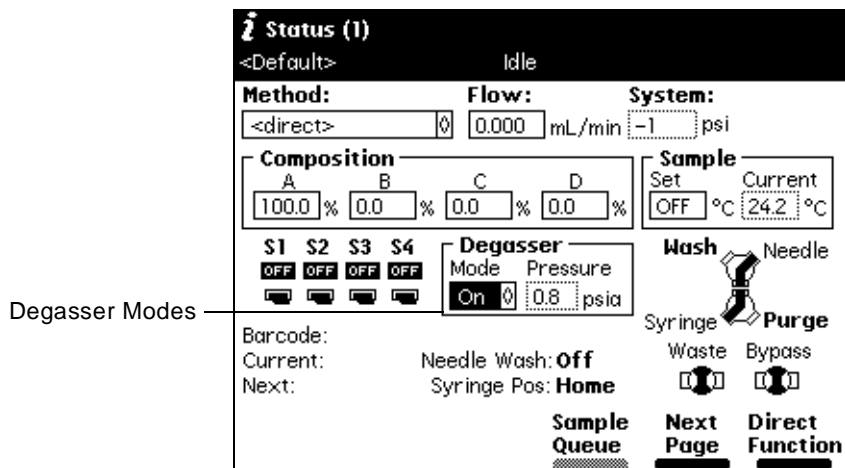
Degasser Modes ——

Figure 1-15 Degasser Modes on Status Screen

4. Select the desired operating mode, then press **Enter**.

The Vac pump field displays the current status of the degasser vacuum pump. The Pressure field displays the current vacuum level in psi, bar, or kPa.

1.5.3 Priming the Plunger-Seal-Wash Pump

The plunger-seal-wash solvent lubricates the solvent management system plunger and flushes away solvent or precipitated salts forced past the plunger seal from the high-pressure side of the solvent piston chamber (see the *Waters 2795 Separations Module Operator's Guide*, Figure 7-2).

Required Materials

You need the following materials:

- Tubing adapter (Startup Kit)
- 30-mL syringe (Startup Kit)
- Seal-wash solution

Note: Ensure the plunger-seal-wash supply line (labeled "Seal Wash In") is in the plunger-seal-wash solvent bottle and that the plunger-seal-wash waste line is in an appropriate waste container.

Procedure

To prime the plunger-seal-wash pump:

1. Disconnect the inlet filter from the plunger-seal-wash inlet tube.

2. Connect the tubing adapter to the syringe.

3. Fill the syringe with plunger-seal-wash solution, then connect the syringe assembly to the plunger-seal-wash inlet tube.

4. In the Main screen, press the **Diag** screen key, then press the **Prime SealWsh** screen key. The Prime Seal Wash dialog box appears (Figure 1-16).

   Note: When the Prime Seal Wash procedure is not running, the dialog box reads "Idle."
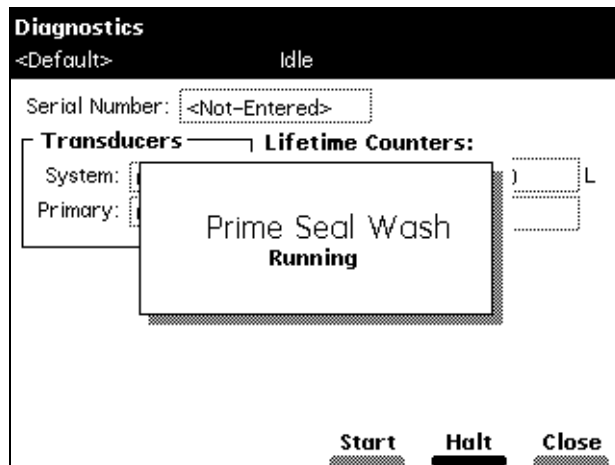
Figure 1-16 Prime Seal Wash Dialog Box

5. Press the **Start** screen key to begin the seal-wash priming procedure, then push on the syringe plunger to push plunger-seal-wash solvent through the system.

6. When the plunger-seal-wash solvent is flowing out of the plunger-seal-wash waste tube, press the **Halt** screen key.

   If you do not press **Halt**, the seal-wash pump priming procedure stops after 15 minutes.

7. Press the **Close** screen key to return to the Diagnostics screen, then press **Exit** to return to the Main screen.

> **Note:** *You can use the same solvent for both the purge solvent and the plunger seal wash solvent, depending on your application.*

8. Remove the syringe and adapter, reconnect the filter, and place the plunger-seal-wash inlet tube into the purge/plunger-seal-wash solvent reservoir.

For more information on performing diagnostics procedures, see the *Waters 2795 Separations Module Operator's Guide*, Chapter 8, Diagnostics and Troubleshooting.

1.5.4 Priming the Solvent Management System

Prime the solvent management system using the:

- Dry prime direct function when the solvent lines are dry (see Section 2.2.1, Dry Priming)
- Wet prime direct function when you want to switch from one solvent to another (see Section 2.2.2, Wet Priming)

1.6 Preparing the Sample Management System

Prepare the sample management system for operation after you have prepared the solvent management system (Section 1.5, Preparing the Solvent Management System). Preparing the sample management system involves:

1. Wash sequence (Section 1.6.1)
2. Priming the needle-wash pump (Section 1.6.2)
3. Refreshing the syringe (Section 1.6.3)
4. Loading the sample plates (Section 1.6.4)

Before using the sample management system for the first time, become familiar with the procedures in the *Waters 2795 Separations Module Operator's Guide,* Section 4.2, Performing Direct Functions, and Section 4.3, Monitoring System Status.

1.6.1 Wash Sequences

A new wash *sequence* option is part of the separation method for 2795 Separations Modules beginning with firmware version 2.00. Refer to Figure 1-17.

By default, the wash sequence is set to Wash-Purge sequence. The alternative is the Purge-Wash-Purge sequence in which an additional purge of the syringe is performed before the Needle-Wash. This option uses more degassed purge solvent, and can be useful in applications where the samples are in serum or salt-containing matrices.
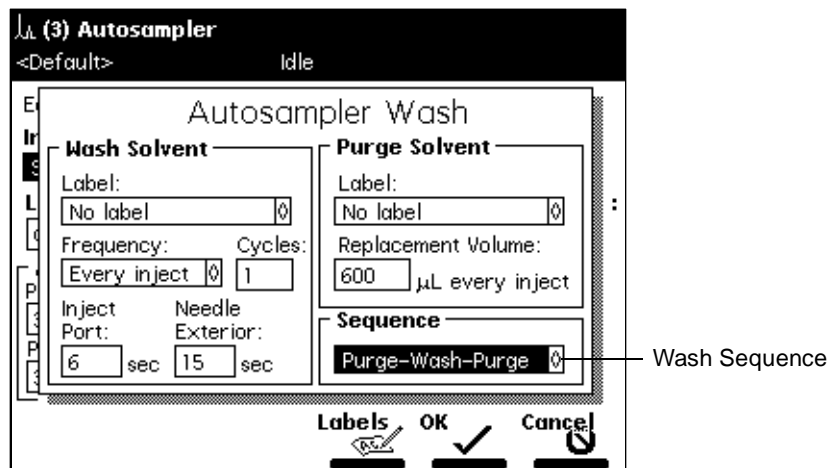
Figure 1-17 AutoSampler Wash

MassLynx Control

Versions 3.4 and 3.5

When operating the 2795 Separations Module with MassLynx versions 3.5 and 3.4, you can program the Purge-Wash-Purge sequence by selecting that option in the 2795 Default method. For MassLynx version 3.5 only, you must also select **Wash-Purge** in the Inlet Editor.

Millennium[32] Control

With Millennium[32] version 3.2, you can program the 2795 Separations Module to use the purge-wash-purge sequence by selecting that option in the 2795 Default method along with entering a value of 0 in the Purge Cycles field of the 2795 instrument method editor in Millennium[32] software.

1.6.2 Priming the Needle-Wash Pump

The needle-wash pump flushes the needle in the sample management system with wash solvent, preventing carryover of sample between injections. The needle wash also extends the life of the inject port seat by removing buffered mobile phase and sample from the needle.

Prime the needle-wash pump when one or both of the following occurs:

• There is a lack of flow in the needle-wash system.

• You are changing the wash solvent.

Selecting the Needle-Wash and Purge Solvents

Use a needle-wash solvent based on the sample and mobile phase chemistries, making sure all solutions/buffers are miscible and soluble. Table 1-5 lists some needle-wash and purge solvents recommended for use with certain mobile phase conditions. High sample concentrations may require other needle wash solvents.

Table 1-5 Suggested Needle-Wash and Purge Solvents

Chromatographic Condition	Wash Solvent	Purge Solvent
Buffered aqueous, reversed-phase	50 to 100% MeOH or ACN	90% H_2O, 10% MeOH or ACN to 50% H_2O, 50% MeOH or ACN
Nonaqueous, reversed-phase	100% MeOH	Mobile phase
Normal phase	Mobile phase	Mobile phase
GPC	Mobile phase	Mobile phase
Ion exchange	90% H_2O, 10% MeOH	H_2O

Required Materials

You need the following materials:

• 30-mL syringe (Startup Kit)
• Tubing adapter (Startup Kit)
• Wash solvent

Note: Be sure the needle-wash fluidic lines and reservoirs are installed properly (see the Waters 2795 Separations Module Operator's Guide*, Section 2.4.5, Installing the Wash Reservoirs).*

Procedure

To prime the needle-wash pump:

1. Remove the inlet filter from the needle-wash inlet tube.
2. Connect the syringe adapter to the 30-mL syringe.
3. Fill the syringe with wash solvent and connect it to the wash-solvent inlet tube.
4. Press the **Diag** screen key in the Main screen. The Diagnostics screen appears.

5. Press the **Prime NdlWash** screen key. The Prime Needle Wash Solvent dialog box appears (Figure 1-18).
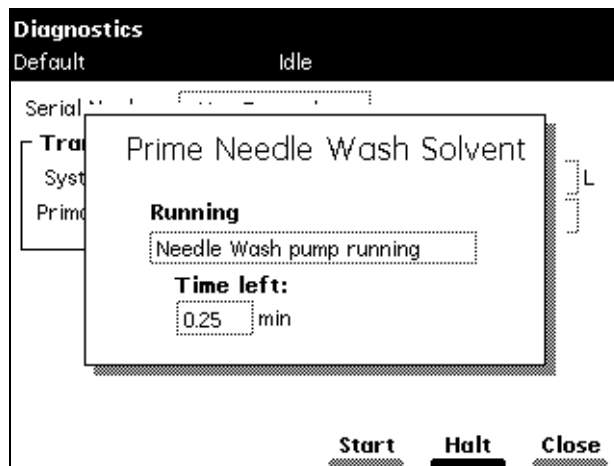
Figure 1-18 Prime Needle Wash Solvent Dialog Box

6. Press the **Start** screen key to begin the needle wash, then press lightly on the syringe plunger to push needle-wash solvent through the needle-wash system. If solvent does not flow out of the needle-wash-waste tubing within 30 seconds, press **Start** a second time.

   If solvent still does not flow out of the waste line, check for leaks in the tubing or sample management compartment.

7. When needle-wash solvent flows out of the needle-wash waste tube, press the **Halt** screen key.

   If you do not press **Halt**, the needle-wash prime procedure stops after 30 seconds.

8. Press the **Close** screen key to return to the Diagnostics screen, then press **Exit** to return to the Main screen.

9. Remove the syringe and adapter, reconnect the filter, and place the solvent line back in the needle-wash solvent reservoir.

For more information on performing diagnostics procedures, see the *Waters 2795 Separations Module Operator's Guide*, Chapter 8, Diagnostics and Troubleshooting.

1.6.3 Refreshing the Syringe

Refresh the syringe whenever you do any of the following:

- Prime the solvent management system
- Change solvents
- See bubbles in the syringe
- Start using the 2795 Separations Module at the beginning of each day

STOP **Attention:** *To prevent degradation to the syringe and valves, avoid the use of salt buffers in the purge solvent. Use a low concentration (0% to 50%) organic-aqueous solvent that is similar to the mobile phase, initial gradient conditions, or sample diluent (see Table 1-5).*

Note: *When you purge the syringe for the first time or when you change the purge solvent, set the number of syringe strokes to* **10** *and the replacement volume to* **600** *µL. This volume fills the entire degasser chamber and the associated inlet tubing (with the standard 500-µL syringe installed).*

To refresh the syringe:

1. Press the **Menu/Status** key. The Status screen appears (Figure 1-19).
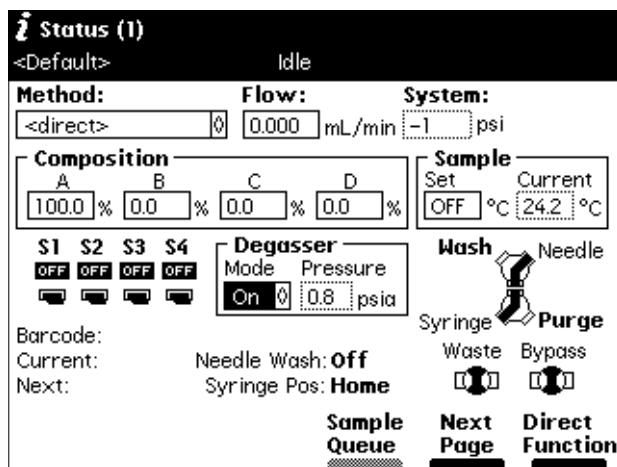
Figure 1-19 Status Screen

2. Turn on the degasser by selecting the **On** mode from the Degasser drop-down list (see the *Waters 2795 Separations Module Operator's Guide*, Section 4.3, Monitoring System Status).

3. Press the **Direct Function** screen key. A list of direct functions appears (Table 2-1).

4. Select the **Refresh Syringe** option, then press **Enter**. The Refresh Syringe dialog box appears (Figure 1-20).
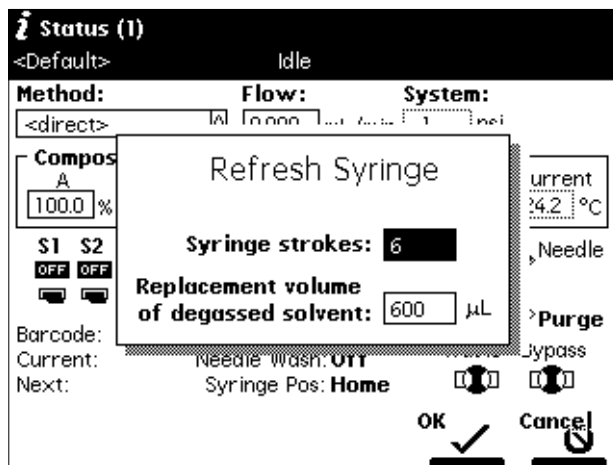
Figure 1-20 Refresh Syringe Dialog Box

5. Enter the number of syringe strokes and the replacement volume of degassed solvent in the appropriate field. Start with **3** syringe strokes and **600** μL replacement volume. To prime a new or dry instrument, or when you change the purge solvent, refer to the previous Note.

6. Press the **OK** screen key. The Refresh Syringe cycle begins.

1.6.4 Loading the Sample Plates

STOP **Attention:** *Using vial caps smaller than 8.8 mm in diameter (outer diameter) can damage the needle or XYZ mechanism.*

The 2795 Separations Module plate carrier holds up to four rectangular plates that you load through the front panel compartment door (Figure 1-21). Load each plate so that position A:1 (or 1) is located in the left-rear corner and the front edge of the plate is behind the guide button on the front edge of the carrier (Figure 1-21).

The plate carrier guides hold each plate loosely in place until the plate is moved to the Inject position. At that time, a plate positioning spring pushes the plate toward the A:1 (left rear) corner and holds it in position.

To move the plate carrier from one position to the next, press the **Next** screen key or the press the color-coded plate number screen key in the Door Is Open dialog box (Figure 1-22).

STOP **Attention:** *The 2795 Separations Module cannot draw or inject samples when the sample compartment door is open.*
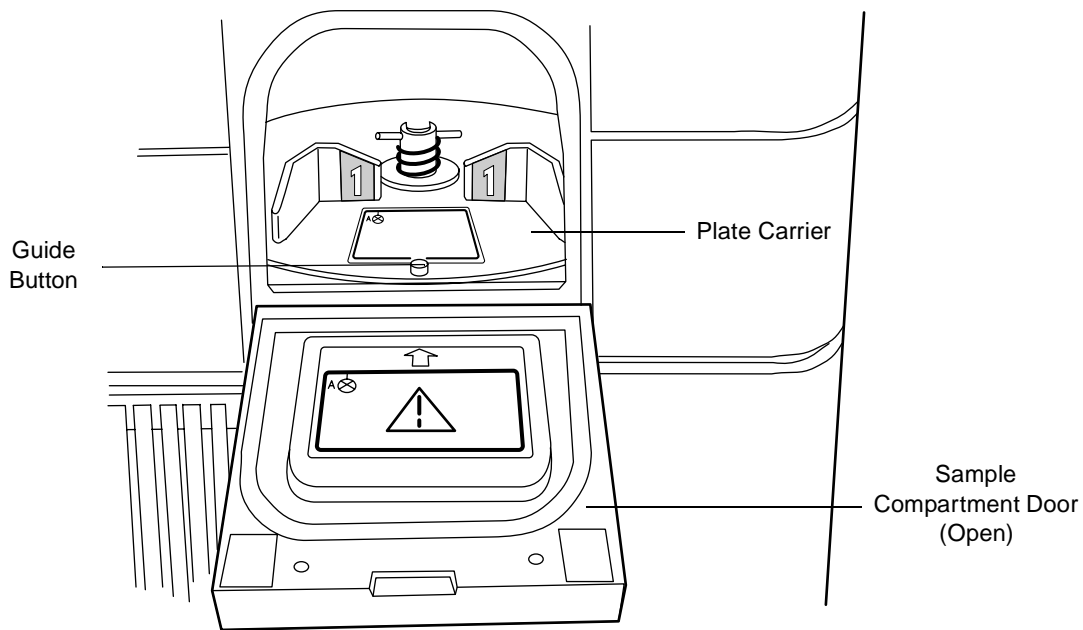
Figure 1-21 Positioning the Sample Plate

Note: *If you power on the 2795 Separations Module with the sample compartment door open, close the door, then reopen it to replace a sample plate on the carrier.*

To load the plates onto the plate carrier:

1. Open the 2795 Separations Module sample compartment door. The Door Is Open dialog box appears (Figure 1-22).

2. Press the **Next** screen key or select the desired plate number to position the plate carrier for loading the appropriate plate.

   Carrier plate positions are numbered 1 through 4, with individual color identification as follows:

   - Position 1 = Blue
   - Position 2 = Yellow
   - Position 3 = Red
   - Position 4 = Green

3. Load the plate into the sample compartment with well A:1 (or position 1) at the left rear position as the plate is loaded into the carrier.

4. Repeat steps 2 and 3 until all plates are loaded.

5. Close the sample compartment door. The Door Is Open dialog box closes and you return to the previous screen.
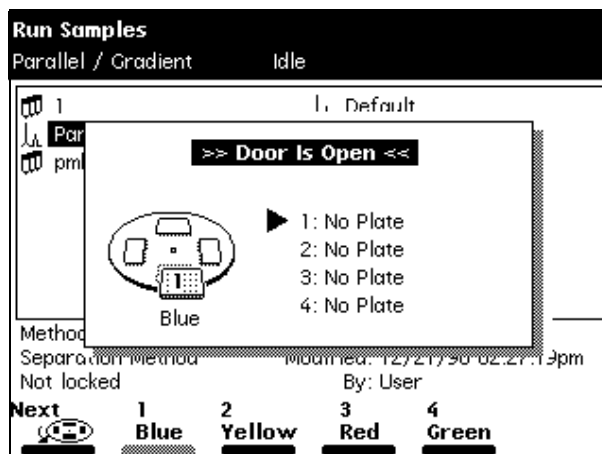
Figure 1-22 Door Is Open Dialog Box

The 2795 Separations Module uses *plate logic* (based on letter labels for rows and number labels for columns) to locate samples on the plates. For example, sample positions on a:
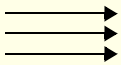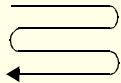
- 24-vial plate are designated A-D:1-6
- 48-well plate are designated A-F:1-8 or A-H:1-6

- 96-well plate are designated A-H:1-12
- 384-well plate are designated A-P:1-24

The 2795 Separations Module also uses a simple numerical well location scheme, where well number 1 is located at Row 1, Column 1. You can customize the processing order (also known as the *sequential reference*) in the Plate Type Configuration dialog box. See and .

Position A:1 (or 1) is always located at the left rear of a plate as it is loaded into the carrier. ().

Table 1-6 Sequential Well Location Schemes for Sample Processing Order

Processing Order	Icon	Function	Example (48 Wells)
Left to right, top to bottom		Increasing columns, then increasing rows. Also called Discontinuous (horizontal).	``` 1 2 3 4 5 6 7 8 A 1 2 3 4 5 6 7 8 B 9 10 11 12 13 14 15 16 C 17 18 19 20 21 22 23 24 D 25 26 27 28 29 30 31 32 E 33 34 35 36 37 38 39 40 F 41 42 43 44 45 46 47 48 ```
Top to bottom, left to right		Increasing rows, then increasing columns. Also called Discontinuous (vertical).	``` 1 2 3 4 5 6 7 8 A 1 7 13 19 25 31 37 43 B 2 8 14 20 26 32 38 44 C 3 9 15 21 27 33 39 45 D 4 10 16 22 28 34 40 46 E 5 11 17 23 29 35 41 47 F 6 12 18 24 30 36 42 48 ```
Horizontal serpentine		Left to right until end of row, then right to left starting at next row. Also called Continuous (horizontal).	``` 1 2 3 4 5 6 7 8 A 1 2 3 4 5 6 7 8 B 16 15 14 13 12 11 10 9 C 17 18 19 20 21 22 23 24 D 32 31 30 29 28 27 26 25 E 33 34 35 36 37 38 39 40 F 48 47 46 45 44 43 42 41 ```
Vertical serpentine		Top to bottom until end of column, then bottom to top starting at the next column. Also called Continuous (vertical).	``` 1 2 3 4 5 6 7 8 A 1 12 13 24 25 36 37 48 B 2 11 14 23 26 35 38 47 C 3 10 15 22 27 34 39 46 D 4 9 16 21 28 33 40 45 E 5 8 17 20 29 32 41 44 F 6 7 18 19 30 31 42 43 ```

For more information on configuring sample plates, see Section 1.2.5, Configuring Plate Types. For more information on selecting individual well locations or grouping wells, see Section 2.2.8, Selecting Wells for Making Injections.

1.7 Preparing the 2795 Separations Module for Operation

This section outlines in flowchart form the steps to prepare the 2795 Separations Module for operation starting from three different conditions:

- New or dry
- Idle or powered off with no solvent flow
- Changing solvents (for example, from a buffered to an organic solvent)

The priming, purging, equilibrating, and other procedures outlined here are described in detail in Section 2.1, Accessing Direct Functions.

Figure 1-23 outlines how to prepare a new or dry 2795 Separations Module for operation.

```
┌─────────────────┐
│      Start       │
└─────────────────┘
         │
         ▼
┌─────────────────┐      ┌─────────────────┐      ┌─────────────────┐
│ Fill the Solvent│      │    Prime the     │      │ If Running a    │
│ Reservoirs with │      │ Plunger-Seal-Wash│      │   Gradient (for │
│ Fresh Solvent   │      │      Pump         │      │ Example, A:B), Set│
│ Filtered and    │      │ (Section 1.5.3)   │      │ the A and B     │
│ Degassed by     │      │                  │      │ Composition Fields│
│ Vacuum Filtration│     │                  │      │ in the Status   │
│ (Section 1.5.2)ᵃ │      │                  │      │ Screen to 50%,  │
│                  │      │                  │      │ Then Wet Prime  │
└─────────────────┘      └─────────────────┘      │ for 2 min. at   │
         │                        │                │ 5.00 mL/min.    │
         ▼                        ▼                │ (Section 2.2.2) │
┌─────────────────┐      ┌─────────────────┐      └─────────────────┘
│ Set the Degasser│      │ Perform a Wet   │               │
│       to On      │      │ Prime (for One of│              ▼
│ (See "Equilibrating│    │ the Solvents) for 10│   ┌─────────────────┐
│ Solvents in the  │      │ min at 5.00 mL/min│     │ Equilibrate for a│
│ Vacuum Degasser" │      │ (Section 2.2.2)   │     │ Minimum of 10   │
│ in Section 2.2.5)│      │                  │      │ Column Volumes  │
└─────────────────┘      └─────────────────┘       │ (Section 2.2.5) │
         │                        │                └─────────────────┘
         ▼                        ▼                         │
┌─────────────────┐      ┌─────────────────┐                ▼
│ Perform a Dry   │      │ Prime the Needle-│      ┌─────────────────┐
│ Prime to Fill Each│     │   Wash Pump      │      │ Refresh the Syringe│
│ Solvent Line and │      │ (Section 1.6.2)   │      │ for a Minimum of 80│
│ Degasser Chamber │      │                  │      │ Cycles and a    │
│ (Section 2.2.1)  │      │                  │      │ Replacement     │
└─────────────────┘      └─────────────────┘       │ Volume of 600 μL│
         │                        │                │ (Section 1.6.3) │
         ▼                        ▼                └─────────────────┘
┌─────────────────┐      ┌─────────────────┐                │
│ Equilibrate the │      │ Repeat the Wet  │                ▼
│ Solvent in the  │      │ Prime at the Same│      ┌─────────────────┐
│ Degasser Chamber│      │ Rate and Time for│      │ System Is Ready │
│ for 5 min at a Flow│    │ Each of the     │      └─────────────────┘
│ Rate of 0.000   │      │ Remaining Solvents│
│ mL/minᵃ (See    │      └─────────────────┘
│ "Equilibrating  │
│ Solvents in the │
│ Vacuum Degasser"│
│ in Section 2.2.5)│
└─────────────────┘
```

a. To maximize degasser efficiency, Waters recommends that all five degassing chambers be filled with eluent. If you choose water as an "unused" solvent, change the water weekly to prevent bacterial contamination.

Figure 1-23 Preparing a New or Dry 2795 Separations Module for Operation

Figure 1-24 outlines how to prepare for operation a 2795 Separations Module that is idle or powered off with no solvent flow.

```
┌─────────────────────────┐
│          Start          │
└─────────────────────────┘
              │
              ▼
┌─────────────────────────┐
│ Set the Degasser to     │
│ On                      │
│ (See "Equilibrating     │
│ Solvents in the         │
│ Vacuum Degasser"        │
│ in Section 2.2.5)       │
└─────────────────────────┘
              │
              ▼
┌─────────────────────────┐
│ Perform a Wet Prime     │
│ (for One of the         │
│ Solvents) for 2 min at  │
│ 7.500 mL/min            │
│ (Section 2.2.2)         │
└─────────────────────────┘
              │
              ▼
┌─────────────────────────┐
│ Repeat the Wet          │
│ Prime at the Same       │
│ Rate and Time for       │
│ Each Solvent            │
└─────────────────────────┘

┌─────────────────────────┐
│ Equilibrate the         │
│ Solvent in the          │
│ Degasser Chamber        │
│ for 5 min at a Flow     │
│ Rate of 0.000           │
│ mL/min (See             │
│ "Equilibrating          │
│ Solvents in the         │
│ Vacuum Degasser"        │
│ in Section 2.2.5)       │
└─────────────────────────┘
              │
              ▼
┌─────────────────────────┐
│ If You Are Running a    │
│ Gradient (for           │
│ Example, A:B), Set      │
│ the A and B             │
│ Composition Fields      │
│ in the Status Screen    │
│ to 50%, Then Wet        │
│ Prime for 3 min at      │
│ 5.00 mL/min             │
│ (Section 2.2.2)         │
└─────────────────────────┘

┌─────────────────────────┐
│ Equilibrate for a       │
│ Minimum of 10           │
│ Column volumes          │
│ (Section 2.2.5)         │
└─────────────────────────┘
              │
              ▼
┌─────────────────────────┐
│ Refresh the Syringe     │
│ for a Minimum of        │
│ 3 Cycles and a          │
│ Replacement             │
│ Volume of 600 μL        │
│ (Section 1.6.3)         │
└─────────────────────────┘
              │
              ▼
┌─────────────────────────┐
│    System Is Ready      │
└─────────────────────────┘
```
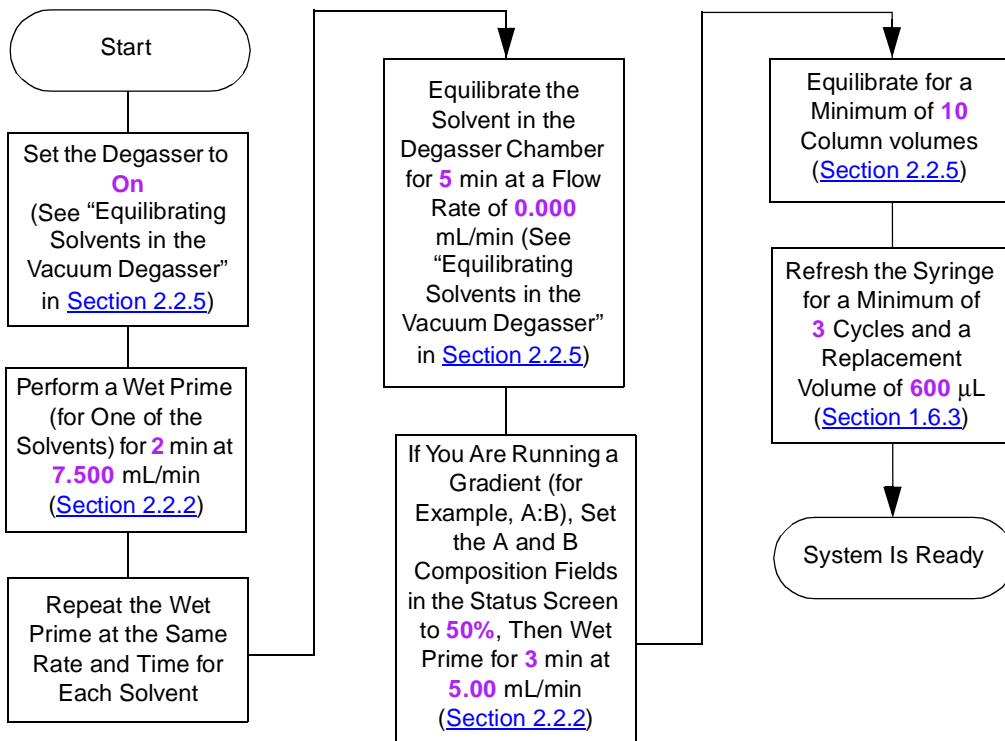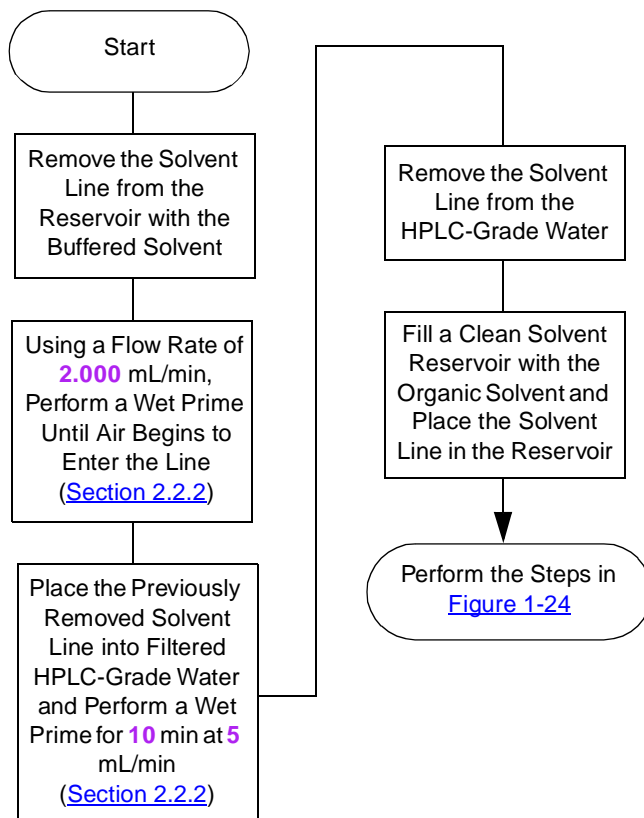
Figure 1-24 Preparing an Idle or Powered-Off 2795 Separations Module for Operation

[Figure 1-25](#) outlines how to change from a buffered solvent to an organic solvent.

Figure 1-25 Changing from a Buffered Solvent to an Organic Solvent

STOP ***Attention:*** *To avoid precipitating salts in the 2795 Separations Module, use an intermediate solvent such as water when you change from buffers to high-organic-content solvents. For more information, refer to the* Waters 2795 Separations Module Operator's Guide*, Section C.3, Solvent Miscibility.*

Chapter 2
Performing Direct Functions

You can perform many functions through the front panel controls when the 2795 Separations Module is in System Controller mode or No Interaction mode (see the *Waters 2795 Separations Module Operator's Guide,* Section 3.2.3, Configuring the Operating Mode*)*.

You perform direct functions as needed during operation. You can also specify these functions be performed automatically as part of a separation method (see the *Waters 2795 Separations Module Operator's Guide*, Section 5.1, Creating and Editing Separation Methods).

Direct functions are:

- Dry priming (Section 2.2.1)
- Wet priming (Section 1.2.2)
- System preparation (Section 1.4)
- Refreshing the syringe (Section 1.6.3)
- Purging the 410 or 2410 (if RI detector is installed) (Section 2.2.3)
- Washing the needle (Section 2.2.4)
- Equilibrating the system (Section 2.2.5)
- Conditioning the column (Section 2.2.6)
- Injecting samples (Section 2.2.7)

Figure 2-1 outlines the procedure for using the 2795 Separations Module front panel to perform direct functions.

Note: To perform direct functions when the 2795 Separations Module is controlled by the MassLynx software, you need to exit MassLynx control and configure the 2795 Separations Module for No Interaction mode (see the Waters 2795 Separations Module Operator's Guide*, Section 3.2.3, Configuring the Operating Mode).*

For information on configuring the MassLynx software, see the *MassLynx Data Acquisition Guide* or the *Waters Alliance LC/MS System Quick Start Guide*.
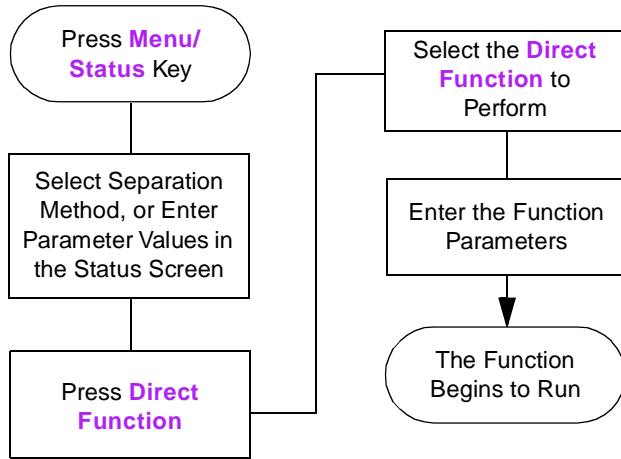
Figure 2-1 Steps to Setting Up a Direct Function Control Run

STOP *Attention:* *If you make any changes to an existing separation method in the Status screen, the Method field changes to <direct>, the separation method name in the banner area is enclosed within brackets (< >), and the current isocratic conditions (with no timed events) are applied.*

2.1 Accessing Direct Functions

To access direct functions:

1. Press **Menu/Status** to display the Status screen (Figure 1-19).

2. Select an existing separation method or enter parameter values.

3. Press the **Direct Function** screen key. A list of direct functions appears (Figure 2-2). Table 2-1 describes the direct functions.
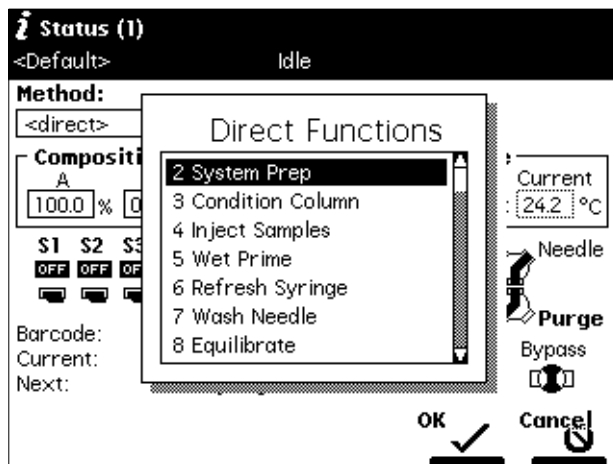
Figure 2-2 Direct Functions List

4. Select a function, then press **Enter**. Follow the instructions for each function, as described in the following sections. See the *Waters 2795 Separations Module Operator's Guide*, Section 3.1.2, Using the Keypad, the "Entering and Editing Values" discussion, if necessary.

Table 2-1 Direct Functions

Direct Function	Description	Reference
Dry Prime	Opens the fluidic path (from the selected solvent reservoir to the prime/vent valve) to replace air with solvent, then performs a prime.	Section 2.2.1, Dry Priming
Wet Prime	Replaces solvent in the fluidic path from the reservoirs through the Prime port of the inject valve to waste. Use to change the solvent(s) in the system.	Section 2.2.2, Wet Priming
Refresh Syringe	Refills the syringe with fresh, degassed, purge solvent.	Section 1.6.3, Refreshing the Syringe
Purge 410/2410 (if applicable)	Purges the Waters 410 or 2410 RI detector reference cell.	Section 2.2.3, Purging the 410 or 2410 Reference Cell

Table 2-1 Direct Functions *(Continued)*

Direct Function	Description	Reference
Wash Needle	Washes both the outside and inside of the needle using wash solvent.	Section 2.2.4, Washing the Needle
Equilibrate	Selects a column position (if applicable) and specifies the system equilibration time.	Section 2.2.5, Equilibrating the System
Condition Column	Selects a column position (if applicable) and delivers mobile phase using the gradient table specified in the current separation method.	Section 2.2.6, Conditioning the Column
Inject Samples	Injects a sample one or more times from the specified well(s) using the selected method.	Section 2.2.7, Injecting Samples
System Prep	Used to prepare the 2795 Separations Module for operation. Supported in both direct and sample set modes.	Section 1.4, System Preparation Function

2.2 Using Direct Functions

2.2.1 Dry Priming

Use the dry prime option to prime the system when the fluidic path in the solvent management system is filled with air.

Required Material

You need a 30-mL syringe (Startup Kit).

Procedure

To perform a dry prime:

1. Set up the reservoirs as described in Section 1.5.1, Preparing Solvent Reservoirs.
2. Insert the solvent tubing into the appropriate reservoir(s). Be sure the detector waste line and the sample loop waste line drain into an appropriate container.
3. Gently shake the filters in the reservoirs to remove any bubbles that may be trapped.
4. Attach an empty syringe to the prime/vent valve, as shown in Table 2-3, then open the valve by turning it counterclockwise 1/2-turn.
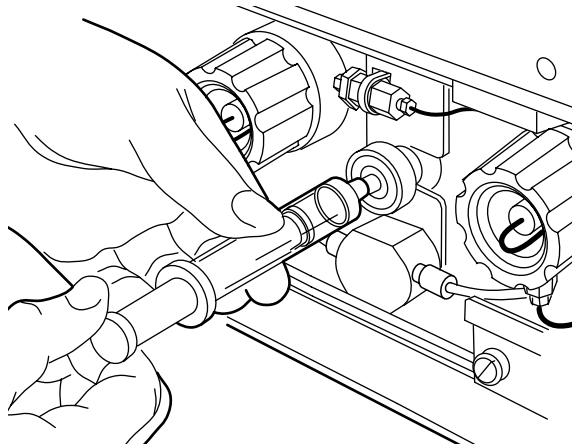5. Press **Menu/Status** to display the Status screen (Figure 1-19).

Figure 2-3 Prime/Vent Valve with Syringe

Note: *The syringe does not lock onto the prime/vent valve; hold it in place while you pull on the plunger.*

6. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).

7. Select **Dry Prime**, then press **Enter**. The Dry Prime dialog box appears (Figure 2-4).

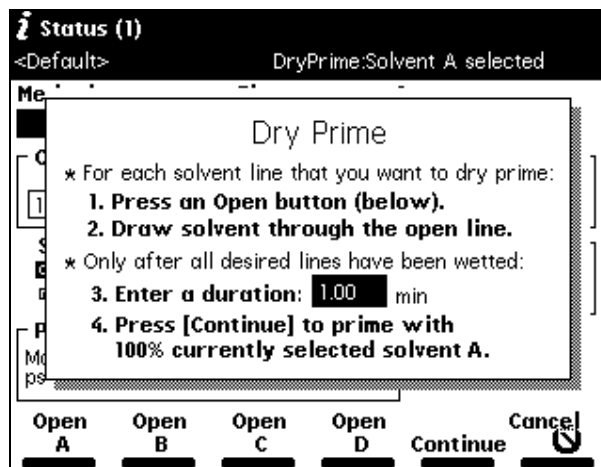Figure 2-4 Dry Prime Dialog Box

8. Press the screen key corresponding to the solvent line you want to prime.

9. Withdraw the syringe plunger to pull solvent through the tubing. You may need to exert force to pull the air and solvent through the system. Continue until you pull all the air through the solvent line into the syringe.

10. Repeat steps 7 to 9 for each solvent line you want to prime, then close the prime/vent valve.

11. Press a screen key corresponding to the solvent line with which you want to prime the system.

    Note: *Waters recommends that you prime using the solvent with the lowest viscosity to help purge air from the lines.*

12. Enter the length of time (in minutes) to prime the solvent management system in the Enter a duration field. Start with a value of **5** minutes. Press the **Continue** screen key.

    The solvent management system starts the priming function. At the end of the priming period, the solvent management system turns off and the 2795 Separations Module enters the Idle mode.

13. Perform a wet prime, as described next.

2.2.2 Wet Priming

STOP **Attention:** *To avoid damaging the plunger seals, perform a wet prime only when there is solvent in the solvent management system fluidic path.*

Perform a wet prime:

- When you want to change reservoirs or solvents
- If the 2795 has been in an idle mode
- If you have just completed a dry prime

The wet prime replaces solvent in the path from the reservoirs through the Prime position of the inject valve to waste. If the solvent lines are dry, perform the dry prime procedure before continuing (see Section 2.2.1, Dry Priming).

STOP **Attention:** *To avoid having salts precipitate in the 2795 Separations Module, use an intermediate solvent such as water when you change from buffers to high-organic-content solvents. Refer to the* Waters 2795 Separations Module Operator's Guide*, Section C.3, Solvent Miscibility, for information on solvent miscibility.*

To perform a wet prime:

1. Press **Menu/Status** to display the Status screen (Figure 1-19).
2. In the Composition field, enter **100%** for the solvent you want to use for wet priming.

   Note: Waters recommends that you start the wet prime using the solvent with the lowest viscosity to help purge air from the lines.

3. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).
4. Select **Wet Prime**, then press **Enter**. The Wet Prime dialog box appears (Figure 2-5).
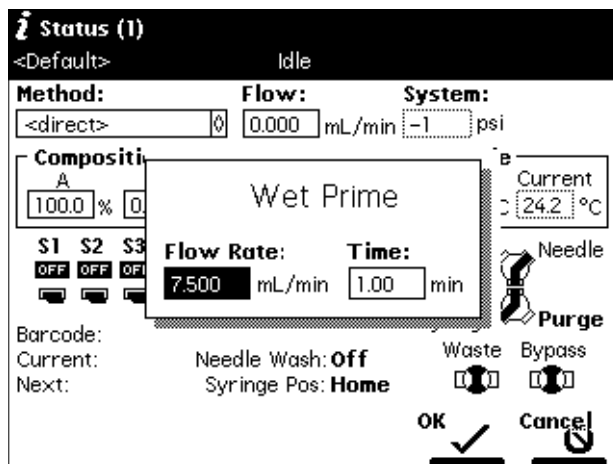
Figure 2-5 Wet Prime Dialog Box

5. Gently shake the filters in the reservoirs to remove any bubbles that may have formed.

6. Enter the flow rate and time (duration) to prime (using the composition entered in the Status screen), then press the **OK** screen key. The solvent management system begins the priming function.

    At the end of the time period you specify, the solvent management system returns to the previous conditions, and the 2795 Separations Module enters the Idle mode.

7. Repeat steps 1 to 6 for each solvent, as appropriate.

Equilibrating Solvents in the Vacuum Degasser

Wet priming occurs at flow rates that do not allow for thorough solvent degassing. Therefore, after you perform a wet prime, the solvent remaining in the degasser chamber lines needs to be degassed before solvent flow resumes. This is accomplished by setting a zero flow rate for a short time period.

For more information on vacuum degassing, refer to the *Waters 2795 Separations Module Operator's Guide*, Section 1.2.2, Solvent Management System Features, the "Inline Vacuum Degasser" discussion.

To equilibrate solvents in the inline vacuum degasser after you have wet primed the system:

1. Press **Menu/Status** to display the Status screen (Figure 1-19).

2. Enter the initial solvent composition for the run.

3. Set the Degasser Mode parameter to **On**.

4. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).

5. Select **Wet Prime**, then press **Enter**. The Wet Prime dialog box appears (Figure 2-5).

6. Enter a flow rate of **0.000** mL/min. and time of **5** minutes, then press the **OK** screen key. At the end of the time period, the solvents are degassed and the 2795 Separations Module enters the Idle mode.

The solvents in the inline vacuum degasser are now equilibrated and the 2795 Separations Module is prepared to run.

2.2.3 Purging the 410 or 2410 Reference Cell

Purge the 410 or 2410 RI detector reference cell (and fluidic path) when you change solvents or experience an unexpected loss in sensitivity due to excess noise or drift.

To purge the 410 or 2410 reference cell:

1. Press **Menu/Status** to display the Status screen (Figure 1-19).

2. Enter the flow rate and composition with which to purge the 410 or 2410 reference cell.

3. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).

4. Select **Purge 410/2410**, then press **Enter**. The Purge 410/2410 dialog box appears (Figure 2-6).

5. Enter the amount of time (in minutes) to pump fresh solvent through the reference cell, then press the **OK** screen key. The timer counts down as the solvent is pumped through the detector reference cell.
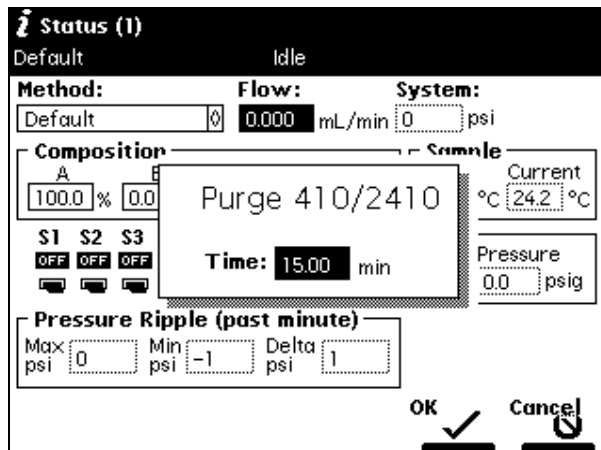
Figure 2-6 Purge 410/2410 Dialog Box

2.2.4 Washing the Needle

The Wash Needle function:

- Washes the inject port and the interior and exterior of the needle
- Flushes the wash solvent out of the needle and inject port and replaces it with degassed purge solvent

Use the Wash Needle function to:

- Ensure that the needle and inject port are clean before the start of a new method
- Verify that the needle-wash system is primed and operating properly by checking for flow through the waste tube

Note: If the 2795 Separations Module does not detect that a needle wash was performed since the last time the 2795 Separations Module was powered on, it performs an automatic needle wash before beginning any injections, either those specified in a sample set or by using the Inject Samples direct function.

Note: Waters recommends using a high percentage organic wash solvent that is compatible with your mobile phase and sample diluent, such as methanol or acetonitrile. See Table 1-5.

STOP *Attention: Make sure the needle-wash solvent line is primed before you perform a needle wash (see Section 1.6.2, Priming the Needle-Wash Pump).*

To wash the needle:

1. Press **Menu/Status** to display the Status screen (Figure 1-19).
2. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).
3. Select **Wash Needle**, then press **Enter**. The Wash Needle dialog box appears (Figure 2-7).
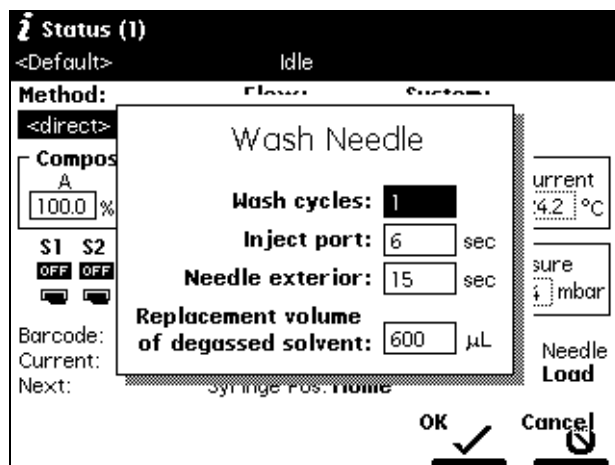

Figure 2-7 Wash Needle Dialog Box

4. Enter the number of wash cycles in the Wash cycles field (the default is 1).
5. Enter the amount of time (in seconds) to wash the inject port and the needle exterior in the appropriate fields.
6. Enter the replacement volume of degassed solvent in the appropriate field. Start with **600** μL replacement volume.
7. Press the **OK** screen key. The Wash Needle cycle begins.

   If necessary, press the **Stop WashNdl** screen key or the **Stop Flow** key to suspend the wash cycle. When solvent flow stops, press the **Abort WashNdl** screen key to end the Wash Needle cycle.

At the end of the final wash cycle, the Wash Needle function automatically flushes out the needle-wash solvent and replaces it with degassed purge solvent.

2.2.5 Equilibrating the System

During equilibration of the HPLC or LC/MS system, the 2795 delivers solvents and maintains separation parameters (such as sample and column temperature, degasser

operation, and so on) at the conditions shown in the Status screen. The time needed to equilibrate the system depends on environmental and application-specific factors.

To equilibrate the system:

1. Press **Menu/Status** to display the Status screen (Figure 1-19).

2. Enter the desired initial conditions. Alternatively, in the Method field, select the separation method that contains the initial conditions for equilibration.

3. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).

4. Select **Equilibrate**, then press **Enter**. The Equilibrate dialog box appears (Figure 2-8).
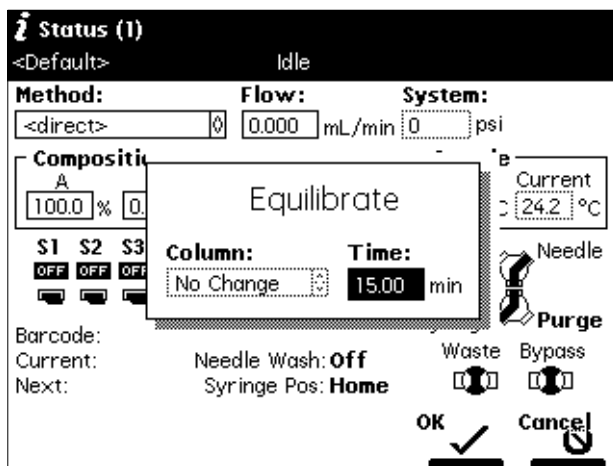
Figure 2-8 Equilibrate Dialog Box

5. Enter the amount of time to equilibrate the system and the column position to equilibrate (if applicable).

6. Press the **OK** screen key. When the equilibration period ends, the 2795 enters the Idle state. The flow rate remains at the value in the Flow field in the Status screen.

2.2.6 Conditioning the Column

Conditioning the column involves running a solvent gradient through the column without injecting samples or running the Events table. You select a separation method (see the *Waters 2795 Separations Module Operator's Guide*, Chapter 5, Creating and Editing Separation Methods and Sample Sets) that contains the gradient parameters you want to use, then the 2795 Separations Module runs the gradient.

Note: *To run timed events without injecting a sample, select the **Inject Samples** option from the Direct Functions list and enter an injection volume of **0** (see Section 2.2.7, Injecting Samples).*

To condition the column:

1. Press **Menu/Status** to display the Status screen.
2. In the Method drop-down list, select the separation method with the gradient parameters you want to use to condition the column, then press **Enter**.
3. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).
4. Select **Condition Column**, then press **Enter**. The Condition Column dialog box appears (Figure 2-9).
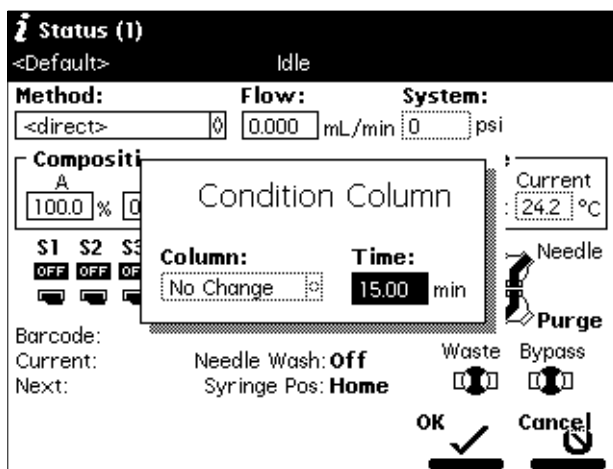
Figure 2-9 Condition Column Dialog Box

5. In the Column field, enter the column position to condition (if applicable).
6. In the Time field, enter the amount of time to condition the column. Ensure that the time is equal to or greater than the time for the gradient defined in the selected separation method plus any reequilibration time.
7. Press the **OK** screen key. The solvent management system runs the column conditioning procedure for the specified time.

2.2.7 Injecting Samples

Using the front panel, you can inject one or more samples from one or more wells in a sample plate. If no separation method is selected, the 2795 Separations Module uses the

instrument conditions displayed in the Status screen. You can use gradients and timed events by selecting a separation method in the Method field. The current separation method is displayed in the banner area.

STOP *Attention: If you make any changes to an existing method in the Status screen, the Method field changes to <direct>, the separation method name in the banner area is enclosed within angle brackets (< >), and the current isocratic conditions (with no timed events) are applied.*

To inject a sample:

1. Place the prepared sample(s) or vial(s) into a plate, then insert the plate in the sample compartment (see the *Waters 2795 Separations Module Operator's Guide*, Section 3.4.3, Loading the Sample Plates).

2. Close the sample compartment door.

3. Press **Menu/Status** to display the Status screen (Figure 1-19).

4. Enter the initial conditions. Alternatively, you can select **Method**, then select the separation method that contains the conditions to perform the injection.

5. Press the **Direct Function** screen key. The Direct Functions list appears (Figure 2-2).

6. Select **Inject Samples**, then press **Enter**. The Inject Samples dialog box appears (Figure 2-10), displaying six parameter fields. Table 2-2 describes the parameters in the Inject Samples dialog box.
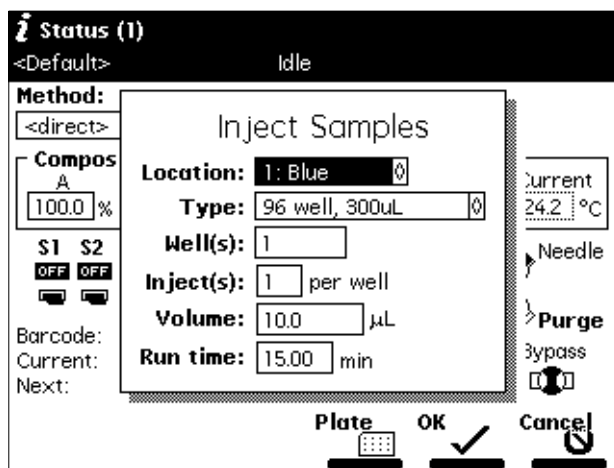
Figure 2-10 Inject Samples Dialog Box

7. Enter a value in each field, then press **Enter**.

8. Press the **OK** screen key to begin the injection(s), or press **Cancel** to return to the previous screen without injecting any samples.

Table 2-2 Inject Samples Dialog Box Parameters

Parameter	Description	Value Range
Location	Specifies the plate carrier location.	1 to 4
Type	Specifies the plate type.	One of up to 15 (see <u>Section 1.2.5, Configuring Plate Types</u>)
Well(s)	Specifies the well number or range from which you want to make injections. Alternatively, you can press the **Plate** screen key to select one or more wells using the Well Selector (see <u>Section 2.2.8</u>, Selecting Wells for Making Injections). When entering a well range, use the • key to separate entries. For example, to inject samples from wells 1 to 20, enter **1.20**.	Depending on plate type, 1 to 24, 48, 96, or 384
Inject(s)	Specifies the number of injections per well.	1 to 99
Volume	Specifies the volume for each injection. See the *Waters 2795 Separations Module Operator's Guide*, Section 5.3.7, Setting Sample Set Parameter Values, the "Injection Volume" discussion.	Maximum injection volume depends on the size of the sample loop or 60% of syringe volume, whichever is smaller. *Note: If the most recently applied separation method specifies a Full Loop, then the Volume field is not editable. See the* Waters 2795 Separations Module Operator's Guide, *Section 5.1, Creating and Editing Separation Methods.*
Run time	Specifies the run time for each injection	0 to 999.99 minutes

2.2.8 Selecting Wells for Making Injections

You can specify the well(s) from which to make injections by using the Well Selector function. This function is available from any screen that displays the **Plate** screen key (Configuration, Sample Set, and Run Samples screens).

To access the Well Selector when you are performing an Inject Samples direct function (Figure 2-10), press the **Plate** screen key. The Well Selector is displayed (Figure 2-11).
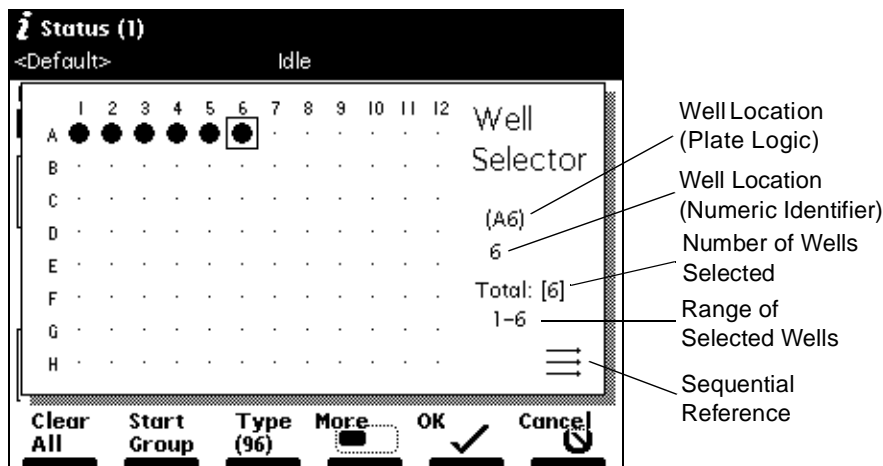

Figure 2-11 Well Selector

To select the plate type:

1. Press the **Type** screen key, then select from the drop-down list of available plate types.

2. Press **Enter** to accept the selected plate, or press the **Cancel** screen key to return to the Well Selector without making a selection.

To select a single well:

1. Press the **Set by Number** screen key, then enter the well number. Alternatively, you can use the arrow keys to move to the specific well you want to specify.

2. Press • to set the selected well, or press **Clear** to clear the selected well. To switch a well to its opposite state (selected to deselected or vice versa), press **Enter**.

To select all the wells on the selected plate, press the **Select All** screen key. The key name changes to **Clear All**.

To deselect all selected wells on the selected plate, press the **Clear All** screen key. The key name changes to **Select All**.

To select a group of wells (two or more) in a rectangular area of the selected plate:

1. Press the **Start Group** screen key.
2. Use the arrow keys to outline the group of wells.
3. Press • to set all the selected wells, or press **Clear** to clear the selected wells. To switch each well in a group to its opposite state (selected to deselected or vice versa), press **Enter**.

Press the **Print** screen key to print the Well Selector page currently displayed.

Press the **OK** screen key after you have selected a group of wells to return to the Inject Samples dialog box or the sample set table, or press the **Cancel** screen key to return to the Inject Samples dialog box without specifying one or more wells graphically.

Note: When a collection contains noncontiguous wells, the wells are indicated in the ***Well(s)*** *field of the Inject Samples dialog box or the Sample Set table by the total number of wells in square brackets ([…]). See the* Waters 2795 Separations Module Operator's Guide*, Section 5.3, Creating and Editing Sample Sets, for more information.*

Note: The 2795 Separations Module provides predefined as well as custom plate types. For more information, see Section 1.2.5, Configuring Plate Types.

Chapter 3
Creating Methods

This chapter provides an overview of creating methods when the 2795 Separations Module is in a standalone mode (System Controller or No Interaction). To create methods when the 2795 Separations Module is under control of the MassLynx software, refer to the *Waters Alliance LC/MS Quick Start Guide*.

The term *method* refers to the instructions that define how your samples are chromatographically separated. There are two kinds of methods:

- **Separation method** – A set of time-based and nontime-based conditions that influence the separation.
- **Sample set** – A set of instructions in table format that applies functions to separation methods.

For complete information on creating and editing separation methods and sample sets, see the *Waters 2795 Separations Module Operator's Guide*, Chapter 5.

Separation Methods

Some of the conditions you can specify in a separation method are:

- Mobile phase composition and flow rate
- Sample temperature
- Column temperature
- I/O parameters
- Column selection
- Normal or high-throughput injection modes
- Autosampler parameters
- Full and partial loop control

Sample Sets

A sample set applies the following functions to a separation method:

- Inject samples or standards
- Auto standards
- Refresh syringe
- Prime

- Condition column
- Equilibrate
- Wash needle
- Pool
- Auto add
- System Prep

Note: The Pool and Auto Add functions are available only when the 2795 Separations Module is configured in a standalone mode (System Controller or No Interaction).

You can use multiple separation methods in a sample set.

You create and edit separation methods and sample sets using the Methods screen (Figure 3-1) and six associated screens. In the Methods screen, separation methods are identified by a chromatogram icon ⌡ and sample sets are identified by a multiple vial icon ▯ .
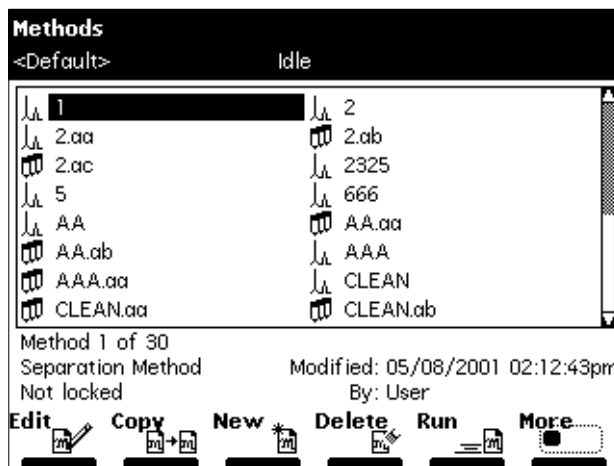
Figure 3-1 Methods Screen

3.1 Creating Separation Methods

To create a separation method using the 2795 Separations Module front panel:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears (Figure 3-1).

2. Press the **New** screen key. The New Method dialog box appears (Figure 3-2).
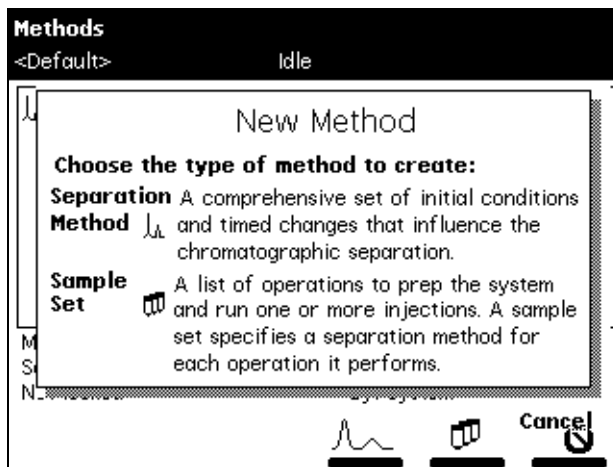
Figure 3-2 New Method Dialog Box

3. Press the chromatogram icon ⌡ₐ (separation method) screen key. The Separation
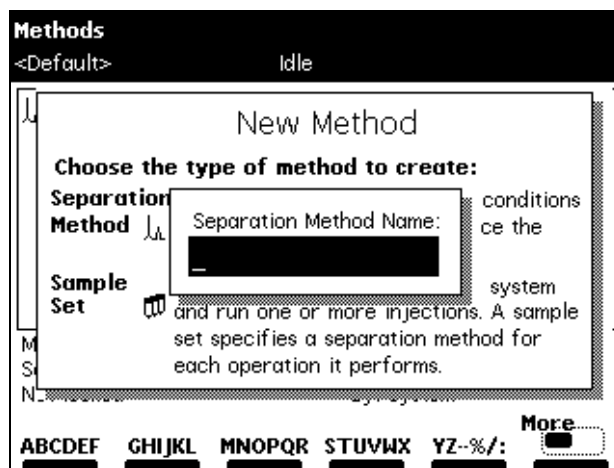   Method Name dialog box appears (Figure 3-3).

Figure 3-3 Separation Method Name Dialog Box

4. Use the screen keys to enter a name of up to 30 characters (see the *Waters 2795 Separations Module Operator's Guide*, Section 3.1.2, Using the Keypad, the "Entering an Alphanumeric String" discussion).

5. Press **Enter**. The Mobile Phase screen appears (Figure 3-4). This screen is one of six parameter screens. The other five parameter screens are Sample, Autosampler, Column, I/O, and Detectors.
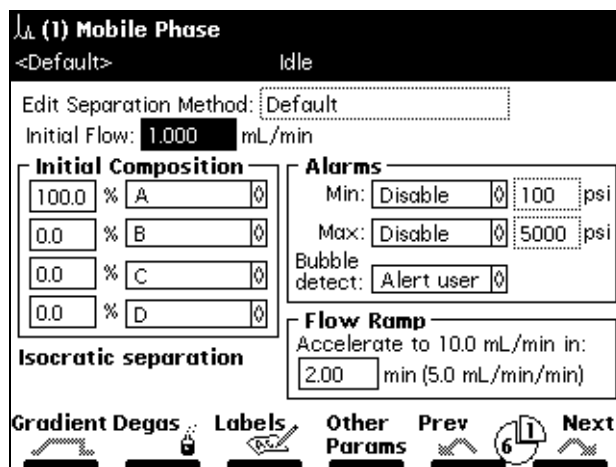
Figure 3-4 Mobile Phase Screen

6. Select values for the parameter fields in the Mobile Phase screen, using the arrow keys to move between fields (see the *Waters 2795 Separations Module Operator's Guide*, Section 5.2, Setting Separation Method Parameters).

7. Press the **Next** or **Previous** screen key to navigate among the six method parameter screens.

8. Select values for the parameter fields in each of the other five separation method parameter screens. Refer to the *Waters 2795 Separations Module Operator's Guide*, Section 5.2, Setting Separation Method Parameters, for details on specifying parameter values in separation methods.

9. To save the new method, press **Exit**. When prompted to save, press the **Yes** screen key.

3.2 Creating Sample Sets

To create a sample set:

1.   Press the **Develop Methods** screen key in the Main screen. The Methods screen appears (Figure 3-1).

2.   Press the **New** screen key. The New Method dialog box appears (Figure 3-2).

3.   Press the multiple vial icon 🔲 (sample set) screen key. The Sample Set Name dialog box appears.

4.   Enter a name of up to 30 characters (see the *Waters 2795 Separations Module Operator's Guide*, Section 3.1.2, Using the Keypad).

5.   Press **Enter**. The Sample Set screen appears (Figure 3-5).

Note: When the Sample Set screen appears initially, it is in the Functional View (see the Waters 2795 Separations Module Operator's Guide*, Section 5.3.6, Viewing Sample Sets).*

Figure 3-5 Sample Set Screen (Functional View)

6.   Enter parameter values in the sample set table as appropriate (see the *Waters 2795 Separations Module Operator's Guide*, Section 5.3.7, Setting Sample Set Parameter Values, and Section 5.3.8, Setting Sample Set Functions).

7. When you finish entering values and functions in the sample set table, press **Exit**, then press the **Yes** screen key to save the sample set.

   To exit without saving the sample set, press the **No** screen key.

   To return to the sample set without entering any changes, press the **Cancel** screen key.

Chapter 4
Making Automatic Runs

This chapter provides an overview of the steps to running methods and sample sets, performing preventive maintenance, and powering off the 2795 Separations Module. For information on high-throughput applications, see the *Waters 2795 Separations Module Operator's Guide*, Section 6.2, Using the 2795 High-Throughput Capabilities.

4.1 Making Automatic Runs in a Stand-Alone Mode

Figure 4-1 summarizes the steps for making an automatic run in a stand-alone mode (System Controller or No Interaction).
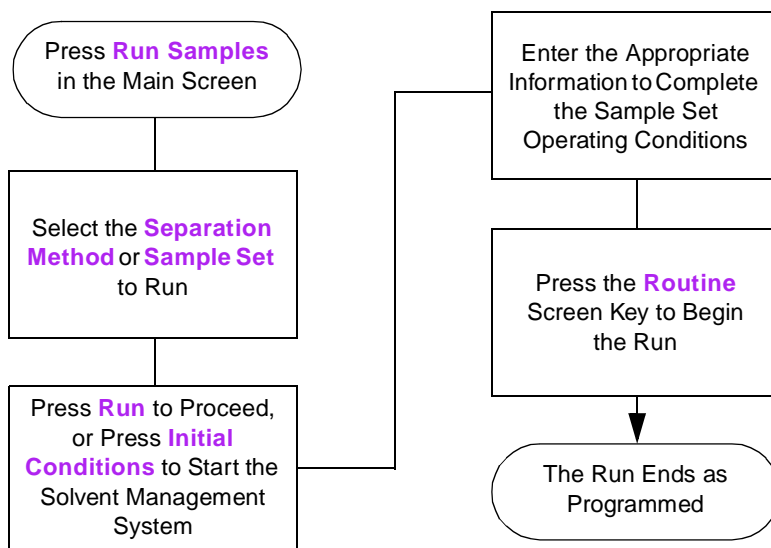
Figure 4-1 Making an Automatic Run in a Stand-Alone Mode

4.1.1 Running a Sample Set

Note: Refer to the Waters 2795 Separations Module Operator's Guide*, Chapter 5, Creating and Editing Separation Methods and Sample Sets, for information on creating, storing, and importing separation methods and sample sets.*

To run a sample set in a stand-alone (System Controller or No Interaction) mode:

1. Press the **Run Samples** screen key in the Main screen. The Run Samples screen appears (Figure 4-2), listing the available separation methods and sample sets.
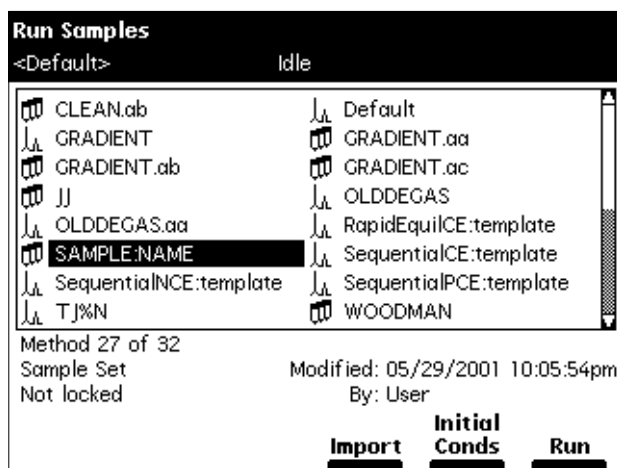

Figure 4-2 Run Samples Screen

2. Select a sample set or separation method, then press one of the following screen keys (or press the **Import** screen key to import a separation method or sample set from a floppy disk):

    *   **Initial Conds** – Starts the solvent management system at the initial conditions specified in the first line of the sample set and begins equilibrating the temperature in the sample heater/cooler and column heater. When the system is equilibrated, press the **Run** screen key.
    *   **Run** – Displays the selected sample set table (to view or edit) as shown in Figure 4-3. Use the screen keys to modify the table.

Figure 4-3 Run Samples Screen (Functional View)

> **Note:** *You can modify the sample set before you press the **Start** screen key.*

3. Load the samples into the plate(s), according to the locations specified in the sample set (see Section 1.6.4, Loading the Sample Plates). Use the Injection, Loading, and Plate Loading views to view the sample set (see the *Waters 2795 Separations Module Operator's Guide*, Section 5.3.6, Viewing Sample Sets).

    If necessary, modify the sample set. Press the **Save Method** screen key to save the modified sample set as a new method.

> **Note:** *You can edit a sample set only in the Functional view.*

4. Press the **Start** screen key. The Requirements screen appears (Figure 4-4). Check that the information in this screen is correct.

5. If a printer is connected to your system, press the **Print** screen key to print the contents of the Requirements screen and a listing of the injections.

6. Press the **Routine** screen key to start a normal run, or press the **STAT** screen key to make a STAT run.

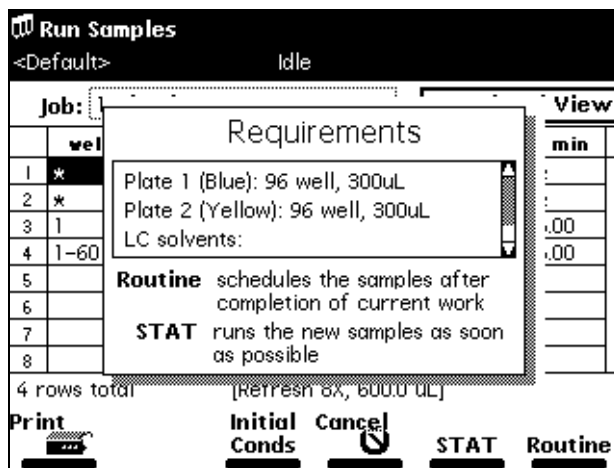   To make a STAT run, or to modify a run in progress, refer to Section 4.1.2, Modifying a Sample Set During a Run.

Figure 4-4 Requirements Screen

4.1.2 Modifying a Sample Set During a Run

You can modify operating conditions during a run in a stand-alone mode by:

- Performing a STAT run
- Editing the separation method

Performing a STAT Run

Use the STAT run function to interrupt a run in progress, perform one or more injections after the current injection is done, then continue with the scheduled run. Performing a STAT run is most useful when the separation methods for the STAT run and the scheduled run use identical solvents and temperatures.

STOP *Attention: If the 2795 Separations Module sample management system is drawing or injecting a sample when you start a STAT run, the plate carrier cannot move and a warning message is displayed if a plate position change is specified. Wait for the sample injection to finish before continuing.*

To perform a STAT run while a sample set is running:

1. Load the STAT run samples into the sample plate, then place the plate in the sample compartment and close the sample compartment door.

2. Press the **Run Samples** screen key in the Main screen. The Run Samples screen appears (Figure 4-2).

3. Select the separation method or sample set to use for the STAT run, then press the **Run** screen key.

4. Enter the well position(s) and other parameters in the appropriate fields.

5. Press the **Start** screen key. The Requirements screen appears (). Check that all information in this screen is correct.

6. Press the **STAT** screen key.

   After the STAT run is complete, the original run resumes immediately.

Modifying a Separation Method During a Run

You can modify a separation method while a sample set is running.

If the separation method you are modifying is being used for the current injection, the current injection is not affected by the changes. You need to save the changes to the separation method before you can apply the modified separation method to any subsequent injections. Use the procedures in the *Waters 2795 Separations Module Operator's Guide*, Section 5.1.2, Editing Separation Methods, to modify and save the separation method.

4.1.3 Stopping a Run

To stop a run in progress, press one of the following keys in the Status screen:

- **Stop (Function)** screen key – Stops the current function and changes the Status screen keys to **Abort** and **Resume** (see later).

- **Hold (Function)** screen key – Allows the current function to finish, suspends operation of subsequent functions, and displays screen keys that allow you to abort or resume the sample set.

- **Hold Gradient** screen key – Maintains the current gradient conditions. A screen key allows you to resume the gradient.

- **Stop Flow** keypad key – Stops the flow of solvent, suspends operation of the current function, and displays screen keys that allow you to abort or resume the function.

If any alarm condition specified in the separation method or sample set is set to "Stop Function" or "Stop Flow" and the alarm condition occurs, a dialog box appears indicating the alarm condition and displaying the following screen keys:

- **Abort** – Cancels the current function
- **Resume** – Resumes the current function

4.2 Performing Preventive Maintenance

To assess the need for preventive maintenance on your 2795 Separations Module:

1. Press the **Diag** screen key in the Main screen. The Diagnostics screen appears (Figure 4-5) with the following fields:

   - Transducers fields – Display the current pressure readings for the two transducers.
   - Lifetime Counters fields – Display total values for the volume of eluent and the number of injections.

   Use this information to assess the requirements for preventive maintenance (see the *Waters 2795 Separations Module Operator's Guide*, Section 7.1, Maintenance Considerations, the "Recommended Preventive Maintenance Schedule" discussion).

2. Press the screen key corresponding to the part of the 2795 Separations Module you want to troubleshoot.

Figure 4-5 Diagnostics Screen

4.3 Powering Off the 2795 Separations Module

Before Powering Off

Before you power off the 2795 Separations Module for more than a few days, remove any buffered mobile phase present in the fluidic path.

STOP **Attention:** *To avoid damaging the column, remove the column before performing the following procedure.*

To remove buffered mobile phase from the fluidic path:

1. Replace the buffered mobile phase with HPLC-quality water and wet prime the system for 10 minutes at 3 mL/min (see Section 2.2.2, Wet Priming).
2. Replace the water in the fluidic path with a solution of 90% water:10% methanol, and flush the system for 10 minutes at 3 mL/min.

Powering Off

To power off the 2795 Separations Module:

1. Ensure that the system has been flushed (see the "Before Powering Off" discussion above).
2. Press the power switch to the (Off) **0** position. The time and date of the power-off are entered in the log file.