Waters 2795
Separations Module

Operator's Guide

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Note: When you use the instrument, follow generally accepted procedures for quality control and methods development.

If you observe a change in the retention of a particular compound, in the resolution between two compounds, or in peak shape, immediately determine the reason for the changes. Until you determine the cause of a change, do not rely on the separation results.

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

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

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

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

Avvertenza: eventuali modifiche o alterazioni apportate a questa unità e non espressamente approvate da un ente responsabile per la conformità annulleranno l'autorità dell'utente ad operare l'apparecchiatura.

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

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

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

주의 : 기기 검교정 담당자의 승인 없이 무단으로 기기를 변경 또는 수정하는 경우에는, 그 기기 운영에 대한 허가가 취소될 수 있습니다.

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

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

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

Attention : *Soyez très prudent en travaillant avec des tuyaux de polymères sous pression :*

- *Portez toujours des lunettes de protection quand vous vous trouvez à proximité de tuyaux de polymères.*
- *Eteignez toutes les flammes se trouvant à proximité.*
- *N'utilisez pas de tuyau de Tefzel fortement abîmé ou déformé.*
- *N'utilisez pas de tuyau de Tefzel avec de l'acide sulfurique ou nitrique, ou du tétrahydrofurane (THF).*
- *Sachez que le chlorure de méthylène et le sulfoxyde de diméthyle peuvent provoquer le gonflement des tuyaux de Tefzel, diminuant ainsi fortement leur pression de rupture.*

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

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

Precauzione: prestare attenzione durante le operazioni con i tubi di polimero sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Estinguere ogni fonte di ignizione circostante.
- Non utilizzare tubi Tefzel soggetti a sollecitazioni eccessive o incurvati.
- Non utilizzare tubi Tefzel contenenti tetraidrofurano (THF) o acido solforico o nitrico concentrato.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamento nei tubi Tefzel, che riducono notevolmente il limite di pressione di rottura dei tubi stessi.

Advertencia: manipular con precaución los tubos de polímero bajo presión:

- Protegerse siempre los ojos en las proximidades de tubos de polímero bajo presión.
- Apagar todas las llamas que estén a proximidad.
- No utilizar tubos Tefzel que hayan sufrido tensiones extremas o hayan sido doblados.
- No utilizar tubos Tefzel con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- No olvidar que el cloruro de metileno y el óxido de azufre dimetilo dilatan los tubos Tefzel, lo que reduce en gran medida la presión de ruptura de los tubos.

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下几點：

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓瘪或嚴重彎曲的特氟隆 (Tefzel) 管線。
- 不要在特氟隆 (Tefzel) 管線中使用四氫呋喃 (THF) 或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致特氟隆 (Tefzel) 管線膨脹，大大降低管線的耐壓能力。

警告：当在有压力的情况下使用聚合物管线时，小心注意以下几点

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

경고 : 폴리머재질의 튜빙을 압력하에서 사용할 때는 다음 사항에 유의하십시오.

- 압력을 받은 폴리머 튜빙 부근에서는 반드시 보호안경을 착용할 것
- 모든 화기의 접근을 금함
- 눌리거나 뒤틀린 Tefzel 튜빙은 사용하지 말 것
- Tefzel 튜빙을 테트라히드로퓨란 (THF)이나 염산 및 황산과 함께 사용하지 말 것
- 디클로로메탄(methylene chloride)와 디메틸설폭시드(dimethyl sulfoxide)는 Tefzel 튜빙을 팽창시켜 쉽게 파열되므로 주의할 것

警告：ポリマーチューブに圧力をかけて取り扱う場合は、次のように注意してください。

- 加圧したポリマーチューブの付近では、常に保護めがねを着用してください。
- 付近の火はすべて消してください。
- 激しい応力やねじれを受けたTefzelチューブは使用しないでください。
- テトラヒドロフラン (THF)、濃硝酸、あるいは濃硫酸には、Tefzelチューブを使用しないでください。
- メチレン-クロライドやジメチルスルホキシドはTefzelチューブを膨張させ、チューブの破断圧力を大幅に低下させますので、注意してください。

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

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

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

Precauzione: *l'utente deve essere al corrente del fatto che, se l'apparecchiatura viene usta in un modo specificato dal produttore, la protezione fornita dall'apparecchiatura potrà essere invalidata.*

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

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

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

경고 : 제조사가 지정한 것 이외의 방법으로 기기를 사용하는 경우에는, 사용자가 위험으로부터 보호될 수 없는 경우가 발생할 수 있음에 유념하십시오.

警告：ユーザは製造業者が指定していない方法で装置を使用した場合は装置が提供する保護が損なわれることがあるということを承知しているものとします。

Caution: *To protect against fire hazard, replace fuses with those of the same type and rating.*

Attention : *Remplacez toujours les fusibles par d'autres du même type et de la même puissance afin d'éviter tout risque d'incendie.*

Vorsicht: *Zum Schutz gegen Feuergefahr die Sicherungen nur mit Sicherungen des gleichen Typs und Nennwertes ersetzen.*

Precauzione: *per una buona protezione contro i rischi di incendio, sostituire i fusibili con altri dello stesso tipo e amperaggio.*

Advertencia: *sustituya los fusibles por otros del mismo tipo y características para evitar el riesgo de incendio.*

警告：為了避免火災的危險，應更換同种類型及規格的保險絲。

警告：为了避免火灾的危险，应更换同种类型及规格的保险丝。

경고 : 화재를 방지하기 위해서는 퓨즈 교체 시 같은 종류, 같은 등급의 것을 사용하십시오.

警告：火災の危険防止のために、ヒューズの交換は同一タイプおよび定格のもので行なってください。

Caution: *To avoid possible electrical shock, disconnect the power cord before servicing the instrument.*

Attention : *Afin d'éviter toute possibilité de commotion électrique, débranchez le cordon d'alimentation de la prise avant d'effectuer la maintenance de l'instrument.*

Vorsicht: *Zur Vermeidung von Stromschlägen sollte das Gerät vor der Wartung vom Netz getrennt werden.*

Precauzione: *per evitare il rischio di scossa elettrica, scollegare il cavo di alimentazione prima di svolgere la manutenzione dello strumento.*

Precaución: *para evitar descargas eléctricas, desenchufe el cable de alimentación del instrumento antes de realizar cualquier reparación.*

警告：要避免觸電，請在修理或保養器材前把電源線拔出。

警告：为避免可能引起得触电危险，在修理前请切断电源连接。

경고: 전기 충격의 가능성을 피하기 위해서는, 기기를 수리하기 이전에 전원 코드를 차단하십시오.

警告：感電の危険性を避けるために、装置の保守を行う前には装置の電源コードを引き抜いてください。

Commonly Used Symbols

|	
$---$	Direct current Courant continu Gleichstrom Corrente continua Corriente continua 直流電 直流电 직류 直流
$\sim$	Alternating current Courant alternatif Wechselstrom Corrente alternata Corriente alterna 交流電 交流电 교류 交流
⏚	Protective conductor terminal Borne du conducteur de protection Schutzleiteranschluss Terminale di conduttore con protezione Borne del conductor de tierra 保護的導線端子 保护性的接地端 보호 도체 단자 接地

⏚	Frame or chassis terminal Borne du cadre ou du châssis Rahmen- oder Chassisanschluss Terminale di struttura o telaio Borne de la estructura o del chasis 結構或底盤端子 机架或底盘接地端 프레임 또는 틀 단자 フレームまたはシャーシアース
⚠	Caution or refer to manual Attention ou reportez-vous au guide Vorsicht, oder lesen Sie das Handbuch Prestare attenzione o fare riferimento alla guida Actúe con precaución o consulte la guía 小心或查閱手冊 小心或查阅手册 경고 또는 사용설명서 참조 警告またはマニュアルを参照
♨	Caution, hot surface or high temperature Attention, surface chaude ou température élevée Vorsicht, heiße Oberfläche oder hohe Temperatur Precauzione, superficie calda o elevata temperatura Precaución, superficie caliente o temperatura elevada 警告，熱表面或高溫 警告,热表面或高温 경고, 뜨거운 표면 또는 고온 警告、熱くなっている面、あるいは高温

	Caution, risk of electric shock (high voltage) Attention, risque de commotion électrique (haute tension) Vorsicht, Elektroschockgefahr (Hochspannung) Precauzione, rischio di scossa elettrica (alta tensione) Precaución, peligro de descarga eléctrica (alta tensión) 警告，小心觸電（高壓電） 警告,小心触电(高压电) 경고, 전기충격의 위험 (고압) 警告、電気ショックの危険性 (高電圧)
	Caution, risk of needle-stick puncture Attention, risques de perforation de la taille d'une aiguille Vorsicht, Gefahr einer Spritzenpunktierung Precauzione, rischio di puntura con ago Precaución, riesgo de punción con aguja 警告，小心尖狀物刺傷 警告,小心尖狀物刺伤 경고, 뾰족한 것으로부터의 상해 위험 警告、ニードルで穴をあける危険性
	Caution, ultraviolet light Attention, rayonnement ultrviolet Vorsicht, Ultraviolettes Licht Precauzione, luce ultravioletta Precaución, emisiones de luz ultravioleta 警告，紫外光 警告,紫外光 경고, 자외선 警告、紫外線

Commonly Used Symbols *(Continued)*

⊏══⊐	Fuse Fusible Sicherung Fusibile Fusible 保險絲 保险丝 퓨즈 ヒューズ
1	Electrical power on Sous tension Netzschalter ein Alimentazione elettrica attivata Alimentación eléctrica conectada 開啓電源 接通电源 전원 켜기 電源オン
0	Electrical power off Hors tension Netzschalter aus Alimentazione elettrica disattivata Alimentación eléctrica desconectada 關閉電源 切断电源 전원 끄기 電源オフ

2795 Separations Module Information

Intended Use

The Waters 2795 Separations Module can be used for in-vitro diagnostic testing to analyze many compounds, including diagnostic indicators and therapeutically monitored compounds. When developing such methods, follow the "Protocol for the Adoption of Analytical Methods in the Clinical Chemistry Laboratory," *American Journal of Medical Technology*, 44, 1, pages 30–37 (1978). This protocol covers good operating procedures and techniques necessary to validate system and method performance. These required criteria must be satisfied and in some cases monitored to ensure the validity of any quantitative results.

Biological Hazard

When analyzing physiological fluids, take all necessary precautions and treat all specimens as potentially infectious. Precautions are outlined in "CDC Guidelines on Specimen Handling," *CDC–NIH Manual*, 1984.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate the standard curve. The concentration range for the standard curve should include the entire concentration range of quality-control samples, typical specimens, and atypical specimens. This practice ensures a linear response for all concentrations being analyzed and provides the basis for analyzing quality-control samples.

Quality Control

Routinely run at least three quality-control samples and monitor them daily. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision day to day and run to run. Such results provide a guide to determine the basic chromatographic integrity of the analysis. If a chromatographic anomaly is present, repeat the analysis and, in those cases where it is needed, troubleshoot the cause of the anomaly. Data collected when quality-control samples are out of range may not be valid. Use these data only after ensuring that chromatographic system performance is acceptable.

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Preface

The *Waters 2795 Separations Module Operator's Guide* describes the procedures for unpacking, installing, using, maintaining, and troubleshooting the Waters® 2795 Separations Module. It also includes appendixes for specifications, spare parts, and solvent considerations.

Organization

This guide contains the following:

Chapter 1 describes the Waters 2795 Separations Module, including features and options.

Chapter 2 describes how to unpack and install the 2795 Separations Module, and how to make power, fluidic, and signal connections

Chapter 3 describes how to power on the 2795 Separations Module, prepare the solvent and sample management systems for operation, perform daily clean-up, and power off the 2795 Separations Module.

Chapter 4 describes how to control the 2795 Separations Module from the front panel.

Chapter 5 describes how to create separation methods and sample sets.

Chapter 6 describes how to make automatic runs in stand-alone and remote-control modes and how to use the 2795 Separations Module high throughput options.

Chapter 7 describes routine maintenance procedures.

Chapter 8 describes troubleshooting procedures for the Waters 2795 Separations Module.

Appendix A describes the specifications of the Waters 2795 Separations Module.

Appendix B list of recommended and optional spare parts.

Appendix C provides information on solvent compatibility, miscibility, degassing, and UV cutoff wavelengths.

Related Documentation

***Waters Licenses, Warranties, and Support*:** Provides software license and warranty information, describes training and extended support, and tells how Waters handles shipments, damages, claims, and returns.

***Waters 2795 Separations Module Quick Start Guide*:** Describes the basics of how to use the 2795 Separations Module in a brief, easy-to-read format.

Waters Alliance LC/MS System Quick Start Guide: Provides useful information when operating a 2795 Separations Module under MassLynx™ software control.

Millennium^{32} Read Me First: Provides useful information when operating a 2795 Separations Module under Millennium®^{32} software control.

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*

Related Adobe Acrobat Reader Documentation

For detailed information about using Adobe® Acrobat® Reader, see the *Adobe Acrobat Reader Online Guide*. This guide covers procedures such as viewing, navigating, and printing electronic documentation from Adobe Acrobat Reader.

Printing This Electronic Document

Adobe Acrobat Reader lets you easily print pages, page ranges, or the entire document by selecting **File > Print**. For optimum print quantity, Waters recommends that you specify a PostScript® printer driver for your printer. Ideally, use a printer that supports 600 dpi print resolution.

Documentation Conventions

The following conventions can be used in this guide:

Convention	Usage
Purple	Purple text indicates user action such as keys to press, menu selections, and commands. For example, "Click **Next** to go to the next page."
Italic	Italic indicates information that you supply such as variables. It also indicates emphasis and document titles. For example, "Replace *file_name* with the actual name of your file."
Courier	Courier indicates examples of source code and system output. For example, "The SVRMGR> prompt appears."

Convention	Usage
`Courier Bold`	Courier bold indicates characters that you type or keys you press in examples of source code. For example, "At the `LSNRCTL>` prompt, enter **`set password oracle`** to access Oracle."
<u>Underlined Blue</u>	Indicates hypertext cross-references to a specific chapter, section, subsection, or sidehead. Clicking this topic using the hand symbol brings you to this topic within the document. Right-clicking and selecting **Go Back** from the shortcut menu returns you to the originating topic. For example, "The column selection valves used with the 2795 Separations Module are described in <u>Section 1.4, Options and Accessories</u>."
Keys	The word *key* refers to a computer key on the keypad or keyboard. *Screen keys* refer to the keys on the instrument located immediately below the screen. For example, "The A/B screen key on the 2414 Detector displays the selected channel."
…	Three periods indicate that more of the same type of item can optionally follow. For example, "You can store *filename1*, *filename2*, … in each folder."
>	A right arrow between menu options indicates you should choose each option in sequence. For example, "Select **File > Exit**" means you should select **File** from the menu bar, then select **Exit** from the File menu.

Notes

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

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

Attentions

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

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

Cautions

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

Caution: *To avoid burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.*

Caution: To avoid electrical shock and injury, unplug the power cord before performing maintenance procedures.

Caution: To avoid chemical or electrical hazards, observe safe laboratory practices when operating the system.

Chapter 1
Introduction to the Waters
2795 Separations Module

1.1 Overview

The Waters® 2795 Separations Module (<u>Figure 1-1</u>) 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.

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
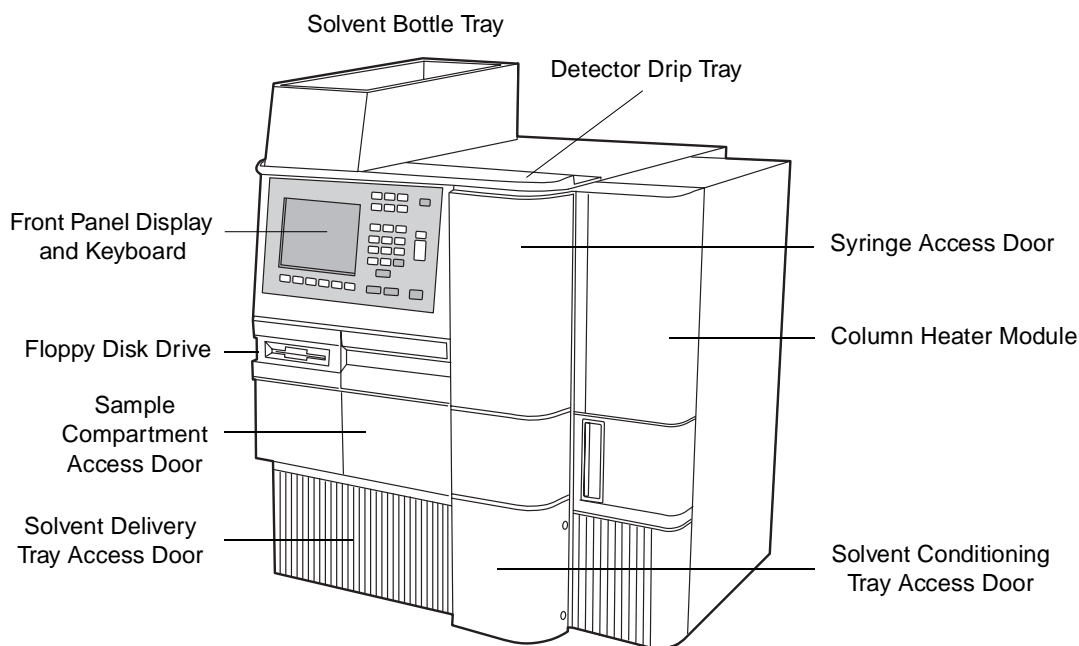
TP01354

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

Components

The 2795 Separations Module consists of:

- Three-axis (XYZ) sample management system
- HPLC solvent delivery system
- Five-channel inline vacuum degasser (one channel reserved for sample management purge solvent)
- Integral plunger seal-wash system
- LCD display
- Keyboard
- Floppy disk drive (using 3.5-inch floppy disks)
- Column heater (optional)
- Sample heater/cooler (optional)
- Column selection valve (optional)
- Bar code reader (optional)

When the 2795 Separations Module with the optional sample heater/cooler is installed with the 2795 column heater, it is known as the 2795 XE. Refer to Section B.3, 2795 Separations Module Options, for information about the 2795 Separations Module options.

Operating Modes

The 2795 Separations Module can operate as a stand-alone HPLC system controller, as an HPLC system controlled by Millennium®[32] software, or as a component of an HPLC or LC/MS system controlled by MassLynx™ software.

- As a stand-alone HPLC system controller, the 2795 Separations Module manages a variety of sample plates while controlling one or more detectors. Optionally, the 2795 Separations Module can also control a 3-column selection, 6-column selection, or column regeneration valve.
- As an HPLC system controlled by Millennium[32] software (version 3.2 or higher), the 2795 Separations Module performs sample and solvent management, column selection, and system equilibration.
- As a component of an LC/MS system controlled by MassLynx (version 3.5 with change note number 343), the 2795 Separations Module performs sample and solvent management, column selection, and system equilibration.

  An LC/MS system that includes the 2795 Separations Module, the Waters ZQ Mass Detector, and MassLynx software is designated a Waters Alliance® HT LC/MS System (Figure 1-2).
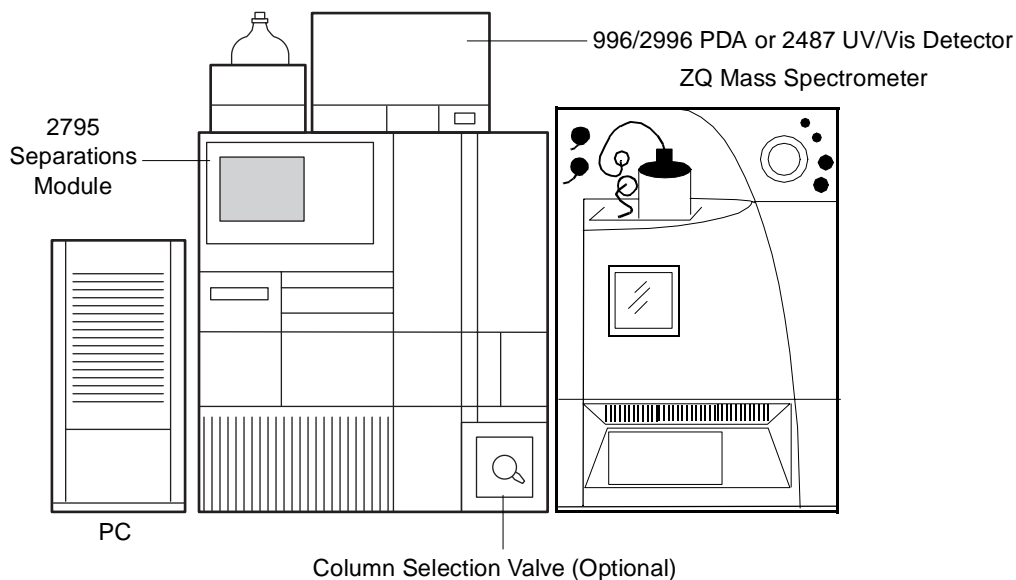
Figure 1-2 Waters Alliance HT LC/MS System

System Configurations

The 2795 Separations Module supports IEEE-488 and I/O connections within a variety of HPLC and LC/MS system configurations.

The 2795 Separations Module can be configured in a stand-alone mode to provide control of up to three UV or RI detector channels as follows:

- Up to two UV detector channels (a Waters 2487 Dual λ Detector, single or dual-channel, and/or a Waters 486 Tunable Absorbance Detector)
- One RI detector channel (Waters 2410 or 410 Differential Refractometer)

The 2795 Separations Module can be configured under Millennium[32] software. You use a Millennium[32] Chromatography Manager workstation to create methods, which control the Separations Module and other HPLC system components. All detectors that are supported by Millennium[32] software can be used with the 2795 Separations Module. Data from the detectors are sent over the IEEE-488 interface bus to the Millennium[32] software for processing.

In an Alliance HT LC/MS system controlled by MassLynx software, MassLynx can control up to two of the following (one of each):

- Waters ZQ Detector
- Waters ZMD Detector
- Waters 2487 Detector
- Waters 996/2996 Detector

- Micromass® single- or triple-quadrupole or time-of-flight mass spectrometer
- Waters SAT/IN™ Module

Parameter Control

In a stand-alone HPLC system, the 2795 Separations Module can control:

- Method programming
- Solvent composition
- Flow rate
- Plunger seal-wash flow
- Sample injection
- External events
- Advanced sample management functions including:
  - Rapid equilibration
  - Parallel sample processing
  - Sample pooling
- Operation of detectors over the IEEE-488 interface bus
- Inline degassing
- Column heating (optional)
- Sample heating/cooling (optional)
- Column selection valve (optional)
- Bar-code reading of sample plates (optional)

With the 2795 Separations Module, you can make parameters such as pressure limits or syringe draw rates part of a separation method. You can program the values of these parameters to change from method to method without having to set up the instrument before each run.

Spill Protection

All fluid-handling areas of the 2795 Separations Module have spill protection; spilled solvent is routed to waste line connectors below the front panel. A drip tray is provided for the top cover if you place a detector on top of the 2795 Separations Module. A solvent bottle tray provides storage for up to four 1-L solvent reservoirs.

Methods Storage and Retrieval

You can store up to 60 *methods* (methods include separation methods and sample sets) in the 2795 Separations Module. When the number of stored methods approaches the maximum (60), the delay when you create and save methods increases to more than 30 seconds. When 60 methods have been stored, a warning message appears indicating that storage capacity has been reached.

The 2795 Separations Module can export and import separation methods and sample sets from standard PC-formatted, 3.5-inch floppy disks using the built-in floppy disk drive.

The 2795 Separations Module also supports import of sample sets created and exported using a PC-based spreadsheet application or text editor in ASCII format.

Record-Keeping Functions

The 2795 Separations Module automatically records:

- Total number of injections
- Total solvent usage (pumped through the system)
- System errors
- Programmed operating conditions for each run
- Column selection

This information can be:

- Printed using a connected serial printer
- Transferred to an external device, such as the Waters 746 Data Module
- Exported to a 3.5-inch floppy disk as an ASCII file

Sample Heater/Cooler

The 2795 Separations Module can be shipped with the optional sample heater/cooler that maintains the sample compartment at temperatures from 4 to 40 °C. The sample heater/cooler is factory-installed on the 2795 XE Separations Module.

Column Heater

The 2795 Separations Module Column Heater maintains the column at temperatures from 5 °C above ambient (minimum of 20 °C) to 60 °C. You can enable an alarm to warn you if the temperature varies outside the range you specify. The procedure to install the column heater is described in Section 2.3, Installing the Column Heater. The 2795 XE includes the column heater.

The 2795 Column Heater is shipped with either no valve or with one of three optional column-selection valves and the associated tubing and connectors. See Section 1.4, Options and Accessories, for information on the column selection valves used with the 2795 Separations Module.

For more information on column heater options, see Section B.3, 2795 Separations Module Options.

1.2 Solvent Management System Overview

The 2795 Separations Module solvent management system blends and delivers solvents from the reservoir bottles to the sample management system in a pulse-free, low-dispersion flow path. In addition, Synchronized Composition Control™ (SCC™) of the gradient proportioning valve (GPV) ensures that the 2795 Separations Module delivers excellent gradient composition accuracy and precision.

1.2.1 Solvent Management System Fluidic Path

Solvent flows in the solvent management system as described below and as shown in Figure 1-3:

1. Solvent degassed by the inline vacuum degasser enters the gradient proportioning valve where the solvents are blended.

2. The blended solvents flow through the inlet check valve into the primary piston chamber while the accumulator piston delivers solvent under pressure to the system pressure transducer.

3. Just before the accumulator chamber empties, the solvent in the primary piston chamber is precompressed to the pressure indicated by the system pressure transducer.

4. When the accumulator piston chamber is empty, the primary piston delivers solvent under pressure through the primary pressure transducer, refills the accumulator piston chamber, and delivers solvent under pressure through the system pressure transducer, while maintaining a constant flow through the system. The cycle repeats, beginning at step 2.

5. The system pressure transducer measures the operating pressure. A firmware algorithm compares the primary head pressure and the system pressure readings and regulates the precompression step to balance the pressures, providing a smooth, ripple-free flow.

6. Flow continues from the system pressure transducer outlet to the prime/vent valve and inline filter and then to the sample management system.

For a detailed view of the solvent management system, see Figure 7-1.

1.2.2 Solvent Management System Features

Solvent Blending

The gradient proportioning valve (GPV) blends up to four solvents in any combination you choose. The GPV produces predictable gradient segments regardless of solvent compressibility and system back-pressure. Solvent selection and proportioning take place on the low-pressure (intake) side of the solvent delivery system. Solvents continue to blend under high pressure in each of the piston chambers.

Gradient
Proportioning
Valve

Solvent
A

Solvent
B

Accumulator
Piston
Chamber

System
Pressure
Transducer

Prime/Vent
Valve

Inline
Filter

Primary
Piston
Chamber

Primary
Pressure
Transducer

Solvent
D

Solvent
C

Check Valve

Check Valve
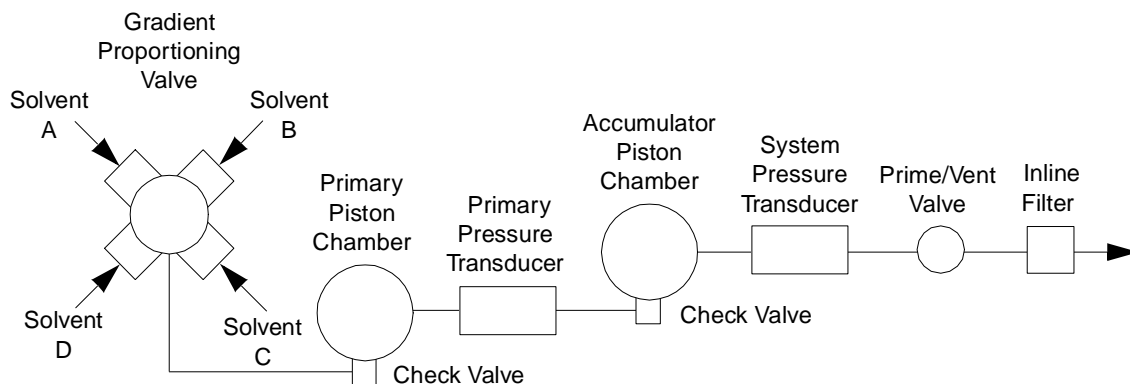
Figure 1-3 Solvent Management Flow Path

Solvent Bottle Tray

The solvent bottle tray holds up to four 1-L reservoirs and one 250-mL wash reservoir, and can contain a 2-L spill. The tray fits on the drip tray at the top of the 2795 Separations Module (Figure 1-1).

Prime/Vent Valve

Attaching a syringe to the prime/vent valve allows you to draw solvent through the solvent management system.

Plunger Seal-Wash System

The seal-wash solvent lubricates the plunger and flushes away any solvent or dried salts forced past the plunger seal from the high-pressure side of each piston chamber. When the solvent management system is delivering solvent, the plunger-seal-wash-pump intermittently supplies wash solvent according to a time interval you select.

Loss of Prime Protection

The 2795 Separations Module automatically stops when the operating pressure remains below 25 psi (1.7 bar, 172 kPa) for 50 cycles of the solvent delivery system. This is likely to occur only when there is a loss of prime because of an empty solvent reservoir.

Preferred Plunger Stroke Volume

Based on comprehensive testing, the Separations Module uses discrete programmable stroke volumes that provide optimal flow delivery and solvent blending. To improve performance for specific applications, you can override the default (preprogrammed) stroke volume of 130 μL by selecting a preferred stroke volume of 25 μL, 50 μL, or 100 μL,

as shown in <u>Table 1-1</u>. A larger stroke volume is recommended when using solvents which may require additional mixing (e.g., trifluoroacetic acid [TFA] gradients) which is afforded by a larger stroke volume. This additional mixing, unlike a static or dynamic mixer, occurs without added delay volume.

Table 1-1 Alternate Stroke Volumes

Flow Range (ml/min)	Stroke Volume (µL)
0.010 - 0.530	25
0.531 - 1.230	50
1.231 - 3.030	100
3.031 - 5.000	130

PerformancePLUS Degasser

The 2795 Separations Module features five vacuum degasser chambers enabling you to degas eluents A, B, C, and D as well as the purge solvent.

The design of the PerformancePLUS™ Degasser combines a variable-speed, continuously operating vacuum pump with high efficiency, low internal volume (< 500 uL) degasser chambers. This technology dramatically shortens instrument priming and equilibration times, allowing the degasser to become operational quickly from an idle state or following a solvent change.

Degasser Considerations

Degassing efficiency is based on the solvent gas load and the length of time that the solvent remains in its respective vacuum chamber. Gas removal efficiency decreases as the solvent flow rate increases because the solvent is in the vacuum chamber for less time. Using a normal analytical flow rate range of 0.000 to 5.000 mL/min, the degasser removes most of the dissolved gasses. Because you can perform dry and wet priming in the Separations Module at flow rates greater than 5.000 mL/min, you need to expose the solvents to the degasser vacuum for a short time (after priming) at zero flow rate. This solvent equilibrating process is described in <u>Section 4.2.3, Wet Priming</u>.

Vacuum Degasser Pressure Transducers

Waters 2795 Separations Modules include an absolute pressure transducer (APT). The APT, located in the degasser vacuum chamber, is unaffected by altitude or barometric changes and therefore provides accurate pressure readings.

1.3 Sample Management System Overview

The 2795 Separations Module sample management system:

• Holds and positions sample plates

• Injects samples into the solvent flow

The sample management system can accommodate up to four sample plates of varying capacities. The system allows you to combine different types of sample plates and vial holders during a sample run. The plate carrier moves the plates to the injection station in the sample compartment.

Five valves, two pumps, and a syringe control the flow of sample, purge solvent, and needle-wash solvent. The valves are designated:

- Inject
- Waste
- Bypass
- Wash
- Purge

The pumps are designated:

- Drain
- Wash

Note: You can monitor the position of each valve using the Status screen (see Section 4.3, Monitoring System Status).

1.3.1 Sample Management System Fluidic Path

The mobile phase and wash solvent paths through the sample management system (Figure 1-4) vary according to what procedures are being performed. When the 2795 Separations Module is:

- **Loading a sample** – The Load position of the inject valve (Figure 1-4) isolates the sample loop from the solvent fluidic path to the column and allows solvent to flow through the inject valve to the column.

- **Injecting a sample** – The Inject position of the inject valve (Figure 1-4) connects the sample loop to the solvent flow. Two types of injection are available, full loop and partial loop.

    For each injection type, the system provides one or more user-programmable needle-wash and purge cycles to ensure that each injection is free from sample residue.

    When the 2795 Separations Module is performing a:

    – **Needle Wash**, the wash cycle washes the interior and exterior of the sample needle and drains excess solvent from the sample management system. This cycle is user-selectable and is recommended for optimum carryover performance.

– **Purge**, the wash cycle includes a purge that draws the purge solvent through the inline vacuum degasser, then through the needle and the inject valve, ensuring a clean degassed flow path for each injection. The replacement volume is user-selectable and is recommended for optimum system performance.

• **Bypassing solvent flow** – During priming of the solvent management system and during rapid equilibration, solvent flows through a bypass valve to waste (Figure 1-4).

1.3.2 Sample Management System Features

Parallel Processing

The 2795 Separations Module offers an advantage over traditional (sequential) chromatography cycle times by performing chromatographic operations in parallel. Parallel processing maximizes throughput by minimizing cycle time. While one sample is running, a second sample can be drawn and injected immediately after the preceding injection is complete. You can specify that a sample be:

• Drawn while the current sample is being injected

• Drawn and pre-loaded into the injector while the current separation is being completed.

Parallel Processing with Rapid Equilibration

The 2795 Separations Module also provides for rapid equilibration by switching the column off-line, purging the system at a high flow rate, then loading the next sample during column reequilibration. By using parallel processing with rapid equilibration, you can significantly increase throughput for some applications.

For more information on parallel processing, see Section 6.2, Using the 2795 High-Throughput Capabilities.
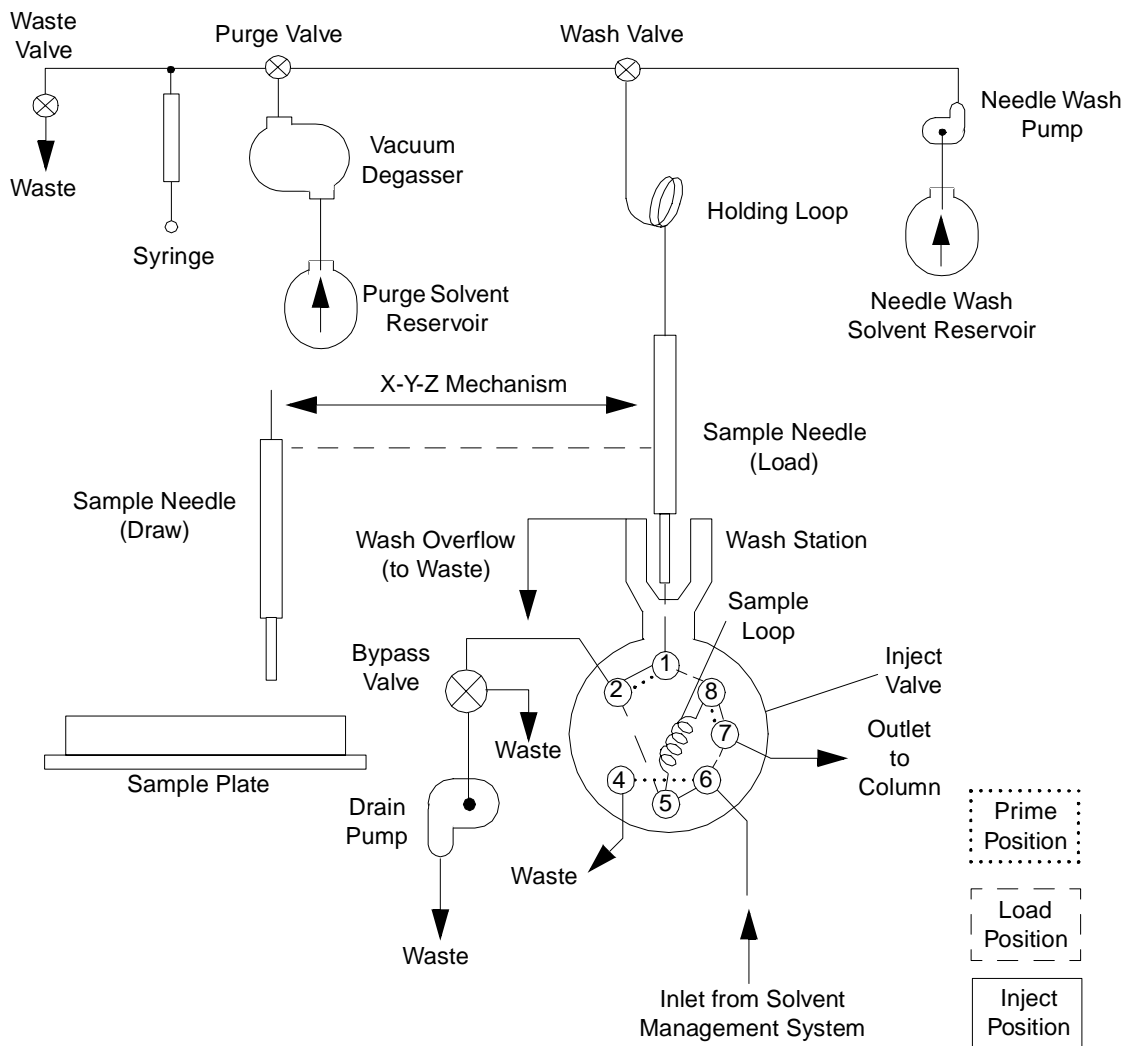
Figure 1-4 Sample Management Fluidic Path

Sample Pooling

The 2795 Separations Module sample management system allows you to pool samples, that is, to collect aliquots from two or more samples, "pool" them in the sample loop, then inject the pooled sample after a specified period of time. For more information on Sample Pooling, see Section 5.3.8, Setting Sample Set Functions, the "Specifying the Pool Function" discussion.

1.3.3 Sample Management System Components

STOP *Attention: Waters recommends you consult Table B-5 before configuring plates for the 2795 Separations Module. Ensure you have read all the recommendations in the footnotes to Table B-5.*

XYZ Needle Assembly

The three-axis (XYZ) needle assembly of the 2795 Separations Module sample management system can process samples from up to four of any of the following types of sample plates:

- 24-position rectangular vial-holder plates for 2-mL autosampler vials or for 1.5-mL microcentrifuge (Eppendorf®-style) tubes
- 48-position rectangular vial-holder plates for:
- 2-mL autosampler vials (6 rows × 8 columns)
- 0.50-mL microcentrifuge (Eppendorf-style) tubes (8 rows × 6 columns)
- 96-well microtiter sample plate (tall and standard)
- 384-well microtiter sample plate
- Open access plates (see Section 1.4, Options and Accessories, the "Open Access Plates" discussion)

You can customize any of the standard plates as described in Section 3.2.2, Configuring Plate Types.

STOP *Attention: Use of plates with adhesive-backed sealing foils and films may interfere with the operation of the needle and inject port.*

STOP *Attention: To avoid damaging the needle or XYZ mechanism, do not use vial caps less than 8.8 mm in diameter (OD).*

The three-axis (XYZ) sampling mechanism (Figure 1-5) uses opto-sensors to detect the depth of the first sample well or vial in a sample set or list, or you can specify the well depth. Use of pre-slit septa and non-adhesive-backed sealing foils or film prevents needle coring and degradation.
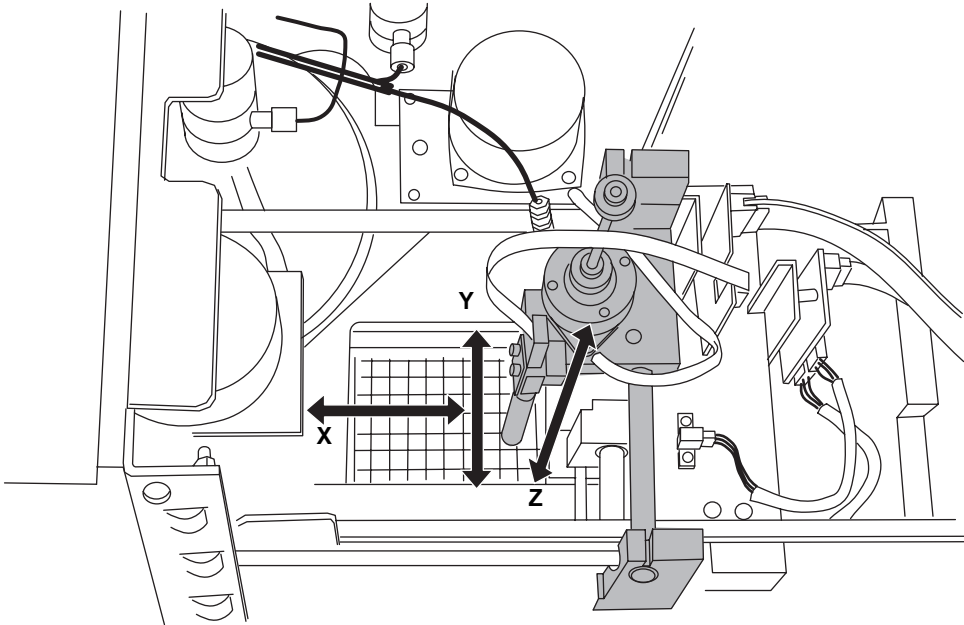
TP01581

Figure 1-5 XYZ Needle Assembly Mechanism

STOP *Attention:* *To prevent damage to the needle, do not use plate covers made of hard or thick material.*

Plate Carrier

The rotating sample plate carrier holds up to four plates (Figure 1-6). Plates must be: 5-inch × 3 3/8-inch (128 mm × 86 mm) with a height of up to 1.9 inches (48.2 mm).

The plate carrier is locked into place under the X-Y field of operation. Each plate is dynamically held in position on the carrier by a plate-positioning spring that engages the plate as it moves into the sampling position (Figure 1-6).

For information on loading the sample plates and plate carrier, see Section 3.4.3, Loading the Sample Plates.

For information on maintenance of the sample plate carrier, see Section 7.3.7, Cleaning the Sample Compartment and Plate Carrier.

For information on editing well locations on the 2795 Separations Module front panel, see Section 4.2.9, Selecting Wells for Making Injections.
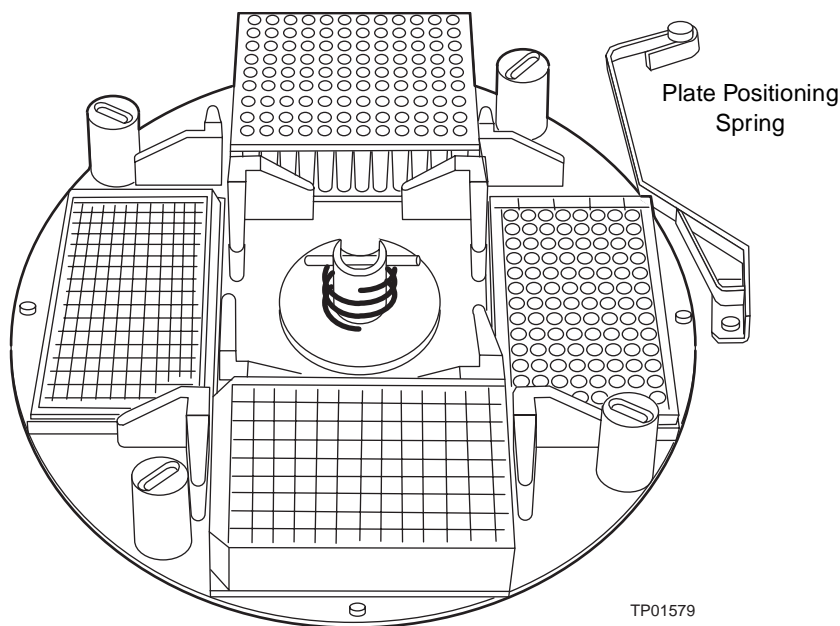
Figure 1-6 Sample Plate Carrier

Syringe Assembly

The syringe assembly (Figure 1-7), located behind a front panel door, uses a standard 500-µL syringe. As an option, you can install a 100-µL, 250-µL, 1000-µL, or 2500-µL syringe (see Section 7.3.2, Replacing the Syringe). Note that the 2795 Separations Module serial number is located below the syringe mounting plate.

Table 7-5 shows the relationship between syringe volume and the sample and holding loop sizes available for the 2795 Separations Module. See also the "Sample Loop, Holding Loop, and Syringe Options" discussion next.
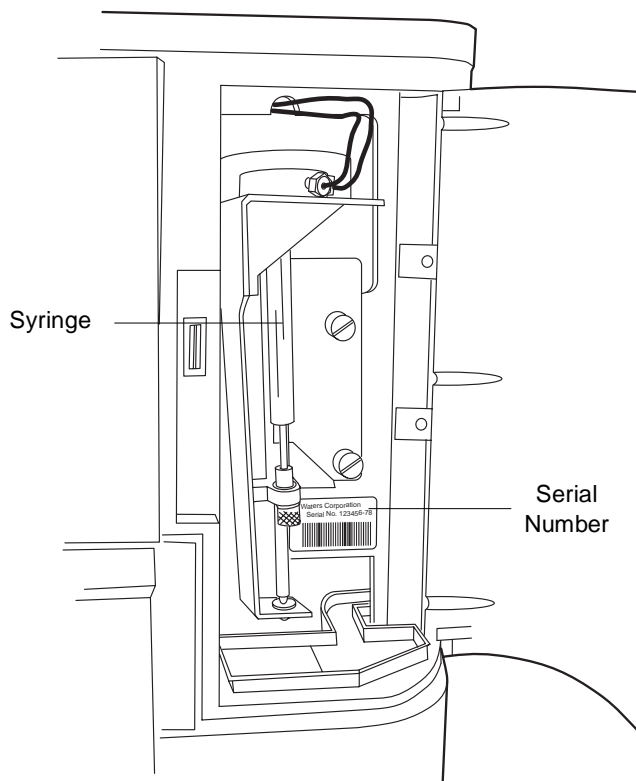
Figure 1-7 Syringe Assembly

Sample Loop, Holding Loop, and Syringe Options

A wide variety of sizes of sample loops, holding loops, and syringes are available as options for the 2795 Separations Module (see Table 7-5). This variety allows you to customize the configuration of the sample management system according to the needs of your applications. See also Section 1.4, Options and Accessories, and Section B.2, Sample Management System Spare Parts.

The standard configuration for the 2795 Separations Module consists of the 50-μL sample loop, the 500-μL syringe, and the 128-μL holding loop. This configuration provides good injection accuracy and repeatability for typical sample volumes (5 to 50 μL).

When you want to optimize for a certain aspect of your chromatography (for example, volume repeatability, cycle time, or injection-volume accuracy), you can use other combinations of syringe, sample loop, and holding loop, according to the following guidelines:

- If you can, use the sample loop size as your first choice to achieve a desired performance characteristic that the standard configuration cannot achieve. The sample loop size is the most versatile configurable option.

- Improve the repeatability of small (for example, 5-μL) injections over the standard configuration performance by:

  - Installing a smaller sample loop and using the full-loop injection mode (see Table 5-24).

  - Installing a smaller sample loop *and* a smaller-volume syringe (see Table 7-5). The smaller syringe decreases the significance of syringe drive variability but also adds to cycle times by requiring more syringe draws (5 to 10) to purge the needle wash solvent from the needle and holding loop.

- Optimize cycle time by:

  - Installing a larger (for example, 2500-μL) syringe. Because less than one stroke of the large syringe purges the needle and holding loop, the cycle time is greatly reduced. Although this configuration increases the significance of syringe drive variability, and, therefore, the variability in injection volumes, using the full-loop injection mode can help to keep variability minimal.

- Make large-volume injections by:

  - Installing a larger sample loop, a larger syringe, and a holding loop of compatible size (see Table 7-5). Ensure that the sample size does not exceed the capacity of the holding loop. If it does, the sample can contaminate the syringe and the rest of the sample management system.

1.4 Options and Accessories

Pressing the Options screen key in the Configuration screen (Figure 3-6) displays the hardware options installed in your 2795 Separations Module. Refer to Section 3.2, Configuring the 2795 Separations Module, for information on the Configuration screen. Other options and accessories for the 2795 Separations Module are also described.

Bar Code Reader

The Waters 2795 Separations Module Bar Code Reader (part number 176000156) allows you to scan bar-coded sample plates to automate recording of samples. The Waters 2795 Separations Module Bar Code Reader is installed at the customer site by Waters Technical Service.

The bar code reader is programmed to decode any of three bar code types:

- Code 128
- Code 39
- Code 2 of 5

The bar code reader can decode an alphanumeric sequence of up to 20 characters, depending on the bar code used and the length of the label. Certain features on microtiter plates, such as robotic contacts, may restrict the maximum length of the label and the number of characters encoded on the label. The labels are mounted as shown in Figure 1-8.
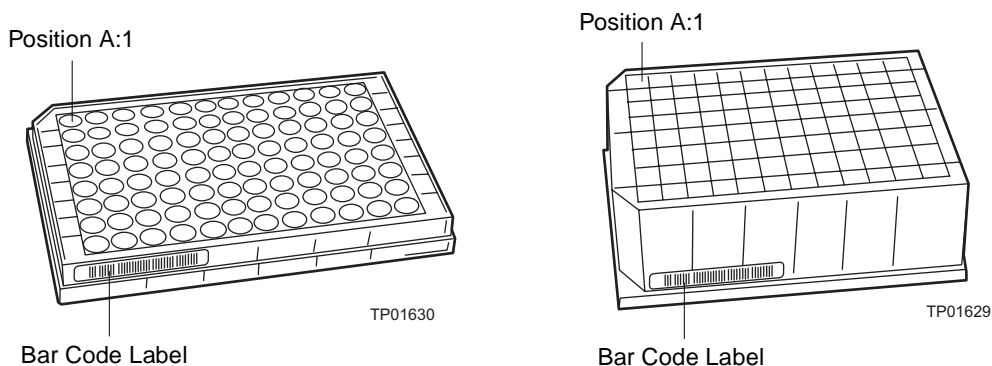
Figure 1-8 Mounting Location for Bar Code on 96-Well Plates

The bar code label system is designed for a minimum bar width of 0.010 in. (0.2 cm), achievable on most common laser or transfer printers. You can print your own labels, then attach them to the plates before use.

Syringes

You can remove the standard 500-µL syringe and install a 100-µL, 250-µL, 1000-µL, or 2500-µL sample syringe in the 2795 Separations Module. For more information, refer to Section 1.3.3, Sample Management System Components, the "Sample Loop, Holding Loop, and Syringe Options" discussion, and to Section 7.3.2, Replacing the Syringe.

Holding Loops

You can replace the installed 128-µL holding loop with a 500-µL or 3000-µL loop. For more information, refer to Section 1.3.3, Sample Management System Components, the "Sample Loop, Holding Loop, and Syringe Options" discussion, and Section 7.3.5, Replacing the Holding Loop.

Sample Loops

You can replace the standard 50-µL sample loop with a larger (100-µL, 200-µL, 500-µL, or 2000-µL) or smaller (5-µL or 20-µL) loop to inject full-loop sample volumes other than 50 µL. For more information, refer to Section 1.3.3, Sample Management System Components, the "Sample Loop, Holding Loop, and Syringe Options" discussion, and Section 7.3.6, Replacing the Sample Loop.

Column Selection Valves

A column selection valve allows you to switch the solvent flow to:

- One of several columns
- A restrictor bypass to a mass spectrometer
- Waste

The column-selection valve is mounted in the 2795 Column Heater (Figure 1-9), and is factory-installed.

The 2795 Separations Module has three column-selection valve options:

- 3-column
- 6-column
- Column regeneration

The 2795 Separations Module firmware automatically detects the presence and type of column selection valve at power on. You can specify column selection:

- Using the front panel (Section 4.2, Performing Direct Functions)
- Through the separation method (Section 5.1, Creating and Editing Separation Methods)
- As part of a function in the sample set method (Section 5.3, Creating and Editing Sample Sets)

The 2795 Separations Module Status screen shows the currently selected column or flow path.
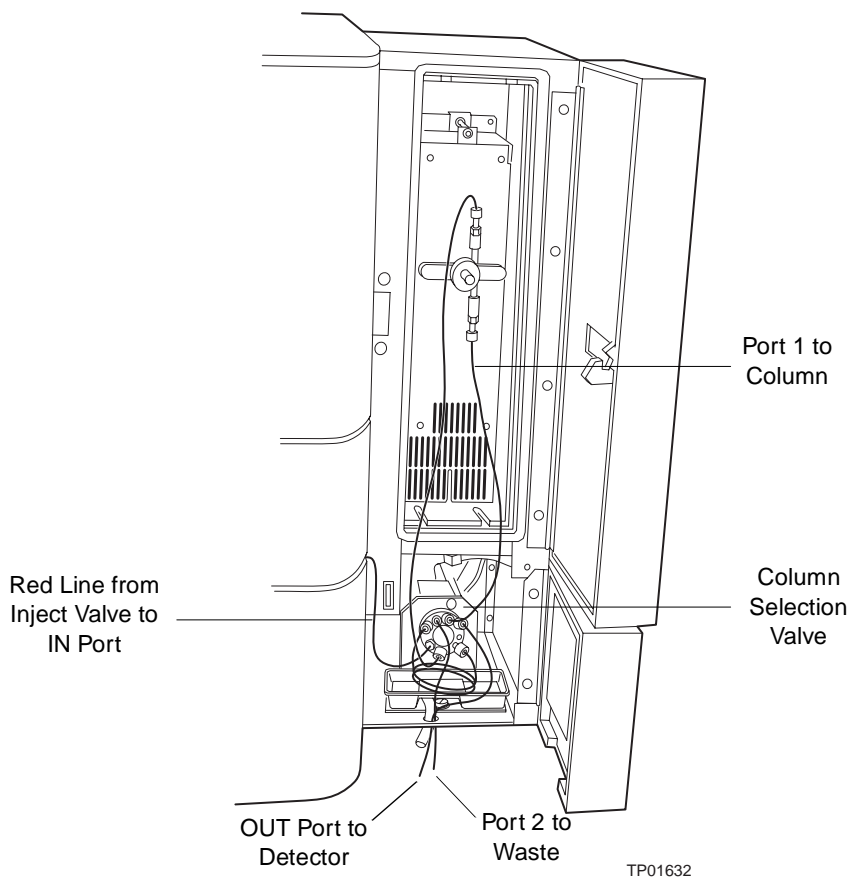
Figure 1-9 2795 Column Heater with 3-Column Selection Valve Installed

Three-Column Selection Valve

The 3-column selection valve allows you to select any of three column positions. The 3-column selection valve is preconfigured before shipment, but you can reconfigure it. For more information, see Section 2.4.7, Connecting the Column-Selection Valve.

Six-Column Selection Valve

The 6-column selection valve allows you to select any one of six column positions, and you can specify any position as bypass or waste. For more information, see Section 2.4.7, Connecting the Column-Selection Valve.

Column Regeneration Valve

The Column Regeneration valve allows you to perform LC operations using one column while the second column is undergoing regeneration by a second solvent delivery system. For more information, see Section 2.4.7, Connecting the Column-Selection Valve.

Open Access Plates

The Waters Open Access Plate Kit (part number 200000114) is an option for a stand-alone 2795 Separations Module, a 2795 Separations Module controlled by Millennium^{32} software, and an Alliance HT LC/MS system controlled by MassLynx software. The kit consists of four 24-well plates (for 2-mL vials) that are beveled to allow access to the sample wells without removing the plates from the carrier.

The plates are color-coded (blue, yellow, red, and green) to match the colors on the sample carrier and in the Open Access software option for Millennium^{32} software. Open access plates help you place the sample vials in the correct locations. For more information, see Section 3.4.3, Loading the Sample Plates.

A High-Capacity Open Access Kit (part number 200000123) is available with color coding, and has 48 vial positions in each of four plates.

Chapter 2
Installing the 2795
Separations Module

This chapter describes how to unpack and install the Waters 2795 Separations Module and add hardware options to the 2795 Separations Module.

2.1 Installation Overview

Figure 2-1 summarizes the major steps in installing the 2795 Separations Module.

```
   ┌─────────────┐                    ┌──────────────┐
   │    Start    │                    │  Make Power  │
   │ Installation│                    │ Connections  │
   └─────────────┘                    └──────────────┘
          │                                   │
   ┌─────────────┐                    ┌──────────────┐
   │Select        │                   │ Make Fluidic │
   │Appropriate   │                   │ Connections  │
   │Site          │                   └──────────────┘
   └─────────────┘                           │
          │                           ┌──────────────┐
   ┌─────────────┐                    │ Make Signal  │
   │ Unpack and  │                    │ Connections  │
   │  Inspect    │                    └──────────────┘
   └─────────────┘                           │
          │                           ┌──────────────┐
   ┌─────────────┐                    │ Installation │
   │ Install Bar │                    │  Complete    │
   │ Code Reader │                    └──────────────┘
   │ (Optional)  │
   └─────────────┘
          │
   ┌─────────────┐
   │Install Column│
   │Heater and    │
   │Selection Valve│
   │(Both Optional)│
   └─────────────┘
```
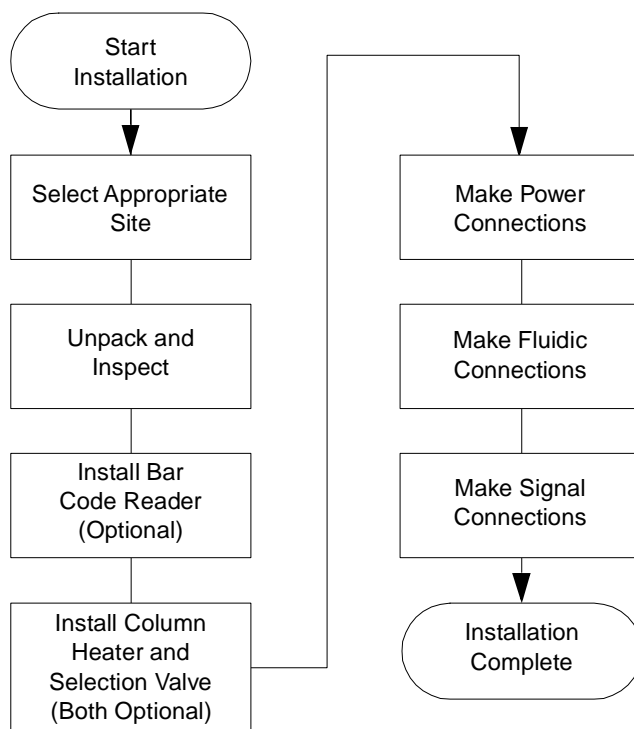
Figure 2-1 Steps to Install the 2795 Separations Module

Selecting a Site

Select a site for the 2795 Separations Module that meets the specifications indicated in Table 2-1.

Table 2-1 Installation Site Requirements

Factor	Requirement
Temperature	4 to 40 °C (39 to 104 °F)
Relative humidity	20 to 80%, noncondensing
Bench space	Width: 23.0 in. (58.4 cm) Depth: 25.5 in. (64.8 cm) Height: 22.5 in. (57.2 cm) Level to within ±2°
Bench-supportable weight	136 lbs. (62 kg)
Vibration	Negligible
Clearance	At least 2 in. (5 cm) at rear for ventilation
Static electricity	Negligible
Power	Grounded AC, 85 to 264 Vac, 47 to 63 Hz
Electromagnetic fields	No nearby source of electromagnetic noise, such as arcing relays or electric motors

Firmware and Software Requirements

Check that the Waters detectors (and remote data system, if any) meet the firmware and software version requirements described in Table 2-2.

Table 2-2 Minimum Firmware and Software Requirements

System Component	Required Firmware/Software Version
Waters 2487 Dual λ Absorbance Detector	Version 1.00 (486-emulation mode) Version 1.01 (2487 mode) or higher
Waters 486 Tunable UV-Vis Absorbance Detector	Version 4.10 or higher
Waters 2410 Differential Refractometer	Version 5.10 or higher
Waters 410 Refractive Index Detector	Version 4.2 or higher
Millennium®^{32} Chromatography Manager	Version 3.2 or higher
MassLynx Software	Version 3.3 or higher

2.2 Unpacking and Inspecting

The components of the 2795 Separations Module are packed and shipped in two or more cartons that contain the following items:

- Waters 2795 Separations Module
- Column heater (optional, with optional selection valve installed)
- Certificate of structural validation/certificate of compliance
- Power cord
- Release notes
- Waters 2795 Separations Module Startup Kit

To unpack the Waters 2795 Separations Module and its components:

1. Remove the bands securing each carton to the pallet. Remove the cartons, the solvent bottle tray, the Startup Kit, and packing material.

2. Check the contents of each carton against the packing slip to confirm that all items are included.

> ⚠ **Caution:** *To avoid injury, ensure that at least two persons lift the 2795 Separations Module from the pallet to the bench.*

3. Lift the 2795 Separations Module from the pallet and place it at the site you have chosen for installation.

4. Check that the instrument serial number (found in the syringe compartment below the syringe mounting plate) corresponds to the number on the validation certificate.

5. Check that the contents of the Startup Kit match the Startup Kit list. In addition, check that the Startup Kit includes the following:

   - *Waters 2795 Separations Module Operator's Guide*
   - *Waters 2795 Separations Module Quick Start Guide*
   - *Waters Alliance LC/MS System Quick Start Guide*

   Note: *For Alliance HT systems operating under MassLynx software control, the* MassLynx Data Acquisition Guide *and the* MassLynx User's Guide *are required; the* MassLynx BioLynx & ProteinLynx User's Guide *and/or the* MassLynx OpenLynx User's Guide *are optional.*

6. If you detect any damage to the contents of any of the cartons, refer to the Waters *Licenses, Warranties, and Support* document.

7. Save the shipping cartons for future transport or shipment.

If you detect any discrepancy in the contents of the order, contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only.* Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

Continue the installation by following the procedures in .

STOP **Attention:** *Do not power on the 2795 Separations Module until you have installed the column heater and completed all fluidic and signal connections.*

Note: *The 2795 bar code reader is an option installed in the field by Waters Field Service Engineers.*

2.3 Installing the Column Heater

STOP **Attention:** *To avoid possible damage to components, power off the 2795 Separations Module before you connect or disconnect the column heater cable.*

STOP **Attention:** *Before you can install the column heater, you need to remove the 2795 Separations Module side panel and the foam restraints that secure the X-Y-Z needle assembly in the shipping position.*

Figure 2-2 shows the dimensions of the 2795 XE (with the column heater installed).
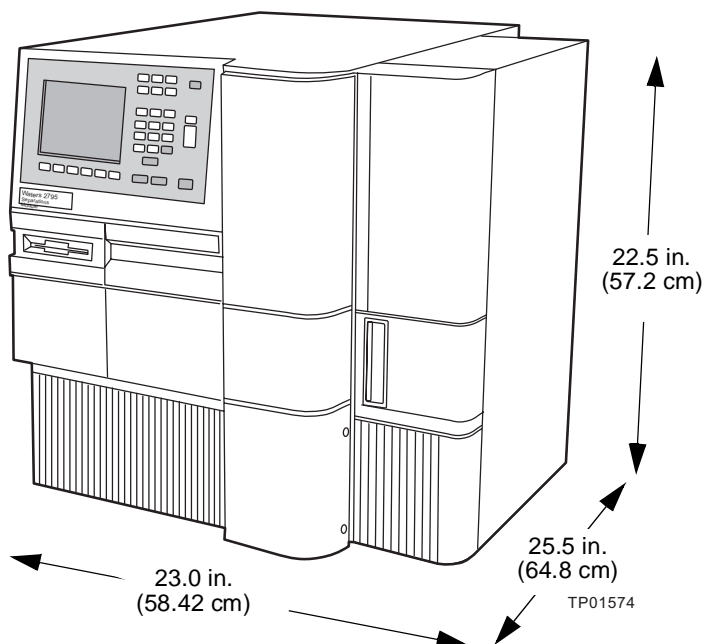
Figure 2-2 Waters 2795 XE Separations Module Dimensions (with Column Heater)

STOP ***Attention:*** *To avoid overheating the 2795 Separations Module, provide at least 2 inches (5 cm) of clearance at the rear of the Separations Module. Provide at least 6 inches (10 cm) clearance to the right of the column heater for room to open the column heater door.*

The column heater is mounted on the right side panel of the 2795 Separations Module with three screws (Figure 2-3). A connector on the rear panel (Figure 2-4) provides power and signal connections to the column heater.
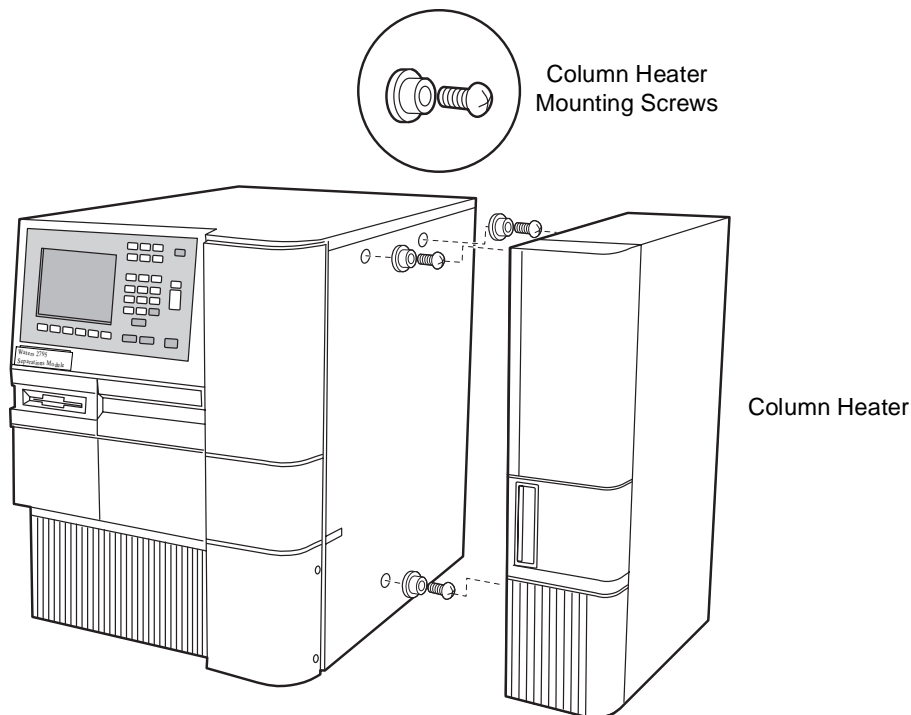
Figure 2-3 Installing the Column Heater

Required Materials

- #2 Phillips-head screwdriver
- Screws and retaining washers, three (Startup Kit)
- Column heater cable
- Fittings and tubing if equipped with an optional column-selection valve (Startup Kit)

To install the column heater:

1. Power off the 2795 Separations Module.
2. Install the three screws and retaining washers on the right side panel of the 2795 Separations Module (see Figure 2-3).
3. Mount the column heater on the three screws on the right side panel.
4. Connect one end of the column heater cable to the rear of the column heater. Connect the other end of the cable to the connector on the rear panel of the 2795 Separations Module (Figure 2-4).

Column Heater
Connector

B A

I/O Connectors
(A, B)

RS-232 Connectors
(A, B)

Power
Connector

B A

IEEE-488
Connector
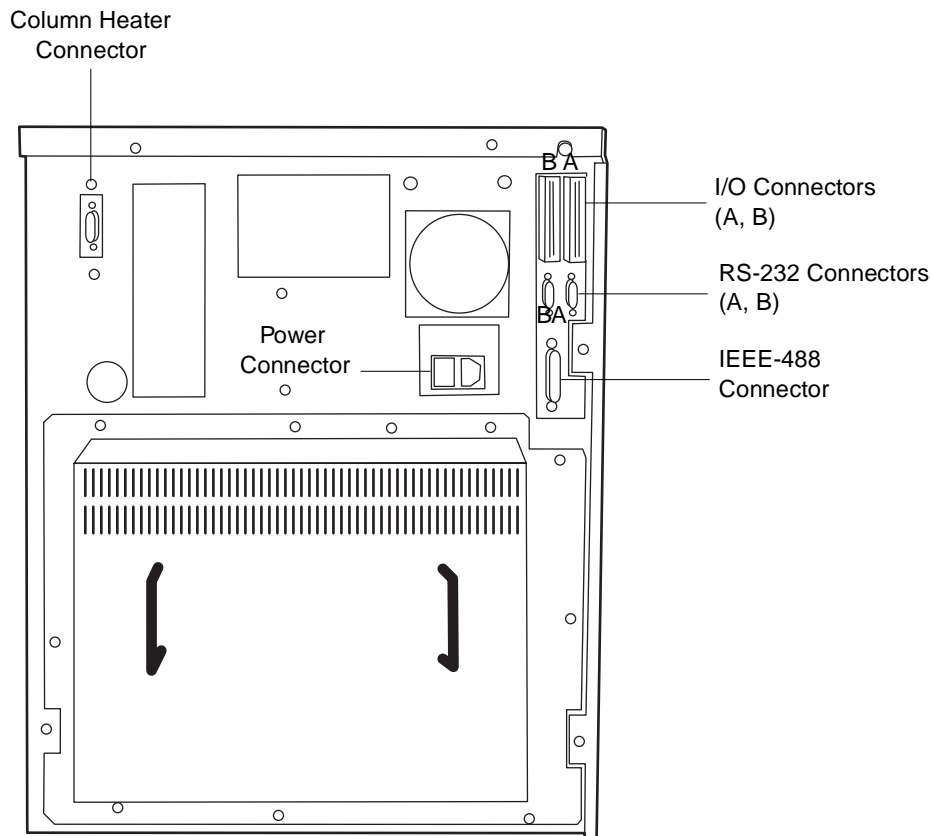
TP01582

Figure 2-4 2795 Separations Module Rear Panel Connections

STOP ***Attention:*** *Do not power on the 2795 Separations Module until you have installed the column heater and completed all fluidic and signal connections.*

2.4 Making Fluidic Connections

Figure 2-5 summarizes the major steps in making fluidic connections to the 2795 Separations Module.
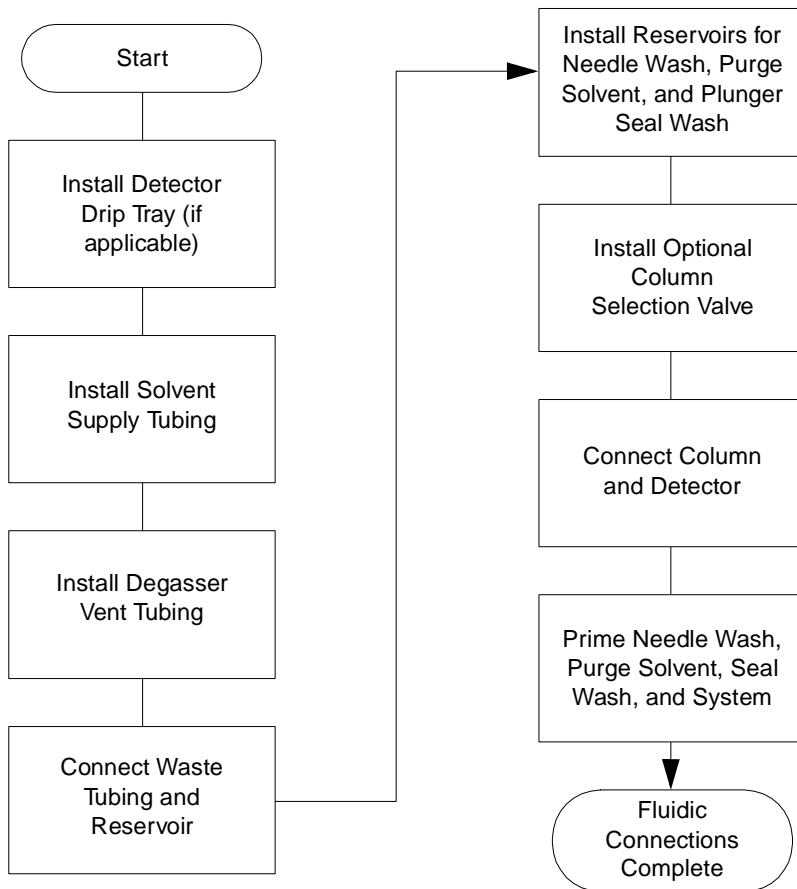
Figure 2-5 Steps to Making Fluidic Connections

Figure 2-6 is a schematic of the 2795 Separations Module fluidic connections. It does not show routing of fluidic lines.
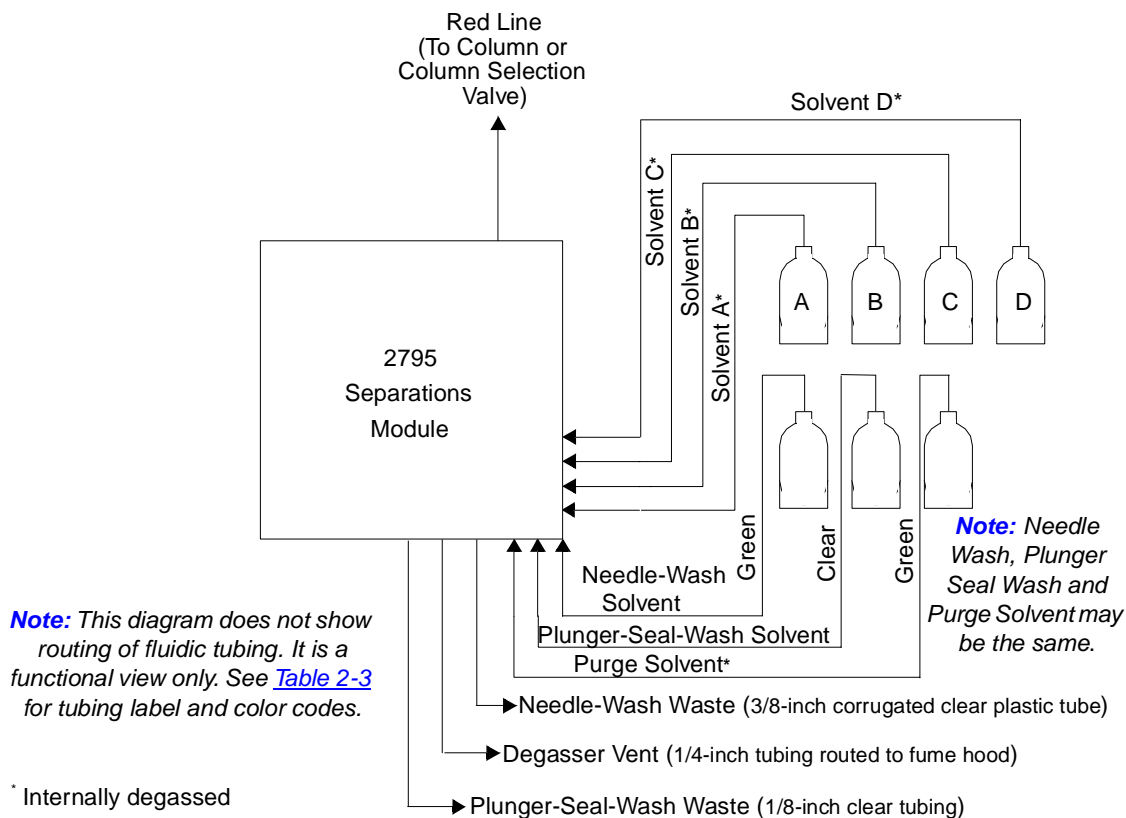
Figure 2-6 2795 Separations Module Fluidic Connections (Functional Diagram)

Required Materials

- Razor blade or tubing cutter
- 3/16-inch ID tubing (Startup Kit)
- 3/16-inch barbed fitting, if installing the detector drip tray (Startup Kit)
- 90° elbow, 2 each (Startup Kit)
- 0.005-inch ID tubing (Startup Kit)
- Purse clips (Startup Kit)

Before You Begin

Before you can make any tubing connections:

1. Uncoil the solvent tubes stored behind the solvent conditioning tray access door (Figure 2-7).

2. Unclip the tubing clips and install the tubing in the two holding clips. Close the clips to secure the tubing (Figure 2-8).

3. Route the tubing so that the solvent conditioning tray door closes over the tubing.
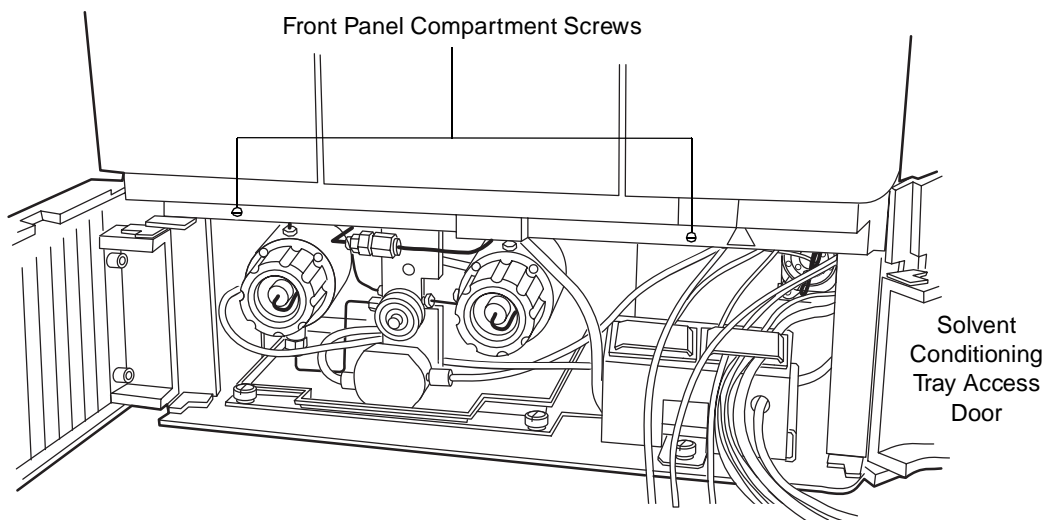
Figure 2-7 Solvent Conditioning Tray Compartment
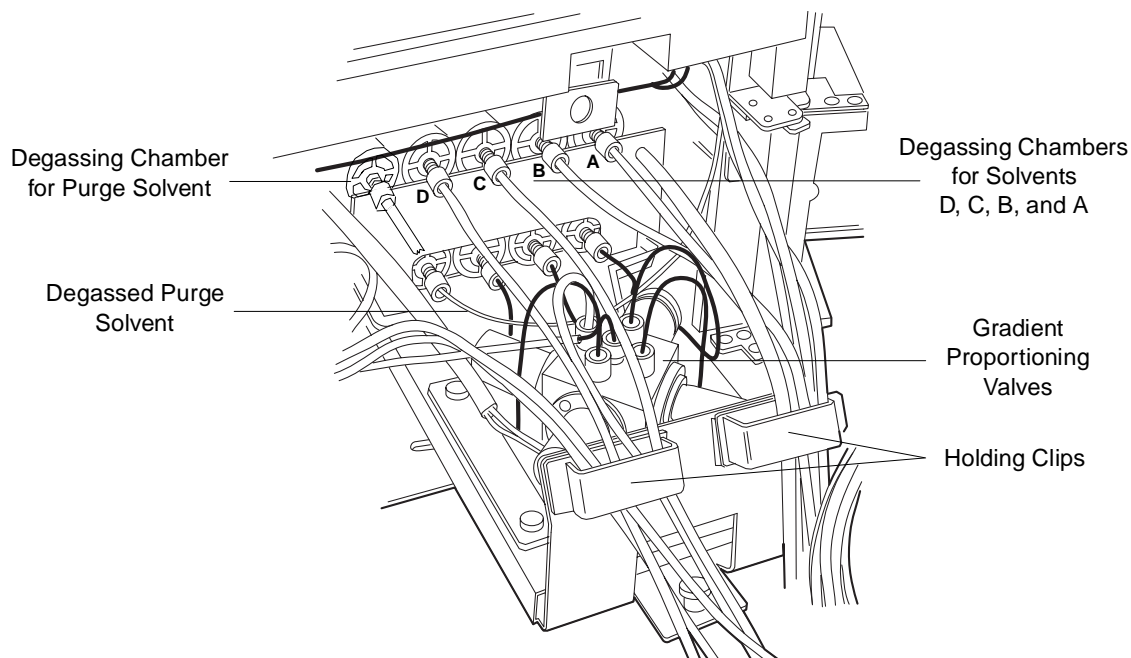
Figure 2-8 Degasser and Gradient Proportioning Valves

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 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.*

4. Route the tubing under the 2795 Separations Module, then up the rear of the unit through the purse clips to the 2795 Separations Module solvent reservoirs.

2.4.1 Installing the Detector Drip Tray and Waste Tubing

STOP *Attention: To ensure proper drip protection, be sure the retaining screws in the solvent delivery and solvent conditioning trays are tightened before you operate the system.*

If you want to position a detector on top of the 2795 Separations Module:

1. Finger-tighten the 3/16-inch barbed fitting into the drain hole in the bottom of the drip tray.
2. Remove the backing from the adhesive pads on the bottom of the drip tray.
3. Open the syringe access door (Figure 1-1) and pass the barbed fitting through the hole in the top panel and into the syringe compartment.
4. Mount the drip tray so the adhesive pads adhere to the top cover of the 2795 Separations Module.
5. Position the solvent bottle tray on top of the 2795 Separations Module (left side) as shown in Figure 1-1. You can place up to four 1-L reservoirs and one 250-mL reservoir in the solvent bottle tray.

2.4.2 Installing Solvent Supply Tubing

The tubing connecting the 2795 Separations Module to the solvent reservoirs is stored behind the solvent conditioning tray access door on the front panel (Figure 2-7). One end of each solvent tube is connected (at the factory) to the inline vacuum degasser which is connected to the GPV (Figure 2-8).

Each tube is identified with a letter (corresponding to each solvent reservoir) and a color-coded label. Table 2-3 indicates the letter and color code for each of the four solvent tubes.

Table 2-3 Solvent Supply Tubing Identification

Solvent Tubing Label	Solvent Tubing Color	Function	Routing
A	Yellow	Degassed internally	After degassing, to Gradient Proportioning Valve
B	Blue	Degassed internally	After degassing, to Gradient Proportioning Valve
C	Red	Degassed internally	After degassing, to Gradient Proportioning Valve
D	Green	Degassed internally	After degassing, to Gradient Proportioning Valve
Purge Solvent	Green	Degassed internally	After degassing to Purge Valve

To install the solvent tubing in the solvent reservoirs:

1. Remove the filter from each solvent tube and insert the free end of each tube through the appropriate reservoir cap.

2. Reinstall a filter on the end of each solvent tube.

3. Install each reservoir cap on the appropriate reservoir. Make sure each solvent filter is at the bottom of the reservoir.

2.4.3 Installing the Degasser Vent Tubing

Solvent vapors drawn off by the degasser may condense to form droplets in the degasser exhaust system. The degasser exhausts these gases and droplets, if any, through a vent tube.

⚠ *Caution: To avoid exposure to solvent vapors, connect the degasser exhaust tube to a suitable fume hood. Check local building and health codes for specific requirements regarding the venting of solvent vapors.*

To install the degasser vent tubing, place the free end of the degasser vent tube in a waste container that is in a properly functioning fume hood.

🛑 *Attention: Do not run the 2795 Separations Module in the degasser "ON" mode without having solvent flowing through the supply tubes. Without solvent flow, excess solvent vapor may condense in the degasser vacuum chamber walls, which could lead you to suspect a vacuum chamber leak.*

2.4.4 Installing the Waste Tubing

Waste tubes are needed to drain the:

- Spill tray
- Waste from the sample compartment
- Needle wash

Note: Installation of waste tubing from the detector drip tray is described in Section 2.4.1, Installing the Detector Drip Tray and Waste Tubing.

Spill Tray Waste Tubing

The spill tray waste outlet is located under the front panel of the 2795 Separations Module, below the solvent conditioning tray access door (Figure 2-7).

Required Materials

- 3/16-inch ID tubing (Startup Kit)
- Razor blade or tubing cutter
- 90° elbow (Startup Kit)

To install the spill tray waste tube:

1. Cut a length of 3/16-inch ID tubing long enough to reach from the spill tray outlet to the front of the bench.
2. Connect one end of the tubing to the spill tray outlet.
3. Connect a 90° elbow to the other end of the tubing.
4. Cut another length of 3/16-inch ID tubing long enough to reach from the elbow fitting to the waste reservoir.
5. Connect the elbow and waste reservoir with the tubing.

Sample Compartment Waste Tubing

The waste outlet from the sample compartment is located under the front panel of the 2795 Separations Module, to the right of the spill tray outlet. Spills from the sample plates and condensate from the sample heater/cooler drain through this waste outlet.

Required Materials

- 3/16-inch ID tubing (Startup Kit)
- Razor blade or tubing cutter
- 90° elbow (Startup Kit)

To install the sample compartment waste tube:

1. Cut a length of 3/16-inch ID tubing (from the Startup Kit) long enough to reach from the sample compartment waste outlet to the front of the bench.

2. Connect one end of the tubing to the waste outlet.

3. Connect a 90° elbow (from the Startup Kit) to the other end of the tubing.

4. Cut another length of 3/16-inch ID tubing long enough to reach from the elbow fitting to the waste reservoir.

5. Connect the elbow and waste reservoir with the tubing.

Needle Wash Waste Tubing

The needle wash, purge (mobile phase), and bypass valve waste tubes are combined inside the 2795 Separations Module and flow into the clear 3/8-inch corrugated plastic needle wash waste tube. Route this tube to a waste reservoir before using the 2795 Separations Module. See Table 2-4.

Note: Place the waste reservoir at least 20 inches below the bench level of the 2795 Separations Module so the solvent waste drains properly.

Plunger-Seal-Wash Waste Tubing

Place the plunger-seal-wash waste tube (1/8-inch, clear) in a suitable waste container. See Table 2-4.

2.4.5 Installing the Wash Reservoirs

The 2795 Separations Module requires at least two wash reservoirs to supply the three wash lines:

• Needle wash

• Purge

• Plunger seal wash

The tubing for the wash reservoirs is located behind the solvent conditioning tray access door on the front panel (see Figure 2-7). Each wash tube is labeled and/or color-coded as indicated in Table 2-4. The plunger-seal-wash inlet tube has a diffuser attached to one end.

STOP *Attention: To prevent damage to the 2795 Separations Module, be sure to use solutions that are miscible with the mobile phase and with the sample diluent you are using. For more information, see Appendix C, Solvent Considerations.*

Table 2-4 Needle Wash, Plunger Seal Wash, and Purge Tube Color Codes

Wash Tube	Label	Color Code	Outer Diameter (inches)
Needle Wash Inlet	WASH SOLVENT	Green Tag	1/8
Purge Inlet	PURGE SOLVENT	Green Tag	1/8
Plunger Seal Wash Inlet	SEAL WASH IN	Green Tag	1/8
Needle Wash Waste	No Label (corrugated plastic)	Clear	3/8
Plunger Seal Wash Waste	No Label	Clear	1/8

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 above the laboratory bench.*

To install the wash reservoirs:

1. Place the reservoirs at least 20 inches above the laboratory bench.

2. Remove the diffuser from the needle wash inlet tube (labeled WASH SOLVENT), insert the tube through a reservoir cap, reinstall the diffuser at the end of the tube, and install the cap on the needle wash reservoir.

3. Remove the diffuser from the purge inlet tube (labeled PURGE SOLVENT), insert the tube through a reservoir cap, reinstall the diffuser at the end of the tube, and install the cap on the purge reservoir.

4. Remove the diffuser from the plunger seal wash inlet tube (clear, labeled SEAL WASH IN), insert the tube through a reservoir cap, reinstall the diffuser on the end of the tube, and install the cap on the purge/plunger seal wash reservoir (the purge solvent and plunger seal wash solvent can be the same).

2.4.6 Connecting the Column

The red outlet tubing from the inject valve of the sample management system is located behind the right side panel and connects to the column inlet (**Figure 2-9**) or to the column-selection valve (if installed). See Section 2.4.7, Connecting the Column-Selection Valve.

To connect the column directly to the red outlet tubing:

1. Pass the red outlet tubing through the opening in the middle of the right side panel.

2. Connect the red outlet tubing to the column inlet and tighten the fitting until snug.

Note: To optimize results using microbore columns or high-throughput methods, Waters recommends that you replace the red outlet tubing with an appropriate length of 0.005-inch ID tubing.

Figure 2-9 shows column connections when no column-selection valve is installed in the column heater.
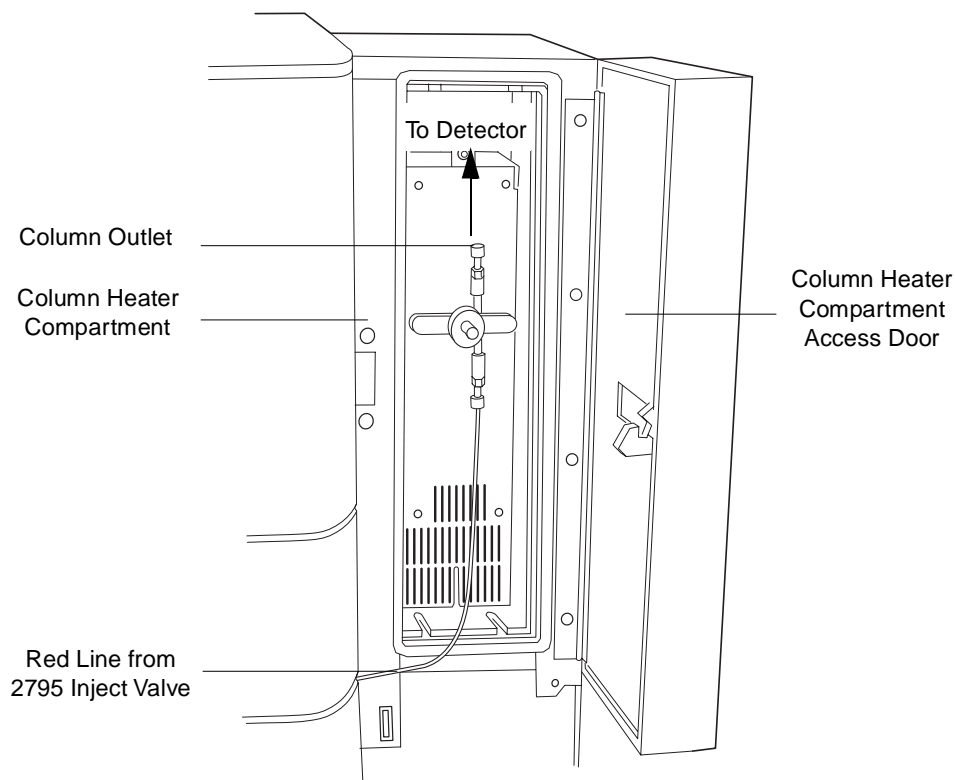
Figure 2-9 Connecting the Column

2.4.7 Connecting the Column-Selection Valve

The 2795 Separations Module is available with three optional column-selection valves that allow you to switch the flow path among multiple columns:

• 3-column selection valve

• 6-column selection valve

• Column regeneration valve

Three-Column Selection Valve

The 3-column selection valve (Figure 2-10) is an eight-port, three-position valve preconfigured at the factory as follows:

- Port 1 – Route to the column (2.1 mm × 50 mm column is supplied)
- Port 2 – Route to waste
- Port 3 – Route through a restrictor bypass to the detector
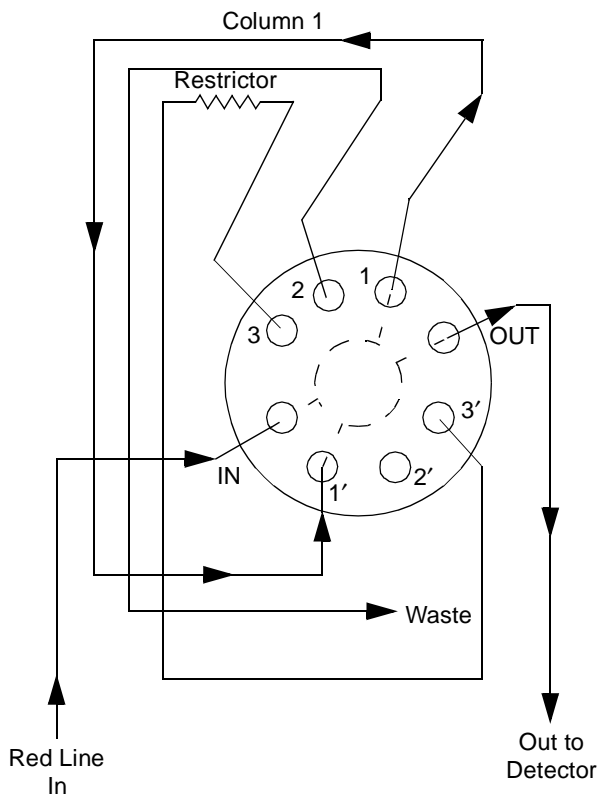
Figure 2-10 Three-Column Selection Valve Configuration (As Shipped)

You can complete installation of the 3-column selection valve after you have installed the column heater (see Section 2.3, Installing the Column Heater).

Required Material

5/16-inch open-end wrench

Procedure

To connect the 3-column selection valve to the sample management system fluidic path:

1. Use the 5/16-inch wrench to connect the red line (from the 2795 Separations Module inject valve) to the IN port on the column selection valve.

2. Use the tubing and fittings (supplied with the 3-column selection valve) to connect the OUT port of the column selection valve to the detector inlet.

3. Replace the stainless steel union shipped inside the column heater with the column (supplied with the valve, or another column of your choice).

4. Route the waste line (installed) from Port 2 of the column selection valve through the hole in the bottom of the column heater to a suitable waste reservoir.

You can reconfigure the 3-column selection valve as appropriate for your application.

Six-Column Selection Valve

The 6-column selection valve is shipped separately with a coil of 0.010-in PEEK tubing, a 0.0025-inch ID restrictor tube, and Upchurch Sealtight™ fittings. Connect as appropriate for your application (Figure 2-11).

Note: Any of the six column positions can be used for a restrictor or for waste as in the three-column selection valve (see the example in Figure 2-10).

2
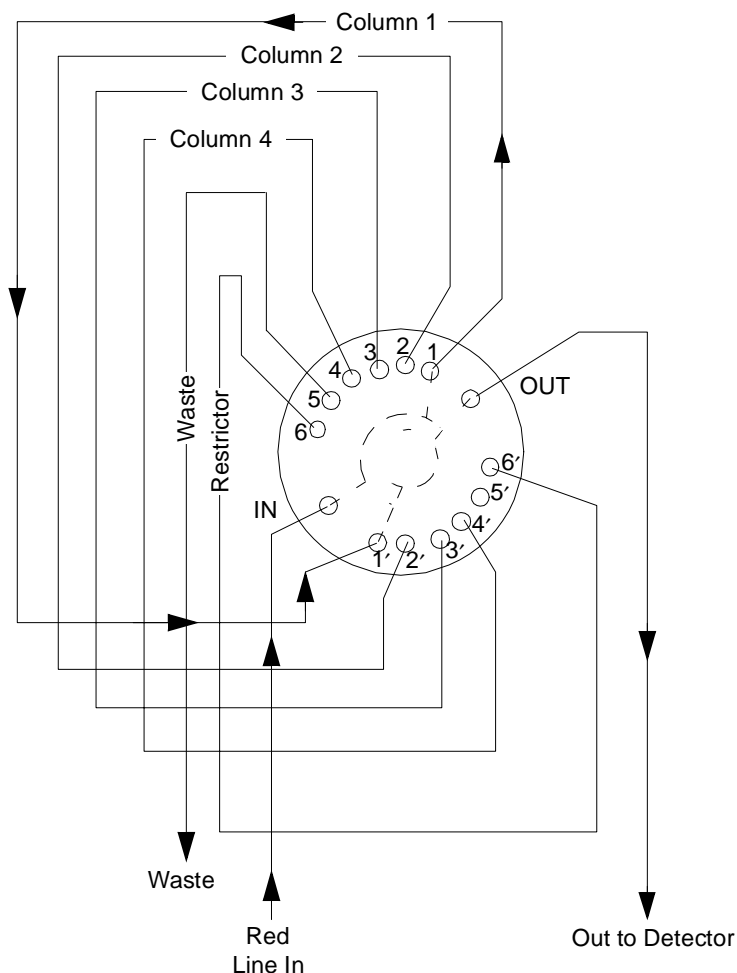
Figure 2-11 Six-Column Selection Valve Connections (Example)

Column Regeneration Valve

The column regeneration valve is shipped separately with a coil of 0.010-inch PEEK tubing, a 0.0025-inch ID restrictor tube, and Upchurch Sealtight fittings. Connect as appropriate for your application (see the example in). Contact Waters Technical Service for more information.
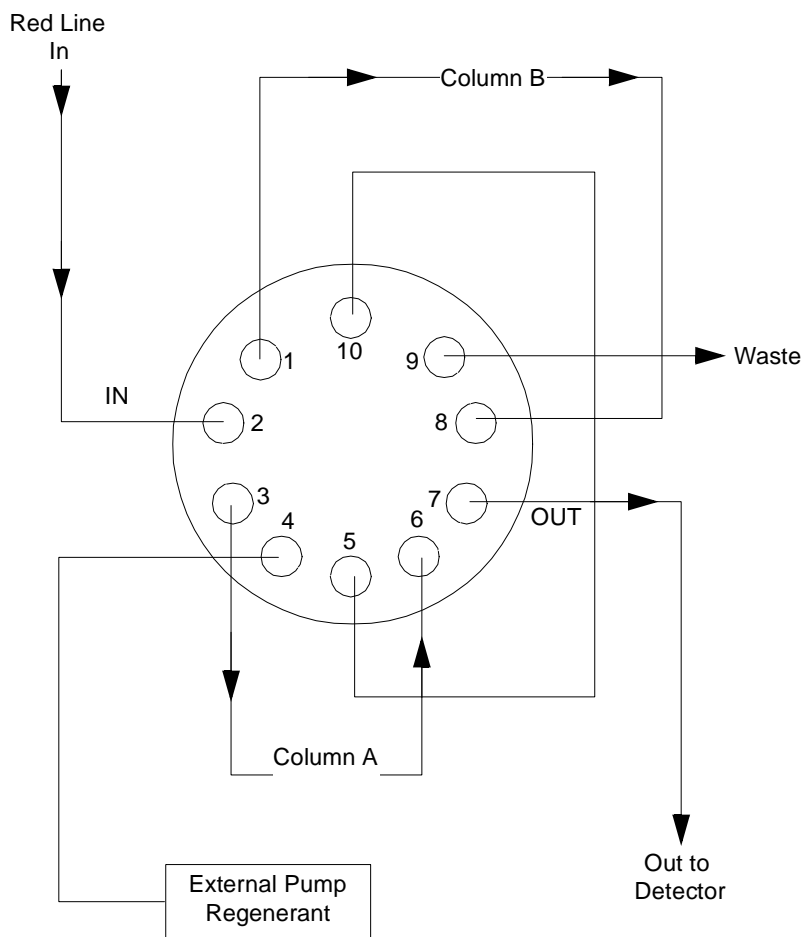
Figure 2-12 Column Regeneration Valve Connections (Example)

2.4.8 Connecting the Detector

You can place a Waters detector on either the top or the right side of the 2795 Separations Module. Place the Waters ZQ or other mass spectrometer as shown in Figure 1-2. If you place a detector on top of the 2795 Separations Module, be sure to install the drip tray using the procedure in Section 2.4.1, Installing the Detector Drip Tray and Waste Tubing.

Required Materials

- 0.010-inch ID PEEK tubing (Startup Kit)

- Razor blade or tubing cutter

- 3/16-inch ID tubing (Startup Kit)

- Waste container

To connect the detector:

1. Cut a length of 0.010-inch ID tubing just long enough to reach from the column outlet to the detector inlet.

   Note: To optimize results using microbore or high-throughput methods, Waters recommends that you replace the detector inlet tubing with an appropriate length of 0.005-inch ID tubing.

2. Connect the outlet of the column to the inlet of the detector with the tubing.

3. Connect the detector outlet to a suitable waste container with a length of 3/16-inch tubing.

STOP *Attention: To avoid spill damage, use a drip tray below the fluidic connections on the detector.*

2.5 Making Signal Connections

2.5.1 Configuration Overview

This section describes the analog (I/O) and digital signal connections required in an HPLC or LC/MS system with a 2795 Separations Module. The configuration mode (of the 2795 Separations Module) depicted in each figure is indicated in parentheses.

- When the 2795 Separations Module is part of an Alliance HT LC/MS system (controlled by MassLynx software), make signal connections as indicated in Figure 2-13.

- When the 2795 Separations Module is part of an LC system (controlled by Millennium[32] software), make signal connections as indicated in Figure 2-14.

- When the 2795 Separations Module functions as the system controller, make signal connections with:

  – A Waters detector, as shown in Figure 2-15.

  – A third-party detector, as shown in Figure 2-16.

  – A Waters or a third-party detector operating with Millennium[32] data acquisition control, as shown in Figure 2-17.

Analog Cable for 2487 Signal

IEEE-488 Cable

Waters 996/2996 or 2487 Detector (Optional)

2795 Separations Module

MassLynx Data System

Port A

Inject Start

RS-232 Cable

MassLynx Proprietary Interface Cable

Waters ZQ or Other Mass Spectrometer

Serial Printer (optional)
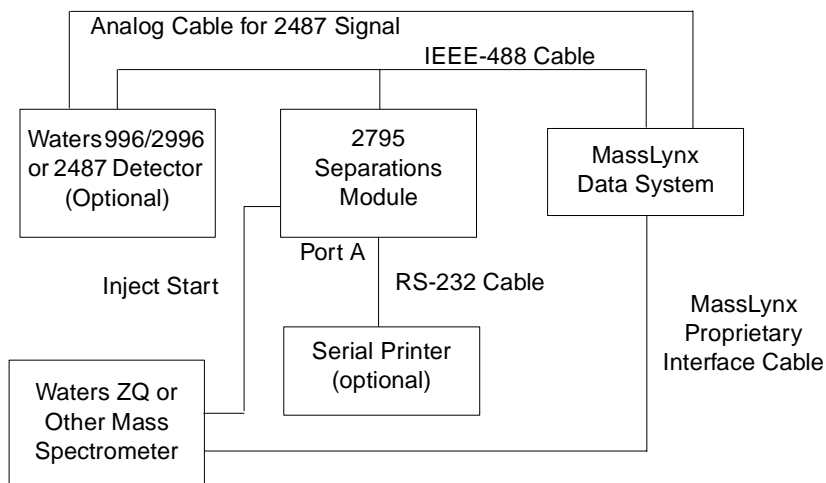
Figure 2-13 Signal Connections in an Alliance HT LC/MS System (Controlled by MassLynx Software)

IEEE-488 Cable

Waters 996/2996 or 2487 Detector (Optional)

2795 Separations Module

Millennium[32] Chromatography Manager

Port A

RS-232 Cable

Serial Printer (optional)
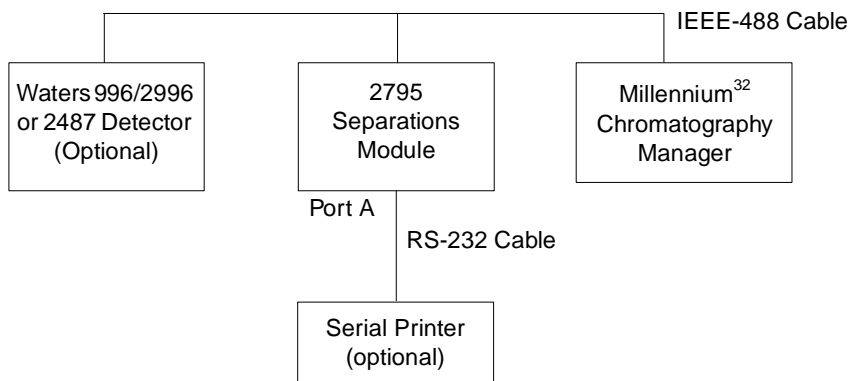
Figure 2-14 Signal Connections in an Alliance HPLC System (Controlled by Millennium[32] Software)

Waters 486, 2487, 410, or 2410 Detector

IEEE-488 Cable

2795 Separations Module

RS-232 Cable

Port A

Serial Printer or Waters 746 Data Module (optional)
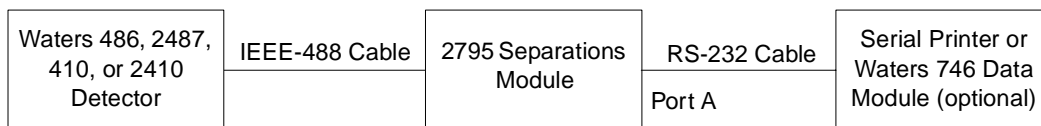
Figure 2-15 Signal Connections in an Alliance HT System with a Waters Detector (System Controller Mode)

Installing the 2795 Separations Module   79

```
┌─────────────────┐     ┌─────────────┐  Analog Cable    ┌─────────────────┐
│ Non-Waters Data │─────│ Non-Waters  │  (Inject Start)  │ 2795 Separations│
│     System      │     │  Detector   │──────────────────│     Module      │
└─────────────────┘     └─────────────┘                  └─────────────────┘
```
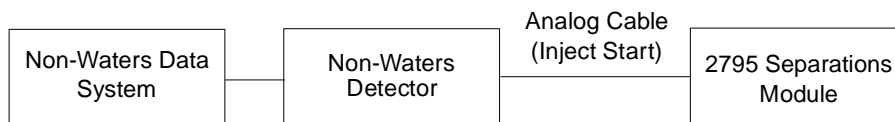
Figure 2-16 Signal Connections in an HPLC System with Non-Waters Components
(No Interaction Mode)
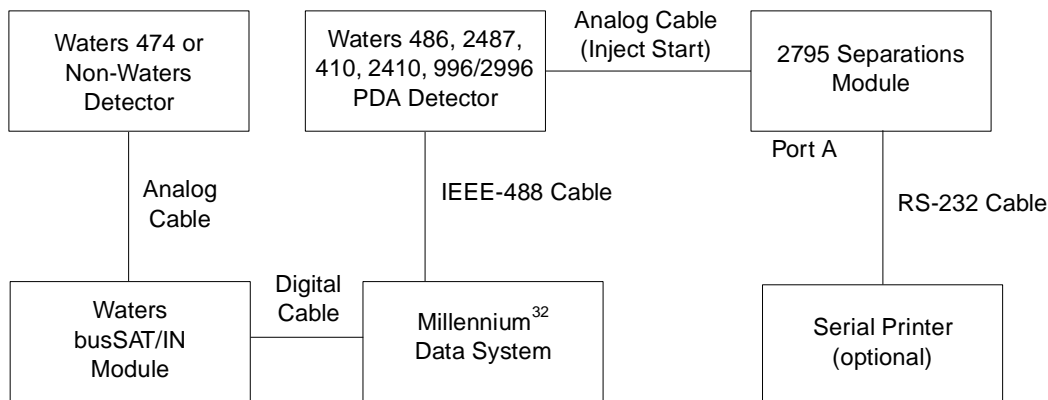
Figure 2-17 Signal Connections in an Alliance HT System Controlled by
Millennium[32] Software (No Interaction Mode)

2.5.2 Making Analog Signal Connections

The 2795 Separations Module rear panel includes two (A, B) removable connectors
(Figure 2-18) that hold the screw terminals for analog signals. These connectors are
keyed so that they can be inserted only one way. The analog signals function as I/O or
event signals.

STOP ***Attention:*** *To avoid the risk of damaging other components, do not remove the keys in the
A and B connectors. These keys ensure that the A connector installs only in the right slot
and the B connector installs only in the left slot.*
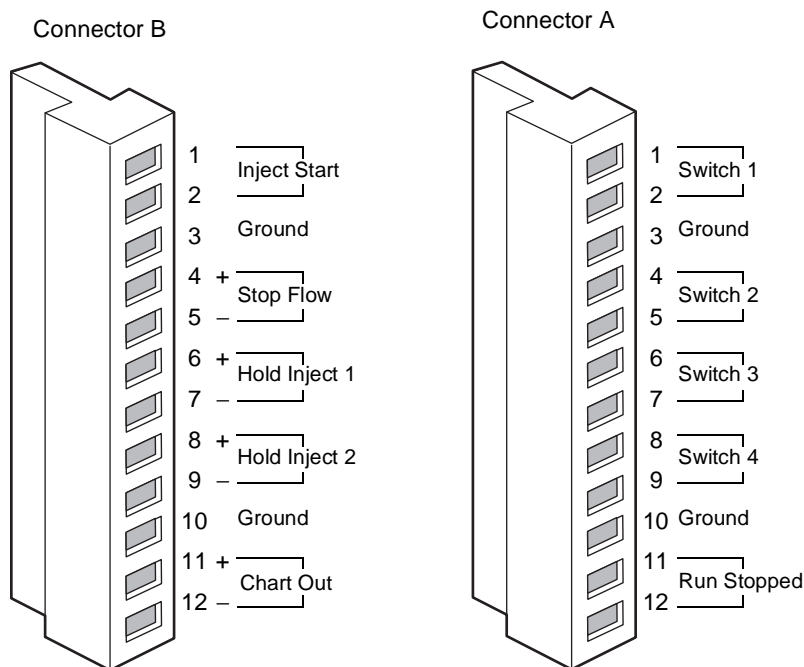
Figure 2-18 I/O Signal Connectors

I/O Signals

Table 2-5 describes each of the signals available on the I/O connectors of the 2795 Separations Module. Refer to Appendix A, Specifications, for details on the electrical specifications for each signal.

Table 2-5 I/O Signals

Terminal	Signal Description
Inject Start	An output (contact closure) to indicate that an injection has begun. Connect a wire pair to the two Inject Start terminals.
Stop Flow	An input signal from other LC or MS components to stop the solvent flow from the 2795 Separations Module. Using the Events In screen key, you can specify the signal conditions to stop the solvent flow. Connect the positive input wire to the Stop Flow+ terminal and the negative input wire to the Stop Flow– terminal.

Table 2-5 I/O Signals *(Continued)*

Terminal	Signal Description
Hold Inject 1 and 2	Input signals from other LC components to delay the injection. Using the Events In screen key, you can specify the signal conditions that prevent the 2795 Separations Module from making an injection. Connect the positive input wire to the appropriate Hold Inject + terminal and the negative input wire to the appropriate Hold Inject – terminal.
Chart Out	An output signal (selected in the Configuration screen) to a device such as an integrator or strip chart recorder (see Section 5.2.6, Setting I/O Parameter Values). You can select one of the following parameters as the Chart Out signal: • Flow rate • System pressure • Composition (%A, %B, %C, %D) • Sample temperature • Column temperature • Degasser vacuum See Table 2-6 for the settings for each of these parameters.
Switch 1–Switch 4	See the "Event Switches" discussion that follows in this section.
Run Stopped	An output signal indicating that the 2795 Separations Module has ceased operation due to an error condition or to an operator request (pressing the Stop Flow button, for example).

Table 2-6 describes each of the signals available on the Chart Out Signal connectors of the 2795 Separations Module. Refer to Appendix A, Specifications, for details on the electrical specifications for each signal.

Table 2-6 Chart Out Signal Conditions

Chart Out Signal	Parameter Setting at 0.0 V (Minimum)	Parameter Setting at 2.000 V (Maximum)
Flow Rate	0.000 mL/min	10.000 mL/min
System Pressure	–50 psi (–3.45 bar, –344.7 kPa)	5500 psi (380 bar, 37,920 kPa)
Composition	0.0 %	100.0 %
Sample Temperature	4 °C	40 °C

Table 2-6 Chart Out Signal Conditions *(Continued)*

Chart Out Signal	Parameter Setting at 0.0 V (Minimum)	Parameter Setting at 2.000 V (Maximum)
Column Temperature	20 °C	60 °C
Degasser Vacuum	0.0 psi	4.91 psi (0.34 bar, 33.85 kPa)

Event Switches

You can use Switches 1 through 4 on Connector A (Figure 2-18) as contact closure switches to control a column selection valve if you do not have the Column Selection option installed (see Section 1.4, Options and Accessories), fraction collectors, or similar external devices. Each pair of switch terminals (the two Switch 1 terminals, for example) is connected directly to an external device. You can operate the switches:

- **Manually** – From the Direct Functions screen (see Section 4.2, Performing Direct Functions)
- **Automatically** – In a separation method using the Events table in the I/O screen (see Section 5.2.6, Setting I/O Parameter Values, the "Setting the I/O Events Table Parameter Values" discussion)

Table 2-7 describes the functions associated with each position of an event switch.

Table 2-7 Event Switch Positions and Functions

Position	Function
On	Closes the switch
Off	Opens the switch
Toggle	Changes the current state of the switch
Pulse	Closes/opens the switch for a user-defined period
No Change	Leaves the switch in its current state

2.5.3 Making Digital Signal Connections

Digital signal communication with the 2795 Separations Module occurs through the:

- RS-232 connector (two ports, each equipped with a DB-9 connector)
- IEEE-488 connector

RS-232 Connections (Port A Only)

When the 2795 Separations Module is operating as the system controller, use Port A (see Figure 2-15) to transmit data to a Waters 746 Data Module or a serial printer. Connect Port A to the 746 data module or the printer using an RS-232 cable.

Press the **Print** screen key to transmit print data to a connected serial printer.

Table 2-8 lists the values of the RS-232 output data parameters that you can select in the Configuration screen. For more information on the Configuration screen, refer to Section 3.2.1, Setting Configuration Parameters.

Table 2-8 RS-232 Output Parameters Displayed in the Configuration Screen

Parameter	Value
Device	None, Waters 746 Data Module, Serial Printer
Baud rate	1200 (for Waters 746 Data Module) 1200, 2400, 9600 (for serial printer)
Output code	Block/Binary

Port B is reserved for future use.

IEEE-488

Use the IEEE-488 interface connector (see Figure 2-13) to connect the 2795 Separations Module to:

- Waters 2487, 486, 2410, and 410 detectors
- A Millennium[32] data system
- A MassLynx data system

The IEEE-488 connector in the 2795 Separations Module connects to a standard IEEE-488 cable.

STOP *Attention: To avoid possible damage to components, power off all instruments connected to the IEEE-488 connector before you connect an IEEE-488 interface cable to an instrument.*

Refer to Figure 2-13 for the signal and electrical connections in an LC/MS system when the 2795 Separations Module is controlled by MassLynx software. Refer to Figure 2-14 for the signal connections in an LC system when the 2795 Separations Module is controlled by Millennium[32] software. Refer to Figure 2-15, Figure 2-16, and Figure 2-17 for the signal connections in a system when the 2795 Separations Module functions as the System Controller or in No Interaction mode.

Setting the IEEE-488 Address

For details on setting the IEEE-488 address for each connected instrument, see Section 3.2, Configuring the 2795 Separations Module, and the documentation for the instrument.

2.6 Making Power Connections

⚠ **Caution:** *To avoid damage to components, power off the 2795 Separations Module before you connect or disconnect the column heater cable.*

STOP **Attention:** *Do not power on the 2795 Separations Module until you have installed the column heater and completed all fluidic and signal connections.*

STOP **Attention:** *Ensure the top cover and side panel are attached before you apply power to the 2795 Separations Module.*

Note: *Proper operation of the 2795 Separations Module requires that the AC power supply is grounded and has no abrupt voltage fluctuations.*

The 2795 Separations Module automatically adjusts for AC input voltages in a continuous range from 85 Vac to 250 Vac, 47 to 63 Hz.

To make the power supply connection:

1. Check that the 2795 Separations Module Power On/Off switch is in the Off (**0**) position.
2. Connect the power cord to the connector on the rear panel of the 2795 Separations Module (Figure 2-4).
3. Connect the other end of the power cord to a properly grounded AC power supply.

Chapter 3
Setting Up the 2795
Separations Module

This chapter describes how to prepare and set up the Waters 2795 Separations Module for operation.

Powering On

STOP **Attention:** *Do not power on the 2795 Separations Module until you have installed the column heater and completed all fluidic and signal connections (Section 2.4, Making Fluidic Connections, and Section 2.5, Making Signal Connections).*

STOP **Attention:** *Ensure the top cover and side panel are attached before you apply power to 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 (ON) **I** position. The startup diagnostics routine begins.

Startup Diagnostics

The startup diagnostics routine performs tests on the:

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

As the startup diagnostics routine continues, the status of each diagnostic test appears in the banner area at the top of the front panel display ().
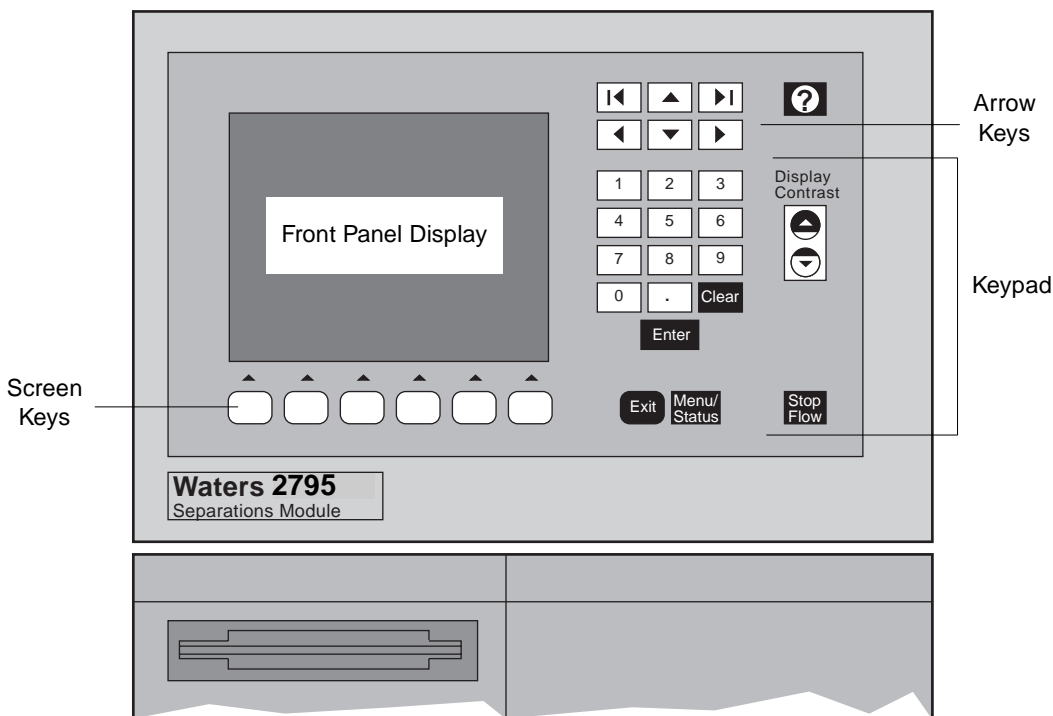
Figure 3-1 2795 Separations Module Display and Keypad

When initial startup diagnostics tests are complete, the Diagnostics Test Results Screen shows the results of the tests (Figure 3-2).


```
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 3-2 Diagnostics Test Results Screen

The Main Screen

If the initial tests of the startup diagnostics routine are successful, the Main screen (Figure 3-3) appears in the front panel display. If the startup diagnostics routine is not successful, refer to Section 8.5, Troubleshooting. When the startup diagnostics routine is complete, the 2795 Separations Module enters the Idle mode.
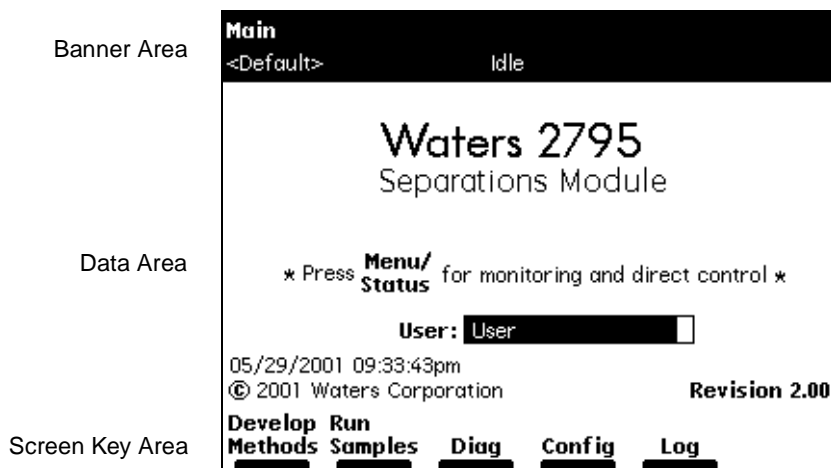
Figure 3-3 Main Screen

3.1 Using the Front Panel Display and Keypad

Whether you operate the 2795 Separations Module under stand-alone or remote control (see Section 1.1, Overview, the "System Configurations" discussion), use the Main screen to set up the instrument for operation.

3.1.1 Screen Display Overview

The Main screen and all other screens display three areas (see Figure 3-3):

- Banner
- Data
- Screen keys

Banner Area

This area of the screen displays the current status of the instrument by indicating:

- Name of the current screen
- **Current function status** – The current state of solvent flow, such as idle, refreshing the syringe, sample, or equilibration
- **Work status –** The number of programmed events that remain to be completed for the current sample set
- **Remote control** – When controlled by Millennium[32] software (version 3.2 or higher) or controlled by MassLynx software (version 3.3 or higher)

- **Current separation method and sample set** – The names of the separation method and sample set currently running

Data Area

This area of the screen shows specific programming and operating information about the instrument. There are two modes of display:

- **Menu (Mode)** – Includes all control features that allow you to perform certain tasks, such as configuring the 2795 Separations Module, or creating or editing separation methods or sample sets.

  The menu screens follow a hierarchical organization (the Main Screen is the top level). Pressing **Exit** allows you to navigate back through many levels of the menu.

- **Status/Control (Mode)** – Shows detailed status and interactive control information, such as flow rate, degassing rate, and solvent composition.

Pressing **Menu/Status** switches between the two display modes.

Screen Key Area

This area of the screen shows the current function of the screen keys located at the bottom of the display. Screen keys are described in more detail in .

Screen Keys in the Main Screen

describes the functions of most of the keys in the Main screen. Each screen key displays a different screen ().

Table 3-1 Screen Keys in the Main Screen

Screen Keys	Function	Reference
Develop Methods	Displays the Methods screen, allowing you to create, edit, view, copy, import, export, delete, and run stored methods. You access the floppy disk drive functions from this screen.	Chapter 5, Creating and Editing Separation Methods and Sample Sets
Run Samples	Displays the Run Samples screen, allowing you to run stored separation methods or sample sets.	Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation
Diag	Displays the Diagnostics screen, allowing you to run instrument diagnostics.	Chapter 8, Diagnostics and Troubleshooting

3

Table 3-1 Screen Keys in the Main Screen *(Continued)*

Screen Keys	Function	Reference
Config	Displays the Configuration screen, allowing you to enter values for instrument parameters.	Section 3.2.1, Setting Configuration Parameters
Log	Displays the error log.	Section 8.2, Using the Error Log

Other Screen Keys

Individual screen keys are described in the section pertaining to their usage. General screen keys that appear frequently are:

- **More** – Displays an additional set of screen keys.
- **Next/Previous** – Cycles through a group of related screens at the same level of the menu hierarchy.
- **OK** – Enters the selected or specified information in a window or dialog box, then proceeds to the next screen.
- **Cancel** – Exits from a dialog box and returns to the previous screen, or used to start over when entering information.
- **Plate** – Opens the Well Selector. Entry may be restricted to one well, depending on where you are in the program when you press the Plate screen key. See Section 4.2.9, Selecting Wells for Making Injections.

3.1.2 Using the Keypad

Table 3-2 describes the functions of the keypad keys on the front panel.

Note: Parameter fields that are not editable for the option you select have a dotted-line border. Screen keys that are not available for use are grayed out.

Table 3-2 Keypad Functions

Key	Function (for Screen Navigation)	Function (for Plate Navigation)[a]
◀	Moves the cursor one field to the left.	Moves the well cursor one or more positions to the left.
▶	Moves the cursor one field to the right.	Moves the well cursor one or more positions to the right.
▲	Moves the cursor to the field above the current field.	Moves the well cursor one or more positions up.

Table 3-2 Keypad Functions *(Continued)*
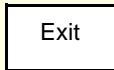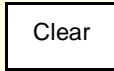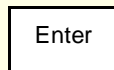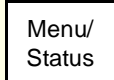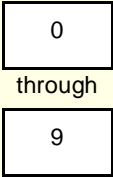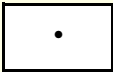
Key	Function (for Screen Navigation)	Function (for Plate Navigation)[a]
▼	Moves the cursor to the field below the current field.	Moves the well cursor one or more positions down.
I◀	Moves the cursor to the left-most field in the current row. Press twice to move the cursor to the first field in the screen.	Moves the well cursor several positions to the left (depending on plate capacity). Also used to move diagonally.
▶I	Moves the cursor to the right-most field in the current row. Press twice to move the cursor to the last field in the screen.	Moves the well cursor several positions to the right (depending on the plate capacity). Also used to move diagonally.
▲	Increases the display contrast.	
▼	Decreases the display contrast.	
?	Displays a context-sensitive Help screen. Press again to display a general Help screen. To close the Help screen, press **Exit** or **Clear**. See the "Using the 2795 Separations Module Help" discussion below.	
Exit	Closes an open menu or dialog box and returns to the previous screen (not used for Menu/Status).	
Clear	Clears the contents of the current field, or enters INIT, OFF, or other information in selected fields.	Clears selection of one well or a group of wells.
Enter	Stores the contents of the current field and advances the cursor to the next field in the screen.	Selects/deselects one well or a group of wells.
Menu/ Status	Switches the display between the Menu mode and the Status/Control mode.	

Table 3-2 Keypad Functions *(Continued)*

Key	Function (for Screen Navigation)	Function (for Plate Navigation)[a]
Stop Flow	Immediately stops the flow of solvent and suspends the current function (sample processing, priming, refreshing the syringe, and so on). Pressing this key also activates the Run Stopped output switch. Press **Resume** to resume or **Abort** to abort the current function.	
0 through 9	Enters the corresponding number into the current field, selects the corresponding gradient curve (0 = curve 10), or positions the cursor to the corresponding entry in a list (0 = tenth item).	
•	Enters a decimal point, selects gradient curve 11, or serves as a separator for well ranges. For example, to enter 2 through 5 in a well field, press **2.5**. Also positions the cursor at the last entry in a list.	Selects one well or a group of wells.

a. See Section 4.2.9, Selecting Wells for Making Injections, for more information on plate navigation.

Entering and Editing Values

Note: Parameter fields display default values until you change them. If you clear a field and do not enter another value, a warning message indicates that an entry in the field is required.

To enter parameter values:

1. Use the screen keys or the Menu/Status key to display the appropriate screen.

2. Use the arrow keys to select (highlight) the parameter field you want to change.

3. A diamond symbol is displayed next to a field that has a drop-down list. Press **Enter** to display the drop-down list of a selected field, use the arrow keys to select a value from the list, then press **Enter**.

4. To edit data fields:

    • Use the numeric keys to enter a number, then press **Enter**.

    • When in alphanumeric entry mode, use a screen key to enter a letter, then press **Enter**. See the "Entering an Alphanumeric String" discussion below.

Selecting/Deselecting Check Boxes

To select a check box, use the arrow keys to move to the check box, then press any numeric key to place an X in the box and activate the option. To deselect a check box, press any numeric key to clear the box and deactivate the option.

Entering an Alphanumeric String

When you select a field that requires an alphabetic entry, such as a user name or method name, each screen key is automatically labeled with a group of letters. Press the **More** screen key for additional selections, such as uppercase and lowercase letters, backspace, insert, or overstrike. Use the numeric keys and the decimal point character from the keypad to enter numbers or a decimal point. Use the arrow keys to position the text cursor. Press the **Clear** key to erase the contents of the field.

Alphabetic Screen Keys
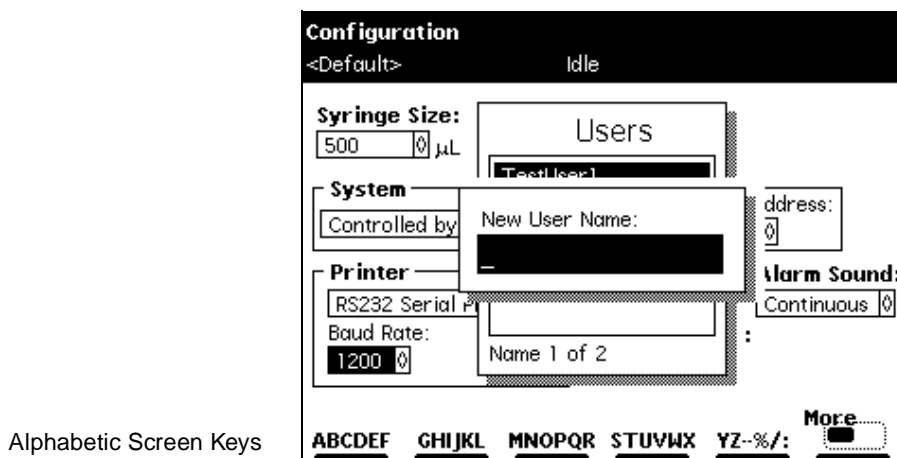
Figure 3-4 Alphabetic Screen Keys

You can enter up to 30 characters in most alphanumeric fields. To end and save an alphanumeric entry, press **Enter**.

Example

To enter the name "User2" in the New User Name field, use the following screen and keypad key sequence:

1. Press **STUVWX**.
2. Press **U**.
3. Press **More**, then press **Lowercase**.
4. Press **stuvwx**.
5. Press **s**.
6. Press **abcdef**.

7. Press **e**.

8. Press **mnopqr**.

9. Press **r**.

10. Press **2** on the front panel keypad.

11. Press **Enter**.

Using the 2795 Separations Module Help

The 2795 Separations Module Help system describes the parameters associated with user-selectable editable fields, check boxes, and drop-down lists in 2795 Separations Module screens and provides information on navigating through the front panel display screens.

When you press the Help key (see Table 3-2), the front panel displays a Help screen (Figure 3-5) with Help information for the selected field in the current screen.
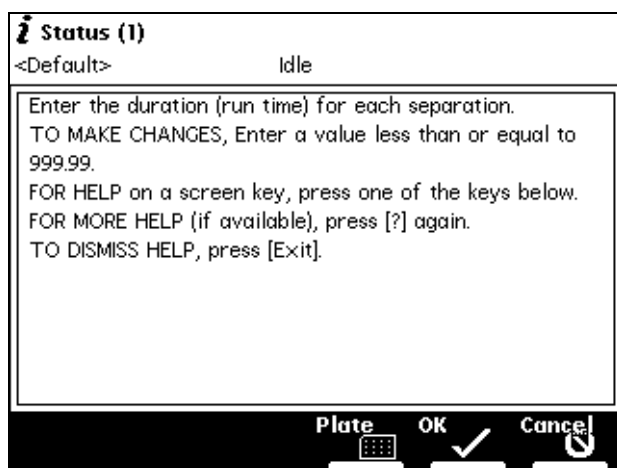
Figure 3-5 2795 Separations Module Help Screen (Example)

The Help information describes the parameter associated with the selected field. If the selected field has a range of available numeric parameter values, the Help screen displays the numeric range of values.

When appropriate, the Help screen provides special instructions associated with the displayed screen or dialog box and its associated field(s), check box(es), and/or drop-down list(s).

If you press Help a second time, the Help screen provides additional information about the current 2795 Separations Module screen.

When the Help screen is open, you have access to the screen keys available in the current 2795 Separations Module screen. Press any screen key to obtain Help about the use of that key.

Use the following keys to navigate the Help system:

- **Up arrow** – Scroll up one line
- **Down arrow** – Scroll down one line
- **Left block arrow** – Page up
- **Right block arrow** – Page down

If you receive a 2795 Separations Module alarm message, press **Help** to obtain information specific to the alarm message.

3.2 Configuring the 2795 Separations Module

Use the Configuration screen (Figure 3-6) to:

- Set up the 2795 Separations Module for the first time
- Change the configuration mode
- Add, change, or delete a user
- Reset the clock

Press the **Config** screen key in the Main screen to display the Configuration screen (Figure 3-6).

Figure 3-6 Configuration Screen (Example)

3.2.1 Setting Configuration Parameters

Table 3-3 describes the parameters you can set for your system configuration.

Table 3-3 Configuration Parameters

Parameter Name	Description
Syringe size/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 Table 7-5).
System	Specifies the operating mode and the IEEE-488 bus address (when required). See Section 3.2.3, 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.*
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). See Figure 3-7.
Pressure units	Specifies psi (pounds per square inch), kPa (kPascal), or bar
Alarm sound	Specifies the type of alarm (continuous or single beep)

3
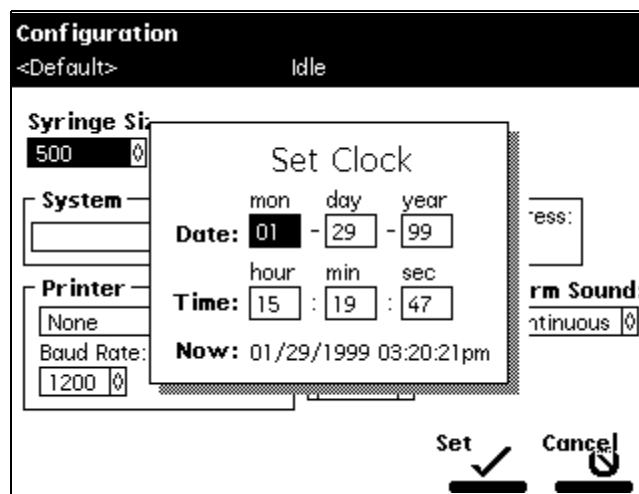
Figure 3-7 Set Clock Dialog Box

Pressing the screen keys in the Configuration screen accesses other parameters whose values you can specify. Table 3-4 describes the functions of these screen keys.

Table 3-4 Screen Keys in the Configuration Screen

Screen Key	Description
User Names	Displays a list of stored user names and allows you to add, change, and remove user names. User names are used for documentation purposes only (not for system or password security).
Detectors	Scans the IEEE-488 bus and displays a list of the Waters detectors connected to the bus, their addresses, and their firmware revision numbers. Active only when the 2795 Separations Module is configured as the System Controller and the detectors are powered on. See Section 3.2.3, Configuring the Operating Mode.
Options	Displays a list of the hardware options installed in the 2795 Separations Module.
Set Clock	Displays the Set Clock dialog box, allowing you to change the current time and date. See Section 3.2.1, Setting Configuration Parameters.
Events In	Specifies the external signal conditions to enable Stop Flow and Inject Hold. See the "Specifying Signal Conditions for Events In" discussion in this section.
More	Alternately displays the two sets of screen keys for the Configuration screen.
Reports	Specifies the type of information that is included in reports and where the reports are to be sent (floppy disk, printer). See the "Specifying Report Options" discussion in this section.
Column Switch	If a column selection valve is installed, allows you to select custom column position labels of up to 12 characters each.
Status Layout	Used to rearrange the status pages by selecting status features for display. See Section 4.3, Monitoring System Status.
Plate Types	Displays the Plate Type Configuration dialog box for managing up to 15 plate types. See Section 3.2.2, Configuring Plate Types.
Auto Shutdown	Specifies the parameters for automatic shutdown of the 2795 Separations Module. See the "Auto Shutdown" discussion in this section.

3

Specifying Signal Conditions for Events In

To define the conditions under which Stop Flow and Hold Inject signals on the I/O connector enable these events:

1. In the Configuration screen, press the **Events In** screen key to display the Events In dialog box (Figure 3-8).
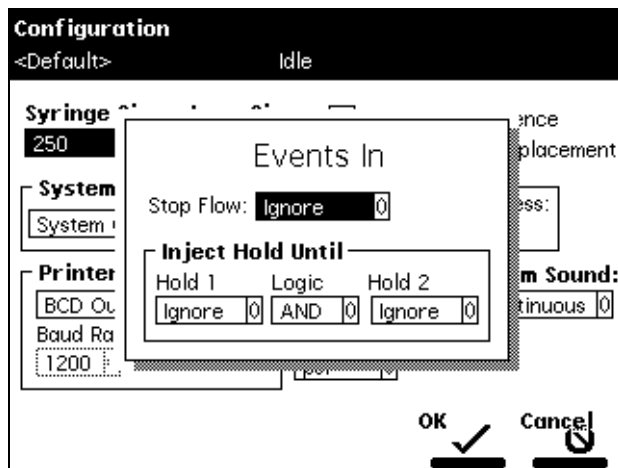
Figure 3-8 Events In Dialog Box

2. Press **Enter** to display the conditions that specify when a signal on the Stop Flow terminals (on the I/O connector) stops the flow of solvent. You can select from:

   • **Ignore** – Disables response to a Stop Flow signal

   • **On High** – Stops flow when the incoming Stop Flow signal is high

   • **On Low** – Stops flow when the incoming Stop Flow signal is low

3. Select the appropriate condition, then press **Enter**.

   When the event in signal condition occurs, the current function is suspended, solvent flow stops, and a dialog box appears, allowing you to abort or resume the function.

4. Select the **Hold 1** field, then press **Enter** to display the conditions under which a Hold 1 input stops the next injection.

5. Select the appropriate condition, then press **Enter**.

6. Repeat steps 4 and 5 for the Hold 2 field.

7. Select the **Logic** field, then press **Enter** to display the combination of Hold 1 and Hold 2 signals that prevents sample from being drawn into the needle. You can select from:

   • **And** – The injection is delayed while the conditions specified on *both* Hold 1 and Hold 2 are in effect.

- **Or** – The injection is delayed while the conditions specified on *either* Hold 1 or Hold 2 are in effect.

8. Select the appropriate logic, then press **Enter**. Sample is drawn into the needle when the specified conditions are no longer in effect.

9. Press **OK** to exit from the Events In dialog box.

Specifying Report Options

Note: Use Port A of the RS-232 connector (Figure 2-4) 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 3-9).
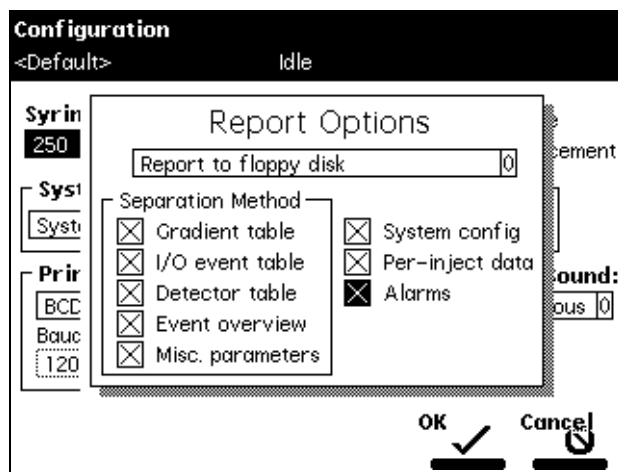
Figure 3-9 Report Options Dialog Box

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 checkbox indicates the option is enabled. The options are:

- **Gradient table** – Generates the gradient table (described in Table 5-4)
- **I/O event table** – Generates the I/O table (described in Table 5-11)
- **Detector table** – Generates the detector table (described in Table 5-17)
- **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 **OK** to exit from the Report Options dialog box.

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

When you are ready to print or to send a report to a 746 data module or floppy disk:

1. Select the printer in the Printer section of the Configuration screen.

2. Press the **Print** screen key.

3. Select the destination of the report in the Print dialog box that appears, then press **OK**.

Figure 3-10 is an example of a report with all options enabled.

3

```
REPORT:              Waters 2795 Separations Module
SERIAL NUMBER:       H96SM4109R
PRINTED:             03/24/2001 01:41:43pm
USER:                User
CONFIGURATION:       Syringe (500 uL), Loop (50 uL)


->Set separation Method: 60/40 MEOH/H2O

(1) MOBILE PHASE
    Low pressure alarm:        Disable
    High pressure alarm:       Disable
    Bubble detect alarm:       Alert user
    Flow Ramp:                 2.00 min (5.0 mL/min/min)
    Solvent names:             A = Methanol
                               B = Water
    Degasser Mode:             On
    Degasser fail alarm:       Alert user
    Pump sealwash period:      1.00
    Preferred stroke volume:   130 uL


    Gradient:    (min)     (mL/min) %A %B%C%DCurve
             1. INIT      1.000     60.0     40.0      0.0      0.0
             2. 45.00     0.000     60.0     40.0      0.0      0.0          11

(2) SAMPLE
    Sample temperature:        Not controlled
    Syringe draw (rate):       Normal (5.00 uL/sec)
                 (depth):      0 mm from bottom of well

(3) AUTOSAMPLER
    Injection mode:            Normal
    Loop option:               Partial loop injection
    preAspirate Air Gap:       0.0 uL
    postAspirate Air Gap:      5.0 uL
    Seek well bottom:          No
    Check plate height:        No
    Pre-column volume:         0.0 uL
    Post-run delay:            0.00 min
```

Figure 3-10 Sample Report

```
Compression check alarm:    Alert user
Wash Solvent:               Methanol/Water
Frequency:          Every inject
Cycles:             1
Inject port:        6 sec
Needle exterior:    15 sec
Purge (degassed) solvent:   Methanol/Water
Every inject:       299 uL
Rapid equilibration:        Off
Column re-equilibration:    0.00 min


(4) COLUMN
Column temperature:         Not controlled
Column 1:                   < blank >

(5) I/O
Chart output:               System pressure

(6) DETECTORS
Abs-1:                      486 Enabled
Abs-2:                      Disabled
410/2410:                   Disabled


   Table:        (min)     Detector   Action                         Param
            1. INIT      486 (1)    Set wavelength          254
            2. INIT      486 (1)    Set filter              1.0
            3. INIT      486 (1)    Set AU full scale       2.000
            4. INIT      486 (1)    Set chart polarity      [+]
            5. INIT      486 (1)    Auto zero
OVERVIEW        (min)     Action
            1. INIT      Column no change
            2. INIT      486(1) 254 nm
            3. INIT      486(1) filter 1.0
            4. INIT      486(1) 2.000 AUFS
            5. INIT      486(1) chart polarity pos
            6. INIT      486(1) auto zero
            7. INIT      1.000 mL/min 60.0%A 40.0%B 0.0%C 0.0%D c6
            8. 45.00     0.000 mL/min 60.0%A 40.0%B 0.0%C 0.0%D c11

->Sample injected at 03/24/1999 01:41:47pm (10.0 uL from 1:A,1)
 Inject 1 of 1, Line 1
 Total points = 383          Minimum   Maximum   Average
 System pressure (psi):9531092964
 Column temperature (deg C):23.423.423.4
 Sample temperature (deg C):24.024.024.0
 Degasser vacuum (psia):  3.03.03.0

```

Figure 3-10 Sample Report *(Continued)*

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 or when system operation is interrupted due to a failed injection or loss of contact with a remote host (Millennium[32] software or MassLynx software). Inactivity is defined as any one of the following:

- No keypad use
- No injections performed
- No changes sent to the 2795 Separations Module from a remotely connected Millennium[32] workstation or a remotely connected MassLynx workstation
- An error condition that suspends the operation of the 2795 Separations Module

Depending on the specified method, the Auto Shutdown feature can be used to:

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

Waters recommends the following guidelines for the Auto Shutdown method:

- Use a flow rate of 0 mL/min.
- Program the idle time to be in excess of the longest run time for which the system will be used.
- Program detectors to set the lamp OFF, after the appropriate idle time.

To enable the Auto Shutdown function:

1. Press **Config** in the Main screen, then press **More**.
2. Press the **Auto Shutdown** screen key. The Auto Shutdown dialog box appears (Figure 3-11).
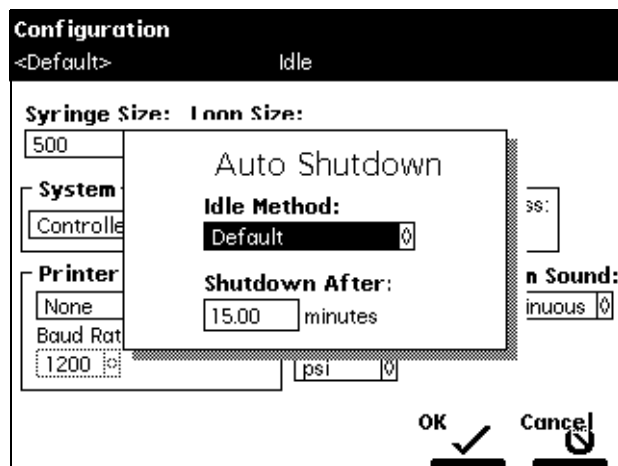
Figure 3-11 Auto Shutdown Dialog Box

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 the Auto Shutdown function).

5. Press **OK**. 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 Section 5.2.6, Setting I/O Parameter Values).

3.2.2 Configuring Plate Types

STOP *Attention: To avoid damaging the 2795 Separations Module, Waters recommends that you consult Table B-5 before configuring plates for the 2795 Separations Module. Be sure to read all the recommendations in the footnotes to Table B-5.*

There is a maximum of 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 3-12).

Figure 3-12 Plate Type Configuration Dialog Box

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

3. In the Sequential reference field, select the processing order (see Table 3-8).

Table 3-5 describes the fields in the Plate Type Configuration dialog box. See Table B-5 for recommended plate types (and their dimensions) for use with the 2795 Separations Module.

STOP

Attention: *Use of incorrect dimensions can cause needle damage.*

Table 3-5 Plate Type Configuration Parameters

Parameter 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 96 8 12 384 16 24
Sequential reference	Direction and sequence of well injections for the selected plate. See Table 3-8.
Well A1 offset (mm) Left Rear	 The distance from the center of well A:1 to the left outer edge of the plate. The distance from the center of well A:1 to the rear outer edge of the plate. The minimum rear offset value is 8.3 mm. See Figure 3-14.
Spacing (mm) Row-to-row Column-to-column	 The distance from the centerline of the wells in one row to the centerline of the wells in an adjacent row. The distance from the centerline of the wells in one column to the centerline of the wells in an adjacent column. See Figure 3-14.
Vertical (mm)[a] Plate height Well depth	 Total plate height The well depth (distance from the top of the plate to the bottom of the well)

a. See Table B-5 for dimensions of the plates recommended for use with the 2795 Separations Module.

3

Configuring Custom Plates

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

Required Material

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 3-13).

2. Enter a custom plate name (see 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).
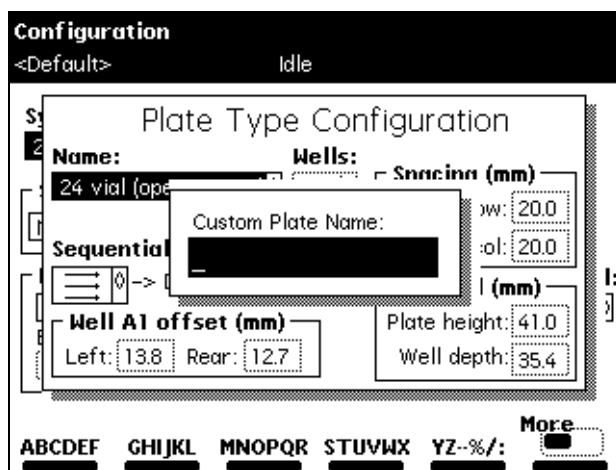
Figure 3-13 Custom Plate Name Dialog Box

3. Using a ruler or straight-edge scaled in millimeters, measure the dimensions for the custom plate as shown in Figure 3-14 and Figure 3-15, then enter each parameter in the appropriate field in the Plate Type Configuration screen.

4. Press **OK** to store the custom plate (or press **Cancel** 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 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" are displayed below the default plate name in the Plate Type Configuration screen.

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 (if a printer is connected to your 2795 Separations Module).

Note: The Needle Depth Offset parameter (Figure 3-15 and Figure 5-9) 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 Section 5.1, Creating and Editing Separation Methods).

Top View Dimensions

Note: Measure the A1 Left and A1 Rear Reference points (the left and rear well offsets) from the outer edge of the plate.
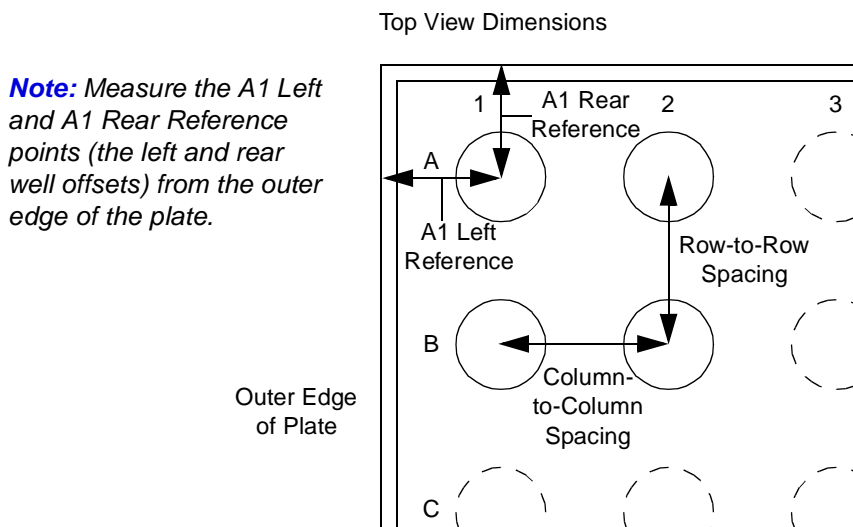
Figure 3-14 Plate Parameters (Top View)

Note: The minimum rear offset value is 8.3 mm. Refer to Section Appendix B, Spare Parts Table B-5.

Side View Dimensions
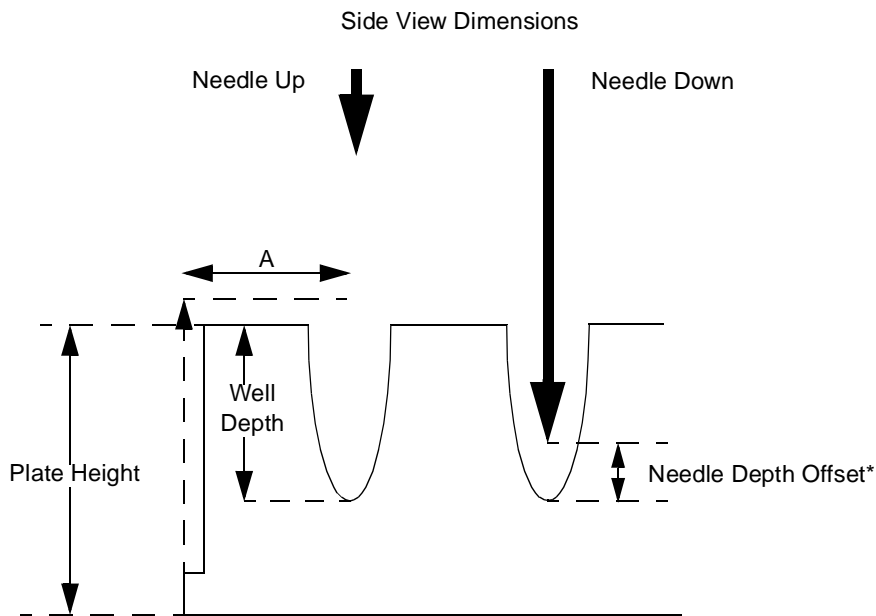
* The Needle Depth Offset parameter is part of the separation
method. See Section 5.1, Creating and Editing Separation

Figure 3-15 Plate Parameters (Side View)

Table 3-6 describes the preconfigured plate types for the 2795 Separations Module. The
plate type names appear as displayed in the Name parameter of the Plate Type
Configuration dialog box.

Table 3-6 Preconfigured Plate Types

Plate Type Name	Plate Type 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 w/700 uL insert	96-well with 700- µL, glass insert plate	186000349
96 well (tall), 350 uL	96-well tall, 350- µL, polypropylene	WAT058943
96 well (tall), 1 mL	96-well tall, 1- mL, polypropylene	WAT058957
96 well (tall), 2 mL	96-well tall, 2- mL, polypropylene	WAT058958

Table 3-6 Preconfigured Plate Types *(Continued)*

Plate Type Name	Plate Type Description	Part Number
96-well, 300 uL (Default)	96-well, 300-µL, polypropylene or polystyrene	Non-Waters[a]
384-well, 80 uL	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 Table B-5.

3.2.3 Configuring the Operating Mode

You can configure the 2795 Separations Module to operate in one of five operating modes (see Figure 3-16):

- **No Interaction** – Use for stand-alone operation with no detector control by the 2795 Separations Module.

- **System Controller** – Use for stand-alone operation when the 2795 Separations Module is controlling Waters detectors (486, 2487, 410, or 2410) over the IEEE-488 interface.

- **Controlled by Millennium 32** – Use for Millennium[32] software control of the 2795 Separations Module (over the IEEE-488 interface).

- **Controlled by MassLynx** – Use for MassLynx software control of the 2795 Separations Module (over the IEEE-488 interface).

- **Operate Gradient by Event In** – Use when an autosampler (external to the 2795 Separations Module) initiates a chromatographic run by transmitting a contact closure signal.

- **Controlled via RS232 (binary)** – Use when a non-Waters data system controls the Separations Module by RS-232 communications, using binary protocol, as specified by the data system.

3
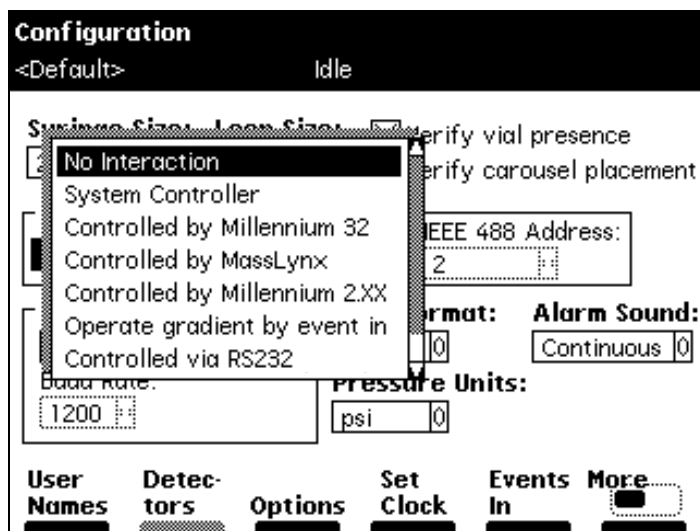
Figure 3-16 Configuring the Operating Mode

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 Section 2.5.2, Making Analog Signal Connections, for the procedure to make I/O connections.

The No Interaction mode is useful when you want to suspend communications with a connected Millennium^{32} workstation or MassLynx NT workstation and operate the 2795 Separations Module and the other HPLC system components from their front panels.

Note: You cannot configure the 2795 Separations Module to No Interaction mode while it is controlled by Millennium^{32} software or MassLynx software.

When the 2795 Separations Module is configured in the No Interaction mode, the Millennium^{32} software or MassLynx software can control a detector using the IEEE-488 interface.

To set the 2795 Separations Module to the No Interaction mode:

1. Remove the 2795 Separations Module from control by Millennium^{32} software or MassLynx software, if applicable.

2. Select **System** in the Configuration screen, then press **Enter** to display the list of operating modes (Figure 3-16).

3. Select **No Interaction**, then press **Enter**.

4. 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).

Refer to 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 Section 2.5.2, Making Analog Signal Connections, for the 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 display the list of operating modes (Figure 3-16).

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 **OK** to return to the Configuration screen.

4. Press **Exit**.

For information on setting detector parameters in a separation method, see Section 5.2.7, Setting Detector Parameters.

Controlled by Millennium 32 Mode

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

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

In this remote-control mode, a Millennium[32] Chromatography Manager workstation running Millennium[32] software controls the operation of the LC system using the IEEE-488 interface bus. Connect the IEEE-488 cable as described in Section 2.5.3, Making Digital Signal Connections (see Figure 2-14).

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

1. Select **System** in the Configuration screen, then press **Enter** to display the list of operating modes (Figure 3-16).

2. Select **Controlled by Millennium 32**, then press **Enter**. The IEEE-488 Address field is highlighted.

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

4. Select an address that is unused by other chromatographic components connected to the workstation, then press **Enter**.

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

Refer to Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation, for more information on making runs under Millennium[32] software control.

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 specific MassLynx software versions.

In this remote-control mode, the Micromass MassLynx software uses the IEEE-488 interface to control the:

- 2795 Separations Module
- Micromass mass spectrometry (MS) detectors
- Waters 996 PDA Detector
- Other Waters detectors, such as the 2487 Dual λ Absorbance detector (analog signal for detection)

For more information, see Figure 2-13.

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

1. Select **System** in the Configuration screen, then press **Enter** to display the list of operating modes (Figure 3-16).

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

3. Press **Enter** to display a list of 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.

Refer to Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation, for more information on making runs under MassLynx control.

Configuring 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 Section 2.5.2, Making Analog Signal Connections, for information on I/O connections.

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

1. Select **System** in the Configuration screen, then press **Enter** to display the list of operating modes (Figure 3-16).

2. Select **Operate Gradient by Event In**, then press **Enter**.

3. Press **Events In** to display the Events In dialog box (Figure 3-8). For information on defining Events In conditions, refer to 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.

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

   - 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.

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 from the Events In dialog box.

3

Controlled via RS-232 Mode

In this remote-control mode, a non-Waters data system (a mass spectrometry system, for example) can become the controller of the 2795 Separations Module using RS-232 communications. The 2795 Separations Module supports **binary** RS-232 communication only. The Separations Module becomes disconnected from the IEEE-488 interface bus when the RS-232 mode is selected.

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

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

3.3 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:

-   Preparing Solvent Reservoirs (Section 3.3.1)
-   Degassing Solvents (Section 3.3.2)
-   Priming the Plunger-Seal-Wash Pump (Section 3.3.4)
-   Priming the Solvent Management System (Section 3.3.5)

For information on preparing the sample management system, see Section 3.4, 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.*

3.3.1 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 Section 2.4, Making Fluidic Connections.

3.3.2 Degassing Solvents

STOP **Attention:** *Do not run in the degasser in the continuous "On" mode unless solvent is flowing through the supply tubes. Without solvent flow, excess solvent vapor may condense in the degasser vacuum chamber walls, which could lead you to suspect a vacuum chamber leak.*

For more information on vacuum degassing, refer to Section 1.2, Solvent Management System Overview, the "PerformancePLUS Degasser" discussion.

To control the vacuum degasser from the 2795 Separations Module front panel (in System Controller or No Interaction modes):

1. Press **Menu/Status**. The Status screen is displayed (Figure 3-17).

2. If the **Degasser Mode** field is not visible, it has been configured to appear on Status page 2. If necessary, press **Next Page** to display the Degasser Mode field.

Figure 3-17 Degasser Modes on Main Status Page

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

- On mode – The degasser is always on (continuous).
- Off mode – The degasser is always off (disabled).

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.

3.3.3 Wash Sequences

A wash *sequence* option is now part of the separation method for 2795 Separations Modules beginning with firmware version 2.00. Refer to Figure 3-18.

By default, the wash sequence is set to Wash-Purge. The alternative is the Purge-Wash-Purge sequence in which an additional purge of the syringe is performed, before to the Needle-Wash. This option uses more degasser purge solvent, and can be useful in applications where the samples are in serum or salt-containing matrices.
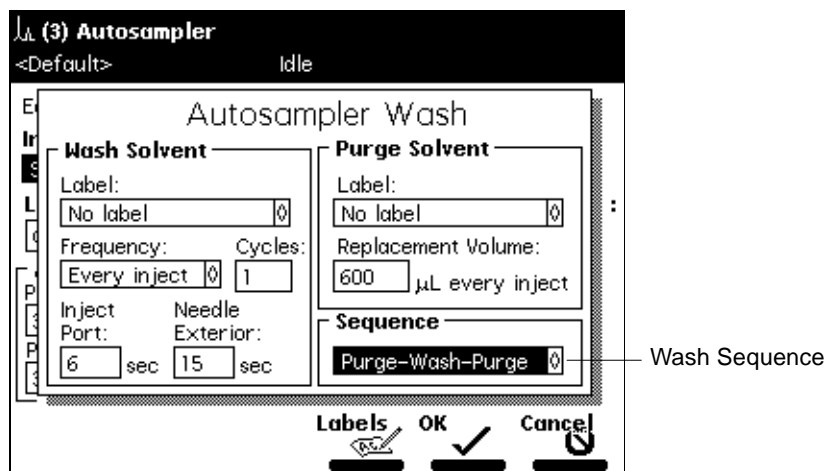
Figure 3-18 AutoSampler Wash

3.3.4 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 Figure 7-2).

Required Materials

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

Procedure

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 (clear) is in an appropriate waste container.

To prime the plunger-seal-wash:

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 3-19).

   Note: When the Prime Seal Wash procedure is not running, the dialog box reads "Idle."
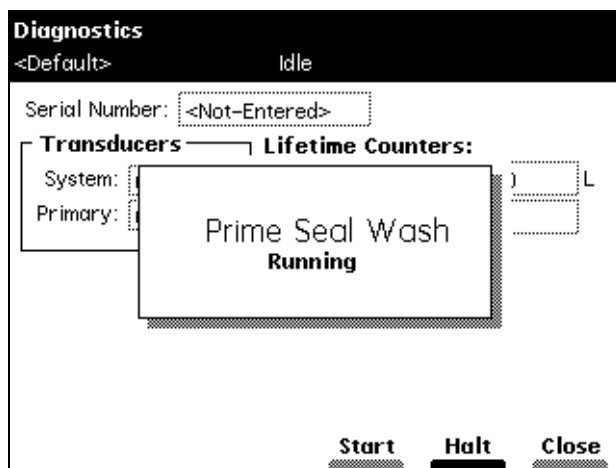
Figure 3-19 Prime Seal Wash Dialog Box

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 Chapter 8, Diagnostics and Troubleshooting.

3.3.5 Priming the Solvent Management System

Prime the solvent management system using the:

- Dry Prime direct function when the solvent lines are dry (see Section 4.2.2, Dry Priming)

- Wet Prime direct function when you want to switch from one solvent to another (see Section 4.2.3, Wet Priming)

3.4 Preparing the Sample Management System

Prepare the sample management system for operation after you have prepared the solvent management system (Section 3.3, Preparing the Solvent Management System). Preparing the sample management system involves:

- Priming the needle-wash pump (Section 3.4.1)

- Refreshing the syringe (Section 3.4.2)

- Loading the sample plates (Section 3.4.3)

Before using the sample management system for the first time, become familiar with the procedures in Section 4.3, Monitoring System Status, and in Section 4.2, Performing Direct Functions.

3.4.1 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:

- 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 3-7 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 3-7 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 50% H_2O, 50% MeOH or 100% H_2O, 0 to 50% MeOH or ACN
Non-aqueous, 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 (ion exchange mobile phase is highly buffered)

Required Materials

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

Procedure

Note: Be sure the needle-wash fluidic lines and reservoirs are installed properly (see Section 2.4.5, Installing the Wash Reservoirs).

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 **Diag** in the Main screen. The Diagnostics screen appears (Figure 8-2).
5. Press the **Prime NdlWash** screen key. The Prime Needle Wash Solvent dialog box appears (Figure 3-20).
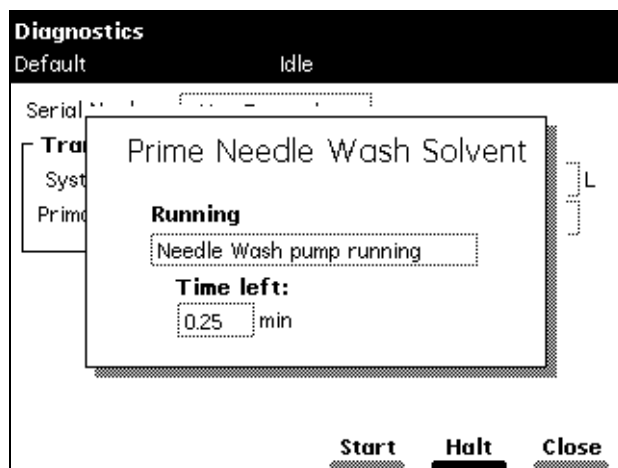
Figure 3-20 Prime Needle Wash Solvent Dialog Box

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 main 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 Chapter 8, Diagnostics and Troubleshooting.

3.4.2 Refreshing the Syringe

Refresh the syringe whenever you:

- 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 3-7).

Note: When you refresh (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. In the Status screen, activate the degasser by selecting the **On** mode in the Degasser field (see Section 4.3, Monitoring System Status).

2. Press the **Direct Function** screen key in the Status screen. The Direct Functions menu appears (see Figure 4-4).

3. Select the **Refresh Syringe** option, then press **Enter**. The Refresh Syringe dialog box appears (Figure 3-21).

4. 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 Note above.

5. Press **OK**. The Refresh Syringe cycle begins.

3
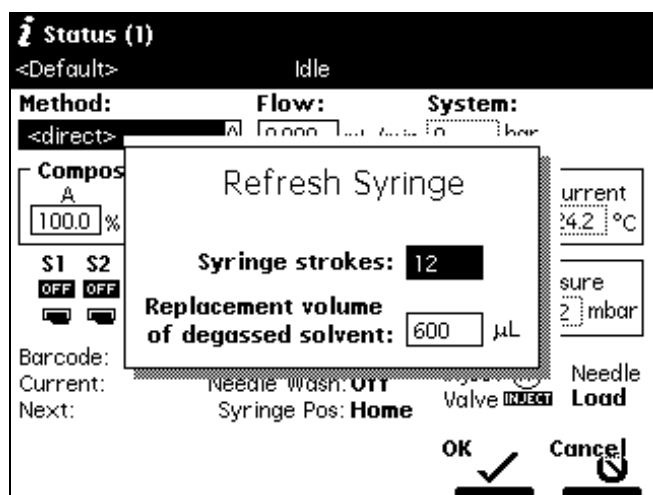
Figure 3-21 Refresh Syringe Dialog Box

3.4.3 Loading the Sample Plates

STOP **Attention:** *To avoid damage to the needle or XYZ mechanism, do not use vial caps less than 8.8 mm in diameter (OD).*

STOP **Attention:** *Use of polyethylene cap mats below 15 °C can cause damage to the needle or XYZ mechanism. If the sample manager is maintained above 15 °C, a polyethylene cap mat for 96 square well plates is acceptable (see Section B.4, Recommended Plate Options). However, at temperatures below 15 °C, the mat may become rigid and cause lifting of the plate from the carrier in the inject cycle. Sample stability may also decrease. If the sample manager is maintained from 4 to 15 °C, a PTFE/silicone cap mat is recommended (see Section B.4, Recommended Plate Options).*

STOP **Attention:** *Some adhesive sealing materials may clog the inject port and needle. You can use thin aluminum foil to seal 96- and 384-well plates. Some heat-sealable foils are also acceptable. See Section B.4, Recommended Plate Options.*

The 2795 Separations Module plate carrier holds up to four rectangular plates that you load through the front panel compartment door (Figure 3-22). 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 3-22).

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 towards the A:1 (left rear) corner and holds it in position.

To move the plate carrier from one position to the next, press **Next** or press the color-coded plate number key in the Door Is Open dialog box (Figure 3-23).

STOP **Attention:** *The 2795 Separations Module cannot draw or load samples when the sample compartment door is open.*
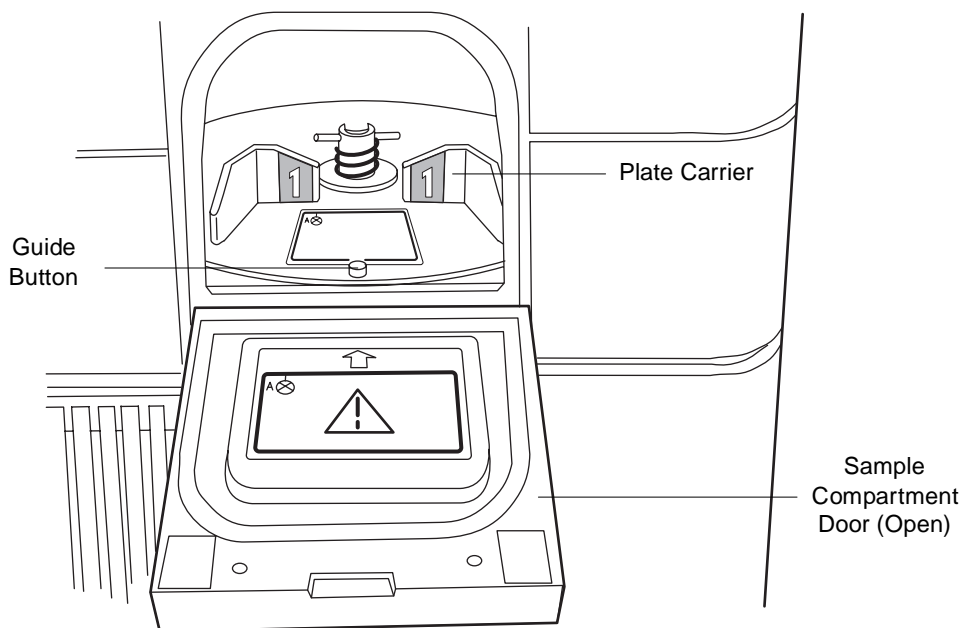
Figure 3-22 Positioning the Sample Plate

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 3-23).

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 ().
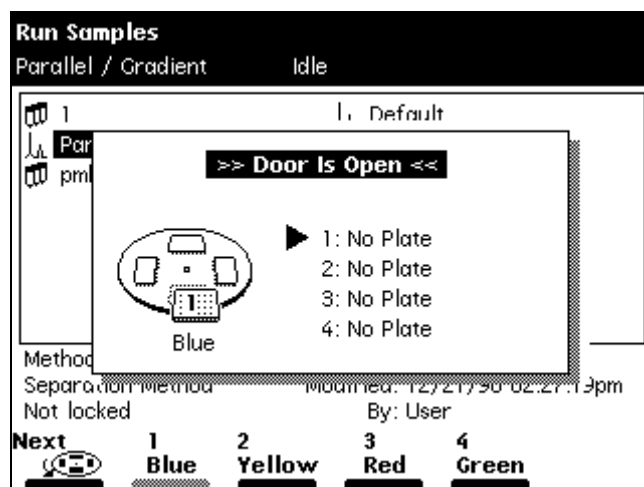
Figure 3-23 Door Is Open Dialog Box
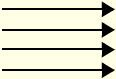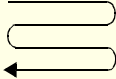
The 2795 Separations Module uses *plate logic* (based on letter labels for rows and number labels for columns) to locate samples on the plates. For example, sample positions on a:

- 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. In the Configuration screen, you can select the numbering scheme for the processing order to be used in the sample set method

([Table 3-8](#)). Position A:1 (or 1) is always located at the left rear of a plate as it is loaded into the carrier ([Figure 3-22](#)).

Table 3-8 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).	
Top to bottom, left to right		Increasing rows, then increasing columns. Also called Discontinuous (vertical).	
Horizontal serpentine		Left to right until end of row, then right to left starting at next row. Also called Continuous (horizontal).	
Vertical serpentine		Top to bottom until end of column, then bottom to top starting at the next column. Also called Continuous (vertical).	

For more information on configuring sample plates, see [Section 3.2.2, Configuring Plate Types](#). For more information on selecting individual well locations or grouping wells, see [Section 4.2.9, Selecting Wells for Making Injections](#).

3.5 Preparing the 2795 Separations Module for Operation

This section outlines the steps to prepare both degasser and sparge-based 2695 Separations Modules for operation using Waters-recommended procedures. Flowcharts provide the sequence of steps to prime, equilibrate, and purge a Separations Module that is in one of the following states:

- A new or dry 2795 Separations Module
- A 2795 Separations Module that has been turned off or left idle for a significant time with no flow
- A 2795 Separations Module that had been recently idle (to quickly refresh the system in between runs)
- A 2795 Separations Module that requires a solvent change (from a buffered solvent to an organic solvent)

The priming, purging, equilibrating, and other procedures outlined here are described in detail in Section 4.2, Performing Direct Functions.

Figure 3-24 outlines how to prepare a new or dry 2795 Separations Module for operation.

3

```
┌─────────────────┐        ┌─────────────────┐        ┌─────────────────┐
│      Start      │        │   Prime the     │        │ The Degasser is │
└────────┬────────┘        │ Plunger-Seal-Wash│       │    set to On    │
         │                 │      Pump       │        │ (See "Equilibrating│
         │                 │ (Section 3.3.4) │        │   Solvents in the │
         ▼                 └────────┬────────┘        │ Vacuum Degasser"  │
┌─────────────────┐                 │                 │  in Section 4.2.3)│
│  Fill the Solvent│                │                 └────────┬────────┘
│   Reservoirs with│                ▼                          │
│   Fresh Solvent  │       ┌─────────────────┐                 ▼
│   Filtered and   │       │  Perform a Wet  │        ┌─────────────────┐
│  Degassed by     │       │ Prime (for Solvents│      │ Equilibrate for a │
│ Vacuum Filtration│       │ A, B, C, D) for 10│       │  Minimum of 10    │
│ (Section 2.4.2)ᵃ │       │   min at 5.00     │       │  Column Volumes   │
└────────┬────────┘        │    mL/min       │        │  (Section 4.2.6)  │
         │                 │ (Section 4.2.3) │        └────────┬────────┘
         ▼                 └────────┬────────┘                 │
┌─────────────────┐                 │                          ▼
│ Set the Degasser to│              ▼                 ┌─────────────────┐
│  ON (See         │       ┌─────────────────┐        │ System Is Ready │
│ "Equilibrating   │       │ Prime the Needle-│       └─────────────────┘
│  Solvents in the │       │   Wash Pump     │
│ Vacuum Degasser" │       │ (Section 3.4.1) │
│  in Section 4.2.3)│      └────────┬────────┘
└────────┬────────┘                 │
```

- Start
- Fill the Solvent Reservoirs with Fresh Solvent Filtered and Degassed by Vacuum Filtration (Section 2.4.2)[a]
- Set the Degasser to **ON** (See "Equilibrating Solvents in the Vacuum Degasser" in Section 4.2.3)
- Perform a Dry Prime to Fill Each Solvent Line (A, B, C, D) and Degasser Chamber (Section 4.2.2)
- Equilibrate the Solvent in the Degasser Chamber for **5** min at a Flow Rate of **0.000** mL/min[a] (See "Equilibrating Solvents in the Vacuum Degasser" in Section 4.2.3)

- Prime the Plunger-Seal-Wash Pump (Section 3.3.4)
- Perform a Wet Prime (for Solvents A, B, C, D) for **10** min at **5.00** mL/min (Section 4.2.3)
- Prime the Needle-Wash Pump (Section 3.4.1)
- Refresh the Syringe for a Minimum of **10** Cycles and a Replacement Volume of **600** μL (Section 3.4.2)
- Set Initial Conditions. If Running a Gradient (for Example, A:B), Set the A and B Composition Fields in the Status Screen to **50%**, Then Wet Prime for **2** min at **5.00** mL/min (Section 4.2.3)

- The Degasser is set to **On** (See "Equilibrating Solvents in the Vacuum Degasser" in Section 4.2.3)
- Equilibrate for a Minimum of **10** Column Volumes (Section 4.2.6)
- System Is Ready

3

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 3-24 Preparing a New or Dry 2795 Separations Module for Operation

Figure 3-25 outlines how to prepare for operation a 2795 Separations Module that is powered off or is idle with no solvent.

```
┌─────────────────┐          ┌─────────────────┐          ┌─────────────────┐
│      Start      │          │  Equilibrate the │          │ The Degasser is set │
└────────┬────────┘          │   Solvent in the │          │       to On       │
         │                   │ Degasser Chamber │          │ (See "Equilibrating │
         │                   │  for 5 min at a  │          │   Solvents in the │
┌────────┴────────┐          │    Flow Rate of  │◄────────►│  Vacuum Degasser" │
│ Set the Degasser│          │   0.000 mL/min   │          │  in Section 4.2.3)│
│       to On     │          │      (See        │          └─────────┬───────┘
│ (See "Equilibrat│          │  "Equilibrating  │                    │
│  ing Solvents   │          │  Solvents in the │          ┌─────────┴───────┐
│  in the Vacuum  │          │ Vacuum Degasser" │          │  Equilibrate for │
│   Degasser"     │          │  in Section 4.2.3)│         │   a Minimum of 10│
│ in Section 4.2.3)│         └─────────────────┘          │  Column Volumes  │
└────────┬────────┘                                        │  (Section 4.2.6) │
         │                   ┌─────────────────┐          └─────────┬───────┘
┌────────┴────────┐          │ If You Are Running│                  │
│ Perform a Wet   │          │   a Gradient (for │         ┌─────────┴───────┐
│  Prime (for One │          │  Example, A:B), Set│        │ Refresh the Syringe│
│ of the Solvents)│          │   the A and B     │         │  for a Minimum of 3│
│  for 2 min at   │          │ Composition Fields│         │   Cycles and a    │
│  7.500 mL/min   │          │ in the Status Screen│       │   Replacement    │
│ (Section 4.2.3) │          │  to 50%, Then Wet │         │  Volume of 600 µL │
└────────┬────────┘          │   Prime for 3 min │         │  (Section 3.4.2) │
         │                   │    at 5.00 mL/min │         └─────────┬───────┘
┌────────┴────────┐          │  (Section 4.2.3)  │                   │
│ Repeat the Wet  │          └─────────────────┘          ┌─────────┴───────┐
│ Prime at the    │                                        │ System Is Ready │
│ Same Rate and   │                                        └─────────────────┘
│ Time for Each   │
│    Solvent      │
└─────────────────┘
```

Figure 3-25 Preparing an Idle or Powered-Off 2795 Separations Module for Operation

[Figure 3-26](#) outlines how to change from a buffered solvent to an organic solvent.
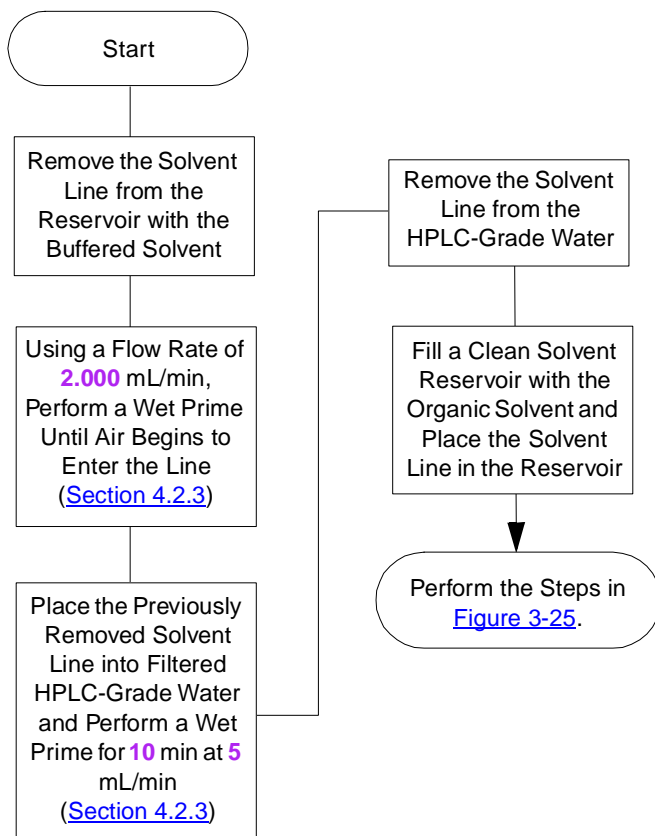
Start

Remove the Solvent Line from the Reservoir with the Buffered Solvent

Using a Flow Rate of **2.000** mL/min, Perform a Wet Prime Until Air Begins to Enter the Line ([Section 4.2.3](#))

Place the Previously Removed Solvent Line into Filtered HPLC-Grade Water and Perform a Wet Prime for **10** min at **5** mL/min ([Section 4.2.3](#))

Remove the Solvent Line from the HPLC-Grade Water

Fill a Clean Solvent Reservoir with the Organic Solvent and Place the Solvent Line in the Reservoir

Perform the Steps in [Figure 3-25](#).

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 [Section C.3, Solvent Miscibility](#), for information on solvent miscibility.*

Figure 3-26 Changing from a Buffered Solvent to an Organic Solvent

3.6 Powering Off

Before Powering Off

Before you power off the 2795 Separations Module for an extended period (longer 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 you perform 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 4.2.3, 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 Separation 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.

3

Chapter 4
Operating the 2795 Separations Module from the Front Panel

Before you create or edit sample sets or separation methods, or perform a sample run, become familiar with the procedures in this chapter.

How you control your 2795 Separations Module system depends on how your system is configured:

- If the 2795 Separations Module is configured in the Controlled by MassLynx mode, refer to Section 6.1, Making Automatic Runs in a Stand-Alone Mode, the "" discussion.

- If the 2795 Separations Module is configured in the Controlled by Millennium^{32} mode, refer to Section 6.1, Making Automatic Runs in a Stand-Alone Mode, the "" discussion.

- If the 2795 Separations Module is configured in the No Interaction or Operate Gradient By Event In mode, you control each HPLC system instrument individually from its front panel.

- If the 2795 Separations Module is configured in the System Controller mode, you control all connected instruments in your HPLC system through the 2795 Separations Module front panel.

This chapter describes how to use the front panel of the 2795 Separations Module (in System Controller or No Interaction mode) to:

- Monitor the operation of your HPLC system
- Prepare for running samples
- Run samples
- Perform functions

4

4.1 Routine Startup

If the 2795 Separations Module is not already powered on, follow the power-on procedure described at the beginning of Chapter 3, Setting Up the 2795 Separations Module.

When the startup diagnostics routine is complete, the 2795 Separations Module Main screen is displayed and the instrument enters the Idle mode (Figure 4-1).
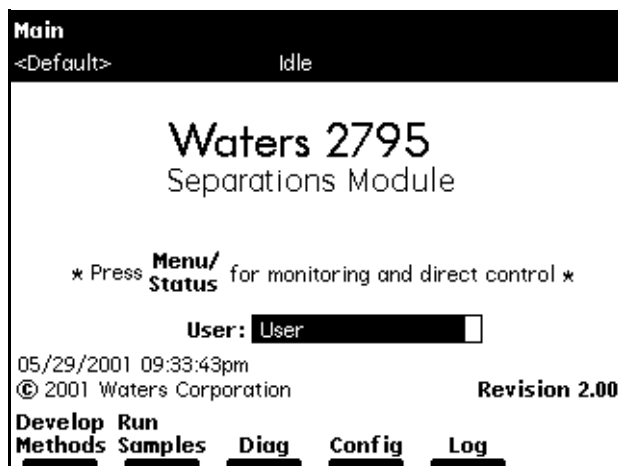
Figure 4-1 Main Screen

STOP **Attention:** *Ensure the top cover and side panel are attached before you apply power to the 2795 Separations Module.*

Updating Your User Name

To update your user name in the reports and methods you plan to generate:

1. In the Main screen, press **Enter**, then select your name in the User list.
2. If your name is not on the list, press **Config** to display the Configuration screen, then press **User Names** to display the Users dialog box.
3. Enter your name using the 2795 Separations Module keypad (see to Section 3.1.2, Using the Keypad, the "Entering an Alphanumeric String" discussion). A user name is not required to operate the 2795 Separations Module.

The time and date the 2795 Separations Module is powered are entered in the log file.

4.2 Performing Direct Functions

You can perform many functions through the front panel controls whether or not the 2795 Separations Module is controlling the chromatographic system (System Controller 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 Section 5.1, Creating and Editing Separation Methods).

Direct functions are:

- System preperation (Section 4.2.1)
- Dry priming (Section 4.2.2)
- Wet priming (Section 4.2.3)
- Refreshing the syringe (Section 4.2.4)
- Washing the needle (Section 4.2.5)
- Equilibrating the system (Section 4.2.6)
- Conditioning the column (Section 4.2.7)
- Injecting samples (Section 4.2.8)

Figure 4-2 outlines the procedure for using the 2795 Separations Module front panel to perform direct functions.
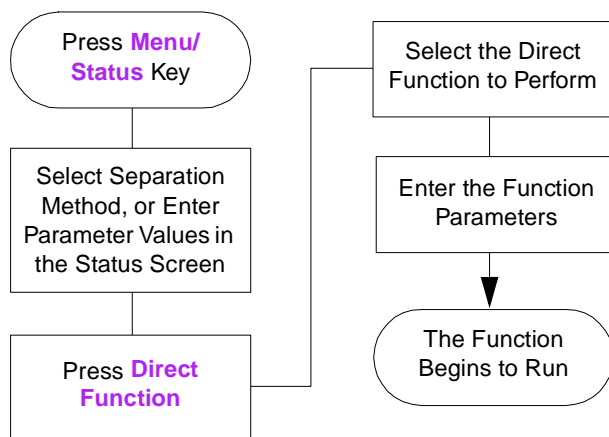
Figure 4-2 Steps to Setting Up a Direct Function Control Run

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.*

To access direct functions:

1. Press **Menu/Status** to display the Status screen (Figure 4-3).
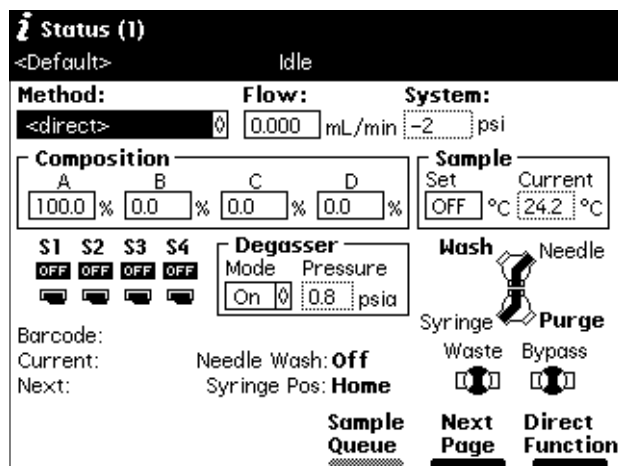
Figure 4-3 Status Screen (Example)

2. Select an existing separation method or enter parameter values in the Status screen.

3. Press the **Direct Function** screen key. A list of direct functions appears (Figure 4-4). Table 4-1 describes the direct functions.
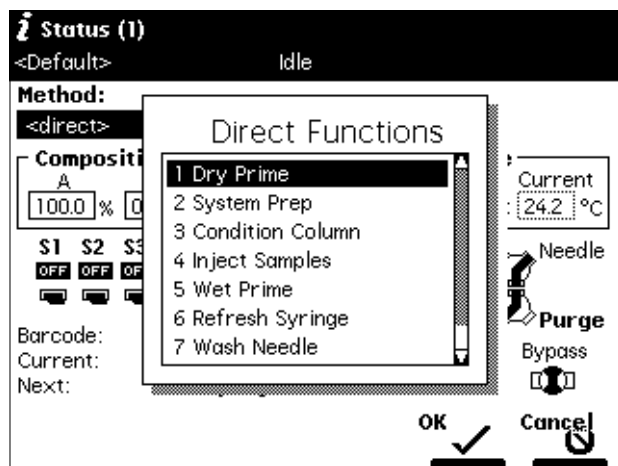
Figure 4-4 Direct Functions Menu

4. Select a function, then press **Enter**. Follow the instructions for each function, as described in the following sections. See [Section 3.1.2, Using the Keypad](#), the "Entering and Editing Values" discussion, if necessary.

Table 4-1 Direct Functions

Direct Function Name	Description	Reference
System Prep	Prepares the 2795 Separations Module for operation.	[Section 4.2.1, System Preparation Function](#)
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 4.2.2, 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 4.2.3, Wet Priming](#)
Refresh Syringe	Refills the syringe with fresh, degassed, purge solvent.	[Section 4.2.4, Refreshing the Syringe](#)
Wash Needle	Washes both the outside and inside of the needle using wash solvent.	[Section 4.2.5, Washing the Needle](#)
Equilibrate	Selects a column position (if applicable) and specifies the system equilibration time.	[Section 4.2.6, Equili-brating 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 4.2.7, Condi-tioning the Column](#)
Inject Samples	Injects a sample one or more times from the specified well(s) using the selected method.	[Section 4.2.8, Injecting Samples](#)

4

4.2.1 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.
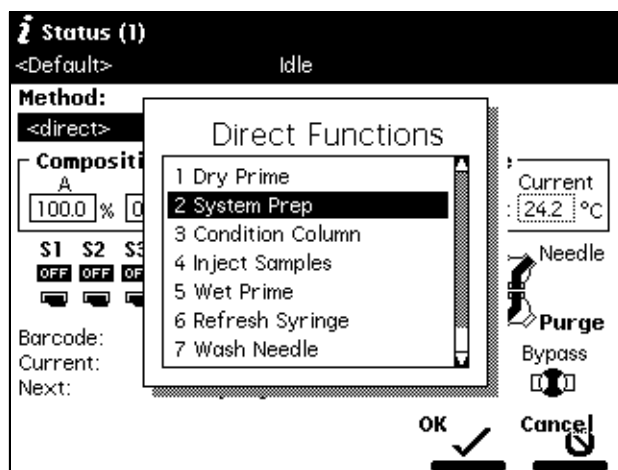
Figure 4-5 System Prep Selection from the Direct Functions Screen
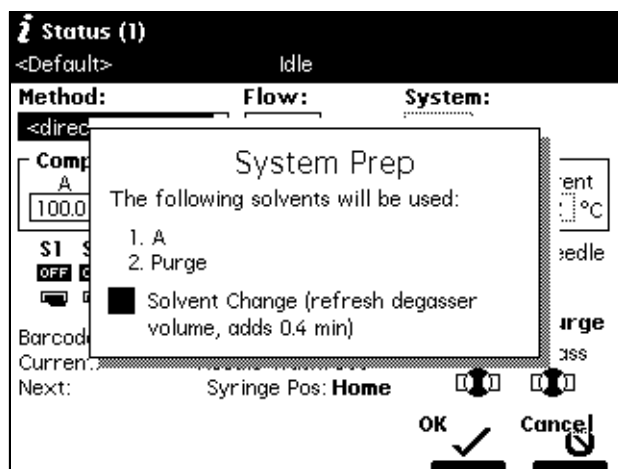
Figure 4-6 System Prep Screen Display

The System Prep functopn performs the following functions:

- 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, which is useful when changing solvent or after extended periods of system inactivity.

- When you select the System Prep function immediately after the powering up 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 that runs simultaneously with the wet primes until complete using the ratios in Table 4-2. In all cases, replacement volume is one stroke.

Table 4-2 Refresh Syringe Ratios

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

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

4. Performs a needle-wash 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 stand-alone mode, or sample list function under control by MassLynx version 3.5.

4.2.2 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

30-mL syringe (Startup Kit)

Procedure

To perform a dry prime:

1. Set up the reservoirs as described in Section 2.4.3, Installing the Degasser Vent Tubing.

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 Figure 4-7, then open the valve by turning it counterclockwise 1/2-turn.

5. Press **Menu/Status** to display the Status screen (Figure 4-3).
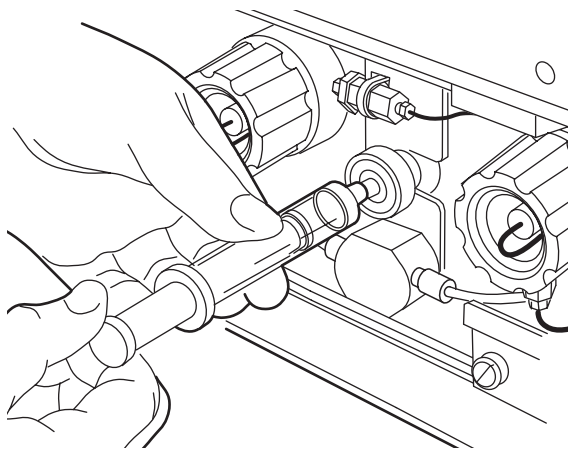
Figure 4-7 Prime/Vent Valve with Syringe

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 on the Status screen. The Direct Functions menu appears (Figure 4-4).

7. Select **Dry Prime**, then press **Enter**. The Dry Prime dialog box appears (Figure 4-8).
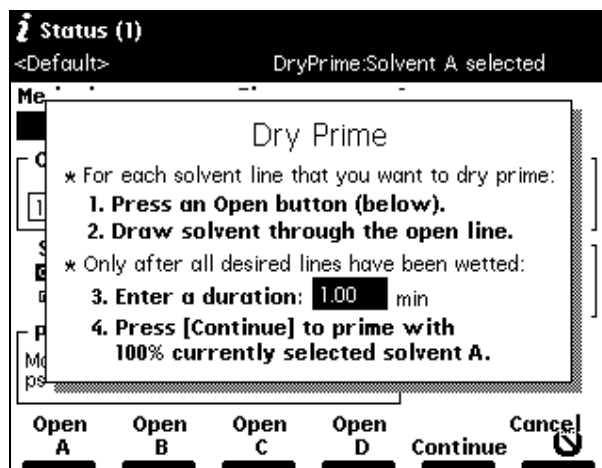
Figure 4-8 Dry Prime Dialog Box

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 8 and 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. Waters recommends that you prime using the solvent with the lowest viscosity to help purge air from the lines, especially if the vacuum degasser is installed.

12. In the Enter a Duration field, enter the length of time (in minutes) to prime the solvent management system. Start with a value of **5** minutes. Press **On**.

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 in Section 4.2.3.

4.2.3 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 Separations Module has been idle for some time
- 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 4.2.2, 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 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 4-3).

2. In the Status screen Composition field, enter **100**% for the solvent you want to use for wet priming. 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 menu appears (Figure 4-4).

4. Select **Wet Prime**, then press **Enter**. The Wet Prime dialog box appears (Figure 4-9).
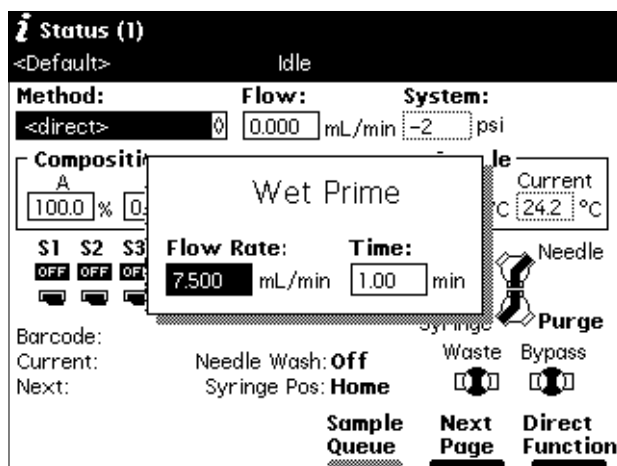
Figure 4-9 Wet Prime Dialog Box

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 2 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 Section 1.2.2, Solvent Management System Features, the "PerformancePLUS Degasser" discussion.

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

1. Press **Menu/Status** to display the Status screen.

2. Enter the initial solvent composition for the run in the Status screen.

3. Set the Degasser Mode parameter in the Status screen to **On**.

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

5. Select **Wet Prime**, then press **Enter**. The Wet Prime dialog box appears (Figure 4-9).

6. Enter a Flow Rate of **0.000** mL/min. and Time of **5** min. At the end of the time period, the solvents are degassed and the 2795 Separations Module enters the Idle mode.

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

4.2.4 Refreshing the Syringe

STOP *Attention: If you are priming the sample management system for the first time or changing the purge solvent, see Section 3.4.1, Priming the Needle-Wash Pump, and Section 3.4.2, Refreshing the Syringe.*

Refresh the syringe to:

• Replace the old solvent in the sample management system with fresh degassed purge solvent

• Remove any traces of solvent in the sample management system from a prior run

• Clear air bubbles from the fluidic path or the syringe

- Change over to a new (miscible) solvent after you perform a wet prime
- Prime a new syringe

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 or sample diluent (see* Table 3-7*). Refer to* Section C.3, Solvent Miscibility, *for more information on solvent miscibility.*

To refresh the syringe:

1. Press **Menu/Status** to display the Status screen (Figure 4-3).
2. Press the **Direct Function** screen key in the Status screen. The Direct Functions menu appears (Figure 4-4).
3. Select the **Refresh Syringe** option, then press **Enter**. The Refresh Syringe dialog box appears (Figure 4-10).
4. 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. For a new (dry) instrument, see Section 3.4.2, Refreshing the Syringe.
5. Press **OK**. The Refresh Syringe cycle begins.
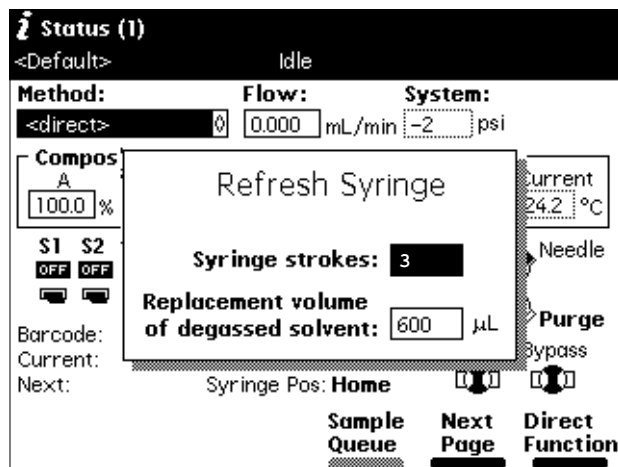
Figure 4-10 Refresh Syringe Dialog Box

4.2.5 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 3-7.

STOP *Attention: Make sure the needle-wash solvent line is primed before you perform a needle wash (see Section 3.4.1, Priming the Needle-Wash Pump).*

To wash the needle:

1. Press **Menu/Status** to display the Status screen (Figure 4-3).
2. In the Status screen, press the **Direct Functions** screen key. The Direct Functions menu (Figure 4-4) appears.
3. Select **Wash Needle**, then press **Enter**. The Wash Needle dialog box appears (Figure 4-11).

Figure 4-11 Wash Needle Dialog Box

4. Enter the number of wash cycles in the Wash cycles field.

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 **OK**. 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.

4.2.6 Equilibrating the System

During equilibration of the HPLC or LC/MS system, the 2795 Separations Module 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 4-3).

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

3.   Press the **Direct Function** screen key.

4.   Select **Equilibrate**, then press **Enter**. The Equilibrate dialog box appears (see Figure 4-12).
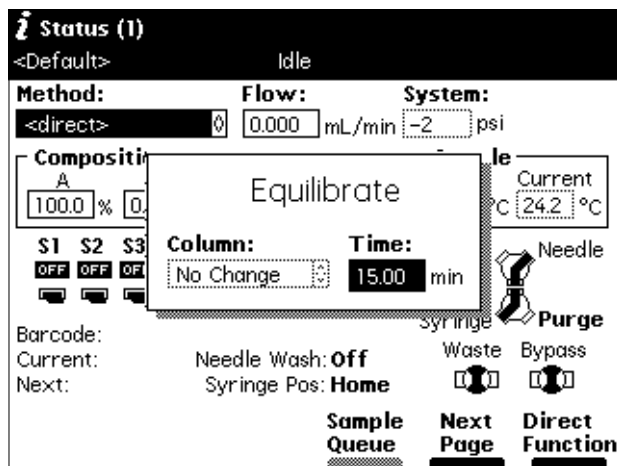
Figure 4-12 Equilibrate Dialog Box

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

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

4.2.7 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 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 menu and enter an injection volume of zero (see Section 4.2.8, Injecting Samples).*

To condition the column:

1. Press **Menu/Status** to display the Status screen (Figure 4-16).

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.

4. Select **Condition Column**, then press **Enter**. The Condition Column dialog box appears (see Figure 4-13).
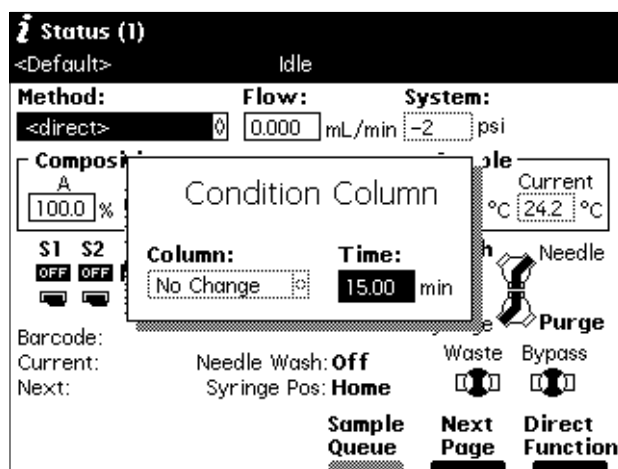
Figure 4-13 Condition Column Dialog Box

5. In the Column field, enter the column position to condition (if applicable).

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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 **OK**. The solvent management system runs the column conditioning procedure for the specified time.

4.2.8 Injecting Samples

Using the 2795 Separations Module 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 a change 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 Section 3.4.3, Loading the Sample Plates).

2. Close the sample compartment door.

3. Press **Menu/Status** to display the Status screen (Figure 4-3).

4. Enter the initial conditions in the Status screen. 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 in the Status screen. The Direct Functions menu appears (Figure 4-4).

6. Select **Inject Samples**, then press **Enter**. The Inject Samples dialog box appears (Figure 4-14), displaying six parameter fields. Table 4-3 describes the parameters in the Inject Samples dialog box.

4
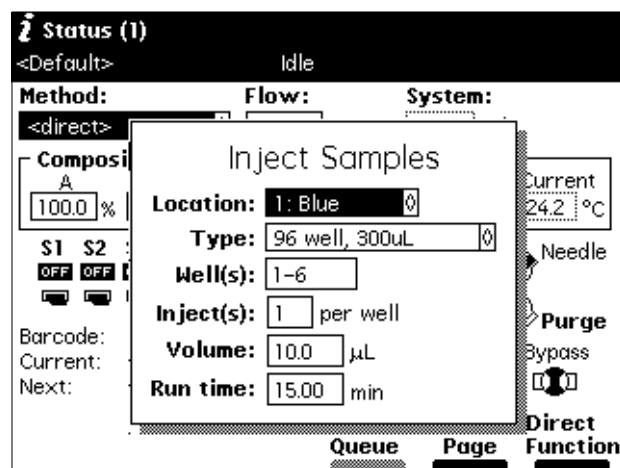
Figure 4-14 Inject Samples Dialog Box

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

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

Table 4-3 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 Section 3.2.2, Configuring Plate Types)
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 Section 4.2.9, Selecting Wells for Making Injections). When entering a well range, use the "•" key to separate entries. For example, to inject samples from wells 1 through 20, enter **1.20**.	Depending on plate type, 1 to 24, 48, 96, or 384

Table 4-3 Inject Samples Dialog Box Parameters *(Continued)*

Parameter	Description	Value Range
Inject(s)	Specifies the number of injections per well	1 to 99
Volume	Specifies the volume for each injection. See Section 5.3.7, Setting Sample Set Parameter Values, the "Injection Volume" discussion.	Injection volume depends on the size of the sample loop or 60% of syringe volume, whichever is smaller, as described in Table 5-24. **Note:** *If the most recently applied separation method specifies a Full Loop, then the Volume field is not editable. See Section 5.1, Creating and Editing Separation Methods.*
Run time	Specifies the run time for each injection	0 to 999.99 minutes

4.2.9 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 4-14), press the **Plate** screen key. The Well Selector is displayed (Figure 4-15).
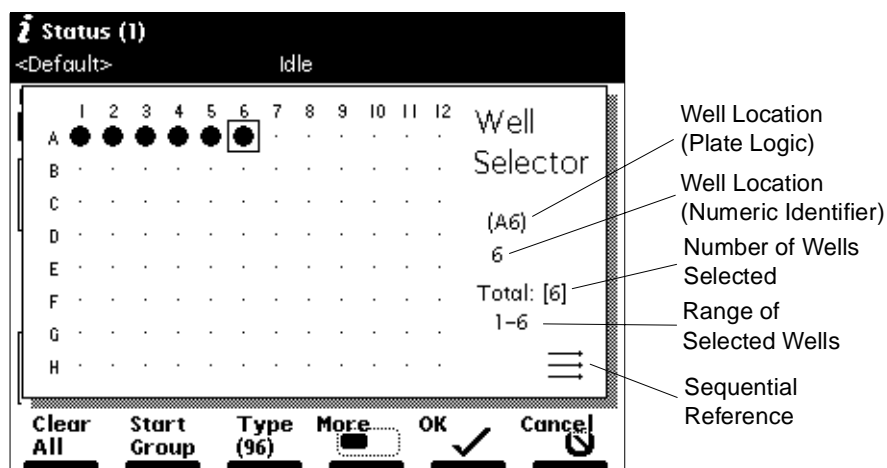
Figure 4-15 Well Selector

Note: You can also access the Well Selector from the Configuration, Sample Set, and Run Samples screens.

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 default screen 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 screen 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** field of the Inject Samples dialog box or the Sample Set table by the total number of wells in square brackets ([…]). See 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 3.2.2, Configuring Plate Types.

4

4.3 Monitoring System Status

Using the Status Screen

Use the 2795 Separations Module Status screen to monitor the status of your HPLC or LC/MS system during a run. Press **Menu/Status** to display the first page of the Status screen (Figure 4-16). Press the **Next Page** screen key to display the second page of the Status screen (Figure 4-17).

The content and layout of the Status screen pages vary with the installed options and with the mode of operation. You can customize the layout of the Status screens (see "Customizing the Status Screen Layout" in this chapter).

Figure 4-16 First Page of Status Screen (Example)

Status Screen Parameters

Table 4-4 describes the parameters in the Status screen pages. Use the 2795 Separations Module keypad arrow keys to move among parameter fields.

Note: In Table 4-4, the Status column provides the parameter names used in the Status Page Layout dialog box. See the "Customizing the Status Screen Layout" discussion.

Figure 4-17 Second Page of Status Screen (Example)

The first three parameters (Method, Flow, and System) in Table 4-4 always appear in the Status screen.

Table 4-4 Status Screen Parameters

Status	Parameter	Description	Values
Method	Method	Indicates the current separation method. When you change any parameter in the Status screen, this field changes to <direct>, indicating that current operating conditions may not correspond to a stored method. To select a stored method, select the Method field, press **Enter**, then select the method from the drop-down list.	All stored separation methods
Flow	Flow	Specifies the current flow rate of the solvent management system in mL/min	0 or 0.010 to 10.000 mL/min
System	System	Indicates the current system pressure in psi, bar, or kPa	Not editable
Composition	Composition	Indicates the current solvent composition from combining up to 4 solvents as a percentage Sum of the four solvent reservoirs = 100%	Each reservoir: 0 to 100%

Table 4-4 Status Screen Parameters *(Continued)*

Status	Parameter	Description	Values
Sample Heater/ Cooler	Sample Set	Specifies the user-specified sample compartment temperature in °C.	OFF or +4 to +40 °C
	Current	Indicates the current actual sample compartment temperature in °C.	Not editable
Column Heater	Column	Indicates the temperature and selection of a column	
	Set	Indicates the user-specified column temperature in °C.	OFF or 20 to 60 °C
	Current	Indicates the current actual column temperature in °C.	Not editable
	Selection	Indicates current column (if column selection valve is installed)	List of configured column position names (if column selection valve is installed)
Switches	S1, S2, S3, S4	Graphically indicate the status of event switches on the I/O connector	On or Off

Table 4-4 Status Screen Parameters *(Continued)*
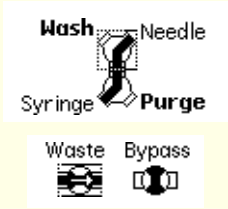
Status	Parameter	Description	Values
Valves	Valves	Graphically indicate the position of each of the four valves in the sample management system See also Section 8.3.5, Motors and Valves Diagnostics, the "Controlling the Wash, Purge, Waste, and Bypass Valves" discussion.	(Shown at left) Wash/Purge 3-way valves: • Syringe to Needle • Wash to Needle • Purge to Syringe • Waste/Bypass 2-way valves: • Open (Waste) • Closed (Bypass)
Degasser	Degasser	Indicates the status of the vacuum degasser	
	Mode	Specifies the mode of operation	On - enabled Off - disabled
	Vac pump	Indicates the status of the vacuum pump	Idle, Active (Not editable)
	Pressure	Indicates the current vacuum level	psia, mbar, kPa (Not editable)
Ripple	Pressure Ripple	Indicates pressure readings in the solvent management system for the most recent minute Max psi – Maximum pressure Min psi – Minimum pressure Delta psi – Peak-to-peak pressure difference	Not editable

4

Table 4-4 Status Screen Parameters *(Continued)*

Status	Parameter	Description	Values
Miscella-neous	Syringe Posi-tion	Indicates the current position of the syringe	Empty – Highest position (to refresh the syringe) Home – Default position Full – Lowest position (to draw in sample) (Not editable)
	Needle Wash	Indicates if the needle-wash pump is on	Not editable
	Sample	Indicates the plate and well number of the current injection	Not editable
	Location Current	Indicates the plate and well number of the sample that is preloaded	Not editable
	Next	Indicates the barcode corresponding to the current plate (if barcode reader is installed)	Not editable
	Barcode		
Needle and Inject Valve	Injector	Indicates the current position of the needle	Up – Home Load – In the inject port Wash – Above the inject port Well – In a sample well (Not Editable)
	Inject Valve	Indicates the current position of the inject valve ![LOAD icon] ![INJECT icon] ![PRIME icon] LOAD INJECT PRIME	Load Inject Prime (Not Editable)

4

Table 4-4 Status Screen Parameters *(Continued)*

Status	Parameter	Description	Values
Abs Detector Ch1/2	486/2487[a]	Indicates the current status of one or two Waters 486 or one or both channels of a 2487 detector, if installed	
	λ	Wavelength	486: 190 to 600 nm
			2487: 190 to 700 nm
	Lamp	Lamp status, ON or OFF	Not editable
	AUFS	Absorbance Units Full Scale	486: 0.001 to 2.000
			2487: 0.0001 to 4.0000
RI Detector	410/2410[a]	Indicates the current status of the Waters 410 or 2410 RI detector, if installed.	
	Cell	Cell temperature	Not editable
	Col 1	External temperature 1	Not editable
	Col 2	External temperature 2	Not editable

[a] These parameters are active only when the 2795 Separations Module is in System Controller mode.

Sample Queue

When you press the **Sample Queue** screen key on the Status Screen, the samples sets to be used for the current run are displayed.

Customizing the Status Screen Layout

To customize the layout of the Status screen pages:

1. From the Main screen, press **Config**, **More**, then **Status Layout**. The Status Page Layout dialog box is displayed (Figure 4-18).

   Each line in the Status Page Layout dialog box consists of a selection box, the name of the system parameter to be displayed in the Status screen(s), and the relative size of the parameter field. By using the screen keys (Raise, Lower), you can specify the order in which the status of each parameter is displayed.

   The Status screen has a grid-like arrangement of six units across and three units down. The measurements next to each parameter in the Status Page Layout dialog box specify the size of each parameter field relative to the full grid. The 2795 firmware attempts to fit all enabled parameter fields on the Status screen in the specified order. However, if you select all parameters, the screen may not have enough room to display them all.
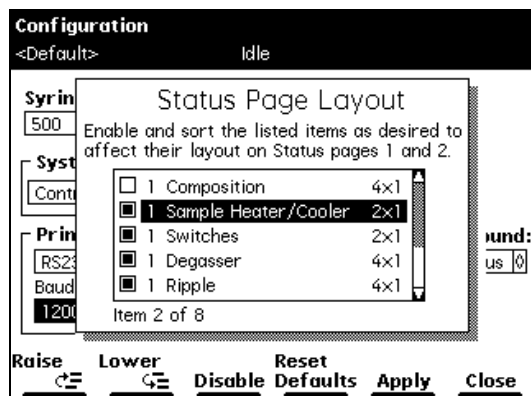
Figure 4-18 Status Page Layout Dialog Box

2. Use the Up/Down arrow keys to select a parameter field on the Status Page Layout dialog box.

3. Press the **Enable** screen key to display the selected parameter field (the selection box is filled in). Press the **Disable** screen key to disable display of a selected parameter field.

   If a parameter field is already enabled, the screen key reads Disable. If a parameter field is disabled, the screen key reads Enable. If the Enable key is grayed out, the parameter is not available for your configuration.

4. Press the **Raise** screen key to move the enabled parameter field higher in the display grid. Press the **Lower** screen key to move an enabled parameter field lower in the display grid.

   Note: Press Reset Defaults to return to system defaults for the Status screen.

5. Press the **Apply** screen key to save the changes without closing the Status Page Layout dialog box. The Status Page number (1 or 2) appears next to each enabled parameter field in the list. If no page number appears next to an enabled parameter, the Status Page Layout does not have enough room to display the parameter as requested.

6. Press the **Close** screen key to exit from the Status Page Layout dialog box.

Note: In the Status screens, you can make changes only in fields that have a solid border.

Chapter 5
Creating and Editing Separation Methods and Sample Sets

This chapter describes how to create and edit the separation methods and sample sets that allow you to make automatic runs when the Waters 2795 Separations Module is in a stand-alone mode. If your LC system is controlled by Millennium[32] software (version 3.2 or higher), refer to Millennium[32] software documentation for information on creating and editing methods using the Millennium[32]software. If your HPLC system is controlled by MassLynx software (version 3.3 or higher), refer to MassLynx documentation for information on creating and editing methods using the MassLynx software.

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 non-time-based conditions that influence the separation
- **Sample set** – A set of instructions in table format that applies functions to separation methods

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
- System Prep

To create or edit a separation method, see Section 5.1.1, Creating Separation Methods, or Section 5.1.2, Editing Separation Methods.

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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

You can use multiple separation methods in a sample set.

You create and edit separation methods and sample sets using the Methods screen (Figure 5-1) and six method parameter screens. In the Methods screen, separation methods are identified by a chromatogram icon ⅃ and sample sets are identified by a multiple vial icon 🔲 . To create or edit a sample set, see Section 5.3, Creating and Editing Sample Sets.
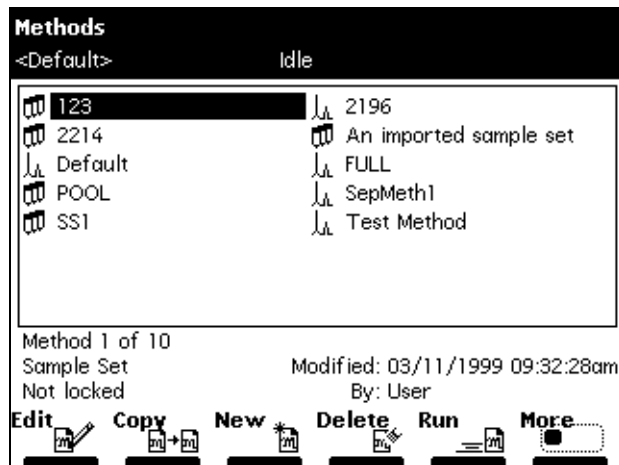
Figure 5-1 Methods Screen

5.1 Creating and Editing Separation Methods

This section provides information on:

- Section 5.1.1, Creating Separation Methods
- Section 5.1.2, Editing Separation Methods
- Section 5.1.3, Copying and Editing Separation Methods
- Section 5.1.4, Locking/Unlocking Separation Methods
- Section 5.1.5, Renaming, Viewing, and Deleting Separation Methods

Note: When you press the Run screen key in the Methods screen, the Sample Set screen appears (Section 5.3, Creating and Editing Sample Sets). For more information on running methods, see Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation.

For information on importing and exporting separation methods, see Section 5.4, Importing and Exporting Methods.

For information on System Prep, see Section 4.2.1, System Preparation Function.

5.1.1 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 (Figure 5-1) appears.
2. Press the **New** screen key. The New Method dialog box appears (Figure 5-2).
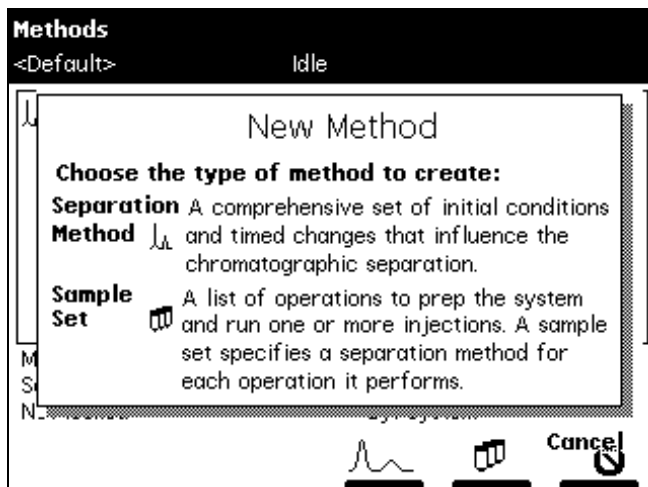
Figure 5-2 New Method Dialog Box

3. Press the chromatogram icon (separation method) screen key. The Separation Method Name dialog box appears (Figure 5-3).
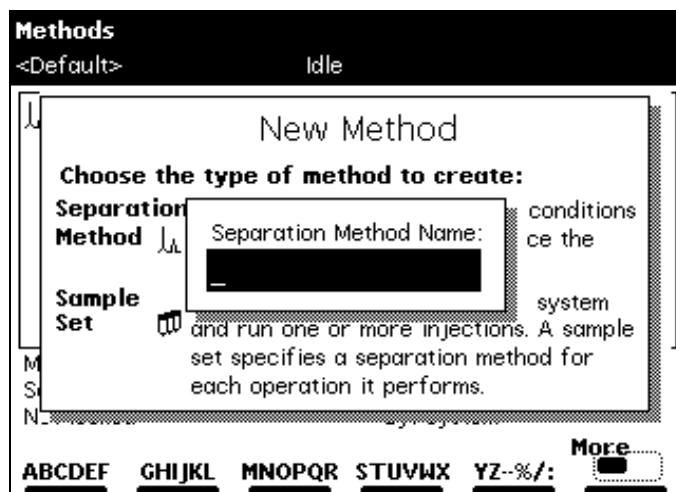
Figure 5-3 Separation Method Name Dialog Box

4. Use the screen keys to enter a name of up to 30 characters (see Section 3.1.2, Using the Keypad, the "Entering an Alphanumeric String" discussion).

5. Press **Enter**. The Mobile Phase screen appears (Figure 5-4). The Mobile Phase screen is one of six parameter screens.
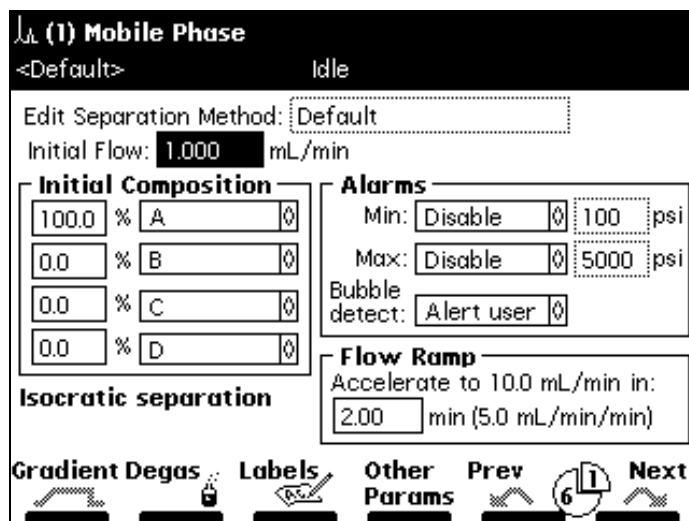
Figure 5-4 Mobile Phase Screen

6. Select values for the parameter fields in the Mobile Phase screen, using the arrow keys to move between fields (see 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 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 **Yes**.

Figure 5-5 summarizes the steps to create a separation method using the 2795 Separations Module front panel.

Figure 5-5 Steps in Creating a Separation Method

Default Separations Method Created Alarm Message

The alarm message, "default separations method created", will appear silently if the firmware needed to create a default separation method. This is a normal occurrence after a Clear-8 or Clear-0 power-up key sequence was used to erase flash memory. However, if this alarm is seen in the log any other time, it indicates that the default method had been corrupted or otherwise lost. Millennium and MassLynx software will ignore this alarm.

5.1.2 Editing Separation Methods

To edit an existing 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 5-1).

5

2. Select the separation method you want to edit, then press the **Edit** screen key. If the separation method is locked, the Edit screen key is grayed out. To unlock a locked separation method, see Section 5.1.4, Locking/Unlocking Separation Methods.

3. Enter the new parameter values in the appropriate screens. For details, refer to Section 5.2, Setting Separation Method Parameters.

4. To save the method:

   • Under its current name, press **Exit**, then, when prompted to save, press **Yes**.

   • Under a new name:

   –Press **Exit**. When prompted to save, press **Save as**. The 2795 firmware automatically creates a new method name by adding an extension to the original method name.

   –Use the alphabetic screen keys to change the name, if desired (see Section 3.1.2, Using the Keypad), then press **Enter**.

5. To exit without saving the changes, press **Exit**. When prompted to save, press **No**.

   To return to the method without entering any changes, press **Cancel**.

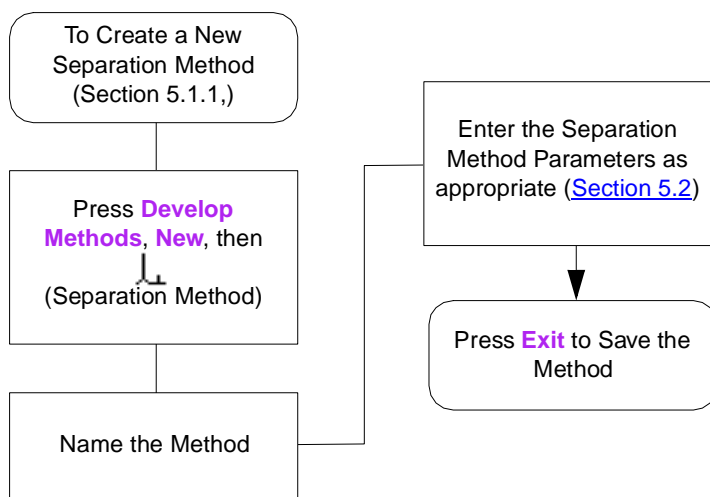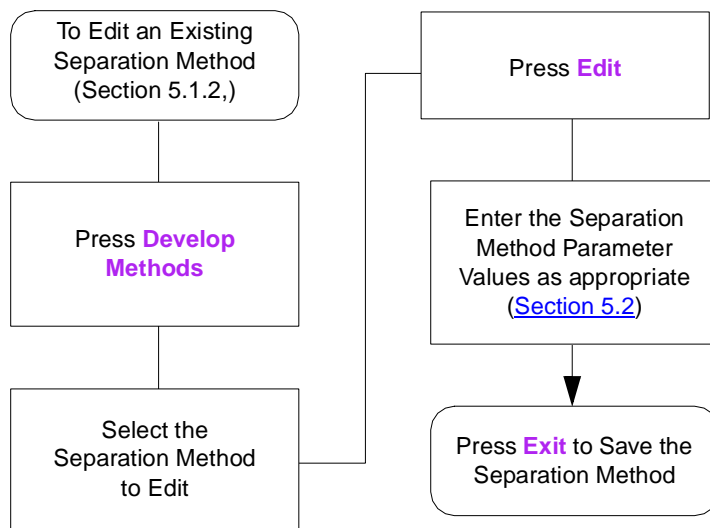Figure 5-6 summarizes the steps to edit a separation method using the 2795 Separations Module front panel.

Figure 5-6 Steps in Editing a Separation Method

5.1.3 Copying and Editing Separation Methods

To copy and then edit an existing separation method, leaving the original method unchanged:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears.

2. Select the separation method you want to copy.

3. Press the **Copy** screen key. To avoid duplicate file names, the 2795 firmware automatically adds an extension to the existing file name.

4. Press **Enter** to accept the default method name, or use the alphabetic screen keys to enter a new name, then press **Enter** (see Section 3.1.2, Using the Keypad).

5. Enter new parameter values in the appropriate parameter screens (Section 5.2, Setting Separation Method Parameters).

6. To save the method, press **Exit**, then press **Yes**. To exit without saving the method, press **No**. To return to the method without entering any changes, press **Cancel**.

5.1.4 Locking/Unlocking Separation Methods

Locking a Separation Method

Locking a method prevents unauthorized changes to the method. To lock a separation method:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears (Figure 5-1).

2. Select the method you want to lock, then press the **Lock** screen key.

3. Enter a password of up to 30 alphanumeric characters (see Section 3.1.2, Using the Keypad), then press **Enter**. The method is locked and cannot be changed, deleted, or renamed until it is unlocked (see below).

   Note: When you copy a locked method and save it with a new name, the copy is not locked.

4. Record the password and keep it in a safe place.

Unlocking a Separation Method

To unlock a separation method:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears (Figure 5-1).

2. Select the separation method you want to unlock, then press **Unlock**.

3. Enter the password, then press **Enter**. The method is unlocked.

5.1.5 Renaming, Viewing, and Deleting Separation Methods

Renaming Separation Methods

To rename a separation method:

1. In the Methods screen, select a separation method to rename.

2. Press the **More** screen key until the Rename screen key is displayed, then press the **Rename** screen key. The Change to Method Name dialog box appears (Figure 5-7).
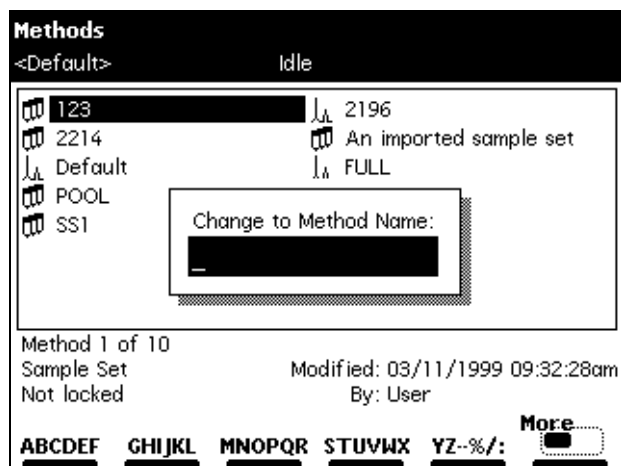
Figure 5-7 Change to Method Name Dialog Box

3. Enter the new method name using the alphanumeric screen keys (see Section 3.1.2, Using the Keypad).

4. Press **Enter** to rename the method.

Viewing Separation Methods Using the View Screen Key

To view a separation method that is locked (not editable):

1. In the Methods screen, select a separation method to view.

2. Press the **More** screen key until the View screen key is displayed, then press the **View** screen key. The first parameter screen (Mobile Phase screen, Figure 5-4) for the selected method appears.

3. Press the **Next** or **Previous** screen key to view the other five parameter screens for the selected method.

Deleting Separation Methods

Note: You cannot delete the Default method or any locked method.

You can delete separations methods using the:

- Delete screen key
- Delete Methods screen key

Using the Delete Screen Key

To delete a separation method using the Delete screen key:

1. In the Methods screen, select a separation method to delete.
2. Press the **Delete** screen key. The Deleting Method dialog box appears.
3. Press the **Yes** screen key to delete the selected separation method.

   Press the **No** screen key to return to the Methods screen without deleting the selected separation method.

Using the Delete Methods Screen Key

To delete a separation method using the Delete Methods screen key:

1. In the Methods screen, press the **More** screen key until the Delete Methods screen key is displayed.
2. Press the **Delete Methods** screen key. The Delete Methods screen appears.
3. For each separation method you want to delete, use the arrow keys to select the method, then press the **Select Method** screen key.

   To select all methods except the previously selected method(s), press the **Invert Choices** screen key.

   To clear all selected methods on the Delete Methods screen, press the **Clear Choices** screen key.

4. To delete the selected method(s), press the **Delete Now** screen key.

5.2 Setting Separation Method Parameters

You enter separation method parameters in six different screens, sequenced as shown in Table 5-1. Press the **Next** or **Previous** screen key to move from one method parameter screen to another. The "pie" icon between the Next and Previous screen keys indicates your location among the six parameter screens.

Table 5-1 Separation Method Parameter Screens

Sequence Number	Screen Name	Reference
1	Mobile Phase	Section 5.2.1, Setting Mobile Phase Parameter Values
2	Sample	Section 5.2.2, Setting Sample Parameter Values

5

Table 5-1 Separation Method Parameter Screens *(Continued)*

Sequence Number	Screen Name	Reference
3	Autosampler	Section 5.2.3, Setting Autosampler Parameter Values
4	Column	Section 5.2.4, Setting Column Parameter Values
5	I/O	Section 5.2.6, Setting I/O Parameter Values
6	Detectors	Section 5.2.7, Setting Detector Parameters

Note: Parameter fields that are not editable for the option you select are surrounded by a dotted line. You cannot use screen keys that are grayed out.

As you change values on the parameter screens, the word "modified" appears in parentheses next to the method name.

When you finish entering or changing values on all six parameter screens, press **Exit**. At the Save prompt, press the **Yes**, **Save As**, **No**, or **Cancel** screen keys as appropriate (see Section 5.1.2, Editing Separation Methods).

5.2.1 Setting Mobile Phase Parameter Values

The Mobile Phase screen (Figure 5-4) appears when you create a new separation method (see Section 5.1.1, Creating Separation Methods), view a locked separation method using the View screen key, or edit a separation method.

To enter parameter values in the Mobile Phase screen:

1. Press the **Next** or **Prev** screen key (if necessary) to display the Mobile Phase screen (Figure 5-4).

2. Enter or select values in the fields, as appropriate. Table 5-2 describes the parameters and screen keys in the Mobile Phase screen.

Table 5-2 Mobile Phase Screen Parameters

Parameter	Function	Value Range
Initial Flow	Specifies the initial flow rate of the method. For isocratic operation, this is the flow rate for the entire separation.	0.000 and 0.010 to 10.000 in 0.001-mL/min increments (typically 50 µL to 5 mL/min)

5

Table 5-2 Mobile Phase Screen Parameters *(Continued)*

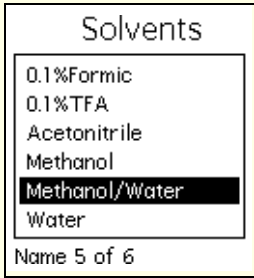| Parameter	Function	Value Range
Initial Composition	Specifies the initial composition of the mobile phase. The sum of the four fields must equal 100%. (Enter, change, or delete solvent names using the Labels screen key. See "Labels" elsewhere in this table.)	0 to 100.0 in 0.1% increments
Alarms: Min	Specifies the system pressure (set in psi, bar, or kPa) below which the alarm condition (selected in the adjacent box) is executed. To enable access to the Pressure fields, set the alarm parameter to any selection other than "Disable."	0 to 4500 in 1-psi increments, 0 to 310 bar in 1-bar increments, or 0 to 31025 kPa in 1-kPa increments (see Section 4.3, Monitoring System Status, the "Status Screen Parameters" discussion). Refer to the "Setting Alarm Responses" discussion below.
Alarms: Max	Specifies the system pressure (set in psi, bar, or kPa) above which the alarm condition (selected in the adjacent box) is executed. Use to detect problems with method conditions and to protect your column from overpressure.	0 to 5000 in 1-psi increments, 0 to 344 bar in 1-bar increments, or 0 to 34473 kPa in 1-kPa increments (see Section 4.3, Monitoring System Status, the "Status Screen Parameters" discussion). Refer to the "Setting Alarm Responses" discussion below.
Alarms: Bubble Detect	Specifies the response to occur when the solvent management system detects a bubble in the flow path.	Refer to the "Setting Alarm Responses" discussion below.

Table 5-2 Mobile Phase Screen Parameters *(Continued)*

Parameter	Function	Value Range
Flow Ramp (see Figure 5-4)	Specifies the time (in minutes) for the solvent delivery system to reach the maximum system flow rate. This limits the rate of change of the flow rate to protect the column from potentially damaging sudden changes in pressure. **Note:** *The 2795 firmware ignores this parameter during rapid equilibration when the column is off-line.*	0.01 to 30 min in 0.01-min increments.
Gradient (screen key)	Displays the Gradient screen, which allows you to build a gradient table.	See the "Setting Gradient Table Parameter Values" discussion below.
Degas (screen key)	Displays the Degasser screen, which allows you to set the Degasser Mode. Refer to Section 3.3.2, Degassing Solvents. On Degasser Error: Specifies the response to occur when an vacuum degasser fault is detected. The degasser is disabled on any fault regardless of the alarm setting you select. Waters recommends that you enable either the "Stop Function" or "Stop Flow" alarm setting.	Off (disabled) On (enabled) Refer to the "Setting Alarm Responses" discussion below.
Labels (screen key)	Displays the Solvents dialog box (the default solvent list is shown at right), which allows you to add, remove, or change the names of solvents used in methods. Use the Add, Remove, and Change screen keys to edit the list of solvents. **Note:** *A, B, C, and D are not valid user-entered solvent names.*	Solvents 0.1%Formic 0.1%TFA Acetonitrile Methanol **Methanol/Water** Water Name 5 of 6

Table 5-2 Mobile Phase Screen Parameters *(Continued)*

Parameter	Function	Value Range
Other Params (screen key)	Displays the Preferred Stroke Volume and Plunger-Seal-Wash fields. The Preferred Stroke Volume field specifies the volume of solvent delivered with each piston stroke. You can override the default stroke volume, but do not exceed the flow rate limits displayed on the screen for each of the stroke volume settings. Refer to the "Preferred Plunger Stroke Volume" discussion in Section 1.2,. The Plunger Seal Wash period specifies the time interval between successive plunger seal-wash pump cycles.	130 µL - default 100 µL 50 µL 25 µL Off, 0.50 to 10.00 in 0.01-min increments

Setting Alarm Responses

During operation, the 2795 Separations Module records and reports errors according to the alarm responses you specify. Each configurable alarm can produce one of five user-selected responses (Table 5-3).

Table 5-3 Alarm Responses

Alarm Response	Function
Disable	All alarm response reporting is disabled.
Log Quietly	The error is entered into the error log without alerting the operator.
Alert User	The error is entered into the error log and the operator is alerted with a dialog box.
Stop Funct	The error is entered into the error log, the operator is alerted with a dialog box, and operation is suspended at the end of the current function. You can abort or resume the operation of the sample set by pressing the appropriate screen key.
Stop Flow	The error is entered into the error log, the operator is alerted with a dialog box, the current function is suspended, and solvent flow is stopped.

5

Setting Gradient Table Parameter Values

The Gradient table allows you to make time-based changes to the composition or flow rate of the mobile phase during a run. You can program up to 25 rows in the Gradient table.

To set parameter values in the Gradient table:

1. Press the **Gradient** screen key in the Mobile Phase screen. The Gradient screen is displayed (Figure 5-8).

Figure 5-8 Gradient Screen

2. Enter values in the Gradient table as appropriate. Table 5-4 describes the parameters in the Gradient table.

   Use the Overview, Insert Row, Delete Row, Sort by Time, Copy Down, More, Reset Table, and Print screen keys as appropriate.

3. When you finish setting parameter values in the Gradient screen, press **Exit** to save the Gradient table and return to the Mobile Phase screen.

Table 5-4 Gradient Table Parameters

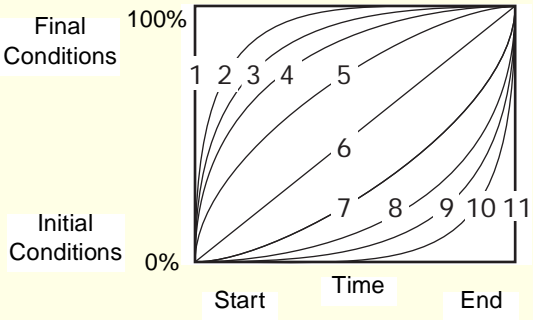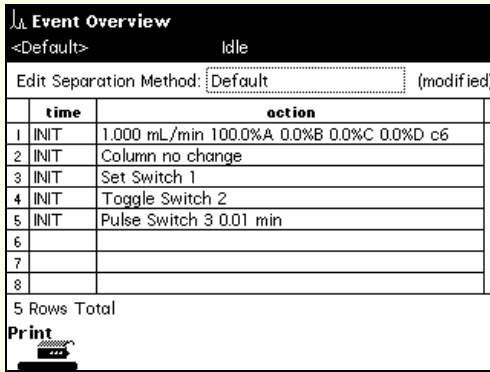| Parameter	Function	Value Range
Time	Specifies the time after the start of the run at which the change is to occur (INIT is allowed only in the first row of this table)	INIT, 0.00 to 999.99 in 0.01-minute increments
Flow	Specifies the flow rate of the solvent delivery system	0.000 and 0.010 to 10.000 in 0.001-mL/min increments

Table 5-4 Gradient Table Parameters *(Continued)*

Parameter	Function		Value Range
%A, %B, %C, %D	Specifies the proportion of each solvent in the mobile phase. The sum of these four fields must equal 100%.		0 to 100 in 0.1% increments
Curve	Specifies the rate profile at which the mobile phase composition and/or flow rate are to change The figure (right) shows the rate profile for each value.		
	Select the desired gradient curve from the list of profiles, or select the curve number by pressing the appropriate numeric key. Press "**0**" for curve 10 and "**.**" for curve 11.		
Overview (screen key)	Displays a time-ordered summary of the events in the Gradient, Detector, and Timed Events tables (as shown below) 		N/A
Insert Row (screen key)	Inserts a row above the current row		N/A
Delete Row (screen key)	Deletes the current row		N/A

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Table 5-4 Gradient Table Parameters *(Continued)*

Parameter	Function	Value Range
Sort by Time (screen key)	Sorts the rows based on time ***Note:*** *The 2795 Separations Module firmware automatically sorts entries when you exit from the Gradient table.*	N/A
Copy Down (screen key)	Copies the contents of the current table cell into all subsequent cells in the same column	N/A
Reset Table (screen key)	Clears the table, leaving the INIT row	N/A
Print (screen key)	Prints the Gradient table to a floppy disk or to a serial printer, if connected	N/A

5.2.2 Setting Sample Parameter Values

To enter parameter values in the Sample screen:

1. Press the **Next** or **Prev** screen key (as appropriate) to display the Sample screen (Figure 5-9).

Figure 5-9 Sample Screen

2. Enter or select values in the Sample screen. Table 5-5 describes the parameters in the Sample screen.

Table 5-5 Sample Screen Parameters

Parameter	Function	Value/Range
Sample Temperature Target	Specifies the temperature of the sample heater/cooler. To turn off the heater/cooler, press **Clear**.	Off, 4 to 40 °C in 1 °C increments
Sample Temperature On error	Specifies the response to occur when the sample compartment temperature is out of the specified range during a run.	See Table 5-3
Sample Temperature Range	Specifies the maximum allowable deviation in sample temperature from the target temperature. If the temperature deviation exceeds the range, the alarm response selected in the On error field occurs.	1 to 20 °C in 1 °C increments
Syringe Draw Rate Nominal Custom	Specifies one of three pre-set syringe draw rates to accommodate viscous samples. The rate changes with the size of the installed syringe (see table below). Specifies a user-selected custom draw rate value. {SEE SUBTABLE BELOW}	Fast (40 µL/sec)[a] Normal (5.0 µL/sec)[a] Slow (2.0 µL/sec)[a] Custom (2 to 40 µL/sec)[a]
Depth (Needle Depth Offset)	Adjusts the depth of the needle tip to accommodate sedimented sample or nonstandard wells. A value of 0 corresponds to the bottom of the well. A value of 10 corresponds to 10 mm from the bottom of the well.	0 to 20 in 1-mm increments

Subtable (within Syringe Draw Rate row):

Syringe Size	µL/min Minimum	µL/min Maximum
100	0.4	8.0
250	1.0	20.0
500	2.0	40.0
1000	4.0	50.0
2500	10.0	50.0

a. The specified syringe draw rates are for the standard 500-µL syringe. The rates corresponding to Fast, Normal, and Slow automatically change in proportion to the syringe size entered in the Configuration screen (see Section 3.2.1, Setting Configuration Parameters).

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5.2.3 Setting Autosampler Parameter Values

To enter parameter values in the Autosampler screen:

1. Press the **Next** or **Prev** screen key (as appropriate) to display the Autosampler screen (Figure 5-10).

Figure 5-10 Autosampler Screen

2. Enter or select values in the Autosampler screen as appropriate. Table 5-6 describes the parameters in the Autosampler screen.

Table 5-6 Autosampler Parameters

Parameter	Function	Value/Range
Injection mode	Specifies one of two injection modes: • **Sequential** – Sample aspiration and loop fill occur at the start of each injection cycle, after completion of the previous injection. • **Parallel** – Sample aspiration and loop fill occur concurrently with other separation method functions for higher throughput (for more information, see Section 6.2,).[a]	Sequential Parallel

Table 5-6 Autosampler Parameters *(Continued)*

Parameter	Function	Value/Range
Loop option	Specifies the way sample is placed in the sample loop: • **Full loop** – Does not allow you to control the injection volume in the Sample Set Editor. • **Partial loop** – Allows you full control of the injection volume in the Sample Set Editor, including the option to revert to Full-loop operation. ***Note:*** *Full-loop injections provide the best peak area reproducibility.*	Full Partial
Full-loop (overfill)	Specifies the excess volume (expressed as a multiple of the sample loop volume) of sample drawn when you select the Full-loop option (see above), or when you override the Partial-loop option in the Sample Set Editor.	1.0 to 99.9× in 0.1× increments Default = 4.0×
STOP	***Attention:*** *You can select either full-loop or partial-loop injection mode in the instrument method in Millennium^{32} software. After saving an instrument method with a full-loop injection mode and a designated number of loop overfills, you cannot make partial-loop injections in Run Samples using that instrument method. Similarly, an instrument method with a partial-loop injection mode cannot be used for full-loop injections in Run Samples. Waters recommends naming instrument methods to designate them as either full- or partial-loop methods.*	

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Table 5-6 Autosampler Parameters *(Continued)*

Parameter	Function	Value/Range
Pre-column (volume)	Specifies the volume of mobile phase delivered to the system after the gradient starts, but before an injection is made. Use this parameter when you use a method from a system that has a delay volume smaller than that of the 2795 Separations Module. You can also use this parameter to reduce the delay volume of the 2795 Separations Module for narrow or microbore columns or for high-throughput separations, because the sample is held in the sample loop (if desired) until the gradient front reaches the sample loop.	0.0 to 10000.0 in 0.1-µL increments
Loop off-line	When the Parallel Injection mode option (see above) is selected, specifies the time after an injection when the next sample is preloaded into the sample loop. Preloading the sample into the loop can result in a small decrease in injection-to-injection cycle time. The values of Off and 0.00 are equivalent and ensure that the next sample will not be preloaded into the sample loop. Values greater than 0.00 and less than or equal to the method run-time will cause the next sample to be preloaded into the sample loop at the given time. However, the time entered should not be less than the time needed for the current sample to leave the sample loop (based on the size of the sample loop and the current flow rate). In Sequential mode, there is no "pre-load" of sample, but the loop can be brought off-line to achieve a decrease in delay volume during the separation.	Off, 0.00 to 999.99 in 0.01-min increments
Post-run delay	Specifies the time after a run that a data system is processing run data. During the delay, the 2795 Separations Module can complete sample management functions, including: needle wash and purge, sample aspiration, and loop loading (but does not perform an injection).	0 to 999.99 in 0.01-min increments

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Table 5-6 Autosampler Parameters *(Continued)*

Parameter	Function	Value/Range
Aspirate Air Pre-sample Post-sample	Specifies a volume of air (air gap) aspirated into the holding loop to minimize sample diffusion. Specifies aspiration of air before the sample is in the needle. Specifies aspiration of air after the sample is in the needle. ***Note:*** *To minimize sample diffusion, incorporate air gaps (3 to 5 µL), pre- and post-sample. These "air gaps" may enhance area precision, but are not recommended for all LC/MS applications.*	 0 to 999.9 in 0.1-µL increments 0 to 999.9 in 0.1-µL increments
Seek well bottom (check box)	When selected, at the vial specified for the first injection from a plate, the needle detects the well bottom location. This location is stored for use during subsequent injections from that plate. When the carrier advances to the next plate, a new well bottom measurement occurs. **STOP** ***Attention:*** *Due to variations in dimensions, do not use the Seek Well Bottom feature with thin-bottom glass inserts (such as part number 186000349) or with the two microcentrifuge sample container plates (part numbers 405000560 and 405000772).*	Selected (X) or Not selected (blank)
Check plate height (check box)	When selected, at the first injection from each plate, the needle moves to a position over the corner of the plate nearest the inject port, and a needle position sensor determines the height of the plate. This value is checked against an acceptable range for the plate type.	Selected (X) or Not selected (blank)
Wash (screen key)	Displays the Autosampler Wash dialog box (Figure 5-11). See the "Setting Autosampler Wash Parameter Values" discussion below.	N/A
Rapid Equil (screen key)	Displays the Rapid Equilibration dialog box (Figure 5-12). See the "Setting Rapid Equilibration Parameter Values" discussion below and Section 6.2.3, Parallel Processing with Rapid Equilibration.	N/A

a. Waters recommends you do not calibrate or do quantitative analysis with your system based on the first of a series of parallel injections. The first injection in a series of parallel injections may differ in timing because the first injection is not coordinated with a previous injection. Results obtained from the first injection may vary from those of subsequent injections.

Setting Autosampler Wash Parameter Values

To set the Autosampler Wash parameters:

1. Press the **Wash** screen key in the Autosampler screen. The Autosampler Wash dialog box (Figure 5-11) appears.
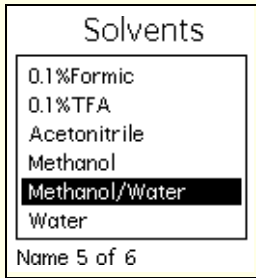

Figure 5-11 Autosampler Wash Dialog Box

2. Enter or select parameter values in the Autosampler Wash dialog box as appropriate. Table 5-7 describes the Autosampler Wash parameters.

Table 5-7 Autosampler Wash Parameters

Parameter	Function	Value/Range
Wash Solvent Label	Specifies the wash solvent from the Solvents drop-down list (example shown at right). To add or delete a solvent in the Solvents list, press the **Labels** screen key, then add or delete as appropriate.	
Frequency	Specifies when a needle wash is to occur.	Every inject Every well Never

Table 5-7 Autosampler Wash Parameters *(Continued)*

Parameter	Function	Value/Range
Cycles	Specifies the number of wash cycles to be performed at each wash	0 to 9 in 1 cycle increments
Inject Port	Specifies the number of seconds to wash the inject port	0 to 9999 in 1 second increments. Default = 6
Needle Exterior	Specifies the number of seconds to wash the exterior of the needle	0 to 9999 in 1 second increments. Default = 15
Purge Solvent Label	Specifies the purge solvent from the Solvents drop-down list (example shown at right). To add or delete a solvent in the Solvents list, press the **Labels** screen key, then add or delete as appropriate.	
Purge Solvent Replacement Volume of Degassed Solvent	Specifies the volume (in μL) needed to replace old sample management system solvent with fresh degassed purge solvent	0 to 9999 in 1-μL increments

3. Press **OK** to enter the needle wash parameter values, or press **Cancel** to return to the Autosampler page without changing the default wash parameter values.

   Note: By default, the wash sequence is set to Wash-Purge. The alternative is the Purge-Wash-Purge sequence that performs an additional purge of the syringe, prior to the Needle-Wash. Refer to Section 3.3.3, Wash Sequences.

Setting Rapid Equilibration Parameter Values

To set Rapid Equilibration parameter values:

1. Press the **Rapid Equil** screen key in the Autosampler screen. The Rapid Equilibration dialog box appears (Figure 5-12).
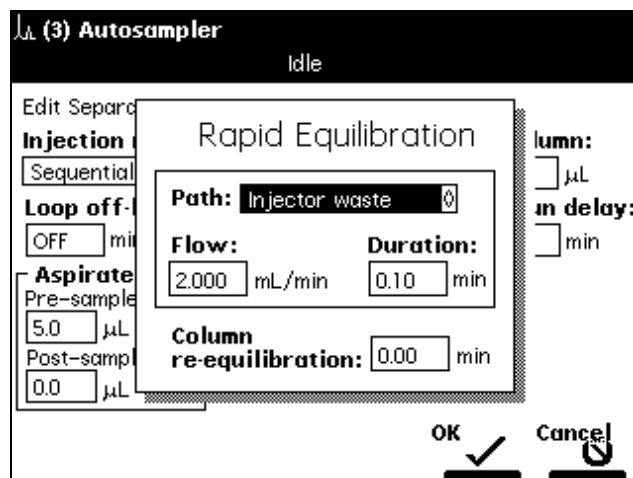
5

Figure 5-12 Rapid Equilibration Dialog Box

2. Enter or select parameter values for the displayed fields. Table 5-8 describes the
    Rapid Equilibration parameters.

Table 5-8 Rapid Equilibration Parameters

Parameter	Function	Value/Range
Path	Specifies the flow path for rapid equilibration *Note: When the 3-column selection valve is installed at Waters, Position 2 is used for waste.*	**Off** – No rapid equilibration **No change** – Uses the same path as previously specified **Injector waste** – Directs flow to waste through the inject valve **Position 1** – Directs flow out through Position 1 of the column selection valve (if installed) **Position 2** – Directs flow out through Position 2 of the column selection valve (if installed) **Position 3** – Directs flow out through Position 3 of the column selection valve (if installed) **Position 4 to 6** – Directs flow out through Positions 4 to 6 of a 6-column selection valve (if installed)

Table 5-8 Rapid Equilibration Parameters *(Continued)*

Parameter	Function	Value/Range
Flow	Specifies the system equilibration flow rate	0.010 to 10.000 mL/min in 0.001 mL/min increments
Duration	Specifies the system equilibration time	0.00 to 999.99 min in 0.01 min increments
Column re-equilibration	Specifies the time after system equilibration is complete to reequilibrate the column. See Table 6-2.	0.00 to 999.99 min in 0.01 min increments Default value – 5.00 min

3. Press **OK** to enter the rapid equilibration parameter values, or press **Cancel** to return to the Autosampler page without changing the default rapid equilibration parameters.

For more information on parallel processing with rapid equilibration, see Section 6.2, Using the 2795 High-Throughput Capabilities.

5.2.4 Setting Column Parameter Values

To enter parameter values in the Column screen:

1. Press the **Next** or **Prev** screen keys (as appropriate) to display the Column screen (Figure 5-13).

Figure 5-13 Column Screen

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2. Enter or select parameter values in the Column screen, as appropriate. describes the parameters.

Table 5-9 Column Parameters

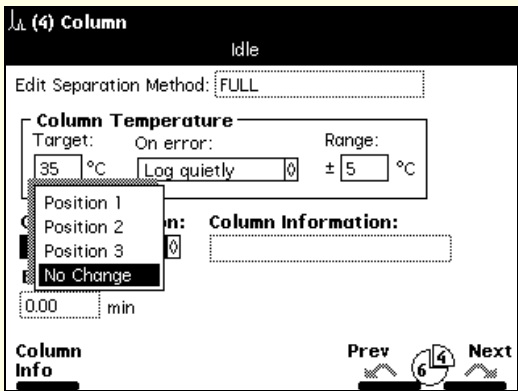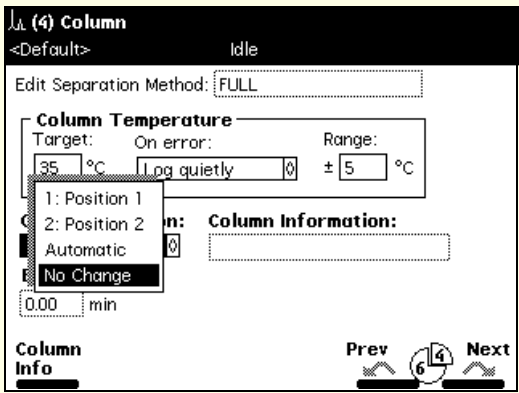| Parameter	Function	Value/Range
Column Temperature Target	Specifies the temperature of the column heater. To turn off the column heater, press the **Clear** key.	Off, 20 to 60 °C (minimum of ambient + 5 °C) in 1 °C increments.
Column Temperature On error	Specifies the response that occurs when the column temperature is outside the specified range.	See Table 5-3
Column Temperature Range	Sets the maximum allowable deviation in column temperature from the target tempera- ture. If the temperature deviation exceeds the range, the alarm response selected in the On error field occurs.	1 to 20 °C in 1 °C increments
Column Selection 3-Column Valve	Selects a column position if a column selection valve is installed. The example below shows the selections for a 3-column valve. *Note: Select **No Change** when you link methods in a sample set and do not want to change the column from the previous method.*	Position 1 through Position 3, or No Change
6-Column Valve	Selects a column position if a 6-column valve is installed.	Position 1 through Position 6, or No Change

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Table 5-9 Column Parameters *(Continued)*

Parameter	Function	Value/Range
Column Selection Column Regeneration Valve	Selects a column position if a column regeneration valve is installed. 	Refer to Section 5.2.5, Setting Up Two-Column Regeneration
Column Info (screen key)	Allows you to enter or modify the column information for each column position.	30 characters each
Column Information	Displays the column information for the column position you select using the Column Info screen key. See the "Setting Column Information Parameter Values" discussion below.	N/A
Equilibration	When a change in column selection occurs at run time, specifies the amount of time that the 2795 Separations Module operates at initial conditions before performing an injection.	0 to 999.99 min

Setting Column Information Parameter Values

To set column information parameter values:

1. Press the **Column Info** screen key in the Column screen (Figure 5-13). The Column Information dialog box appears (Figure 5-14).
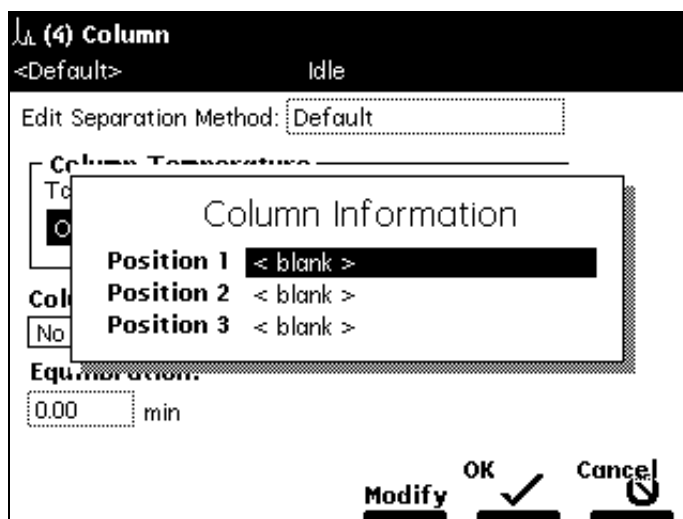
5

Figure 5-14 Column Information Dialog Box

Note: The Column Information dialog box shows three positions for the 3-column selection valve, six positions for the 6-column selection valve, and two positions for the Column Regeneration Valve (see Section 1.4, Options and Accessories, the "Column Selection Valves" discussion).

2. Select a column position to modify, then press the **Modify** screen key. A dialog box that allows you to change the information for the selected column position appears (Figure 5-15).
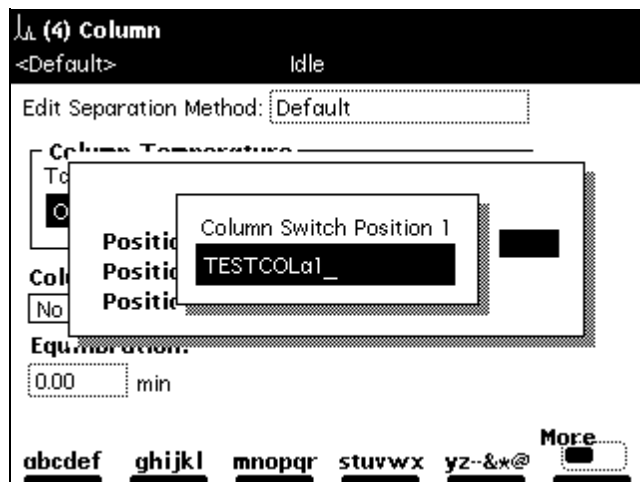

Figure 5-15 Column Selection Position 1 Dialog Box (Example)

3.   Enter the new information, up to 30 characters for each column position, in the Column Switch Position information field (see Section 3.1.2, Using the Keypad).

When you press **Enter**, the new information appears in the selected column position in the Column Information dialog box (Figure 5-16).
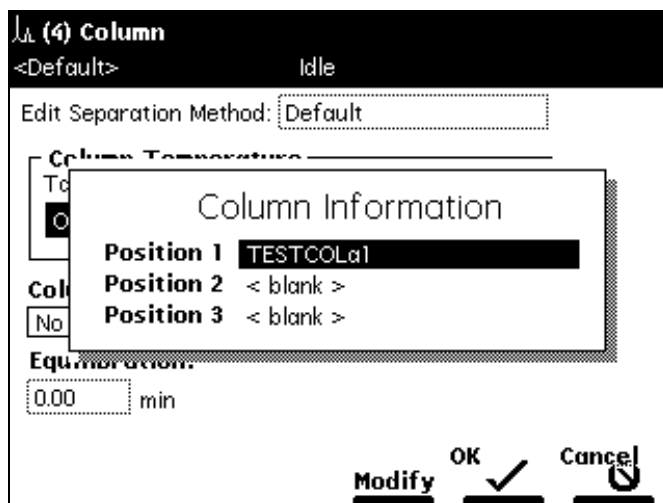

Figure 5-16   Column Information Dialog Box (Example)

4.   Press the **OK** screen key to store the changes and return to the Column screen.

5.2.5 Setting Up Two-Column Regeneration

The automated column regeneration valve operates under 2795 Separations Module stand-alone control (version 1.01 and higher, no interaction mode), Millennium[32] software control (version 3.2 and higher), or MassLynx software control (version 3.4 and higher). MassLynx software cannot control the operation of a regeneration pump within the 2795-based separation method.

In stand-alone mode, the 2795 Separations Module controls the 2-column regeneration valve through inputs on the Column page of the separation method, as follows:

- **1** – Places column 1 in the flow path
- **2** – Places column 2 in the flow path
- **Automatic** – Changes from one position to the other
- **No change**

Millennium[32] software controls the 2-column regeneration valve according to the following selections:

- **1** – Places column 1 in the flow path
- **2** – Places column 2 in the flow path

5

- **Toggle** – Changes from one position to the other

These positions are not documented in Millennium[32] software. Create a custom field if documentation of the valve position and column is needed.

MassLynx software controls the 2-column regeneration valve through the following timed events and choice of initial column position with each method:

- **1** – Places column 1 in the flow path
- **2** – Place column 2 in the flow path
- **Autoregeneration** – Changes from one position to the other.

5.2.6 Setting I/O Parameter Values

Set the I/O parameter values when you want the 2795 Separations Module to notify other instruments of the status of the 2795 Separations Module. For example, you may want the 2795 Separations Module to:

- Notify a detector that an injection has begun
- Turn on a heating plate or a stirring bar in a solvent reservoir
- Notify a third-party data system or integrator that an event has occurred
- Sound an audible alarm

You can also select the analog signal for the system parameter transmitted through the Chart Out terminals.

To enter parameter values in the I/O screen:

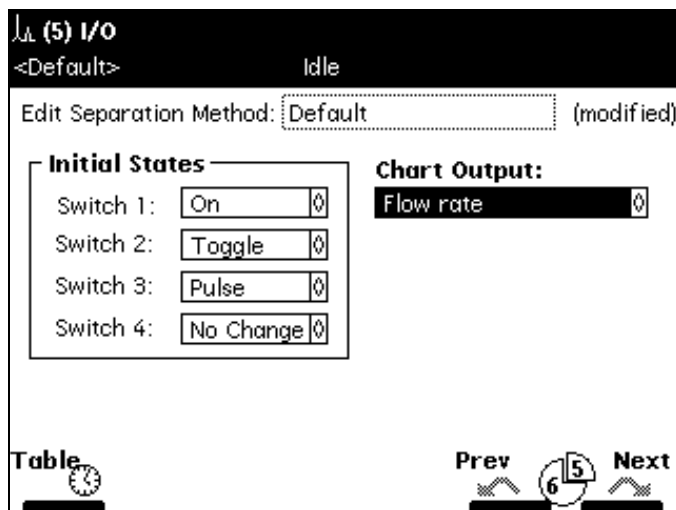1. Press the **Next** or **Prev** screen key to display the I/O screen (Figure 5-17).

Figure 5-17 I/O Screen

2. Enter or select parameter values in the I/O screen (Table 5-10).

Table 5-10 I/O Parameters

Parameter	Function	Value/Range
Initial States	Defines the initial condition for each of the four event switches. At the beginning of each injection cycle, each switch returns to the state defined in this parameter.	On Off Toggle[a] Pulse[b] No Change
Chart Output	Defines the signal sent out on the Chart Out terminals (on the 2795 Separations Module rear panel).	Flow rate System pressure %A, %B, %C, %D Column temperature Sample temperature Degasser vacuum Primary head pressure
Table (screen key)	Displays the I/O Events table.	See the "Setting the I/O Events Table Parameter Values" discussion below

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

Setting the I/O Events Table Parameter Values

The I/O Events table (Figure 5-18) allows you to set the times for the following events to occur during a run:

- Changing the state of event switches

- Setting the temperature of the column or sample compartment

- Selecting column position

- Displaying alerts

Note: You can specify up to 25 events in the I/O Events table.

To specify events in the I/O Events table:

1. Press the **Table** screen key in the I/O screen. The I/O Events table appears (Figure 5-18).

5

Figure 5-18 I/O Events Table

2. Select values in the I/O Events table as appropriate. Table 5-11 describes the parameters in the I/O Events table. Table 5-12 lists the parameters you can use in the Action field in the I/O Events table according to the specified event type.

3. View or edit the I/O Events table using the screen keys. Table 5-13 explains the functions of the screen keys.

4. When you finish specifying parameter values and/or editing the I/O Events table, press **Exit** to return to the I/O screen.

Table 5-11 I/O Events Table Parameters

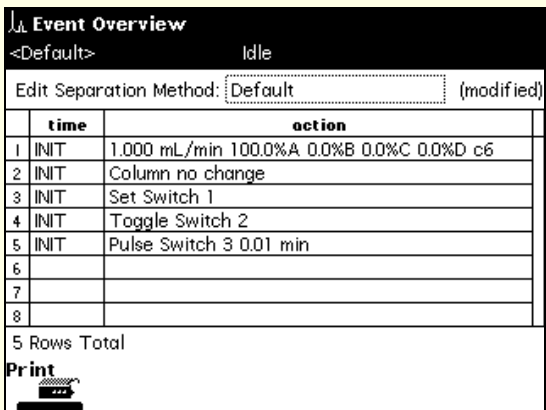| Parameter	Function	Value/Range
Time	Specifies the time after the start of a run at which the event is to occur. Press the **Clear** key to select INIT. Conditions in the INIT line occur when a separation method is applied to the system, while events at time 0.00 occur immediately upon an injection.	INIT, 0.00 to 999.99 in 0.01-min increments
Event type	Specifies the type of event to occur.	Switches 1 through 4 Set temperature Set column Alert See Table 5-12.
Action	Specifies the action to be performed with the specified event.	See Table 5-12.
Parameter	Specifies the value for the action.	See Table 5-12.

Table 5-12 Action Parameters for I/O Events

Event Type	Action	Value Range (Param Column)
Switches 1, 2, 3, or 4 (Contact closures, see Section 2.5.2, Making Analog Signal Connections)	On Off Toggle[a] Pulse[b] No Change	0.01 to 10.00 in 0.01-min increments (Pulse only)
Set Temperature	Sample Column	4 to 40 °C in 1 °C increments 20 (minimum of ambient + 5 °C) to 60 °C in 1 °C increments
Set Column	Position 1–6 No change	N/A
Alert	No action	N/A

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

Table 5-13 Gradient, Detector, and I/O Events Table Screen Keys

Screen Key	Function
Overview	Displays a time-ordered summary of the events in the Gradient, Detector, and I/O Events tables. See the example below.
Insert Row	Inserts a row above the selected row.
Delete Row	Deletes the selected row.
Sort by Time	Sorts the rows based on time. *Note: The 2795 Separations Module software automatically sorts entries by time when you exit from the Gradient, Detector, or I/O Events tables.*
Copy Down	Copies the contents of the current table cell into all subsequent cells in the same column.
Reset Table	Clears the table of all current entries.
Print	Prints the I/O Events, Gradient, or Detector Events table to a floppy disk, or to a serial printer, if installed.

5.2.7 Setting Detector Parameters

With the 2795 Separations Module configured as the System Controller, you can control:

- One Waters 410 or 2410 Differential Refractometer and up to two UV/Vis detector channels (Waters 2487 or Waters 486 detector) using the IEEE-488 interface (see Section 2.5.3, Making Digital Signal Connections).

- One Waters or a third-party detector using I/O connections (see Section 2.5.2, Making Analog Signal Connections).

Note: The 2795 Separations Module software recognizes only detectors connected using the IEEE-488 interface.

To enter parameters in the Detectors screen:

1. Press the **Next** or **Prev** screen key to display the Detectors screen (Figure 5-19).
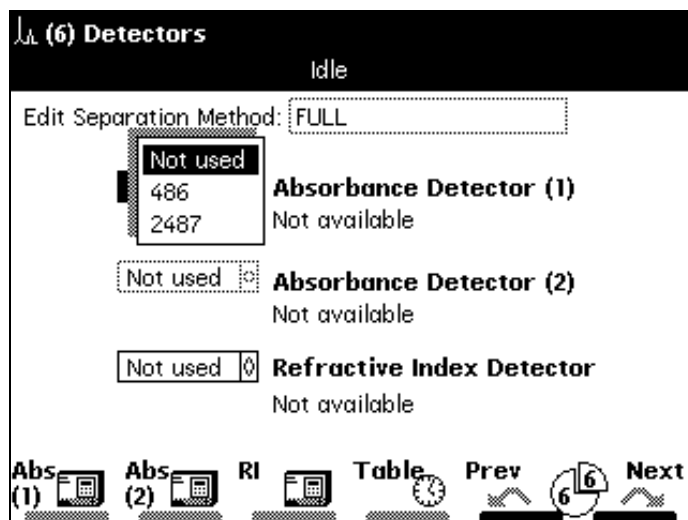

Figure 5-19 Detectors Screen

2. Select the appropriate detector (for example, Absorbance Detector 1), then press **Enter** to display the detector selections drop-down list. Table 5-14 describes the available detector selections.

Table 5-14 Detector Selections

Parameter	Function	Selections[a]
Absorbance Detector (1)	Enables a Waters 486/2487 UV/Vis Detector channel	2487, 486, or Not used
Absorbance Detector (2)	Enables a second Waters 486/2487 UV/Vis detector channel	2487, 486, or Not used
Refractive Index Detector	Enables a Waters 410 or 2410 Differential Refractometer	410/2410 or Not used
Absorbance (1) (screen key)	Displays the 486 or 2487 screen (Figure 5-20)	See Table 5-16
Absorbance (2) (screen key)	Displays the second 2487 channel or second 486 screen	See Table 5-16
RI (screen key)	Displays the 410/2410 RI screen	See Table 6-13
Table (screen key)	Displays the Detector table	See Table 5-17

a. See Table 2-2 for minimum detector firmware requirements.

3. Set the detector parameter values according to your selected detector(s) as described below in the "Setting Absorbance Detector Parameter Values"and "Setting RI Detector Parameter Values" discussions.

Setting Absorbance Detector Parameter Values

To enter control parameters for a Waters 2487 or 486 UV/Vis detector:

1. In the Detectors screen, press the **Abs (1)** or **Abs (2)** screen key to display the appropriate absorbance detector screen. Figure 5-20 shows the screen for a Waters 2487 Dual λ Absorbance Detector.

    Note: You cannot select Absorbance Detector (2) unless you have already selected an Absorbance Detector (1).

2. Enter detector parameter values as required. Table 5-15 describes the parameters for the Waters 486 and the 2487 detectors.

3. Press **OK** to accept the parameter values (or **Cancel** to exit the Detectors screen without accepting the values).

    To program detector events, refer to the "Setting the Detector Events Table Parameter Values" discussion below.
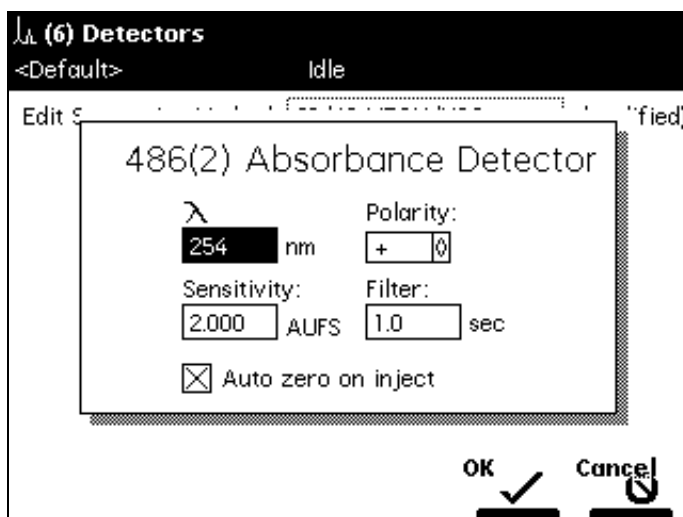
5

Figure 5-20 486 (2) Absorbance Detector Screen

Table 5-15 Waters 486 and 2487 UV/Vis Parameters

Parameter	Function	Value/Range
λ (wavelength)	Specifies the wavelength of the detector.	2487 – 190 to 700 nm in 1-nm increments 486 – 190 to 600 nm in 1-nm increments
Sensitivity	Specifies the sensitivity of the detector.	2487 – 0.0001 to 4.0000 in 0.0001-AU increments 486 – 0.001 to 2.000 in 0.001-AU increments
Filter	Specifies the time constant of the filter in the detector.	2487 – 0.1 to 99.0 in 0.1-sec increments 486 – 0.1 to 5.0 in 0.1-sec increments
Polarity	Specifies the output of the 2487 to create positive or negative peaks.	+, –
Auto zero on inject	Resets the output of the 2487 to 0 volts when the injection begins.	Selected or deselected

Setting RI Detector Parameter Values

To enter control parameters for a Waters 410 or 2410 RI detector:

1. In the Detectors screen, press the **RI** screen key to display the 410/2410 detector screen (Figure 5-21).

Figure 5-21 Waters 410/2410 RI Detector Screen

2. Enter detector parameter values as required. Table 5-16 describes the parameters for the Waters 2410 and 410 RI detectors.

3. Press **OK** to accept the parameter values (or **Cancel** to exit the Detectors screen without accepting the values).

Table 5-16 Waters 410 and 2410 RI Parameters

Parameter	Function	Value/Range
Sensitivity	Specifies the initial sensitivity of the detector	1, 2, 4, …, 512, 1024
Polarity	Specifies the output of the detector to create positive or negative peaks	+, −
Filter	Specifies the time constant of the filter in the detector	0.2, 1, 3, and 10 sec
Temperature: Cell	Temperature of the flow cell in °C	30 to 50 °C in 1 °C increments

Table 5-16 Waters 410 and 2410 RI Parameters *(Continued)*

Parameter	Function	Value/Range
Temperature: External Temp °C 1	Specifies the temperature of external column heater 1 in °C[a]	Ambient to 150 °C in 1 °C increments
Temperature: External Temp °C 2	Specifies the temperature of external column heater 2 in °C[a]	Ambient to 150 °C in 1 °C increments
Autozero on inject	Resets the output of the detector to 0 volts when the injection begins	Selected or deselected

a. The Waters 2410 and 410 Differential Refractometers can control the temperature of one or two external Waters Column Heater Modules (CHM). These devices are separate from the column heater installed with the 2795 Separations Module.

Setting the Detector Events Table Parameter Values

The Detector Events table allows you to program 2487 and 486 detector events to occur during a run.

To enter events in the Detector Events table:

1. Press the **Table** screen key in the Detectors screen. The Detector Events screen appears, displaying the Detector Events table. Figure 5-22 shows the Detector Events table for a Waters 2487 detector using channels 1 and 2.

Figure 5-22 Detector Events Screen

2. Enter or select parameter values in the Detector Events table as appropriate.

Table 5-17 describes the parameters in the Detector Events table. Table 5-18 lists the action parameter values you can use according to the specified action parameter.

Refer to Table 5-13 for information on the Detector Events screen keys.

Table 5-17 2487 and 486 Detector Events Parameters

Parameter	Function	Value/Range
Time	Indicates the time at which the action is to occur. Press the **Clear** key to display INIT. Conditions in the INIT line occur when a separation method is applied to the system, while events at time 0.00 occur immediately upon an injection.	INIT, 0.00 to 999.99 in 0.01-minute increments
Detector	Selects the detector on which to perform the action	2487 (1), 2487 (2) 486 (1), 486 (2)
Action	Selects the action to perform on the selected detector	See Table 5-18
Parameter	Defines the action, where appropriate	See Table 5-18

3. Press **Exit** to return to the Detectors screen.

Table 5-18 describes the actions that you can specify with the Waters 2487 and 486 Tunable Absorbance Detectors.

Table 5-18 2487 and 486 Detector Actions

Action	Function	Value Range
Set wavelength	Specifies the wavelength of the detector	2487: 190 to 700 nm in 1-nm increments. 486: 190 to 600 nm in 1-nm increments.
Set filter	Specifies the time constant of the filter in the detector	2487: 0.1 to 99.0 sec in 0.1-sec increments. 486: 0.1 to 5.0 sec in 0.1-sec increments.
Set AU full scale	Specifies the absorbance units full scale to adjust the sensitivity of the detector	2487: 0.0001 to 4.0000 AU in 0.0001-AU increments. 486: 0.001 to 2.000 AU in 0.001-AU increments.
Set chart mark	Specifies that a chart mark signal be sent to the output terminals	(No values)

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Table 5-18 2487 and 486 Detector Actions *(Continued)*

Action	Function	Value Range
Set chart polarity	Specifies the polarity of the analog output	+, −
Auto zero	Specifies that an autozero be performed on the detector	(No values)
Set lamp	Specifies that the lamp be powered on or off	On, Off

5.3 Creating and Editing Sample Sets

A *sample set* is a set of instructions (displayed in table form) that the 2795 Separations Module uses to perform an automated run in stand-alone mode. During a run, the 2795 Separations Module performs in sequence the function specified in each row of the sample set table. You create and edit sample sets using the Methods screen (Figure 5-1).

Note: The sample set method icon 🗔 *appears to the left of a sample set in the Methods screen.*

This section provides information on:

- Section 5.3.1, Creating Sample Sets
- Section 5.3.2, Editing Sample Sets
- Section 5.3.3, Copying and Editing Sample Sets
- Section 5.3.4, Locking/Unlocking Sample Sets
- Section 5.3.5, Renaming, Viewing, and Deleting Sample Sets
- Section 5.3.6, Viewing Sample Sets
- Section 5.3.7, Setting Sample Set Parameter Values
- Section 5.3.8, Setting Sample Set Functions
- Section 5.3.9, Linking Rows in a Sample Set

*Note: When you press the **Run** screen key in the Methods screen, the Sample Set screen appears. For more information on running sample sets, see Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation.*
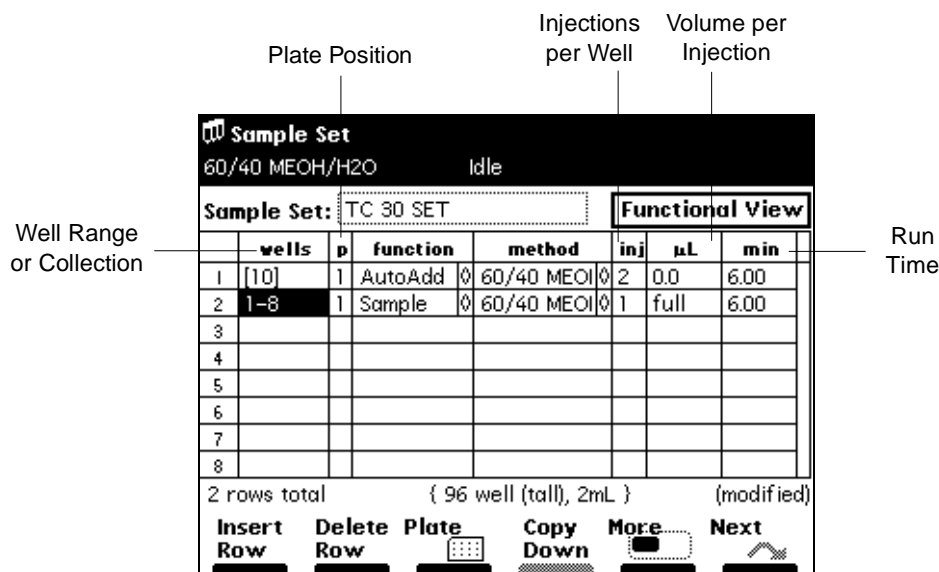
For information on importing and exporting sample sets, see Section 5.4, Importing and Exporting Methods.

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5.3.1 Creating Sample Sets

To create a sample set:

1. Press **Develop Methods** in the Main screen. The Methods screen (Figure 5-1) appears.

2. Press the **New** screen key. The Create New Method dialog box appears (Figure 5-2).

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 alphanumeric characters (see Section 3.1.2, Using the Keypad).

5. Press **Enter**. The Sample Set screen appears (Figure 5-23).

Note: When the Sample Set screen is displayed initially, it appears in the Functional View (see Section 5.3.6, Viewing Sample Sets).

Figure 5-23 Sample Set Screen (Functional View)

6. Enter parameter values in the sample set table as appropriate (see 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 **Yes** to save the sample set. To exit without saving the sample set, press **No**. To return to the sample set without entering any changes, press **Cancel**.

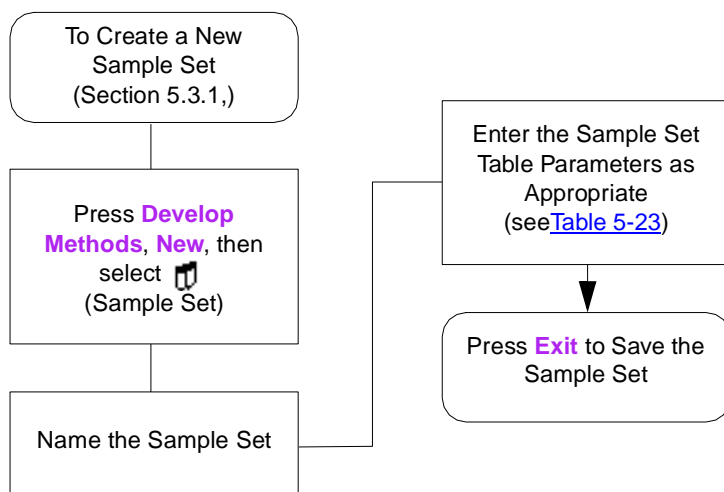Figure 5-24 summarizes the steps to create a sample set using the 2795 Separations Module front panel.

Figure 5-24 Steps in Creating a Sample Set

5.3.2 Editing Sample Sets

To edit an existing sample set:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears (Figure 5-1).

2. Select the sample set you want to edit, then press the **Edit** screen key. The Sample Set screen (Figure 5-23) appears. If the sample set is locked, the Edit screen key is grayed out. To unlock the sample set, see Section 5.3.4, Locking/Unlocking Sample Sets.

3. Enter sample set table parameter values in the Sample Set screen (see Section 5.3.7, Setting Sample Set Parameter Values, and Section 5.3.8, Setting Sample Set Functions).

4. To save the sample set, press **Exit**, then press **Yes**. To exit without saving the sample set, press **No**. To return to the sample set without entering any changes, press **Cancel**.

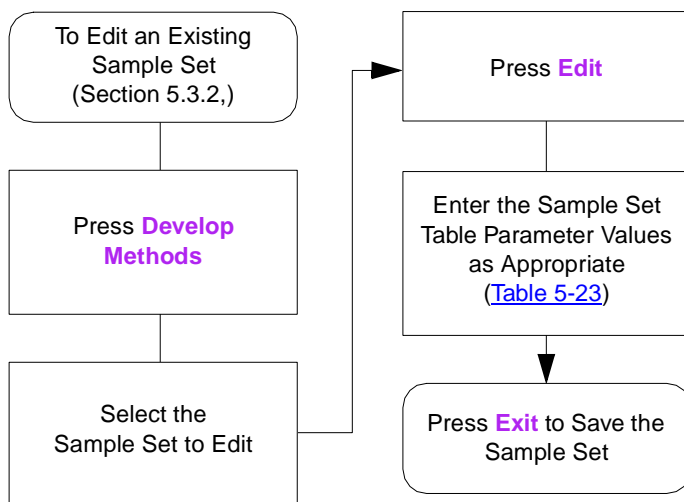Figure 5-25 summarizes the steps to edit a sample set using the 2795 Separations Module front panel.

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Figure 5-25 Steps in Editing a Sample Set

5.3.3 Copying and Editing Sample Sets

To copy and then edit an existing sample set, leaving the original sample set unchanged:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears (Figure 5-1).

2. Select the sample set you want to copy.

3. Press the **Copy** screen key. To avoid duplicate file names, the 2795 Separations Module software automatically adds an extension to the existing file name.

4. Press **Enter** to accept the default method name, or use the alphabetic screen keys to enter a new name, then press **Enter** (see Section 3.1.2, Using the Keypad).

5. Enter new parameter values in the sample set table (see Section 5.3.7, Setting Sample Set Parameter Values and Section 5.3.8, Setting Sample Set Functions).

6. To save the sample set, press **Exit**, then press **Yes**. To exit without saving the sample set, press **No**. To return to the sample set without entering any changes, press **Cancel**.

5.3.4 Locking/Unlocking Sample Sets

Locking a Sample Set

Locking a sample set prevents unauthorized changes to a sample set. To lock a sample set:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears.

2. Select the sample set you want to lock, then press the **Lock** screen key.

3. Enter a password of up to 30 alphanumeric characters (Section 3.1.2, Using the Keypad), then press **Enter**. The sample set is locked and cannot be changed, deleted, or renamed until it is unlocked (see the "Unlocking a Sample Set" discussion below).

   Note: When you copy a locked sample set and save it with a new name, the copy is not locked.

4. Record the password and keep it in a safe place.

Unlocking a Sample Set

To unlock a sample set:

1. Press the **Develop Methods** screen key in the Main screen. The Methods screen appears (Figure 5-1).

2. Select the sample set you want to unlock, then press **Unlock**.

3. Enter the password, then press **Enter**. The method is unlocked.

5.3.5 Renaming, Viewing, and Deleting Sample Sets

Renaming Sample Sets

To rename a sample set:

1. In the Methods screen, select a sample set to rename.

2. Press the **More** screen key until the Rename screen key is displayed, then press the **Rename** screen key. The Change to Method Name dialog box (Figure 5-26) appears.

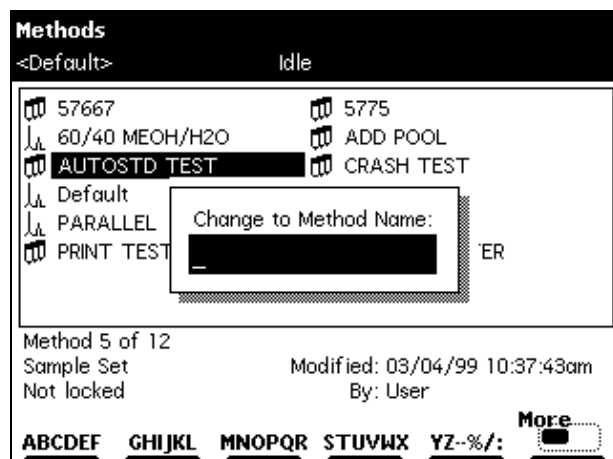Figure 5-26 Change to Method Name Dialog Box

3. Enter the new sample set name (see).

4. Press **Enter** to rename the sample set.

Viewing Sample Sets Using the View Screen Key

To view a sample set using the View screen key:

1. In the Methods screen, select a sample set to view.

2. Press the **More** screen key until the View screen key is displayed, then press the **View** screen key. The Sample Set screen () appears.

   For information on displaying the Functional, Injection, Loading, and Plate Loading views of sample sets, see .

Deleting Sample Sets

Note: You cannot delete a locked sample set.

You can delete a sample set with the:

• Delete screen key

• Delete Methods screen key

Using the Delete Screen Key

To delete a sample set using the Delete screen key:

1. In the Methods screen, select a sample set to delete.

2. Press the **Delete** screen key. The Deleting Method dialog box appears.

3. Press **Yes** to delete the selected sample set. Press **No** to return to the Methods screen without deleting the selected sample set.

Using the Delete Methods Screen Key

To delete a sample set using the Delete Methods screen key:

1. Press the **More** screen key until the Delete Methods screen key is displayed.

2. Press the **Delete Methods** screen key. The Delete Methods screen appears.

3. Press the **Select Method** screen key to select the sample set you want to delete, then press the **Delete Now** screen key.

   If you want to delete all methods except the previously selected one(s), press the **Invert Choices** screen key, then press the **Delete Now** screen key. To clear all selected methods on the Delete Methods screen, press the **Clear Choices** screen key.

5.3.6 Viewing Sample Sets

After you create or edit a sample set, you can display the sample set in any one of four views:

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- **Functional View** – Displays the sample set with one function per line (Figure 5-23). This view gives you a complete overview of the entire run, including well ranges and any linked rows (see Table 5-19 and Table 5-23).

  Note: The Functional View is the only view in which you can make changes to the sample set.

- **Injection View** – Displays the sample set with one injection per line (Figure 5-27). This list corresponds directly to the chromatograms produced by the run (see Table 5-20).

- **Loading View** – Displays the sample set with one well per line (Figure 5-28). This view shows you how to load samples in the plates (see Table 5-21).

- **Plate Loading View** – Displays the plate graphically within the Loading View. Use the Plates screen key (Figure 5-29) to display the Plate Loading view (see Table 5-22).

Table 5-19 describes the functions of the screen keys in the Sample Set screen Functional View. The Functional View is the only view in which you can make changes to the sample set.

Table 5-19 Sample Set Screen Keys (Functional View)

Screen Key	Function
Insert Row	Inserts a row between the selected row and the row above
Delete Row	Deletes the selected row
Plates	Graphically displays the Well Selector and selected wells
Copy Down	Copies the value in the currently selected cell to the corresponding cell in all subsequent rows
Make Link/Break Link	Attaches/detaches the currently selected row to/from its predecessor. Linked rows provide a tool for repeating a series of functions using the well range or collection as a counter. See Section 5.3.9, Linking Rows in a Sample Set
Reset Table	Clears all entries in the sample set table
Row Details	Displays and allows entry of additional parameters for the selected function
Print	Prints the contents of the current screen to a floppy disk or to a serial printer, if connected
Next/Previous	Opens the next view or returns to the previous view

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Numerical Well Numbers

Figure 5-27 Sample Set Screen (Injection View)

Table 5-20 describes the functions of the screen keys displayed in the Injection View.

Table 5-20 Sample Set Screen Keys (Injection View)

Screen Key	Function
Print	Prints the contents of the current screen to a floppy disk or to a serial printer, if connected
Next/Previous	Opens the next view or returns to the previous view
Well Numbers/ Well Alpha	In the Injection and Loading views, switches between displaying the wells numerically or alphanumerically
Scroll	Prompts for a row number to be shown at the top of the table. Displays eight rows at a time. For example, enter 55 to display rows 55 to 62.
Injects Only	Displays only the lines of the Injection View that contain an injection

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Alphanumeric
Well Numbers

Figure 5-28 Sample Set Screen (Loading View)

Table 5-21 describes the functions of the screen keys displayed in the Loading View.

Table 5-21 Sample Set Screen Keys (Loading View)

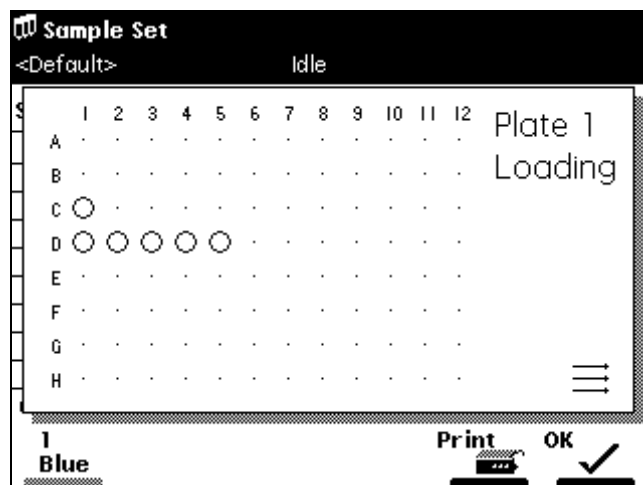| Screen Key	Function
Plates	Graphically displays the Well Selector and selected wells
Print	Prints the contents of the current screen to a floppy disk or to a serial printer, if connected
Next/Previous	Opens the next view or returns to the previous view
Well Numbers/ Well Alpha	In the Injection and Loading views, switches between displaying the wells numerically or alphanumerically
Scroll	Prompts for a row number to be shown at the top of the table. Displays eight rows at a time. For example, enter 55 to display rows 55 to 62

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Figure 5-29 Plate Loading View

Table 5-22 describes the functions of the screen keys displayed in the Plate Loading View.

Table 5-22 Sample Set Screen Keys (Plate Loading View)

Screen Key	Function
Print	Prints the contents of the current screen to a floppy disk or to a serial printer, if connected
1 Blue 2 Yellow 3 Red 4 Green	In the Plate Loading view, one button is displayed for each plate used in the sample set. The button corresponding to the current plate is grayed out (see Section 3.4.3, Loading the Sample Plates)

5.3.7 Setting Sample Set Parameter Values

Table 5-23 provides parameters, functions, and values you use to set up the sample set table in the Functional View.

Table 5-23 Sample Set Parameters (Functional View Only)

Parameter	Function	Value Range
Wells	Specifies the well numbers from which the injection is to be made. Use the "." key to specify a well number range. For example, press 2.5 to enter wells 2 through 5. Use the Plates screen key to display the plate graphically. In the Well Selector, you can select a collection of wells that are noncontiguous. For more information, see Section 4.2.9, Selecting Wells for Making Injections.	1 to 384 (depending on plate type). Use the Plates screen key to specify plate type.
p (plate position)	Specifies the plate position in the carrier (see Section 3.4.3, Loading the Sample Plates)	1 to 4
Function	Specifies the action/process to perform for the row in the Sample Set table (see Section 5.3.8, Setting Sample Set Functions)	See Table 5-25
Method	Specifies the separation method to use with the specified function in the sample set (see Section 5.1, Creating and Editing Separation Methods, for the procedures to create, edit, and store separation methods)	Stored methods
Inj (injections per well)	Specifies the number of injections to perform from each well in the range or collection	1 to 99
µL (volume per injection)	Specifies the amount of sample or standard (in microliters) to inject from each well in the range or collection. Press **Clear** to specify "Full." *Note: This field is not editable if you specify Full Loop injection in the separation method (see Section 5.2.3,).*	Full or 0.1 to 1500.0 in 0.1-µL increments[a]
Min (minutes)	Time in minutes applied to the function (see Table 5-25)	0.1 to 999.99 in 0.01-min increments

a. This parameter is constrained by the sizes of the sample and holding loops and the syringe (see Table 7-5). To enter amounts greater than 50 µL, install a larger sample loop.

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The maximum number of rows in a sample set table is 384 (displayed in the Functional View). You can display eight rows of a table at a time.

Injection Volume

When you are specifying parameters in the sample set table or as a direct function (see Section 4.2.8, Injecting Samples), the injection volume (μL parameter in Table 5-23) depends on the size of the sample loop or 60% of syringe volume, whichever is smaller, as shown in Table 5-24:

Table 5-24 Injection Volume Constraints

Syringe Size (μL)[a]	Injection Volume Range (μL)[b]
100	0 to 60.0
250	0 to 150.0
500	0 to 300.0
1000	0 to 600.0
2500	0 to 1500.0

a. Syringe sizes as available on Configuration screen. You can enter any loop size from 5 to 2000 μL.
b. Or the sample loop size, whichever is smaller. The maximum injection volume shown in Table 5-24 is the sample loop size above which a full-loop injection cannot be performed.

5.3.8 Setting Sample Set Functions

Entries in the "function" column of the sample set table specify the functions you apply to a sample set. Table 5-25 describes the functions available for the 2795 Separations Module, and indicates whether the other columns in the sample set table are editable (Y, N) for each function.

Note: An asterisk in a cell of the sample set table indicates that the cell is not applicable to the selected function and method.

Use these functions in the same way you use the corresponding direct functions described in Section 4.2, Performing Direct Functions.

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Press **Row Details** to enter additional parameters for the functions described in Table 5-25.

Table 5-25 Sample Set Functions

Function Name	Description	Wells	p (plate)	Inj	μL	min
Sample	Injects sample from the selected well(s)	Y	Y	Y	Y[a]	Y
Standard	Injects standard from the selected well(s)	Y	Y	Y	Y[a]	Y
AutoStds	Programs the repetition of standards over the course of a run (see the "Specifying the Auto Standards Function" discussion below)	Y	Y	Y	Y[a]	Y
Prime	Wet primes the solvent management system at the specified rate for the specified period (see Section 4.2.3, Wet Priming)	N	N	N	N	Enter a duration for the Prime function.
Refresh Syringe	Refreshes the solvent in the syringe (see Section 4.2.4, Refreshing the Syringe)	N	N	N	N	N
Wash Needle	Washes the needle and purges the sample management system (see Section 4.2.5, Washing the Needle)	N	N	N	N	N
Equil (Equilibrate)	Delivers solvent through the system for the specified period of time using the initial conditions in the specified separation method (see the "Specifying the Equilibrate Function" discussion below)	N	N	N	N	Enter system equilibration time.
Condition	Delivers to the column the gradient specified in the separation method; conditions the column without injecting samples (see Section 4.2.7, Conditioning the Column)	N	N	N	N	Enter column conditioning time.[b]

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Table 5-25 Sample Set Functions *(Continued)*

Function Name	Description	Wells	p (plate)	Inj	µL	min
Auto Add	Collects sample from a single well and non-sample material from other wells into the sample loop for injection in a specified order (see the "Specifying the Auto Add Function" discussion below)	Y	Y	Y	Y[c]	Y
Pool	Collects multiple samples into the sample loop for injection all at once (see the "Specifying the Pool Function" discussion below)	N	N	Y	N[d]	Y

a. Not editable (N) if the separation method is for Full Loop injection only.

b. Specify sufficient time to deliver the specified gradient to the column.

c. Never "Full."

d. Injection volume is not editable in the sample set table for the Pool function. Volume injected is the total of all volumes entered individually in the Pool Row Details and sample gaps, if any.

Specifying the Auto Standards Function

Use the AutoStds function in the sample set table to run standards repetitively (at specified intervals) throughout the run. You can use up to five standard wells for large runs.

To use the Auto Standards function:

1. In the Sample Set screen, select **AutoStds** from the drop-down list of functions, then press the **Row Details** screen key to display the Auto Standards dialog box (Figure 5-30).

2. In the Run standards every: fields, enter the frequency with which you want to run standards (after how many wells or injections), then select either **wells** or **injects** from the drop-down list.

3. You can select standards wells by either of the following methods:

   • In the Source Well(s) table, enter the location of each standards well in the well column and its "practical capacity" (i.e., the amount that can be removed from the standards well based on the well depth) in the total µL column.

   • Press the **Plate** screen key to graphically select a standards well.

   • Press **OK** after you have completed your selection (or press **Cancel** to exit without making a selection). For more information, see Section 4.2.9, Selecting Wells for Making Injections.
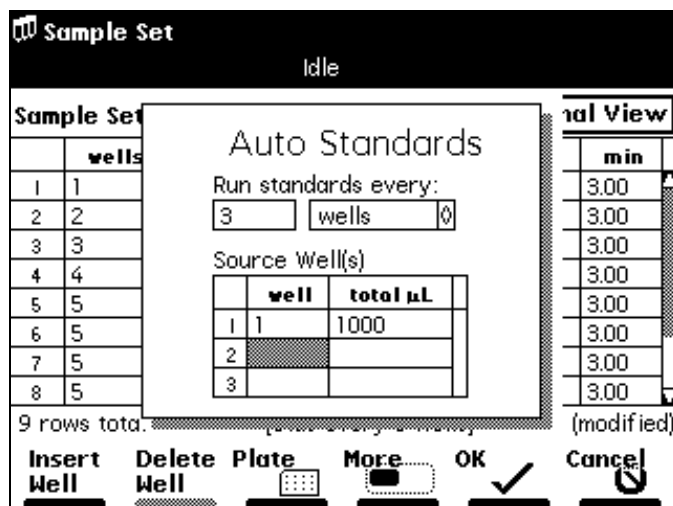
5

Figure 5-30 Auto Standards Dialog Box

When you start the run, the 2795 Separations Module runs the standard. After the specified number of wells or injects has been processed, the standard is run again. This repeats throughout the run to the end.

When the current standards well is exhausted (based on the number of injections, the size of the injections, and the total volume), the 2795 Separations Module automatically advances to the next standards well specified in the Source Well(s) table.

Table 5-26 describes the functions of the screen keys displayed with the Auto Standards dialog box.

Table 5-26 Auto Standards Dialog Box Screen Keys

Screen Key	Function
Insert Well	Allows you to insert a well between the selected well and the well above in the Auto Standards table
Delete Well	Deletes the selected well
Plate	Allows you to graphically display the Well Selector and select individual wells
Clear Table	Clears the Auto Standards table
Injects	Switches the "practical capacity" column (see Step 3 above) of the Source Well(s) table from "total µL" to "injects" to specify the capacity of the standards well in number of injections

Specifying the Equilibrate Function

Use the Equil (Equilibrate) function in the sample set table to deliver solvent through the system for a specified period of time using the initial conditions specified in the separation method.

To use the Equilibrate function:

1. In the Sample Set screen, select **Equil** from the drop-down list of functions, then press the **Row Details** screen key to display the Equilibrate dialog box (Figure 5-31).

Figure 5-31 Equilibrate Dialog Box

2. In the Column: field (if a column selection valve is installed), select the appropriate column selection option from the drop-down list, then press **Enter**.

   Note: You cannot access the Column: field if there is no column selection valve installed.

3. In the Time: field, enter the time in minutes to equilibrate the column.

4. For the typical unattended operation, leave the **Hold when time expires** check box unchecked. If you enable the check box and set a time, the 2795 Separations Module maintains (holds) initial conditions until the set time expires and requires a response to one of two screen keys (Resume Sample or Abort Samples) in the Status screen. Pressing one of these screen keys performs the desired action. Elapsed time continues until you press one of these keys.

5. For more information, see Section 4.2.6, Equilibrating the System.

5

Specifying the Auto Add Function

Use the Auto Add function to make an injection that is composed a single sample aliquot along with up to 16 aliquots from additional wells that may contain diluents, reagents, catalysts, or other non-sample material. You can specify a delay time to allow the aliquots in the sample loop to mix before an injection.

Note: Waters recommends that the total volume of an Auto Add injection be less than one-half the volume of the sample loop.

To use the Auto Add function:

1. In the Sample Set screen, select **AutoAdd** from the drop-down list of functions, then press the **Row Details** screen key to display the Auto Add dialog box (Figure 5-32).

   Note: Only one line of the Auto Add table can be a SAMPLE line. The SAMPLE line is a placeholder for the actual well location used according to the range or collection entered in the wells column of the sample set functional view.

2. Enter the volume (in µL) you want to draw from the SAMPLE well.

3. Repeat steps 1 and 2 for additional aliquots. You can insert aliquots before the SAMPLE line.

4. Enter the delay time (in minutes) to allow the combined aliquots to commingle in the sample loop.

5. Press **OK** to return to the Sample Set screen.

Figure 5-32 Auto Add Dialog Box (Example)

Table 5-27 describes the functions of the screen keys displayed with the Auto Add function dialog box.

Table 5-27 Auto Add Dialog Box Screen Keys

Screen Key	Function
Insert Well	Allows you to insert a well between the selected well and the well above in the Auto Add table
Delete Well	Deletes the selected well
Plate	Allows you to graphically display the Well Selector and select individual wells
Clear Table	Clears all entries in the Auto Add table

Specifying the Pool Function

Use the Pool function to make a single injection that is composed of multiple samples from up to 16 wells in series.

Note: The pooling function is available on the 2795 Separations Module when in the No Interaction mode. Pooling is not supported for Millennium or MassLynx control.

Note: Waters recommends that the total volume of a Pool injection be less than one-half the volume of the sample loop.

To use the Pool function:

1. In the sample set table, select or insert a row in the sample set to specify sample pooling.

2. In the function field of the selected row, select **Pool** from the drop-down list.

3. Press **More**, then press the **Row Details** screen key to display the Pool Samples dialog box (Figure 5-33).

5
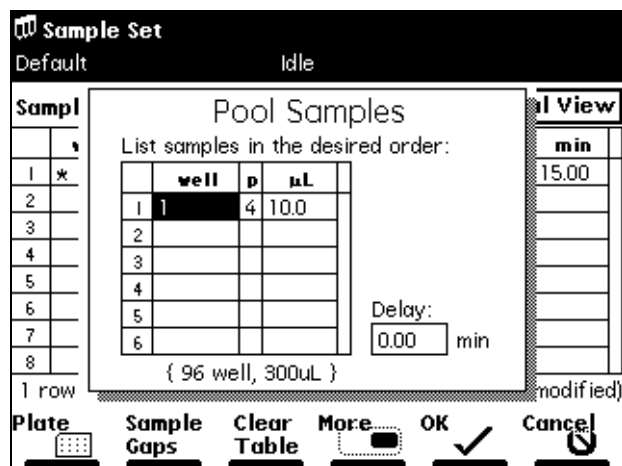
Figure 5-33 Pool Samples Dialog Box

4. In the Pool Samples dialog box, enter the well number, plate number(s), and
    volume (in µL) to draw from each sample well for pooling.

    You can use the Plate screen key to graphically select a collection of sample pool
    wells using the Well Selector (see Section 4.2.9, Selecting Wells for Making
    Injections).

5. In the Delay: field, enter the time (in minutes) to delay the injection to allow the
    pooled sample to commingle in the sample loop.

6. To specify air or liquid gaps between sample aliquots in the pool, press the **Sample
    Gaps** screen key to display the Pool Sample Gaps dialog box (Figure 5-34).

    The Pool Sample Gaps function allows you to insert air or liquid gaps:

    • Before the first sample

    • Between each successive sample

    • After the last sample

    • To enter a liquid or air gap, specify for each gap location:

    • The well number in the well field

    • The plate number in the p field

    • The volume in the uL field

    • For an air gap, enter the well number of an empty well.

5
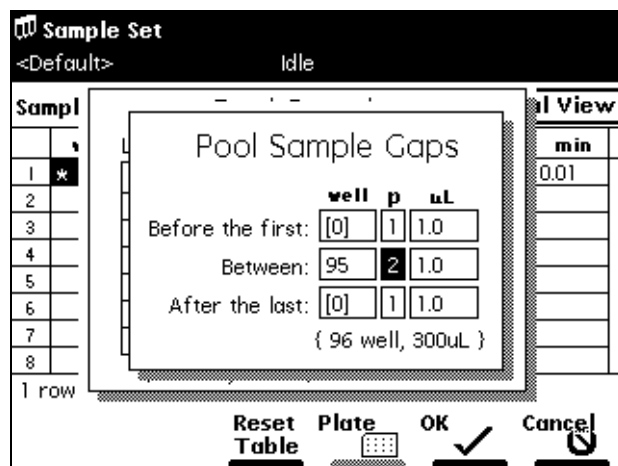
Figure 5-34 Pool Sample Gaps Dialog Box

Note: *You need to enter a volume for the Pool Sample Gaps function to operate correctly.*

You can specify that each sample gap be selected from a different well or plate. All are defaulted to well collection "[0]" meaning that no sample gaps are specified. To change a well field with an entry in it to "[0]," press **Clear**.

7. When you complete the Pool Sample Gaps dialog box, press **OK** (or press **Cancel** to exit from the dialog box without entering any information).

8. When you complete the Pool Samples table, press **OK** (or press **Cancel** to exit from the Pool Samples dialog box without entering any information in the table).

5.3.9 Linking Rows in a Sample Set

You can link two or more rows in a sample set to perform multiple functions for the same set of wells. For example, you can link a Sample function with the Equilibrate and Refresh Syringe functions, as shown in Rows 2, 3, and 4 in Figure 5-35. In this example, one 10-µL injection is made after equilibrating for 2.0 minutes, followed by a Refresh Syringe. The process is repeated for all 16 wells in the collection.

STOP *Attention: When unlinking or deleting linked rows in a sample set, be aware that well numbers in the remaining rows may differ from those originally entered. Make sure that well numbers for all rows are correct before running the sample set.*

5

Linked Rows in Sample Set

Figure 5-35 Linked Rows in a Sample Set

Linking Rows

To link rows in a sample set:

1. In the Sample Set screen, select the row you want to link with the previous row.

2. Press the **More** screen key, then press the **Make Link** screen key. The current row is linked with the previous row.

Unlinking Rows

To unlink rows in a sample set:

1. Select the row you want to unlink from the previous row.

2. Press the **More** screen key, then press the **Break Link** screen key.

5.4 Importing and Exporting Methods

You can use the 2795 Separations Module floppy disk drive to import, store, and retrieve separation methods and sample sets on 1.44-MB PC-formatted floppy disks. By saving reports and tables on 3.5-inch floppy disks, the floppy disk drive can function as an alternative to a serial printer.

You can import and export methods (separation methods and sample sets) from one 2795 Separations Module to another 2795 Separations Module that has the same configuration. You can also import sample sets that were created using a text editor or spreadsheet application on a PC.

5.4.1 Exporting Methods

To export one or more methods to a floppy disk:

1. Insert a pre-formatted 3.5-inch floppy disk into the floppy disk drive.

2. Press **Develop Methods** in the Main screen, then press **More** twice to access the Export Methods screen key.

3. Press the **Export Methods** screen key. The Export Methods screen appears, displaying a list of currently stored methods (Figure 5-36).
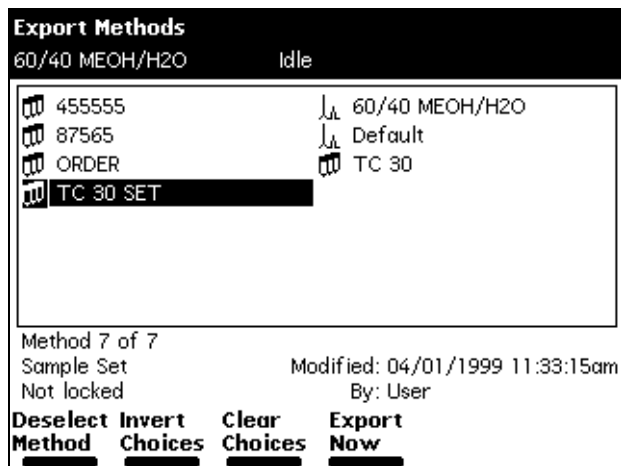
Figure 5-36 Export Methods Screen with Method Selected

4. Select the method you want to export to the floppy disk, then press the **Select Method** screen key. To select an additional method, select the desired method, then press the **Select Method** screen key again.

   To select all methods except the Default method:

   a. Select the **Default** method, then press the **Select Method** screen key.

   b. Press the **Invert Choices** screen key. All methods except the Default method are selected (or press the **Clear Choices** screen key to clear all choices and start again).

5. When you have selected the methods you want to export, press the **Export Now** screen key. The file transfer begins.

5.4.2 Importing Methods

To import one or more methods from a floppy disk:

1. Insert the floppy disk that contains the method(s) into the floppy disk drive.

2. Press the **Methods** screen key in the Main screen, then press the **More** screen key twice to display the Import Methods screen key.

3. Press the **Import Methods** screen key. After a brief standby message, the Import Methods screen displays a list of methods stored on the floppy disk (Figure 5-37). To update the list, press the **Refresh List** screen key.
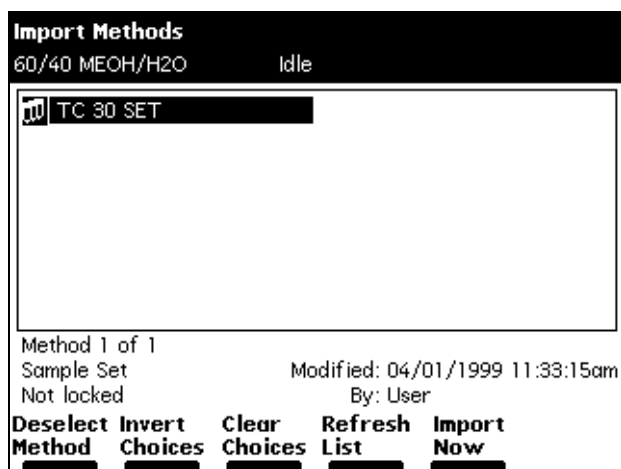
Figure 5-37 Import Methods Screen

4. Using the arrow keys, select the method you want to import into the 2795 Separations Module, then press the **Select Method** screen key. To select additional methods, select each method and press **Select Method** again. When you press the **Select Method** screen key, it changes to **Deselect Method**.

    Use the Invert Choices or Clear Choices screen keys as appropriate.

5. When you have selected all the methods you want to import, press the **Import Now** screen key. The file transfer begins.

> **STOP** **Attention:** *Do not remove the floppy disk from the floppy disk drive before the import is complete; the 2795 Separations Module cannot detect a partially imported method. Waters recommends that you carefully review any imported method before you run it.*

An error message appears if you attempt to import more than 60 methods, or if the storage capacity of the 2795 Separations Module is exceeded.

5.4.3 Importing ASCII Files

You can import ASCII text files that were created on a PC using a text editor or a spreadsheet application.

Note: Add the file extension *.ASC to the ASCII file you want to import. Ensure that the file contains no special formatting characters other than spaces, tabs, carriage returns, and line feeds.

Note: The floppy disk (ASCII sample set file) icon 🖫 *indicates a sample set in the Methods screen when you view the list of methods available for importing from a floppy disk. After importing the sample set, the 2795 Separations Module displays the method with the multiple vial (sample set)* 🛢 *icon.*

For an imported ASCII file to operate correctly, ensure that:

- The ASCII sample set file is formatted with one line of text per well.
- Each line in the file ends with a carriage return (or carriage return followed by a line feed).
- Each row contains the fields and values shown in Table 5-28.

Table 5-28 ASCII Sample Set Fields

Field	Range
Well Number	1 to 384
Plate Number	1 to 4
Function	Sample, Standard
Method	(Separation Method Name)
Number of Injects	1 to 99
Injection Volume	0.0 to 1500.0 µL
Run Time	0.00 to 999.99 minutes

Figure 5-38 provides an example of an ASCII file to be imported into a 2795 Separations Module.

```
# This is a comment

3          1          Sample     Test Method     1          12.0          4.65
10         1          Sample     SepMeth1        1          10.0          6.00
9          1          Sample     SepMeth1        1          10.0          6.00
8          1          Sample     SepMeth1        1          10.0          6.00
```

Figure 5-38 ASCII Text File for Import (Example 1)

In Figure 5-38, the separation method name ("Test Method") is enclosed in quotes, because the method name contains a space.

Ensure that ASCII files to be imported use separation methods already stored in the 2795 Separations Module. If "Test Method" or SepMeth1 in Figure 5-38 does not exist in the

5

2795 Separations Module, the separation method becomes "Default" when the file is imported.

Lines in the ASCII file that begin with the "#" character indicate the start of one of the following instructions:

- DELIMIT
- PLATE
- NAME
- USER
- PRESERVE

If the line beginning with the "#" character contains none of the above instructions, then the information is not imported (it can be used to document the file).

Defining the Delimit Character

You can use either spaces or tabs to separate columns in the ASCII text file. If a spreadsheet application generates a text file that separates fields using another character (such as the semicolon), you need to insert a "#DELIMIT" instruction at the top of the file, as shown in Figure 5-39.

When you define the delimit character (in this example, the semicolon), you do not need to enclose a multi-word separation method name in quotation marks.

# DELIMIT=						
3	1	Sample	Test Method	1	12.0	4.65
10	1	Sample	Test Method	1	10.0	6.00
9	1	Sample	SepMeth	1	10.0	6.00
8	1	Sample	SepMeth	1	10.0	6.00

Figure 5-39 ASCII Text File for Import (Example 2)

Specifying the Plate

When you set up an ASCII file for import into the 2795 Separations Module, use numbers only (not plate logic) to indicate well locations. The plate type configuration defines the number of rows, number of columns, and sequential reference mode (see Section 3.4.3, Loading the Sample Plates).

If you do not specify a plate type in the ASCII file, the 2795 Separations Module uses the default plate type configuration when it imports the file. Figure 5-40 identifies plate position 1 (used on every line of the sample set) as "96 well (shallow)," the default plate type.

You must enter the plate type in the text file exactly as it appears in the 2795 Plate Type Configuration screen (Section 3.2.2, Configuring Plate Types). Alternatively, you can specify the plate type by using the sequence of plate types as they appear in the Plate Type Configuration drop-down list (Figure 3-12). For example, entering "#PLATE1=4" indicates that the plate type in position 1 is the fourth plate type in the Plate Type Configuration screen drop-down list (Figure 3-12).

#PLATE1="96 well (shallow)"

3	1	Sample	"Test Method"	1	12.0	4.65
10	1	Sample	SepMeth1	1	10.0	6.00
9	1	Sample	SepMeth1	1	10.0	6.00
8	1	Sample	SepMeth1	1	10.0	6.00

Figure 5-40 ASCII Text File for Import (Example 3)

Specifying the File Name and User

The 2795 Separations Module assigns the same name you used on the PC to the file when it is imported. The *.ASC file name is limited to eight characters with no spaces. You can override these conventions with a custom file name that can include up to 30 characters and spaces by using the "#NAME" instruction (Figure 5-41). In addition, you can store the imported sample set with a specific user name, which the 2795 Separations Module records as the creator of the sample set (Figure 5-42).

#NAME="An imported sample set"
#USER=Anna

3	1	Sample	"Test Method"	1	12.0	4.65
10	1	Sample	SepMeth1	1	10.0	6.00
9	1	Sample	SepMeth1	1	10.0	6.00
8	1	Sample	SepMeth1	1	10.0	6.00

Figure 5-41 ASCII Text File for Import (Example 4)

When you import ASCII sample sets, the 2795 Separations Module normally merges adjacent lines, defining well ranges or collections as necessary. After import, the sample set in Figure 5-41 becomes a sample set named "An imported sample set" as shown in Figure 5-42.

5

Figure 5-42 Functional View of Sample Set in Examples 1-4

Preventing Row Merging

The methods of creating ASCII files shown in Figure 5-38 through Figure 5-44 result in merging the rows of the sample set table. Merging the rows consolidates repetitive information and conserves storage space in the 2795 Separations Module. If you store many very large sample sets, you can run out of storage space before reaching the 2795 Separations Module limit of 60 stored methods.

However, if you merge rows in the Sample Set table as shown in Figure 5-38 through Figure 5-44, you have less control over the precise sequence of injections. To force the 2795 Separations Module to store a sample set without merging Sample Set table rows, use the "#PRESERVE" instruction at the top of the ASCII file, as shown in Figure 5-43.

```
#PRESERVE

3        1       Sample      "Test Method"       1       12.0      4.65
10       1       Sample      SepMeth1            1       10.0      6.00
9        1       Sample      SepMeth1            1       10.0      6.00
8        1       Sample      SepMeth1            1       10.0      6.00
```

Figure 5-43 ASCII Text File for Import (Example 5)

Figure 5-44 shows the Functional view of the sample set after importing the ASCII file data (in Figure 5-43) into the 2795 Separations Module.

Figure 5-44 Functional View of Sample Set in Examples 1-4

Specifying the List of AutoAdd or Pool Aliquots

The "well", "plate", and "volume" fields in the Sample Set table can include multiple entries separated by commas. The number of entries in the "well" field determines the number of rows in the AutoAdd or Pool Details table. If the number of entries in the "plate" or "volume" column is less than the number of wells, then the plate or volume of the first-listed well is assigned to all wells in the Details table. Any of the following directives affects all subsequent AutoAdd and Pool rows (Table 5-29).

Table 5-29 AutoAdd or Pool Aliquots Specifications

Action	Formula	Variables
Specifying pool sample gaps	#GAPn=p:w,v	$n = \{ 1, 2, 3 \}$ p = plate number $\{ 1, 2, 3, 4 \}$ w = well number (≥ 1) v = volume
Specifying a delay time for AutoAdd and Pool functions	#DELAY=t	t = delay time in min
Specifying the SAMPLE aliquot position	#AASAMPLE=n	n = relative position of the SAMPLE entry in AutoAdd rows[a]

a. For example, if you sip from SAMPLE, from well 4:2, then from well 4:3, n is 1.

shows a typical ASCII text file for import.

```
#GAP1=4:1,3.0
#GAP2=4:2,1.0
#GAP3=4:1,3.0
#DELAY=1.5
#AASAMPLE=2

95,1,96     1      Autoadd      Default           1      5.0,10.0,5.0      6.00
95,2,96     1      Autoadd      Default           1      5.0,10.0,5.0      6.00
95,3,96     1      Autoadd      Default           1      5.0,10.0,5.0      6.00

#DELAY=3.0
2,2,2       1,2,3    Pool         Default           1         10.0          6.00
```

Figure 5-45 ASCII Text File for Import (Example 6)

In this example, four injections are performed. The first is a 10-µL sample taken from well 1:1, which is injected as an AutoAdd, between 5 µL from well 1:95 and 5 µL from well 1:96. The next two injections are similar to the first, except that the sample well is 1:2 for the second and 1:3 for the third. For each injection, the sample is allowed to diffuse with the AutoAdd aliquots for 1.5 minutes before injection.

The fourth injection is a pool of three 10-µL samples from wells 1:2, 2:2, and 3:2. A gap of 3 µL from well 4:1 is drawn before the first sample; 1 µL is drawn from well 4:2 between samples; and 3 µL is drawn from well 4:1 after the final sample. The samples are allowed to diffuse with gaps for 3.0 minutes before injection.

Note: Pooling is not available under control by Millennium[32] software or MassLynx software. AutoAdd is available under control by Millennium[32] software, but is not available under control by MassLynx software.

5.4.4 Compatibility of Imported Sample Sets

When exporting and importing sample sets that use *customized plates*, you must have *identical plate type configuration lists* on the source and target 2795 Separations Module systems.

A *customized plate* is either of the following:

- A factory-default plate for which the sequential reference has been changed to something other than horizontal discontinuous
- A user-named plate type that was created by using the Copy button on the Plate Type Configuration dialog box

An *identical plate type configuration list* means that the customized plate used by the sample set appears in the Plate Type Configuration list on the two systems with the identical name, sequential reference, and dimensions, and *in the identical ordinal location*.

If you cannot be sure that the two 2795 Separations Modules have identical plate type definitions, then after importing the sample set, you must open it for editing and verify the plate type of every plate (1 through 4) used in the sample set. Select a different plate type for each plate as necessary, and then save the modified sample set before running it.

A simple way to compare the plate type configuration lists between two 2795 Separations Modules is to go to the Plate Type Configuration dialog box, insert a formatted floppy disk and press **Print**. This will generate a complete list of plate types, in the critical order. Compare the two plate type configuration files directly in a text editor or by printing and doing a side-by-side review.

A dialog box warning reminder of the important considerations appears when importing a sample set (Table 5-46).
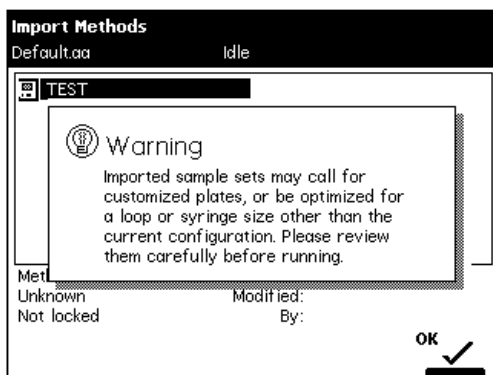
Figure 5-46 Import Methods Warning Reminder

Chapter 6
Making Automatic Runs and Setting Up for High-Throughput Operation

This chapter describes how to set up the Waters 2795 Separations Module for:

- Making automatic runs in a stand-alone mode (Section 6.1)
- Using the 2795 Separations Module high-throughput capabilities (Section 6.2)

To configure the 2795 Separations Module operating modes, see Section 3.2.3, Configuring the Operating Mode.

Note: *Before making any runs with the 2795 Separations Module, become familiar with the procedures in Chapter 5, Creating and Editing Separation Methods and Sample Sets.*

System Controller and No Interaction Modes

If your HPLC system uses the 2795 Separations Module in a stand-alone mode, in System Controller or No Interaction mode (Section 3.2.3, Configuring the Operating Mode), you make automatic runs using stored or imported sample sets. Chapter 5, Creating and Editing Separation Methods and Sample Sets, describes how to create, store, and import separation methods and sample sets.

Controlled by Millennium 32 Mode

If your LC system is controlled by Millennium®^{32} software version 3.2 or higher (see Section 3.2.3, Configuring the Operating Mode), you make automatic runs using the Millennium^{32} project, instrument method, and sample set stored in the Millennium^{32} software. When the 2795 Separations Module is controlled by Millennium^{32} software, the word "Remote" appears in the 2795 Separations Module front panel banner area.

For information on making automatic runs with the 2795 Separations Module under Millennium^{32} software control, refer to the Millennium^{32} system documentation and *Millennium*^{32} *Help*.

Note: *When Millennium*^{32} *software is not running, you can operate the 2795 Separations Module from its front panel (see Section 3.2.3, Configuring the Operating Mode, the "No Interaction Mode" discussion).*

Controlled by MassLynx Mode

If your HPLC or LC/MS system is controlled by MassLynx software (Section 3.2.3, Configuring the Operating Mode), you make automatic runs using the MassLynx sample processing list. The list contains the method(s) that define the operating parameters of the 2795 Separations Module and the detector(s) (other than the mass spectrometer).

When the 2795 Separations Module is controlled by MassLynx software, the word "Remote" appears in the 2795 Separations Module front panel banner area.

For information on making automatic runs with the 2795 Separations Module under MassLynx software control, refer to the *Waters Alliance LC/MS System Quick Start Guide*, the MassLynx system documentation, or the MassLynx Help.

Note: When MassLynx software is not running, you can operate the 2795 Separations Module from its front panel (see Section 3.2.3, Configuring the Operating Mode, the "No Interaction Mode" discussion).

6.1 Making Automatic Runs in a Stand-Alone Mode

Figure 6-1 summarizes the steps for making an automatic run in a stand-alone mode (System Controller or No Interaction).
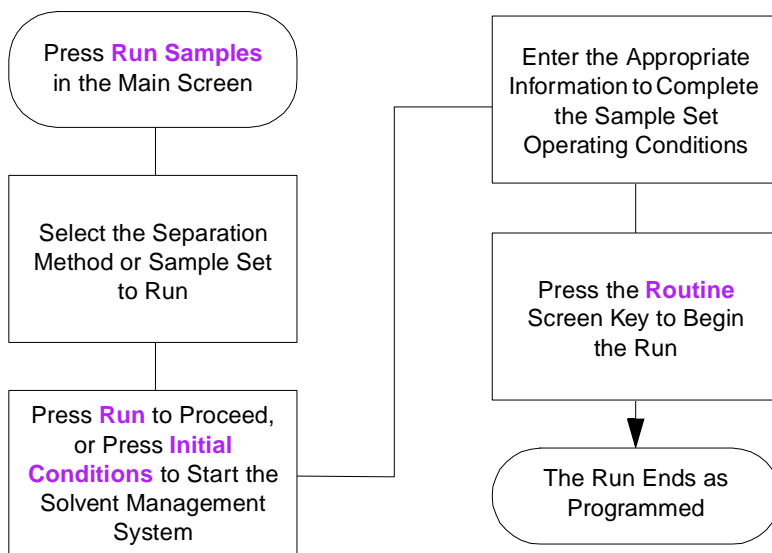
Figure 6-1 Making an Automatic Run in a Stand-Alone Mode

6.1.1 Running a Sample Set

Note: *Refer to 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 **Run Samples** in the Main screen. The Run Samples screen appears (Figure 6-2), listing the available separation methods and sample sets.
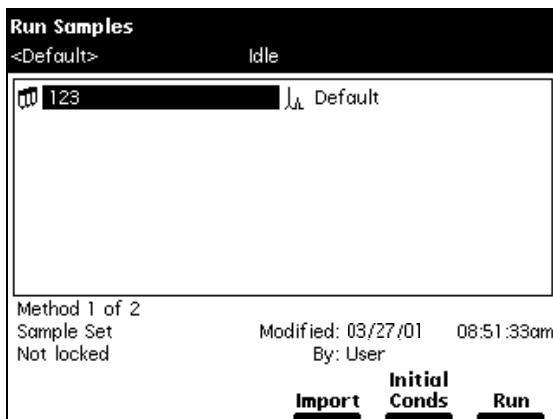
Figure 6-2 Run Samples Screen (Example)

2. Select a sample set, then press one of the following screen keys (or press **Import** to import a 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** – s

Figure 6-3 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 3.4.3, Loading the Sample Plates). Use the Injection, Loading, and Plate Loading views to view the sample set (see 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 Run Samples Requirements screen appears (Figure 6-4). Check that the information in the Requirements screen is correct.
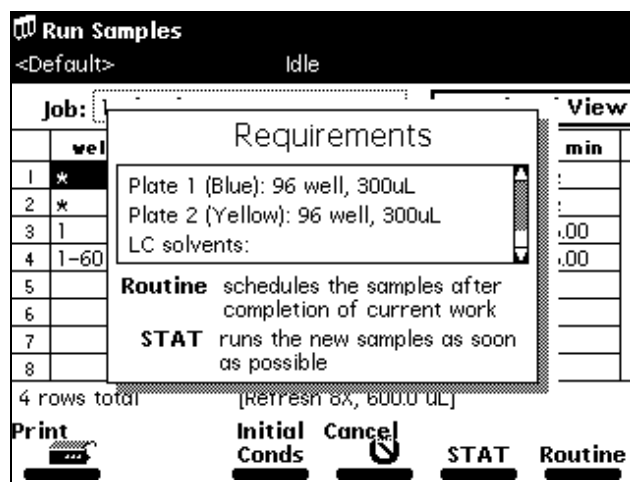
Figure 6-4 Run Samples Requirements Screen (Example)

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 6.1.2, Modifying a Sample Set During a Run.

6.1.2 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 loading 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 **Run Samples** in the Main screen. The Run Samples screen appears (Figure 6-2).

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

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

5. Press the **Start** screen key. The Run Samples Requirements screen appears (Figure 6-4). Check that all information in the Requirements screen is correct.

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

7. 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 Section 5.1.2, Editing Separation Methods, to modify and save the separation method.

6.1.3 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 below).

- **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) – 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

6.2 Using the 2795 High-Throughput Capabilities

Note: Before making any runs with the 2795 Separations Module, become familiar with the procedures in Chapter 5, Creating and Editing Separation Methods and Sample Sets.

When you use gradient separations, you can reduce cycle time and, therefore, significantly increase sample throughput by using the parallel processing capabilities of the 2795 Separations Module. This capability allows the 2795 Separations Module to prepare the next sample for injection before the current sample cycle is complete.

The 2795 Separations Module provides three ways to perform LC applications:

- **Sequential** (traditional) **chromatography** (Figure 6-5) – All operations are performed in sequence (one after the other).
- **Parallel processing** – Some operations are performed concurrently (Figure 6-8).
- **Parallel processing with rapid equilibration** – Some operations are performed concurrently, and the column is taken off-line while the system is equilibrated at a high flow rate (Figure 6-11).

    In addition, if you specify a pre-column volume when using any one of the three processing methods, the solvent management system delivers the gradient to the inject valve in parallel with column equilibration for "just-in-time" gradient delivery.

Figure 6-15 shows a comparison of chromatograms produced using sequential, parallel, and parallel with rapid equilibration processing methods.

Note: Waters recommends you do not calibrate or do quantitative analysis of your system based on the first in a series of parallel injections. The first injection in a series of parallel injections may differ in timing because the first injection is not coordinated with a previous injection. Results obtained from the first injection may vary from those of subsequent injections.

As you develop separation methods for high-throughput applications (see Section 5.1, Creating and Editing Separation Methods and Section 5.2, Setting Separation Method Parameters), you may need to change certain parameter values from those values used in sequential processing.

For parallel processing methods, you may need to make changes to the following Autosampler Page parameters (Figure 5-10):

- Injection mode
- Loop offline time
- Loop option (full or partial)
- Full loop overfill
- Pre-column volume
- Aspirate air, pre- and post-sample

For parallel processing with rapid equilibration methods, you may need to make changes to the above Autosampler Page parameters (Figure 5-10) and the following Rapid Equilibration dialog box (Figure 5-12) parameters:

- Path
- Flow
- Duration
- Column reequilibration

6.2.1 Sequential Processing

In sequential processing (traditional chromatography), most LC system operations occur in series (Figure 6-5). However, in all three processing modes, the needle-wash and purge cycles occur in parallel with the gradient separation.
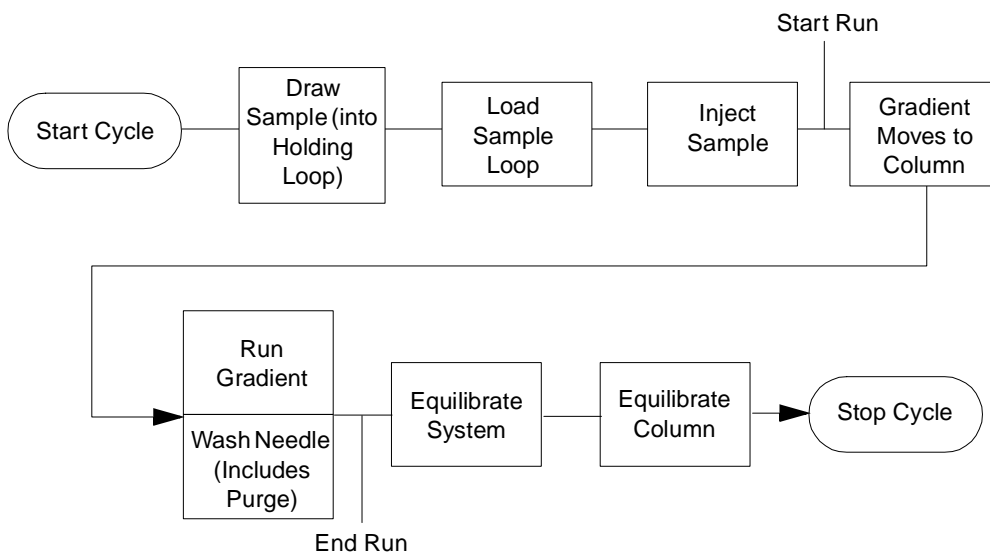
Figure 6-5 Sequential Processing Cycle for Gradient Chromatography

Sequential Processing Example

Figure 6-6 shows the Autosampler screen parameters for the sequential processing in this example. Figure 6-7 shows the chromatography results using a separation method with these parameters.

Figure 6-6 Autosampler Screen Parameter Values for Sequential Processing
Separation Method (Example)
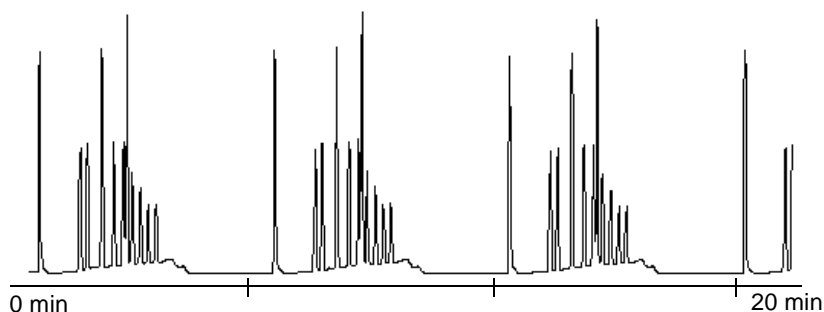
Run time = 5.0 min
Cycle time = 6.2 min
Flow rate = 1mL/min
Gradient = 0 to 100% B in 3 min, hold 0.1 min

Figure 6-7 Sequential Processing Chromatography Results (Example)

6.2.2 Parallel Processing

In parallel processing, the 2795 Separations Module draws the next sample into the holding loop after the needle wash and purge is complete (Figure 6-8). You can specify a time to take the sample loop off-line to load the sample.

Note: Waters recommends you do not calibrate or do quantitative analysis of your system based on the first in a series of parallel injections. The first injection in a series of parallel injections may differ in timing because the first injection is not coordinated with a previous injection. Results obtained from the first injection may vary from those of subsequent injections.
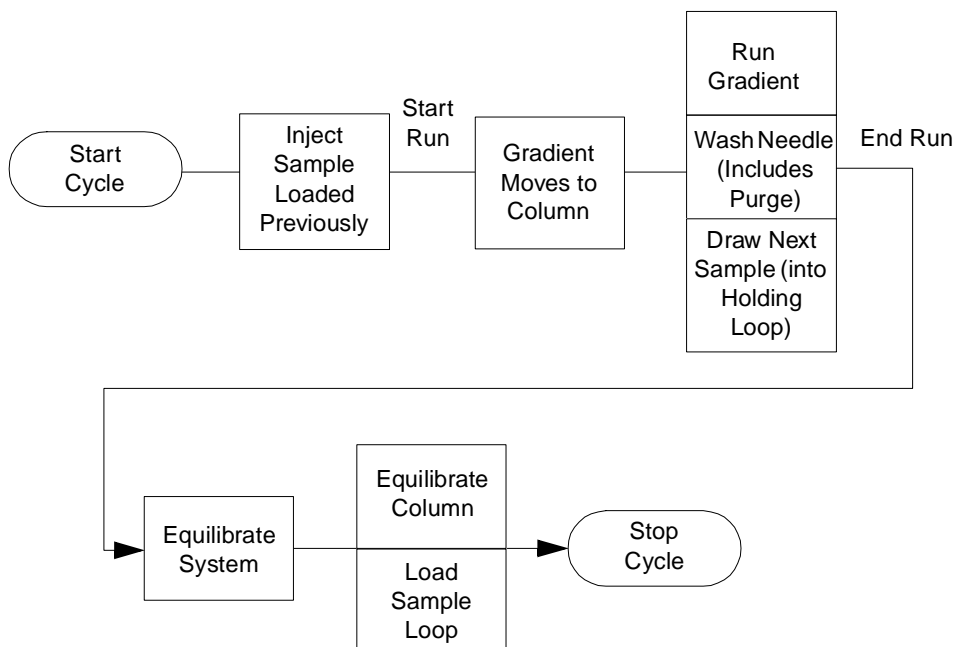
Figure 6-8 Parallel Processing Cycle for Gradient Chromatography

Note: You can also specify a pre-column volume to achieve "just-in-time" delivery of the gradient by the solvent management system (see Section 6.2.3, Parallel Processing with Rapid Equilibration for an example).

Parallel Processing Example

Figure 6-9 shows the Autosampler screen parameter values used to specify parallel processing in this example. Figure 6-10 shows the chromatography resulting from using a separation method with those parameter values.

Note: To minimize sample diffusion with parallel sample processing, incorporate air gaps (3 to 5 μL), pre- and post-sample.

Figure 6-9 Autosampler Screen Parameter Values for Parallel Processing
Separation Method (Example)

0 min   20 min
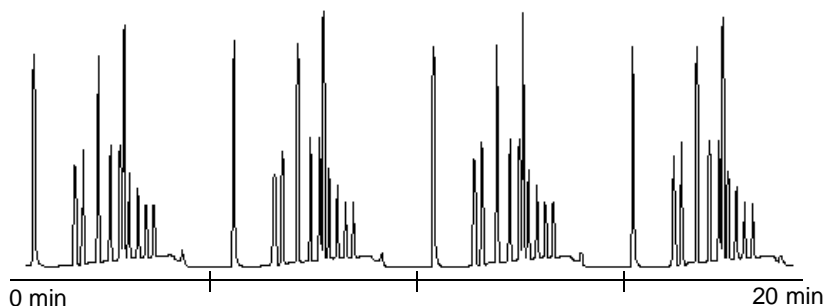
Run time = 5.0 min
Cycle time = 5.1 min
Flow rate = 1mL/min
Gradient = 0 to 100% B in 3 min, hold 0.1 min

Figure 6-10 Parallel Processing Chromatography Results (Example)

The parallel processing example demonstrates the reduction in cycle time compared to
the sequential method (Figure 6-7).

6.2.3 Parallel Processing with Rapid Equilibration

To maximally increase 2795 throughput, you can combine parallel processing options, a fast system equilibration time, and "just-in-time" gradient delivery (Figure 6-11).

In this method, after the gradient is finished, the flow of mobile phase is diverted to waste (using either the column selection valve or the inject valve) and the flow rate is increased (typically, to 5 mL/min) to purge the system more quickly.

When the column returns online, reequilibration occurs at the normal flow rate. During this period, the solvent management system begins gradient delivery. The 2795 Separations Module software times the loading of the sample loop and injection with the delivery of gradient to the inject valve.

Note: Waters recommends you do not calibrate or do quantitative analysis of your system based on the first in a series of parallel injections. The first injection in a series of parallel injections may differ in timing because the first injection is not coordinated with a previous injection. Results obtained from the first injection may vary from those of subsequent injections.
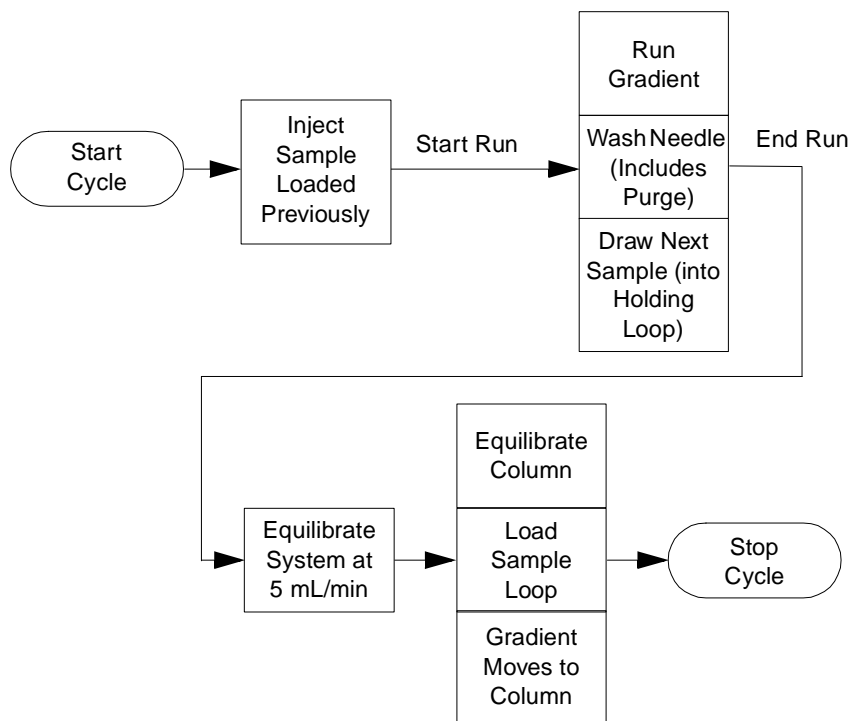
Figure 6-11 Parallel Processing with Rapid Equilibration Cycle for Gradient Chromatography

Parallel Processing with Rapid Equilibration Example

Figure 6-12 and Figure 6-13 show the Autosampler screen and Rapid Equilibration dialog box parameter values that specify parallel processing with rapid equilibration in this example. Figure 6-14 shows the chromatography that results when you use a separation method with these parameter values.

Note: If you specify rapid equilibration parameters in the Autosampler screen, the 2795 Separations Module software ignores the Loop off-line parameter.

Figure 6-12 Autosampler Screen Parameter Values for Parallel Processing with Rapid Equilibration Separation Method (Example)

See the "Determining the Pre-Column Volume for Parallel Processing" and the "Specifying Rapid Equilibration Values" discussions in this section for information about determining pre-column volume and column reequilibration values for your application.
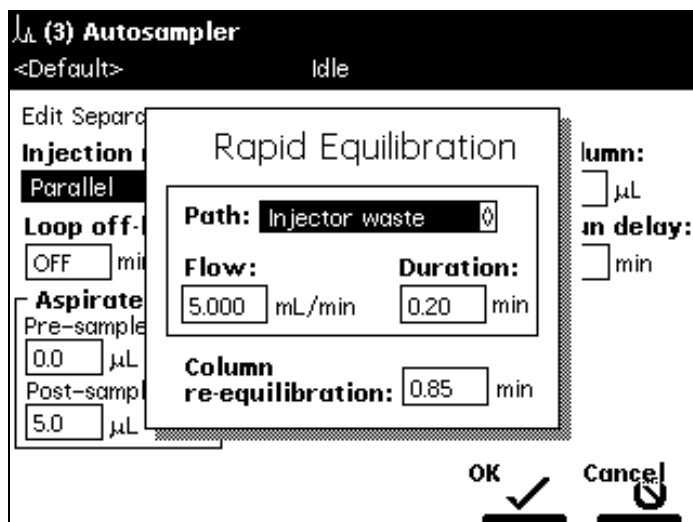
Figure 6-13 Rapid Equilibration Dialog Box Parameter Values (Example)
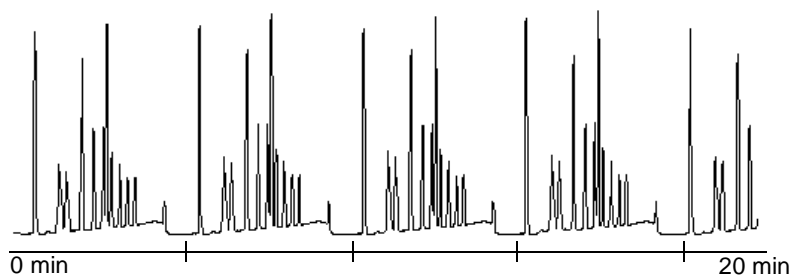
Run time = 3.2 min
Cycle time = 4.4 min
Flow rate = 1mL/min
Gradient = 0 to 100% B in 3 min, hold 0.1 min

Figure 6-14 Parallel Processing with Rapid Equilibration Chromatography Results (Example)

Comparing Cycle Times

shows a comparison of chromatograms from the three processing methods and their cycle times.
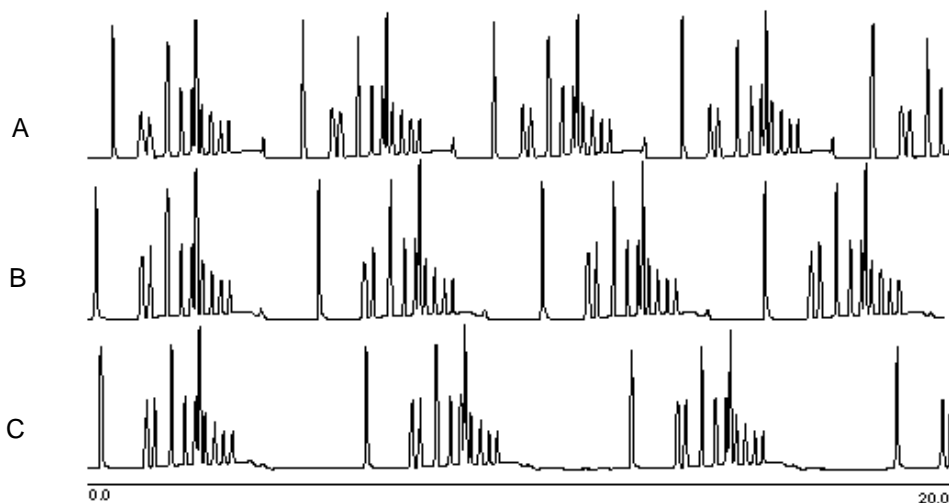
A = Parallel with Rapid Equilibration, Cycle Time = 4.4 min.

B = Parallel, Cycle Time = 5.1 min.

C = Sequential, Cycle Time = 6.2 min.

Figure 6-15 Comparison of Cycle Times

Determining the Pre-Column Volume for Parallel Processing

Use this section to determine the pre-column volume to specify when you use an Alliance HT system for high-throughput gradient chromatography.

Note: The pre-column volume measurement is approximate. Actual pre-column volume values vary depending on your application.

Recommended Materials

- Alliance HT System
- UV detector, λ 254
- Restrictor capable of $>$ 300 psi at 1 mL/min placed where the column connects to the inject valve (or use a short column placed after the detector to produce backpressure)
- Mobile phase A, methanol; B, 1% acetone in methanol

Procedure

To determine the pre-column volume for a high-throughput separation method:

1. Program the following gradient table (see Section 5.2.1, Setting Mobile Phase Parameter Values):

Table 6-1 Gradient Table for Mobile Phase

Time	Flow (mL/min)	% A	% B	Curve
0 min (Initial or Start)	1	100	0	*
2 min	1	0	100	11
5 min	1	100	0	11

2. Equilibrate the system at 100% A until you have a stable baseline.
3. Perform a 0-μL injection with a run time of 10 minutes.
4. Draw the baseline.
5. Expand the chromatogram to enlarge the baseline region.
6. Draw a line tangent to the chromatogram where the absorbance first begins to increase.
7. Read the time where the tangent intersects the baseline, then subtract 2 minutes from this time. The time multiplied by the flow rate (1 mL/min) is the pre-column volume.

Specifying Rapid Equilibration Values

Table 6-2 provides values for the following Rapid Equilibration dialog box parameters adjusted for a wide range of system equipment operational conditions:

1. Flow equal to 5.0 mL/min (recommended)
2. Rapid flush duration based on a 0.4 mL pre-column volume × 3 (use the method in "Determining the Pre-Column Volume for Parallel Processing" discussion above to determine actual volume)
3. Column reequilibration time based on a recommended reequilibration volume of 5× column volume

Table 6-2 assumes the following values for all parameters:

- Pre-column volume – 0.4 mL
- Column equilibration (3× volume) – 1.2 mL
- Rapid flow duration at 5 mL/min – 0.24 min

Note: Column reequilibration and pre-column volume mobile phase solvent are delivered concurrently.

Table 6-2 Rapid Equilibration Parameters for High-Throughput Applications

Column I.D. (mm)	Column Length (mm)	Column Volume[a] (mL)	Column Equil. 5× Vol. (mL)	Flow Rate (mL/min)	Column Reequil. (min)
4.6	250	4.15	20.76	1.391	14.93
3.9	150	1.79	8.95	1.000	8.95
3.9	100	1.19	5.97	1.000	5.97
3.9	75	0.90	4.48	1.000	4.48
3.9	50	0.60	2.98	1.000	2.98
3.9	20	0.24	1.19	1.000	1.19
3.0	75	0.53	2.65	0.591	4.48
2.1	150	0.52	2.60	0.290	8.95
2.1	50	0.17	0.87	0.290	2.98
2.1	30	0.10	0.52	0.290	1.79
1.0	150	0.12	0.59	0.066	8.92
1.0	50	0.04	0.20	0.066	2.97
3.9	50	0.60	2.98	1.000	2.98
3.9	50	0.60	2.98	2.000	1.49
3.9	50	0.60	2.98	3.000	0.99
2.1	50	0.17	0.87	0.290	2.98
2.1	50	0.17	0.87	0.580	1.49
2.1	50	0.17	0.87	1.000	0.87
2.1	30	0.10	0.52	0.290	1.79
2.1	30	0.10	0.52	0.580	0.90
2.1	30	0.10	0.52	0.870	0.60
2.1	30	0.10	0.52	1.000	0.52
2.1	20	0.07	0.35	0.290	1.19

Table 6-2 Rapid Equilibration Parameters for High-Throughput Applications *(Continued)*

Column I.D. (mm)	Column Length (mm)	Column Volume[a] (mL)	Column Equil. 5× Vol. (mL)	Flow Rate (mL/min)	Column Reequil. (min)
2.1	20	0.07	0.35	0.580	0.60
2.1	20	0.07	0.35	0.870	0.40
2.1	20	0.07	0.35	1.000	0.35
2.1	10	0.03	0.17	0.290	0.60
2.1	10	0.03	0.17	0.580	0.30
2.1	10	0.03	0.17	0.870	0.20
2.1	10	0.03	0.17	1.000	0.17
1.0	50	0.04	0.20	0.066	2.97
1.0	50	0.04	0.20	0.132	1.49
1.0	50	0.04	0.20	0.198	0.99

a. $V = \pi R^2 L$ (V = column volume, L = column length, R = column radius)

6.2.4 Separation Methods Templates

The 2795 Separations Module with firmware version 2.00 contains four Separations Module temples that can be used as a guide for developing customized Separations Methods. The names of the Separations Methods are listed below. Waters recommends either using the templates as provided or editing them as necessary and using the "Save as" function to rename them to an unique identifier.

- SequentialNCE:template
- SequentialCE:template
- SequentialPCE:template
- RapidEquilCE:template

These templates (Figure 6-16) provide starting points for medium to high-throughput, generic gradient methods, and incorporate different software capabilities of the 2795 that decrease analysis cycle time. Parameters not listed in Table 6-3 reflect no change from default method parameters. The templates have a common set of assumptions:

- Column: Reverse phase, 2.1 x 50 mm, 3.5 μm particles, 0.17- mL internal volume
- Binary Generic Gradient: 0-100%B in 3 min, return to 0%B at 3.1 min
- System pre-column volume: 350 μL.

- Reequilibration volumes: 3 times pre-column volume and 5 times column volume
- Column selector: none installed

The rate of solvent change of the generic gradient in the template is relatively gradual and serves as a starting point only. Cycle times can be greatly reduced by use of a steeper gradient. See Section 6.2.3 for details on calculating exact system pre-column volume (350 µL is a good approximation to use) and a table of column volumes (Table 6-2) based upon tube diameter and length.

Table 6-3 Template Parameters

	SequentialNCE: template	SequentialCE: template	SequentialPCE: template	RapidEquilCE: template
Comment	Routine method sequence. Elevated column temperature reduces solvent viscosity.	Column reequilibration is part of next sample. Can hide its time behind sample draw and data system reset.	Adds just in time gradient. The isocratic delay due to pre-column volume is eliminated.	Adds rapid flush of system for reequilibration. Next sample is drawn right after needle wash and purge
Injection mode	Sequential	Sequential	Sequential	Parallel
Pre-column volume	0	0	350	350
Rapid equilibration	Off	Off	Off	Flow: 5.0 Duration: 0.2
Column reequilibration time	0	0.85	0.85	0.85
Column temperature	30	30	30	30
Run time (front panel control)	5.0	4.2	3.6	2.8
Cycle time (front panel control)	5.9	5.1	4.9	4.6
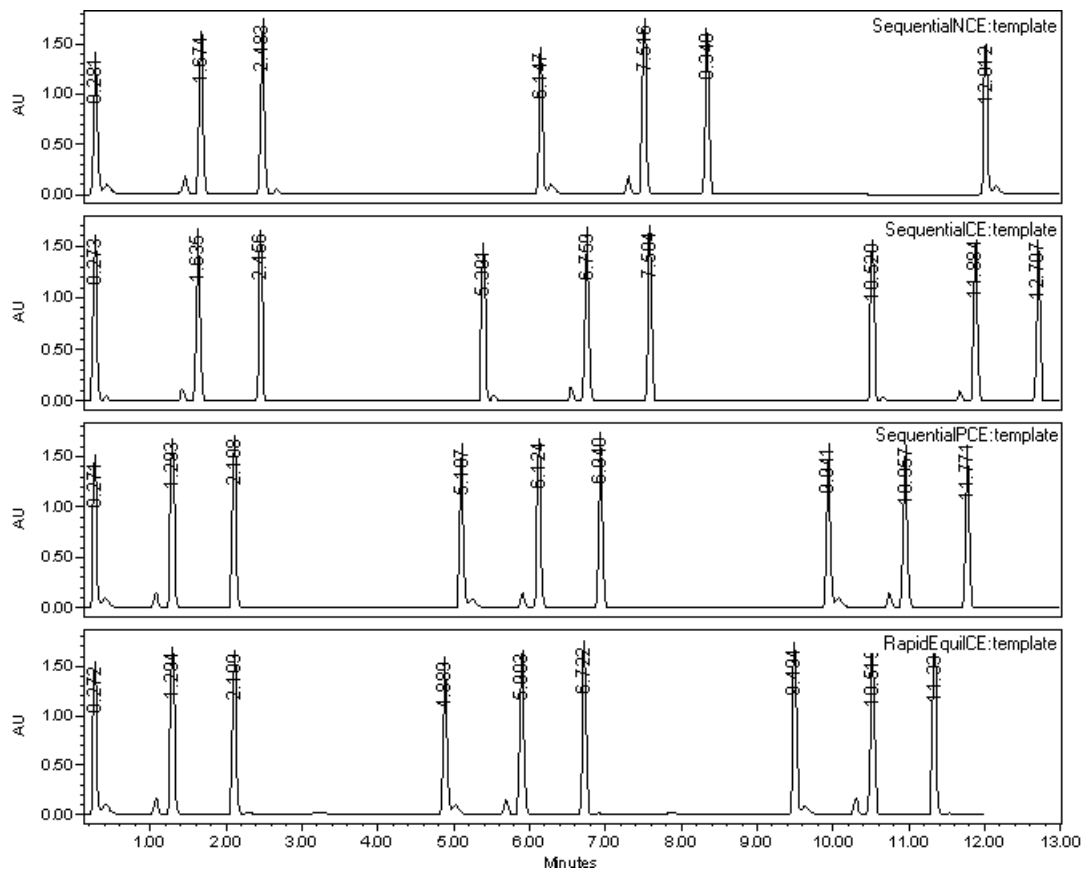
Figure 6-16 2795 Separations Module Templates, Firmware Version 2.00

Chapter 7
Maintenance

This chapter describes routine maintenance procedures you can perform to ensure the Waters 2795 Separations Module consistently provides accurate and precise results. Maintenance procedures are grouped under the solvent management system and the sample management system.

7.1 Maintenance Considerations

Safety and Handling

When you perform maintenance procedures on your 2795 Separations Module, keep the following safety considerations in mind.

STOP **Attention:** *To avoid damaging electrical parts, never disconnect an electrical assembly while power is applied to the 2795 Separations Module. Once power is turned off, wait ten seconds before you disconnect an assembly.*

STOP **Attention:** *To prevent circuit damage due to static charges, do not touch integrated circuit chips or other components that do not specifically require manual adjustment.*

Caution: *To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the 2795 Separations Module. Know the physical and chemical properties of the solvents you use. Refer to the Material Safety Data Sheets for the solvents in use.*

Caution: *To avoid the possibility of electric shock, do not open the power supply cover. The power supply does not contain user-serviceable parts.*

Proper Operating Procedures

To keep your 2795 Separations Module running smoothly, follow the operating procedures and guidelines described in Chapter 4, Operating the 2795 Separations Module from the Front Panel, Chapter 5, Creating and Editing Separation Methods and Sample Sets, and Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation.

Spare Parts

Refer to Appendix B, Spare Parts, for spare part information. Parts not included in Appendix B are not recommended for replacement by the customer.

Recommended Preventive Maintenance Schedule

Table 7-1 lists the recommended schedule of maintenance and diagnostic protocols to follow to ensure uninterrupted operation of the 2795 Separations Module. If you use the system continuously (for example, during nights and weekends), or if you use aggressive or buffered solvents that can cause system components to deteriorate, you may need to perform the following maintenance procedures more frequently than recommended in Table 7-1.

Table 7-1 2795 Separations Module Recommended Maintenance Schedule

Procedure	Frequency			Reference
	Monthly	Semi-Annual[a]	Annual[a]	
Solvent Management System				
Replace the solvent filter	X			Section 2.4, Making Fluidic Connections
Replace the plunger seal		X		Section 7.2.2, Replacing the Plunger Seal and Face Seals
Replace the plunger		X		Section 7.2.4, Cleaning and Replacing a Plunger
Replace the inlet check valve cartridge		X		Section 7.2.5, Replacing an Inlet Check-Valve Cartridge
Replace the inline filter		X		Section 7.2.6, Replacing the Inline Filter
Sample Management System				
Replace the syringe		X		Section 7.3.2, Replacing the Syringe
Replace the inject port seat		X		Section 7.3.3, Replacing the Needle Assembly
Rebuild the injector		X		Appendix B, Spare Parts
Rebuild the 3-column selection valve (if installed)		X		Appendix B, Spare Parts

Table 7-1 2795 Separations Module Recommended Maintenance Schedule *(Continued)*

Procedure	Frequency			Reference
	Monthly	**Semi-Annual**[a]	**Annual**[a]	
Rebuild the 6-column selection valve (if installed)		X		Appendix B, Spare Parts
Rebuild the column regeneration valve (if installed)		X		Appendix B, Spare Parts

a. Refer to *Waters Licenses, Warranties, and Support* document for "warranty period" discussion.

Contacting Waters Technical Service

If you encounter any problems replacing parts in the 2795 Separations Module, contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only.* Other customers, call your local Waters subsidiary or Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

7.2 Maintaining the Solvent Management System

Note: For information about isolating problems in the solvent management system, see Section 8.5, Troubleshooting.

Maintaining the solvent management system involves:

- Section 7.2.1, Removing the Head, Seal-Wash Assembly, and Plunger
- Section 7.2.2, Replacing the Plunger Seal and Face Seals
- Section 7.2.3, Replacing the Seal-Wash Assembly Seals
- Section 7.2.4, Cleaning and Replacing a Plunger
- Section 7.2.5, Replacing an Inlet Check-Valve Cartridge
- Section 7.2.6, Replacing the Inline Filter

For a complete list of spare parts, see Appendix B, Spare Parts.

Perform the procedures in this section according to the recommended schedule in Table 7-1, or as needed when you determine a problem exists with a specific component in the solvent management system. For information about isolating problems in the solvent management system, see Section 8.5, Troubleshooting.

Caution: To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the 2795 Separations Module. Know the physical and chemical properties of the solvents you use. Refer to the Material Safety Data Sheets for the solvents in use.

STOP *Attention: To ensure proper drip protection, be sure the solvent delivery and solvent conditioning drip trays are secured in place with screws before you operate the system. See Section 2.4.1, Installing the Detector Drip Tray and Waste Tubing, for instructions on installing drip trays.*

Solvent Management System Components

Figure 7-1 illustrates the solvent management system components (with the solvent delivery tray pulled out).

Accumulator Piston Chamber · System Transducer · Primary Transducer · Primary Piston Chamber · Inlet Check Valve · Inlet Check Valve · Prime/Vent Valve · Inline Filter · Seal-Wash Pump

TP01366A
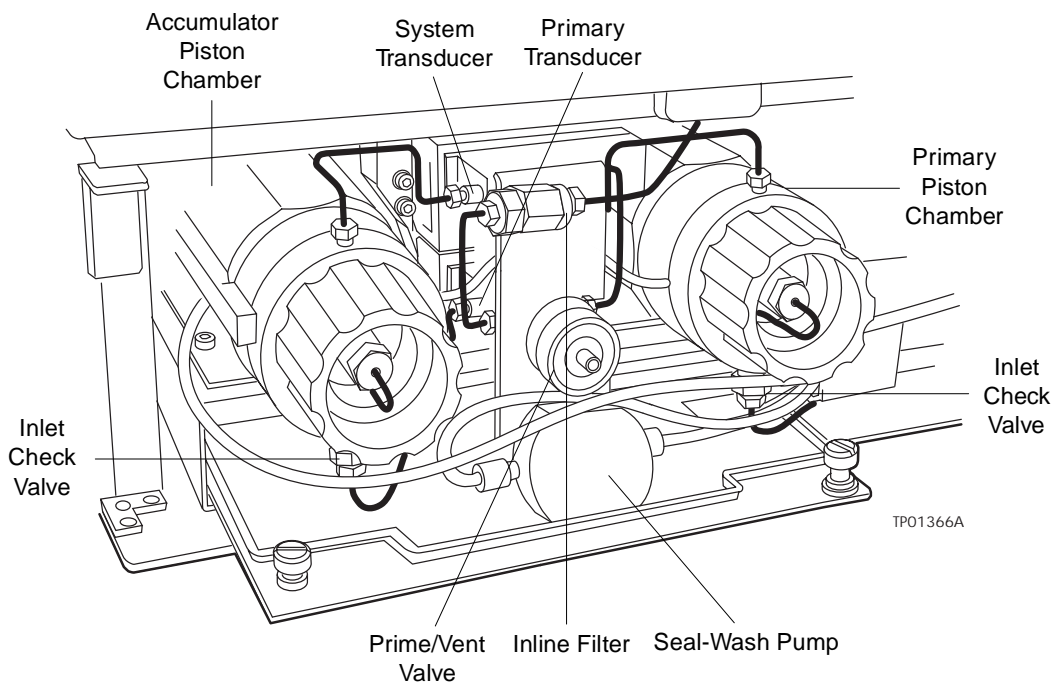
Figure 7-1 Solvent Management System Components

Table 7-2 describes the functions of these components.

Table 7-2 2795 Separations Module Solvent Management System
          Components

Component	Function
Primary Piston Chamber	Draws in/expels solvent as part of serial flow design.
Accumulator Piston Chamber	Draws in/expels solvent as part of serial flow design.
Prime/Vent Valve	Allows solvent purging, priming, and venting.

Table 7-2 2795 Separations Module Solvent Management System
           Components *(Continued)*

Component	Function
Seal-Wash Pump	Provides solvent to wash the seals of the primary and accumulator pistons.
Primary Inlet Check Valve	Maintains flow into the primary piston chamber by opening in one direction only (opens on the plunger intake stroke, closes on the delivery stroke).
Accumulator Inlet Check Valve	Maintains flow into the accumulator piston chamber by opening in one direction only (opens on the plunger intake stroke, closes on the delivery stroke).
Primary Transducer	Senses backpressure developed by resistance to solvent flow in the primary piston chamber.
System Transducer	Senses backpressure developed by resistance to solvent flow in the HPLC system.
Inline Filter	Provides solvent filtering between the solvent management system and the sample management system.

7.2.1 Removing the Head, Seal-Wash Assembly, and Plunger

Remove the head, seal-wash assembly, and plunger whenever you need to:

- Replace the plunger seals
- Service the seal-wash seals
- Clean or replace a plunger

Figure 7-2 shows the head, head nut, plunger, seal-wash assembly, and check valve assembly of each solvent delivery piston chamber. For a list of replacement parts, see Appendix B, Spare Parts.
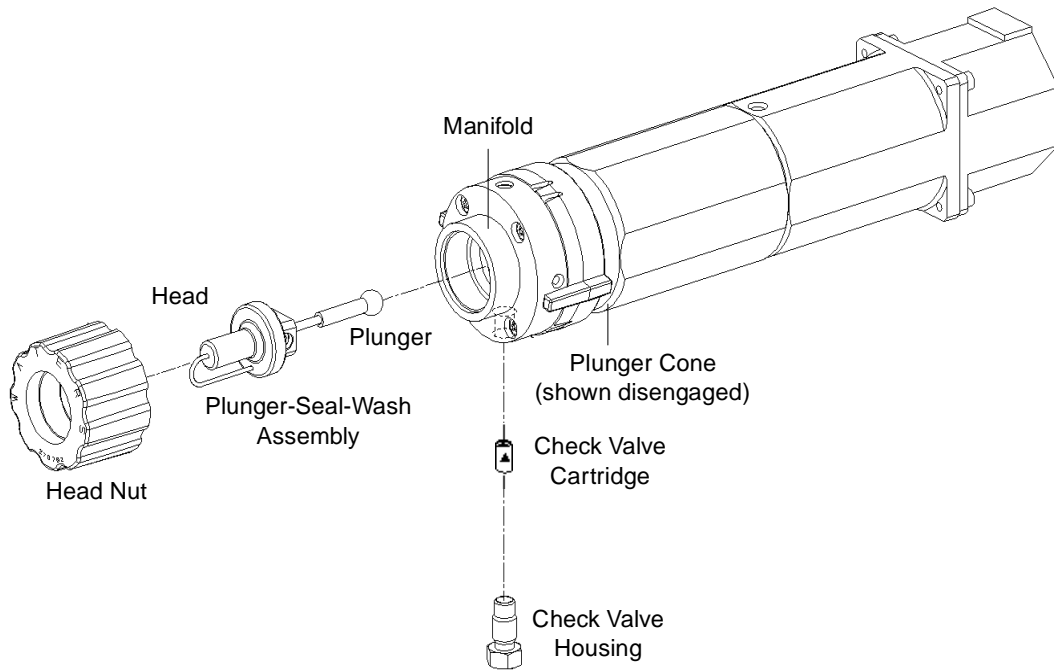
Figure 7-2 Piston Chamber Components

Procedure

To remove the head, seal-wash assembly, and plunger:

1. Press the **Diag** screen key in the Main screen.

2. Press the **Other Diags** screen key in the Diagnostics screen.

3. Select **Head Removal and Replacement** from the list in the Other Diagnostics screen, then press **OK**. The Head Removal and Replacement screen appears (Figure 7-3).

4. Follow the instructions in the screen, removing the head, seal-wash assembly, plunger seals, and plunger as one unit (see Figure 7-6).
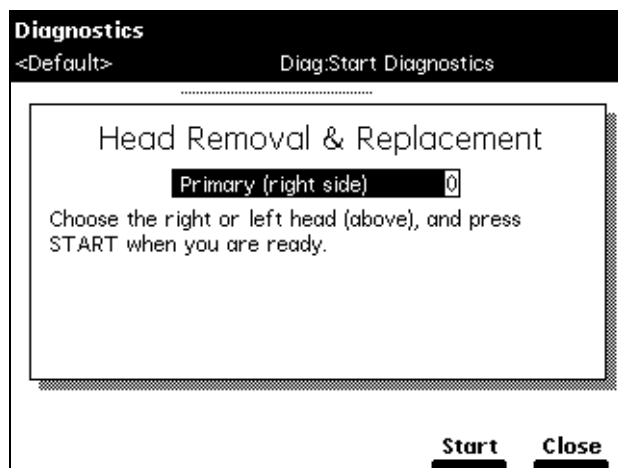
Figure 7-3 Head Removal & Replacement Screen

7.2.2 Replacing the Plunger Seal and Face Seals

Note: To ensure optimum performance, replace the plunger seal in a head when you perform service on the head. Replace the face seals every second or third plunger-seal replacement (see Figure 7-4).

Note: The standard seals in the 2795 Separations Module have excellent chemical resistance to the majority of solvents typically used in HPLC separation methods. If you find that these seals perform unsatisfactorily for your application, contact Waters for alternative plunger seals (part number WAT271066).

Required Materials

- Seal-removal tool (Startup Kit)
- Seal-insertion tool (Startup Kit)
- Replacement seals
- Squeeze bottle with 100% alcohol (isopropanol recommended)
- Clean block, such as a detector cell window (part number WAT097295)
- Syringe (Startup Kit)
- Plastic tweezers or can of compressed air

Replacing the Plunger Seal

STOP *Attention: To avoid damaging the sealing surfaces, use the seal removal tool. Do **not** use a sharp tool to remove or install seals.*

To replace the plunger seal:

1. Remove the head as described in <u>Section 7.2.1, Removing the Head, Seal-Wash Assembly, and Plunger</u>.

2. Use the plastic end of the seal-removal tool to remove the plunger seal from the head (<u>Figure 7-4</u>).
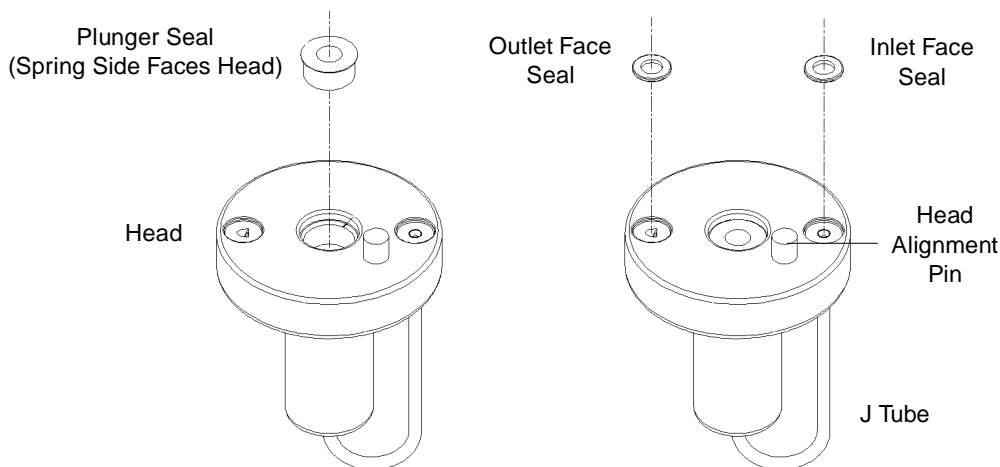
Figure 7-4 Plunger and Face Seal Orientation

3. Wet the insertion tool, seal opening, and new plunger seal with 100% alcohol.

4. Place the new plunger seal on the seal-insertion tool with the spring side facing away from the tool. Insert the tip of the tool into the plunger seal opening in the head (see <u>Figure 7-4</u>), then push firmly to install the seal.

Replacing the Face Seals

STOP *Attention:* *To avoid damage to the sealing surfaces, use the seal removal tool. Do not use a sharp tool to remove or install seals. Replace face seals every second or third plunger-seal replacement.*

To replace the face seals:

1. Remove the head as described in <u>Section 7.2.1, Removing the Head, Seal-Wash Assembly, and Plunger</u>.

2. Direct compressed air into the center of the face seal or use plastic tweezers to remove the inlet and outlet face seals from the seal openings in the head (see <u>Figure 7-4</u>).

3. Wet the new seals and seal openings with 100% alcohol.

4. Place the new face seals in the face seal openings in the head.

5. Use an alcohol-cleaned, flat object, such as a detector window, to firmly press the face seals into the openings on the pump head.

6. After you replace the seals, follow the instructions on the Head Removal and Replacement screen to reinstall the head, seal-wash assembly, and plunger assemblies. To avoid damaging the tube seals (Figure 7-5) and to properly align the seal-wash tubes with the seal-wash assembly, perform steps a through c below. Also, make sure that the head alignment pin is properly oriented and that the "J" tube is facing down (see Figure 7-6).

   a. After finger-tightening the head nut onto the head, loosen the nut approximately 1/2-turn to prepare for the seal-wash tube reconnection.

   b. Inspect the free ends of the seal-wash tubes for damage. If necessary, trim the ends with a razor blade.

   c. Reconnect the seal-wash tubes to the seal-wash inlet and outlet, then fully tighten the head nut.

7. If the 2795 Separations Module does not have solvent in the fluidic path, perform a dry prime to draw solvent into the plunger cavity before you perform a wet prime or start delivery of solvents (see Section 4.2.2, Dry Priming).

7.2.3 Replacing the Seal-Wash Assembly Seals

This procedure involves replacing the:

- Seal-wash face seal
- Two tube seals
- Plunger-seal-wash seal

Required Materials

- Seal insertion tool (Startup Kit)
- Seal removal tool (part number WAT039803)
- Replacement seals
- Squeeze bottle with 100% alcohol (isopropanol recommended)
- Syringe (Startup Kit)
- Plastic tweezers

Procedure

To replace the seal-wash assembly seals:

1. Remove the seal-wash assembly as described in Section 7.2.1, Removing the Head, Seal-Wash Assembly, and Plunger.

> **STOP** **Attention:** *To avoid damaging the sealing surfaces, use the seal removal tool. Do not use a sharp tool to remove or install seals.*

2. Use the plastic end of the seal removal tool to remove the plunger wash seal from its seat. Repeat this procedure for each of the tube seals (Figure 7-5).

3. Use the plastic tweezers to remove the seal-wash face seal (Figure 7-5).
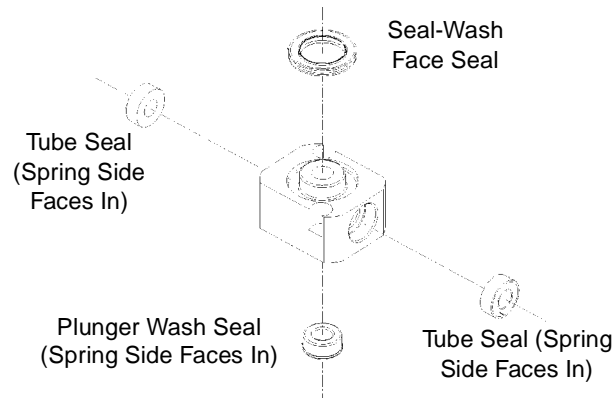
Figure 7-5 Seal-Wash Assembly Seals

4. Wet the seal-insertion tool, the seal opening, and each new seal with 100% alcohol before you install the seal.

5. Place the new plunger-wash seal on the seal-insertion tool with the spring side facing away from the seal-insertion tool (see Figure 7-5). Insert the tip of the tool into the seal opening, then push firmly to install the seal.

6. Repeat this procedure for each of the tube seals and for the seal-wash face seal.

7. Reinstall the seal-wash assembly washer onto the seal-wash assembly (Figure 7-6).

8. Slide the seal-wash assembly with the washer onto the plunger, then insert the full assembly onto the head (Figure 7-6).

9. Follow the instructions in the Head Removal and Replacement screen to reinstall the head, seal-wash assembly, and plunger. To avoid damaging the tube seals and to properly align the seal-wash tubes with the seal-wash housing, perform steps a through c below. Also, make sure that the head alignment pin is properly oriented and that the "J" tube is facing down (see Figure 7-6).

   a. After finger-tightening the head nut onto the head, loosen the nut approximately 1/2-turn to prepare for the seal-wash tube reconnection.

   b. Inspect the free ends of the seal-wash tubes for damage. If necessary, trim the ends with a razor blade.

   c. Reconnect the seal-wash tubes onto the seal-wash inlet and outlet, then fully tighten the head nut.

10. If the 2795 Separations Module does not have solvent in the fluidic path, perform a dry prime to draw solvent into the plunger cavity before you perform a wet prime or start delivery of solvents (see Section 4.2.2, Dry Priming).

7.2.4 Cleaning and Replacing a Plunger

Required Materials

- Pumice, fine
- Cotton swab
- Squirt bottle with 100% alcohol (isopropanol recommended)
- Water, HPLC-grade

Cleaning the Plunger

To clean the plunger:

1. Remove the head, seal-wash assembly, and plunger as described in Section 7.2.1, Removing the Head, Seal-Wash Assembly, and Plunger.

2. Inspect the plunger for damage, excessive wear, or solvent residue without separating it from the pump head and seal-wash assembly (Figure 7-6). If the plunger seal has solvent residue:

   a. Separate the plunger from the head and seal-wash housing assembly.

   b. Clean the plunger with a fine pumice to remove any coating.

   c. Rinse the plunger thoroughly with water to remove all traces of abrasive, then wipe clean.

3. Reinspect the plunger. If the plunger shows excessive wear, replace the plunger (see the "Replacing the Plunger Seal" discussion below).

   If necessary, replace the plunger seal and face seals as described in Section 7.2.2, Replacing the Plunger Seal and Face Seals.
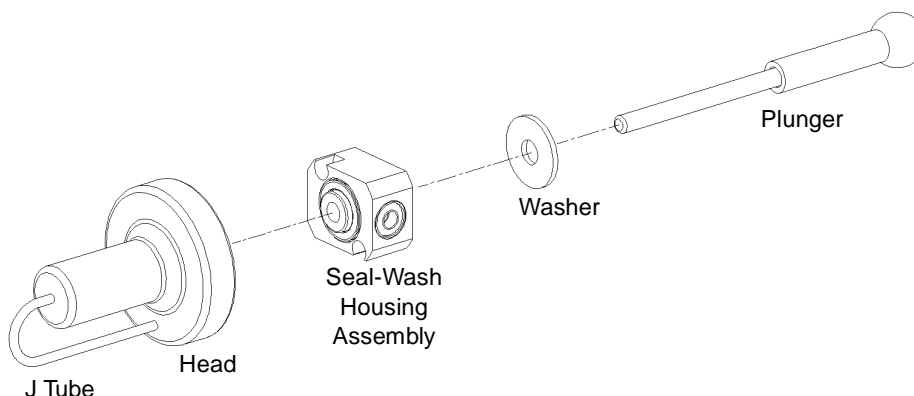
Figure 7-6 Plunger, Seal Wash, and Head

Replacing the Plunger

To replace the plunger:

1. Slide the seal-wash assembly with washer onto the new plunger, then insert the assembly into the head (Figure 7-6).

2. Follow the instructions in the Head Removal and Replacement screen to reinstall the head, seal-wash assembly, and plunger. To avoid damaging the tube seals and to properly align the seal-wash tubes with the seal-wash assembly, refer to steps a through c below. Also, make sure that the head alignment pin is properly oriented and that the "J" tube is facing down (see Figure 7-6).

    a. After finger-tightening the head nut onto the head, loosen the nut approximately 1/2-turn to prepare for the seal-wash tube reconnection.

    b. Inspect the ends of the seal-wash tubes for damage. If necessary, use a razor blade to trim the ends.

    c. Reconnect the seal-wash tubes to the seal-wash inlet and outlet, then fully tighten the head nut.

3. If the 2795 Separations Module does not have solvent in the fluidic path, perform a dry prime to draw solvent into the plunger cavity before you perform a wet prime or start delivery of solvents (see Section 4.2.2, Dry Priming).

7.2.5 Replacing an Inlet Check-Valve Cartridge

Required Materials

- Wrench, 1/2-inch, open-end
- Wrench, 5/16-inch, open-end
- Screwdriver, 1/4-inch, flat-blade

- Check-valve cartridge
- Squeeze bottle with 100% alcohol (isopropanol recommended)
- Syringe (Startup Kit)

Procedure

To replace an inlet check valve on either piston chamber:

1. In the Main screen, press the **Diag screen** key, then press the **Other Diags** screen key.

2. Select **Turn Off GPV** in the drop-down list, then press **OK**. The screen indicates that the GPV is turned off.

3. Loosen the screws that secure the solvent delivery and solvent conditioning drip trays and pull the trays out a few inches to access the check valves (see Figure 7-1).

4. Use the 1/2-inch wrench to hold the inlet check-valve housing while you loosen the inlet check valve compression screw with the 5/16-inch wrench (Figure 7-7).

5. Removing the tubing from the check-valve housing.

6. Use the 1/2-inch wrench to disconnect the check-valve housing from the manifold (Figure 7-7).

Head

Manifold

Check Valve Cartridge

Check Valve Housing

TP01367
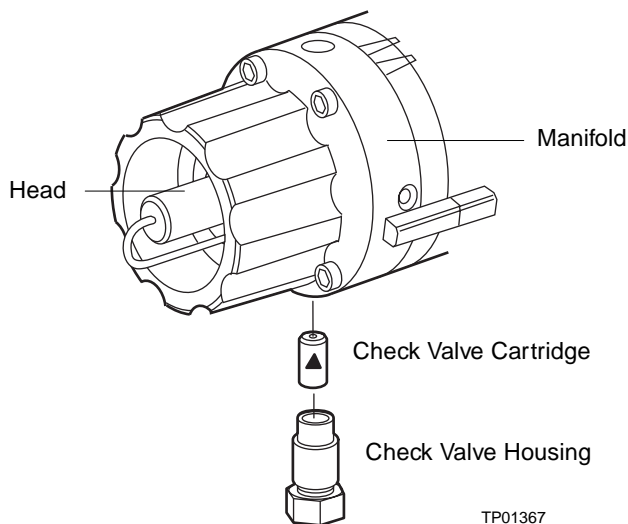
Figure 7-7 Inlet Check Valve

7. Tip the inlet check-valve housing upside down to remove the old check-valve cartridge.

8. Inspect the inlet check-valve housing and clean it with 100% alcohol if necessary. Wet the housing with 100% alcohol.

9. Wet the new check-valve cartridge with 100% alcohol, then insert into the inlet check-valve housing, making sure that the arrow on the check-valve cartridge points toward the piston chamber.

10. Insert and then finger-tighten the inlet check-valve housing into the manifold.

11. Use the 1/2-inch wrench to tighten the inlet check-valve housing 1/8-turn.

12. Use the1/2-inch wrench to hold the check-valve housing while you reinstall the inlet tubing and tighten the compression screw into the check-valve housing with the 5/16-inch wrench.

13. In the Main screen, press the **Diag** screen key, then press the **Sensors, Motors** screen key.

14. Press **Motors** to access the Motors and Valves screen, then set the GPV valve to the **Solvent A** position.

15. If the 2795 Separations Module does not have solvent in the fluidic path, perform a dry prime to draw solvent into the plunger cavity before you perform a wet prime or start delivery of solvents (see Section 4.2.2, Dry Priming).

7.2.6 Replacing the Inline Filter

The inline filter filters solvent as it passes between the solvent management system and the sample management system. Clean and replace the inline filter element when it is the source of elevated backpressure.

Required Materials

- Wrenches, 5/8-inch, open-end (2)
- Wrench, 5/16-inch, open-end
- Squirt bottle with 100% alcohol (isopropanol recommended)
- Absorbent tissue
- Inline filter element (part number WAT088084)

Procedure

To replace the inline filter:

1. Use a 5/8-inch wrench and a 5/16-inch wrench to disconnect the compression screw on the left (inlet) side of the inline filter from the inline filter inlet housing (Figure 7-8). Use an absorbent tissue to absorb the small amount of solvent that may leak.

Inlet Housing

Outlet Housing

Inline Filter Element
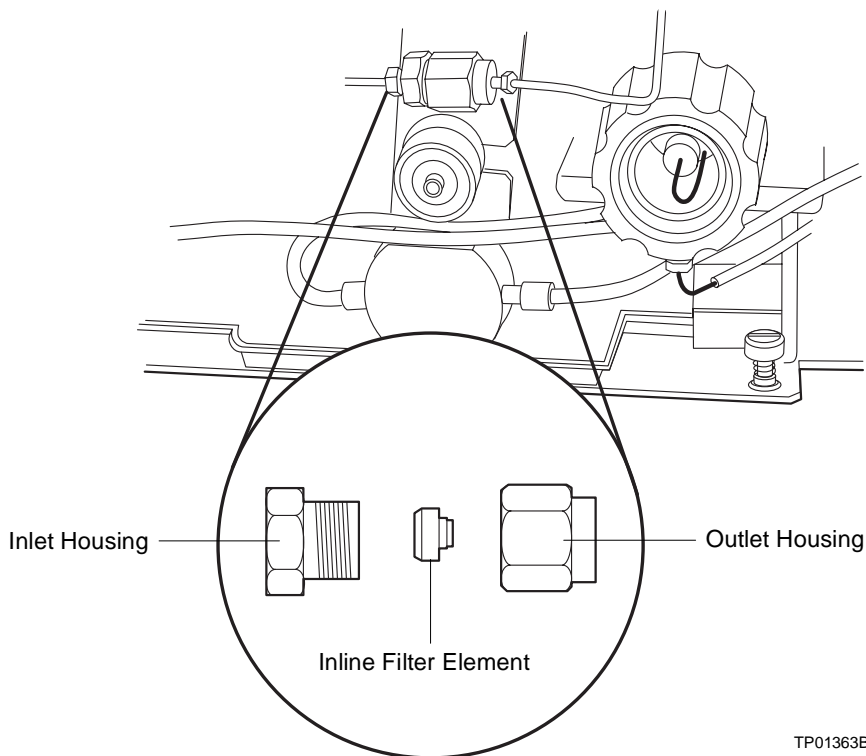
TP01363B

Figure 7-8 Replacing the Inline Filter

2. Use a 5/8-inch wrench to hold the filter outlet housing while you disconnect the inlet housing with another 5/8-inch wrench.

3. Tip the inlet housing upside down to remove the inline filter element.

4. Wet the replacement inline filter element with alcohol.

5. Insert the replacement inline filter element into the inline filter housing.

6. Reconnect the inline filter inlet and outlet housings.

7. Reconnect the compression screw and inlet tubing to the inlet housing.

8. Flush the solvent management system with mobile phase at 1 mL/min for 10 minutes.

9. Inspect the inline filter for leaks, and tighten the fittings as required.

7.3 Maintaining the Sample Management System

Perform the procedures in this section when you determine there is a problem with a component of the 2795 Separations Module sample management system.

Note: For information about isolating problems in the sample management system, see Section 8.5, Troubleshooting.

Note: When you remove the top or right side cover of the 2795 Separations Module for maintenance of the sample management system, the XYZ assembly motors can operate only at very slow speeds. This allows you to safely observe the needle and holding loop. Normal operating speeds resume after you reinstall the covers.

⚠ *Caution: To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the 2795 Separations Module. Know the physical and chemical properties of the solvents you use. Refer to the Material Safety Data Sheets for the solvents in use.*

⚠ *Caution: To prevent injury, keep hands or loose clothing clear of the needle assembly mechanism while it is in motion.*

7.3.1 Overview

Maintaining the 2795 Separations Module sample management system involves:

- Section 7.3.2, Replacing the Syringe
- Section 7.3.3, Replacing the Needle Assembly
- Section 7.3.4, Replacing the Inject Port Seat
- Section 7.3.5, Replacing the Holding Loop
- Section 7.3.6, Replacing the Sample Loop
- Section 7.3.7, Cleaning the Sample Compartment and Plate Carrier

Refer to Appendix B, Spare Parts, for spare part information. Parts not included in Appendix B are not recommended for replacement by the customer.

Figure 7-9 shows a side view of the assemblies and components of the 2795 Separations Module sample management system.

Plate Carrier Motor

XYZ Needle with Stripper

Sample Compartment

Inject Valve

Degasser Assembly

Foam Insert (Location of Optional Bar Code Reader

TP01585
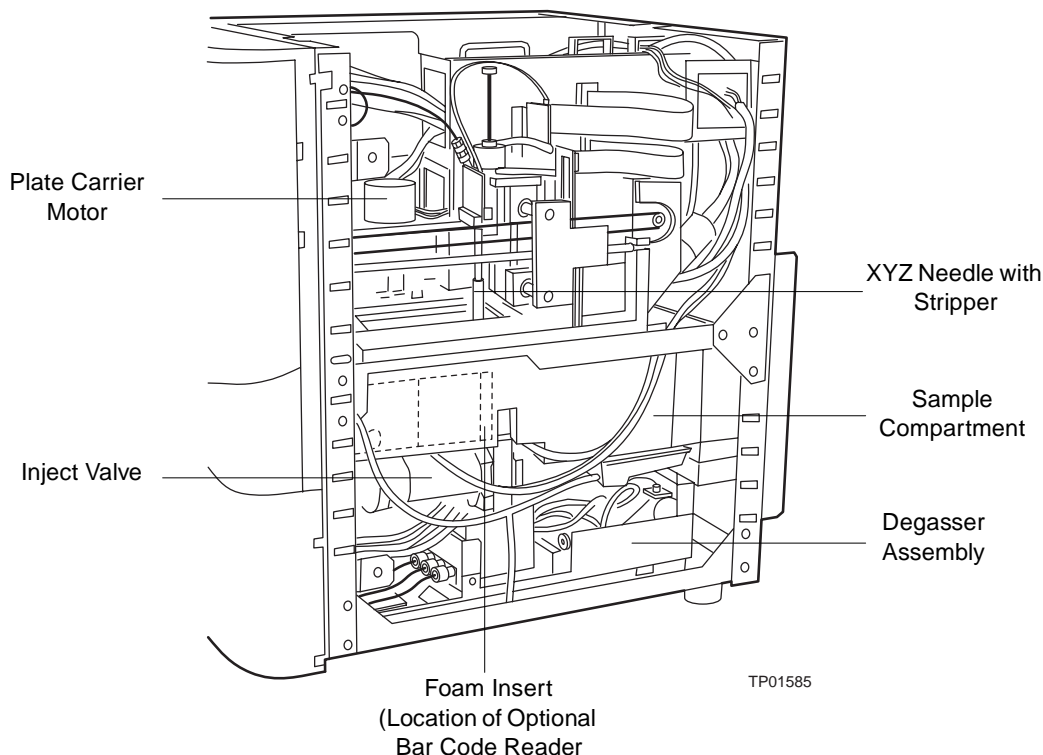
Figure 7-9 Sample Management System Components (Right-Side View)

Table 7-3 describes the functions of each assembly and component of the 2795 Separations Module sample management system.

Table 7-3 2795 Separations Module Sample Management System Assemblies/Components

Assembly/Component	Function
Syringe	Draws sample from the sample vial or well into the sample loop (see Figure 7-10).
Plate Carrier and Plates	Holds up to four plates of types that can hold 24, 48, 96, or 384 samples (see Figure 7-30).
Inject Valve	Accepts sample from the injector needle and switches the sample loop in and out of the high-pressure flow. When in the Prime position (see Figure 1-4), directs solvent flow to waste.

Table 7-3 2795 Separations Module Sample Management System
Assemblies/Components *(Continued)*

Assembly/Component	Function
Sample Loop	Holds up to one sample-loop volume (50 µL standard) of sample (Figure 7-24).
Holding Loop	Holds the sample before injection into the sample loop (Figure 7-23).
Needle Mounting Block	Contains sensors that track upward and downward movement of the injector needle (Figure 7-18).
Needle	Perforates sample plate film or pre-slit septa, draws sample, and injects the sample into the fluidic path through the inject port (see Figure 7-13).
Inject Port Seat	Seals needle to the inject port (see Figure 7-22).

7.3.2 Replacing the Syringe

STOP **Attention:** *To ensure accurate results, use only syringes that are approved by Waters for use in the 2795 Separations Module sample management system. See Appendix B, Spare Parts, for a list of Waters-approved syringes.*

STOP **Attention:** *Changing the syringe to one of a different size may also require changing the sample loop and/or holding loop. Refer to Table 7-5 for more information.*

Replace the syringe:

- If the syringe plunger tip becomes worn or discolored
- To change to another syringe size (100-, 250-, 500-, 1000-, or 2500-µL)

Required Material

- Replacement syringe

Removing the Syringe

STOP **Attention:** *Ensure that no injection is in progress or pending when you remove the syringe.*

To remove the syringe:

1. Open the syringe compartment door (see Figure 1-1).
2. Remove the knurled nut that holds the syringe barrel to the syringe mounting bracket (Figure 7-10).

3. Press the **Diag** screen key in the Main screen. The Diagnostics screen appears.

4. Press the **Sensors, Motors** screen key. The Sensors screen appears.

5. Press **Motors**. The Motors and Valves diagnostics screen appears (Figure 7-11).
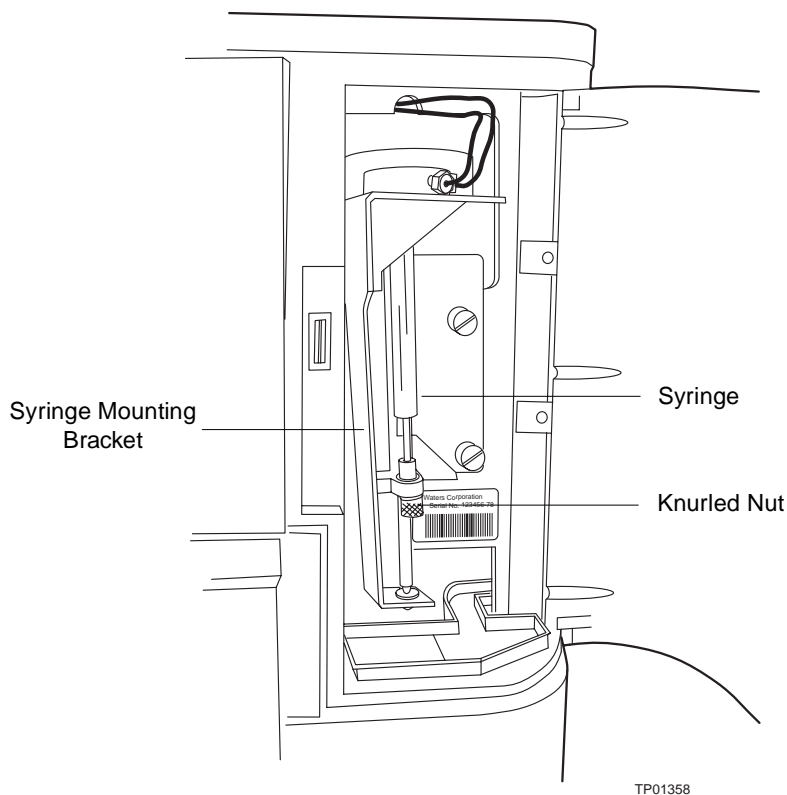
Figure 7-10 Syringe Assembly Components
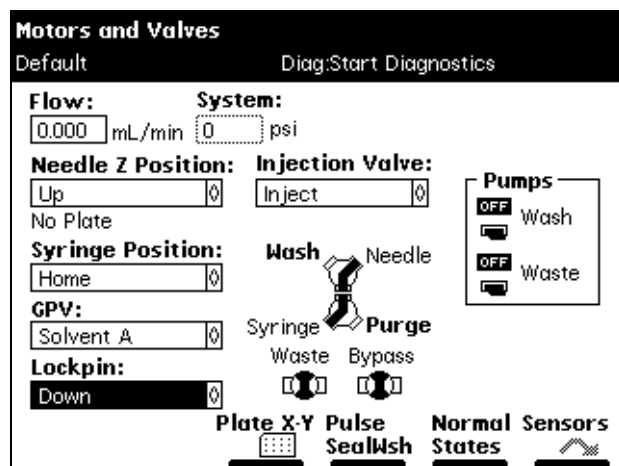
Figure 7-11 Motors and Valves Diagnostics Screen

6. Select the **Syringe Position** field, then press **Enter** to display the drop-down list.

7. Select **Full** from the drop-down list, then press **Enter**. With the knurled nut removed, the plunger tip remains up as the mounting bracket is lowered slowly.

8. When the mounting bracket is fully lowered, grasp the syringe barrel near the mounting bracket, then unscrew the syringe counterclockwise until it is released from the mounting bracket.

Installing a Syringe

If you need to install a syringe different in size from the original syringe, refer to Table 7-4 for available syringe sizes (and to Table B-2 for part numbers). Be sure to enter the new syringe size in the Configuration screen (see the "Modifying Syringe Configuration Parameters" discussion below).

Table 7-4 Syringe Sizes

Standard Size	Optional Sizes
500-μL	100-μL, 250-μL, 1000-μL, 2500-μL

To install a syringe:

1. Partially fill the new syringe (by hand) with purge solvent (to help remove air bubbles).

2. Screw the new syringe partially into the mounting bracket (Figure 7-12). Do not tighten.

3. Push the syringe barrel down so that the threaded end slides through the syringe guide mounting bracket.

4. Finger-tighten the syringe.

5. At the Motors and Valves diagnostics screen (Figure 7-11), select **Syringe Position**, then press **Enter** to display the drop-down list.

6. Select **Home**, then press **Enter**. The mounting bracket moves upward to allow you to install the knurled nut.

7. Press **Exit** to exit from the Motors and Valves screen.

8. Press **Exit** twice to exit from the Diagnostics screen.

9. Install and finger-tighten the knurled nut.

Syringe Assembly

TP01577
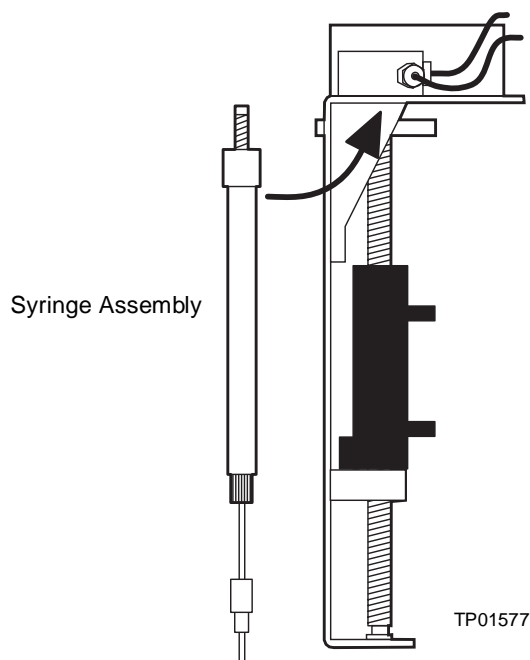
Figure 7-12 Installing the Syringe

Modifying Syringe Configuration Parameters

To configure the 2795 Separations Module for a syringe that is different in size from the original:

1. Press **Configure** in the Main screen to display the Configuration screen.

2. Select **Syringe Size**, select the appropriate syringe size from the drop-down list, then press **Enter**.

3. When you reconfigure the syringe size, you may need to change the syringe draw rate in your separations method.

4. Press **Exit** to return to the Main screen.

Refreshing the Syringe

After you install a syringe, refresh the syringe to remove any bubbles that may exist in the system. If you are priming the sample management system for the first time or changing the purge solvent, see Section 3.4.2, Refreshing the Syringe.

Note: Before refreshing the syringe, ensure the degasser is set to Continuous mode (see Section 3.3.2, Degassing Solvents).

To refresh the syringe:

1. Press the **Menu/Status** key to display the Status screen.
2. Press the **Direct Functions** screen key. The Direct Functions menu appears (Figure 4-4).
3. Select the **Refresh Syringe** option, then press **Enter**. The Refresh Syringe dialog box appears (Figure 4-10).
4. Enter the number of syringe strokes in the Syringe strokes field and the replacement volume in the Replacement volume of degassed solvent field. Start with **12** syringe strokes and **600** μL replacement volume. For a new (dry) instrument, see Section 3.4.2, Refreshing the Syringe.
5. Press **OK**. The Refresh Syringe cycle begins.

7.3.3 Replacing the Needle Assembly

Note: When you remove the top or right side cover of the 2795 Separations Module for maintenance of the sample management system, the XYZ assembly motors can operate only at very slow speeds. This allows you to safely observe the needle and holding loop. Normal operating speeds resume after you reinstall the top or right side cover.

⚠ *Caution: To prevent injury, keep hands or loose clothing clear of the needle assembly mechanism while it is in motion.*

Replace the needle assembly when:

- Contaminant peaks that appear in the chromatogram cannot be corrected by changing the wash solvent in the needle-wash system
- Chromatographic peak heights vary unexpectedly because of any of the following conditions:
  – Bent needle
  – Damaged needle tip
  – Plugged needle
  – Worn inject port seat (see Section 7.3.4, Replacing the Inject Port Seat)

Removing the Needle Assembly

STOP **Attention:** *To prevent damage to the needle during replacement, perform the Position Needle for Replacement procedure before you remove the needle assembly. See [Section 8.4.9, Changing the Needle](#).*

Required Materials

- Wrench, 5/16-inch (2)
- Screwdriver, 1/4-inch flat-blade, short
- Phillips head screwdriver, #2 regular

Procedure

To remove the needle assembly ([Figure 7-13](#)):

1. Power off the 2795 Separations Module.
2. Remove the column heater, then power on the 2795 Separations Module.
3. Remove the top and right side panel of the 2795 Separations Module.

TP01593
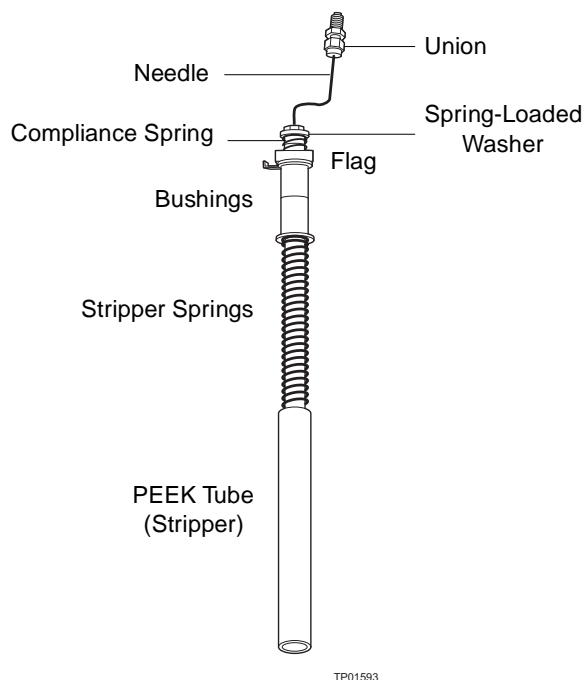
Figure 7-13 2795 Separations Module Needle Assembly

4. In the Main screen, press the **Diag** screen key, then press the **Other Diags** screen key. The Other Diagnostics screen (Figure 7-14) appears.
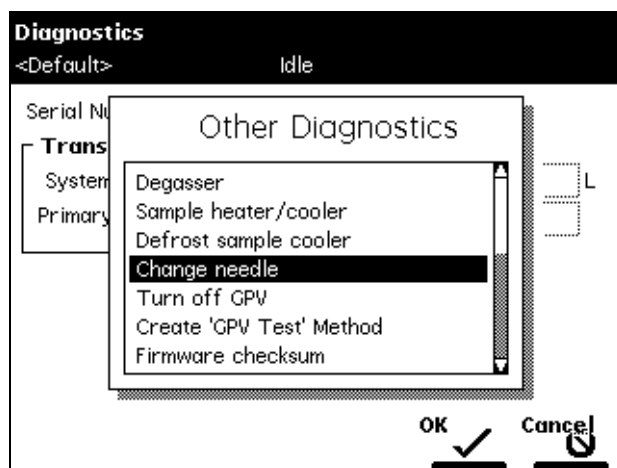
Figure 7-14 Other Diagnostics Screen

5. In the Other Diagnostics drop-down list, select **Change needle**. A message appears requesting that you remove the plates from the carrier.

6. After removing the plates from the carrier, press the **OK** screen key. The screen displays a message stating the needle is being positioned for replacement.

7. When the screen displays a message that the needle is accessible, disconnect the compression nut at the top of the needle assembly (Figure 7-15). This frees the holding loop.

STOP **Attention:** *Take care to avoid damaging the flex cable.*
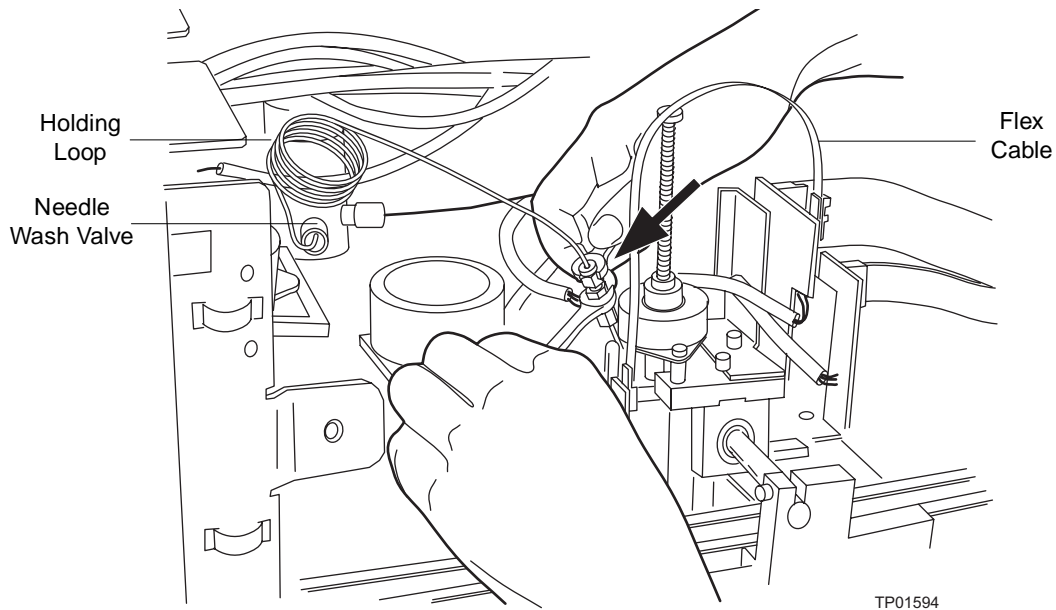
Figure 7-15 Freeing the Holding Loop from the Needle Assembly

8. Use a screwdriver to loosen the two captive screws from the needle holder block ([Figure 7-16](#)).

> ⚠ **Caution:** *To prevent injury or equipment damage, do not touch or press the end of the PEEK tube (stripper, [Figure 7-13](#)). Doing so breaks the needle free from the tubing and can cause a puncture wound..*

Needle Holder
Block

Flex Cable

Needle Mounting Block
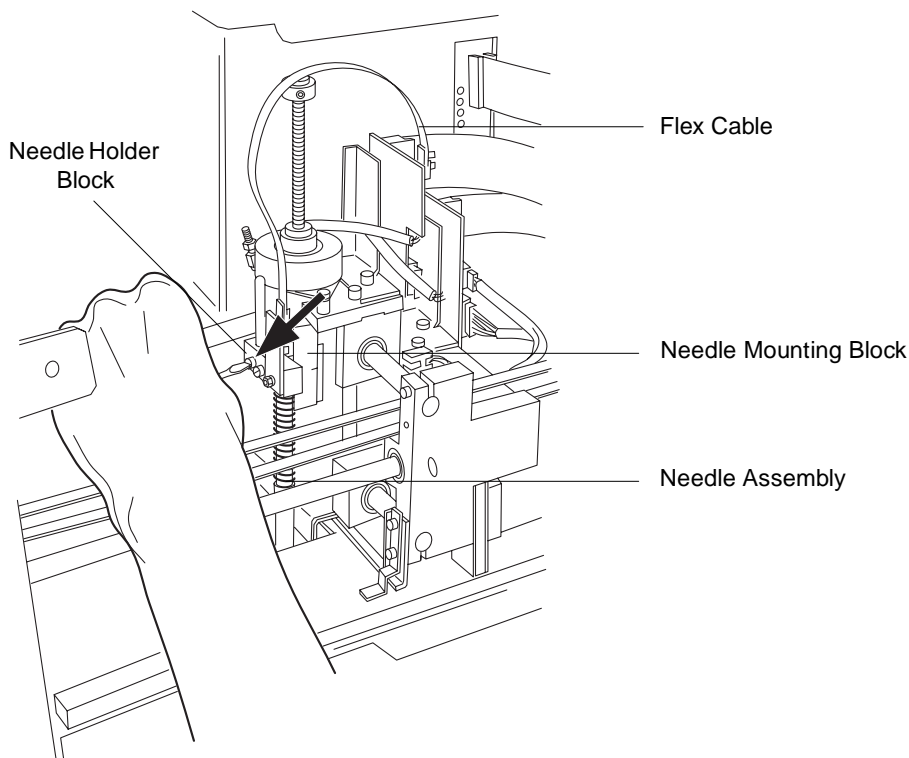
Needle Assembly

TP01595

Figure 7-16 Loosening Two Captive Screws in Needle-Holder Block

Installing the Needle Assembly

Required Materials

- 2795 Separations Module Needle and Housing Assembly (part number 700000388)
- Screwdriver, 1/4-inch flat-blade, short

Procedure

To install the needle assembly:

1. Grasp the new needle assembly below the bushings (but above the PEEK tube) on the assembly (Figure 7-13 and Figure 7-17).

2. Slide the spring-loaded washer under the "fingers" on the needle-mounting block (Figure 7-17). Make sure the flag (Figure 7-13 and Figure 7-17) on the replacement needle assembly is on the right side of the needle (as viewed from the front).

3. Move the replacement needle up slightly, then into the recess in the needle mounting block (Figure 7-18).

Maintenance   273
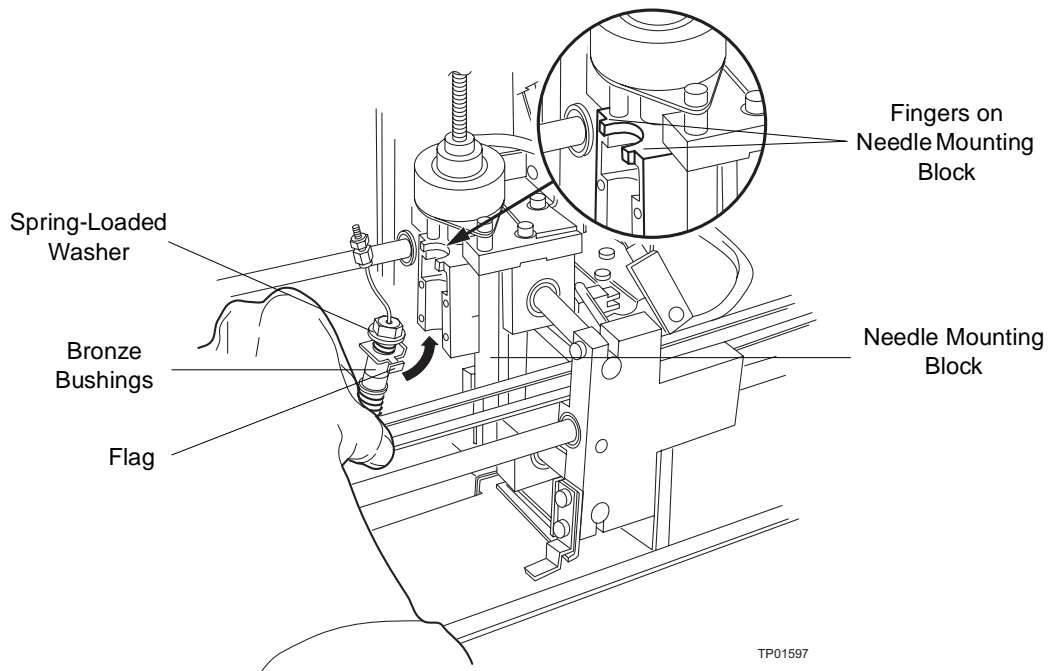
Figure 7-17 Preparing to Insert New Needle Assembly
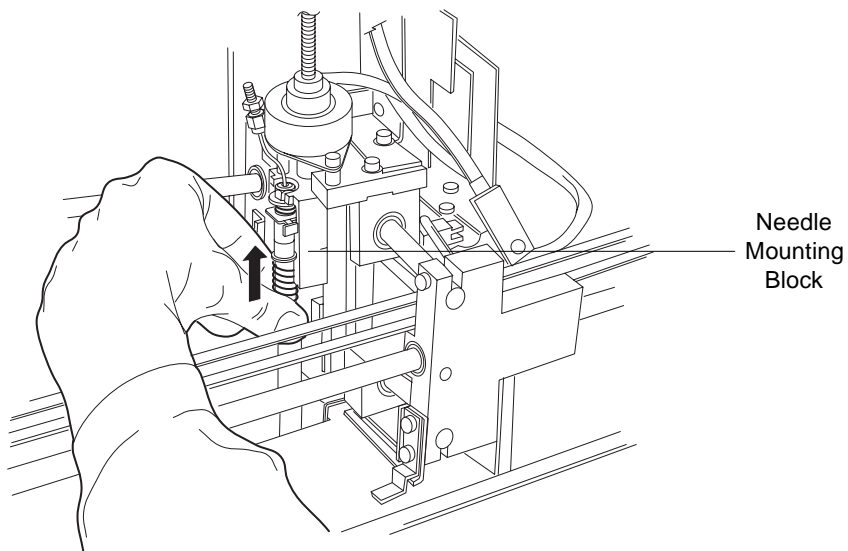
Figure 7-18 Inserting the New Needle into the Needle-Mounting Block

4. Reinstall the needle-holder block; tighten the screws until snug.

5. Reconnect the existing holding loop, or install a new one (see Section 7.3.5, Replacing the Holding Loop).

6. When you complete replacing the needle assembly, press the **OK** screen key to return to the Other Diags screen. The 2795 Separations Module displays a message indicating that it is rehoming the needle.

7.3.4 Replacing the Inject Port Seat

When you observe contaminant peaks that cannot be corrected by changing the wash solvent in the needle-wash system or chromatographic peak heights that vary unexpectedly, a worn inject port seat may be the cause (see Section 7.3.3, Replacing the Needle Assembly, for other possible causes).

Note: Waters recommends that you replace the inject-port-seal washer if it is leaking or blocking the inject port flow path. If you need to replace the inject-port-seal washer, replace it when you replace the inject port seat. See the "Replacing the Inject-Port-Seal Washer" discussion below.

Required Materials

- 2795 Separations Module Inject Port Seat (part number 700000384)
- 2795 Inject Port Seal Washer (part number 700000385)
- Wrench, 5/8-inch, open-end
- T-20 TORX wrench or driver
- Phillips head screwdriver, #2 regular
- Wrench, 1/4-inch, open-end
- Allen wrench, 3-mm

Removing the Inject Port Seat

To remove the inject port seat:

1. Power off the 2795 Separations Module and remove the column heater and right side panel.

2. Remove the foam insulation block (or the optional bar code reader and mounting plate) from the sample compartment (Figure 7-19).

    Note: When the optional bar code reader and its mounting plate are not installed, a foam block occupies the empty space.

3. Use the Allen wrench to loosen the two captive screws on the front panel, then remove the front panel (see Figure 7-25 and Figure 7-26).

Foam Insulation Block
(Removed) or Bar Code
Reader and Mounting Plate

Inject Valve Bracket
Mounting Screw

Rear Check Valve
Mounting Bracket

Inject Valve

TP01601
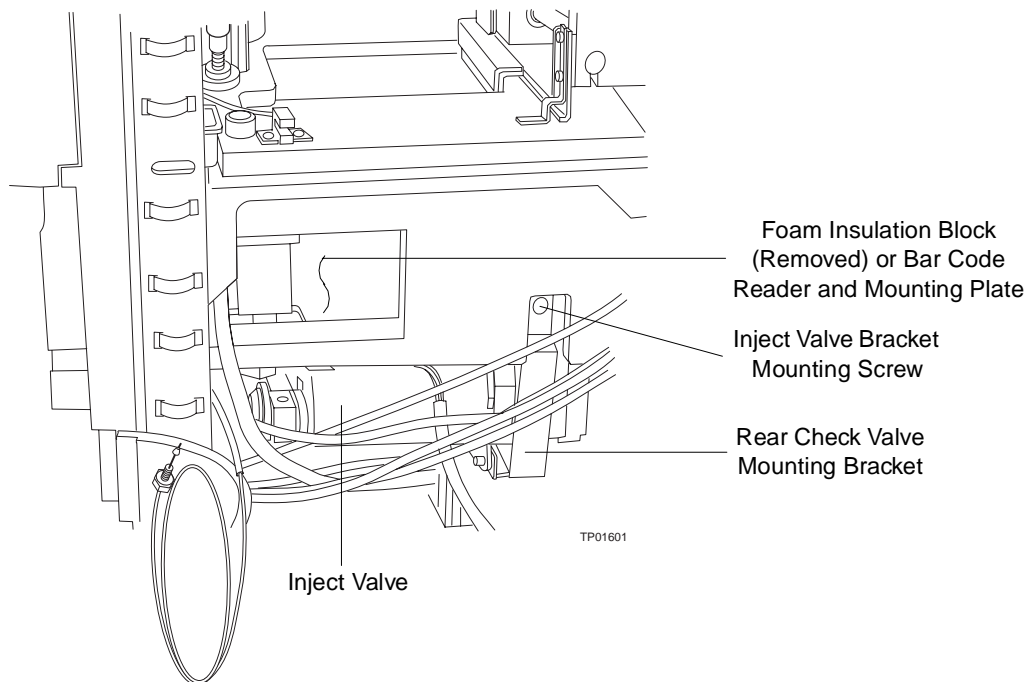
Figure 7-19 Right Side View of Sample Compartment with Foam Insulation Block Removed

4. Use the screwdriver to loosen the captive screw under the sample compartment, then remove the right front panel mounting bracket (Figure 7-20).

5. Use the 1/4-inch open-end wrench to remove the tubing connection from the #6 port on the inject valve (Figure 7-21).

6. Use the 5/8-inch open-end wrench to loosen (clockwise) the *reverse-threaded* nut under the needle-wash housing (Figure 7-20).

7. While holding the inject valve, use the T-20 Torx wrench to remove the inject valve bracket mounting screw at the rear of the valve (Figure 7-19), then lower the inject valve from the housing.

8. Remove the inject port seat from the inject port (Figure 7-22).

Captive Screw on
Right Front Panel
Mounting Bracket

TP01589
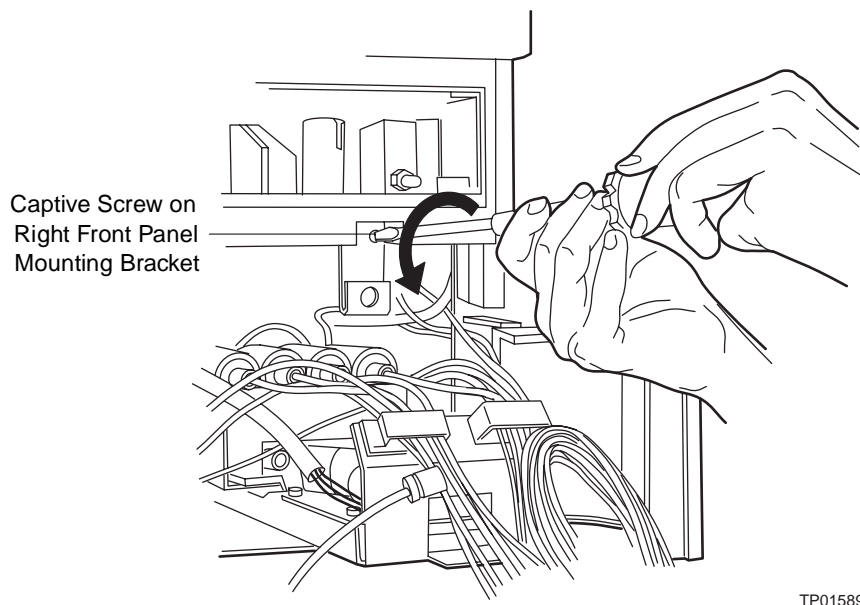
Figure 7-20 Loosening the Screw from the Right Front Panel Mounting Bracket

Turn Clockwise
to Loosen

Reverse-
Threaded Nut

Inject Valve

#6 Port Connection
(Shown Removed)

TP01640
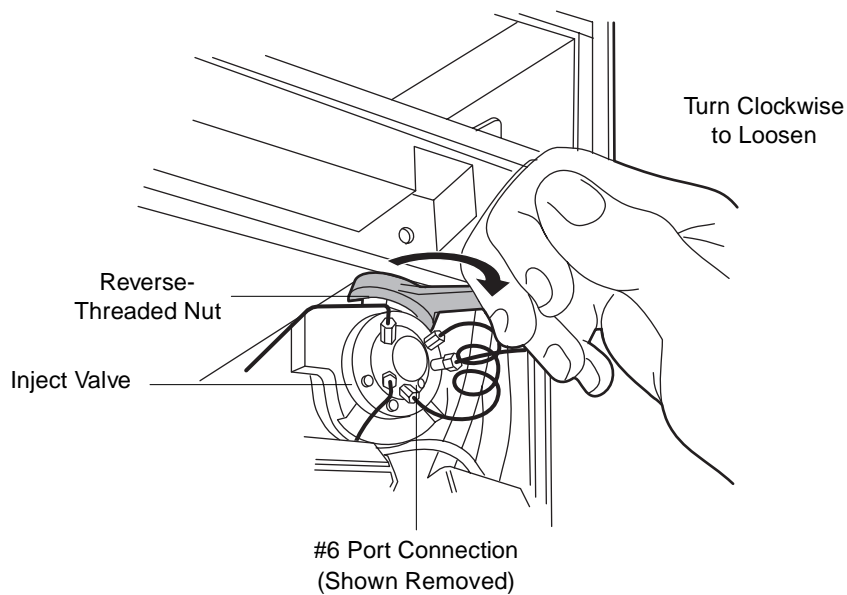
Figure 7-21 Loosening Reverse-Threaded Nut Under Needle-Wash Housing
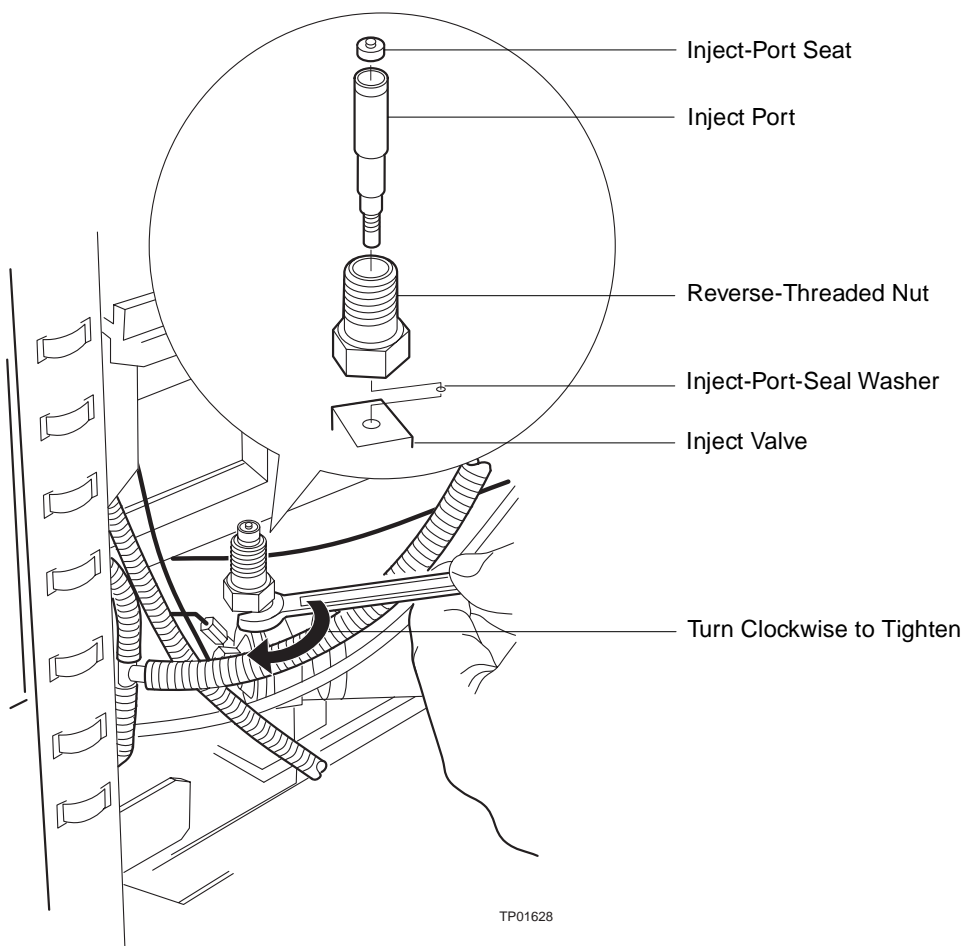
Figure 7-22 Removing the Inject-Port Seat and Inject-Port-Seal Washer from the Inject Port

Installing the Inject-Port Seat

Note: Before you install the inject-port seat, consider replacing the inject-port-seal washer (see the "Replacing the Inject-Port-Seal Washer" discussion below). Waters recommends replacing the inject-port-seal washer when you suspect it is leaking or blocking the inject port flow path.

To install the inject-port seat:

1. Place a new inject-port seat into the inject port (Figure 7-22).

2. Hold the inject valve level to reposition it under the sample compartment, then raise the valve and inject port into the needle-wash housing.

3. While holding the valve and inject port with one hand, thread the reverse-threaded nut up into the wash housing until finger-tight. Use the 5/8-inch open-end wrench to tighten the nut by turning counterclockwise. Do not overtighten.

4. While continuing to hold the inject valve, reinstall the valve bracket mounting screw (Figure 7-19).

5. Use the 1/4-inch wrench to reconnect the tubing to the #6 port on the inject valve.

6. Reinstall the foam insulation block or the bar code reader, as applicable.

7. Reinstall the front panel mounting bracket and the front panel.

8. Reinstall the side panel and the column heater.

9. Power on the 2795 Separations Module.

Replacing the Inject-Port-Seal Washer

Note: Waters recommends replacing the inject-port-seal washer when you suspect it may be leaking or blocking the inject port flow path.

Required Materials

- Wrench, 3/16-inch open-end
- Dental pick or sharp tweezers
- Inject-port-seal washer (see Appendix B, Spare Parts)

Procedure

To remove and replace the inject-port-seal washer:

1. Follow the instructions to remove the inject-port seat above.

2. When you remove the inject valve from the 2795 Separations Module:

   a. Use the 3/16-inch wrench to loosen the inject port, then remove it from the inject valve (Figure 7-22).

   b. Use the dental pick or tweezers to remove the old inject-port-seal washer from the bottom of the inject valve body.

   c. Use your fingers (or a soft-tipped tool) to insert the new seal washer carefully into the inject valve body.

   Note: Inspect carefully to ensure that the washer sits flat on the bottom of the inject valve body cavity.

   d. Reinstall the inject port and inject-port seat as instructed in the "Installing the Inject-Port Seat" discussion above.

7.3.5 Replacing the Holding Loop

STOP *Attention: To avoid sample carryover or contamination of solvent, Waters recommends that you replace the holding loop and sample loop at the same time and according to the size compatibility information provided in Table 7-5.*

Note: To reduce cycle time and sample size, and to optimize accuracy, ensure that the total sample volume never exceeds two-thirds the capacity of the holding loop.

Table 7-5 Recommended Syringe, Sample, and Holding Loop Combinations

Syringe Volume (µL)	Sample Loop Volume (µL)	Holding Loop Volume (µL)	Holding Loop Color	Purge Solvent (Replacement) Volume (µL)	Cycles (# of Syringe Strokes)[a]
500 (standard)	5 20 50	128 128 128	Orange Orange Orange	300 or 600 300 or 600 300 or 600	1 or 2 1 or 2 1 or 2
100	5 20	128 128	Orange Orange	300 or 600 300 or 600	5 or 10 5 or 10
250	5 20	128 128	Orange Orange	300 or 600 300 or 600	2 or 4 2 or 4
1000	5 20 50 100	128 128 128 500	Orange Orange Orange Red	600 600 600 1200	1 1 1 2
2500	5 20 50 100 500 2000	128 128 128 500 3000 3000	Orange Orange Orange Red Tan Tan	600 600 600 1500 6000 6000	<1 <1 <1 1 4 4

a. Entering a purge volume (Replacement Volume of Degassed Solvent) of 600 µL in the Autosampler Wash screen when you are editing a separation method (see Section 5.2.3, Setting Autosampler Parameter Values) thoroughly flushes the needle and holding loop with fresh, degassed purge solvent. However, to minimize cycle time, you can enter 300 µL in the Replacement Volume field because 300 µL provides acceptable injection volume repeatability and reduces cycle time by approximately 15 seconds.

Required Materials

- Wrench, 5/16-inch, open-end (2)
- Screwdriver, 1/4-inch flat-blade, short
- Holding loop (see Table 7-5)

Procedure

To remove and replace the holding loop:

1. Power off the 2795 Separations Module.

2. Remove the column heater, and the top cover and right side panel of the 2795 Separations Module and the bar code reader, if installed.

3. Use two 5/16-in. wrenches to loosen the stainless steel compression nut at the top of the needle assembly (Figure 7-15).

4. Unscrew the plastic compression screw from the Needle Wash valve (see Figure 7-15), then remove the holding loop (Figure 7-23).

TP01591
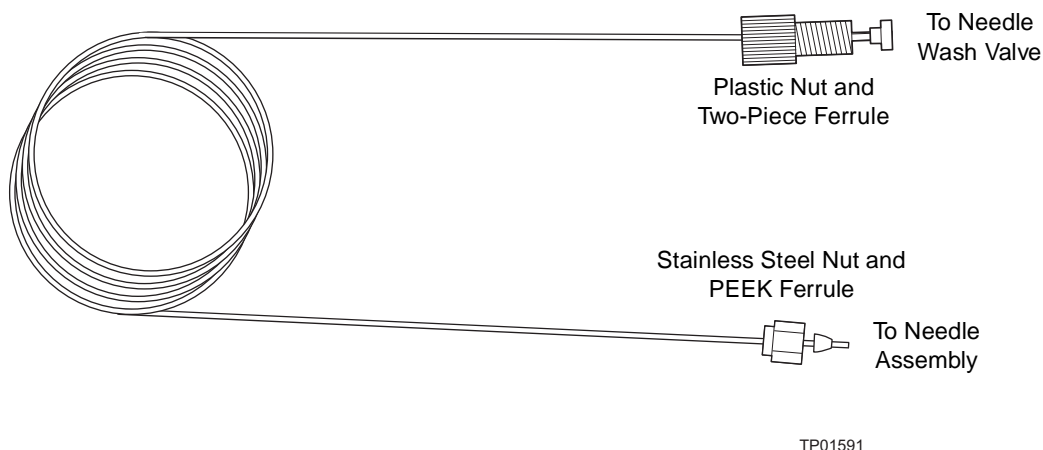
Figure 7-23 Holding Loop (Assembled)

5. Insert the new holding loop (with plastic compression screw and two-piece ferrule) into the Needle Wash valve (see Figure 7-15), then finger-tighten.

6. Remove the plastic cap from the other end of the new holding loop, then use the stainless steel nut to connect the loop end to the stainless steel union at the top of the needle assembly.

7. Use the two 5/16-inch wrenches to tighten the union until snug. Do not overtighten.

7.3.6 Replacing the Sample Loop

STOP ***Attention:*** *To avoid sample carryover or contamination of solvent, Waters recommends that you replace the holding loop and sample loop at the same time and according to the information provided in Table 7-5.*

TP01590
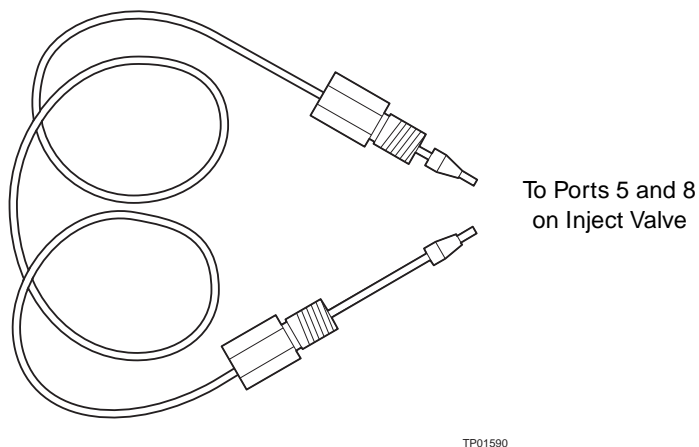
Figure 7-24 Sample Loop (Assembled)

Required Materials

- Allen wrench, 3-mm
- Wrench, 1/4-inch, open-ended
- T-20 TORX wrench or driver
- Stainless steel sample loop and fittings

Procedure

To remove and replace the sample loop:

1. Open the solvent delivery and solvent conditioning tray access doors (Figure 7-25).
2. Use the Allen wrench to loosen the two front panel captive screws and remove the front panel (Figure 7-25 and Figure 7-26).

To Ports 5 and 8
on Inject Valve
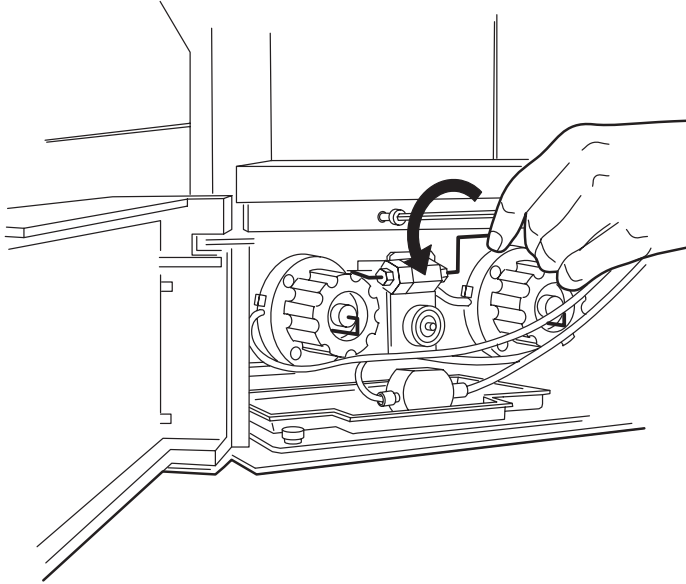
TP01588

Figure 7-25 Loosening the Front Panel Screws
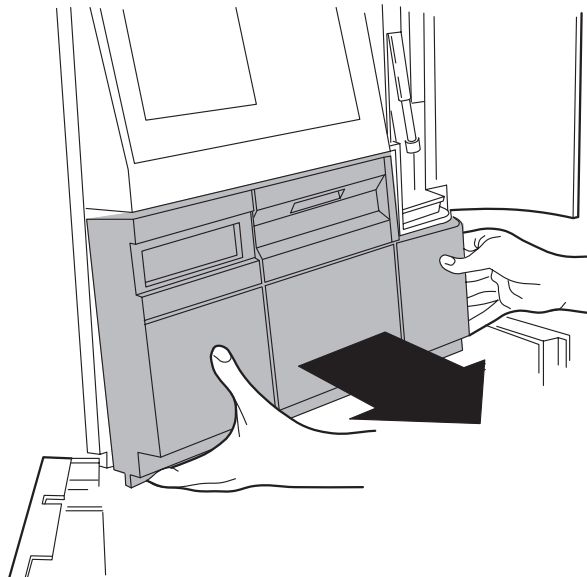
TP01587

Figure 7-26 Removing the Front Panel

3. Use the T-20 Torx wrench to loosen the captive screw securing the holding bracket on the right side of the sample compartment (Figure 7-20), then remove the bracket.

4. Use the wrench to remove the fittings on the #5 and #8 ports of the inject valve (Figure 7-27).

5. Remove the sample loop and its fittings (Figure 7-24).

6. Carefully remove the replacement sample loop and fittings from the packaging.

7. Slide a ferrule and compression screw onto one end of the sample loop as shown in Figure 7-24, then seat the end into the #5 inject valve port.

8. Tighten the fitting with the wrench until snug.

9. Repeat steps 7 and 8 for the other end of the sample loop and for the #8 inject valve port (see Figure 7-27).

10. Reinstall the holding bracket and tighten the captive screw until snug.

11. Reinstall the front panel (open the syringe door when you reinstall the front panel).

STOP *Attention: Ensure the top cover and side panel are attached before you apply power to the 2795 Separations Module.*
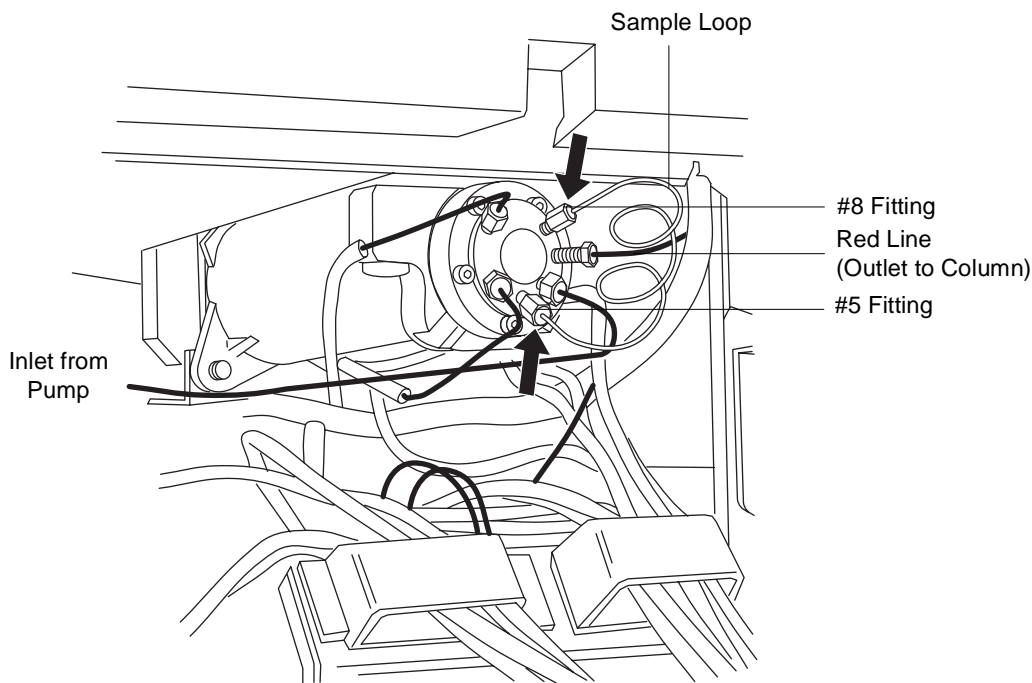
Figure 7-27 Sample Loop Fittings on the Inject Valve

7.3.7 Cleaning the Sample Compartment and Plate Carrier

Clean the sample compartment and plate carrier when:

- The sample plates do not rotate properly
- The plate carrier does not engage
- A spill occurs

Required Materials

- Allen wrench, 3-mm
- Non-abrasive cleaner

Accessing the Sample Compartment

To access the sample compartment:

1. Open the sample compartment door (Figure 7-28).
2. Use the screen keys in the Door Is Open dialog box to move the plate carrier to the #1 (blue) position (see Section 3.4.3, Loading the Sample Plates).

TP01600
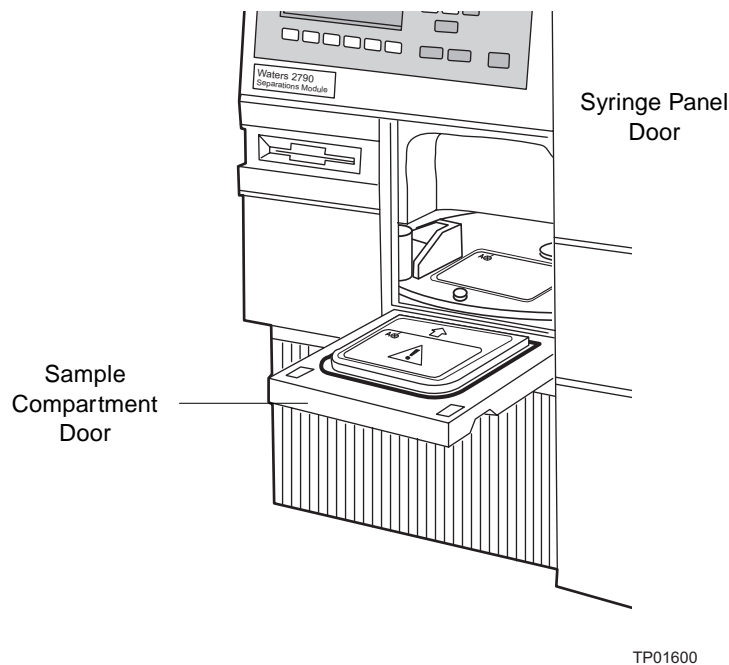
Figure 7-28 Sample and Syringe Compartment Doors

3. In the Main screen, press the **Diag** screen key. The Diagnostics screen appears (Figure 8-2).

4. Press the **Sensors, Motors** screen key. The Sensors screen appears (Figure 8-3).

5. Press the **Motors** screen key. The Motors and Valves screen appears (Figure 8-4).

6. Select the **Lockpin** field, then press **Enter** to display the drop-down list. Select **Up** from the drop-down list.

7. Open the solvent delivery tray and solvent conditioning tray access doors (Figure 7-29).

8. Use the Allen wrench to loosen the two front panel captive screws (Figure 7-28), then remove the front panel (see Figure 7-25 and Figure 7-26).
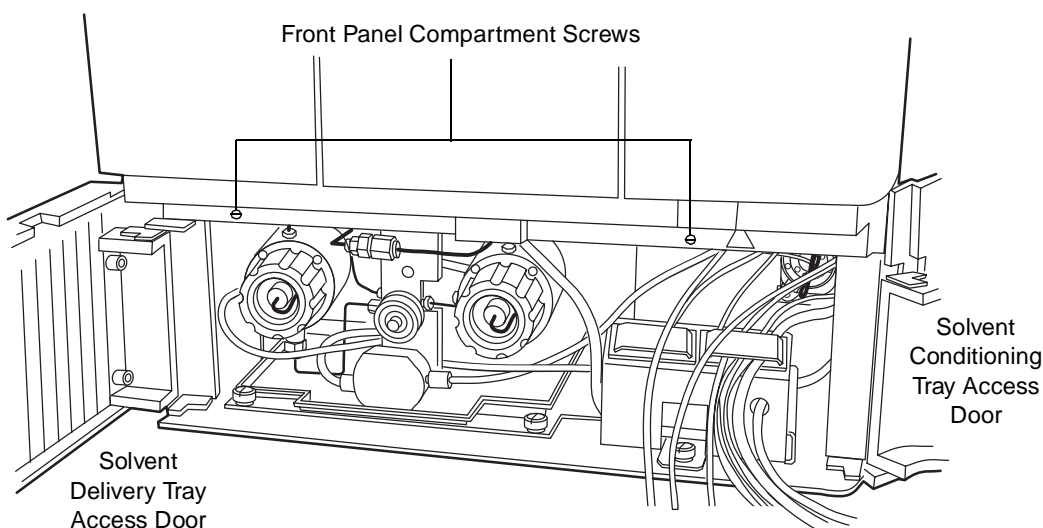
Figure 7-29 Accessing the Sample Compartment

Cleaning the Sample Compartment and Carrier

⚠ **Caution:** *To prevent injury to yourself or damage to the equipment, do not bend or detach the Plate Positioner Spring located in the rear of the sample compartment (Figure 7-30).*

To clean the sample compartment and carrier:

1. Push down about 1/4-inch on the spring-retaining pin in the center of the plate carrier to disengage the carrier from the drive shaft (Figure 7-30).
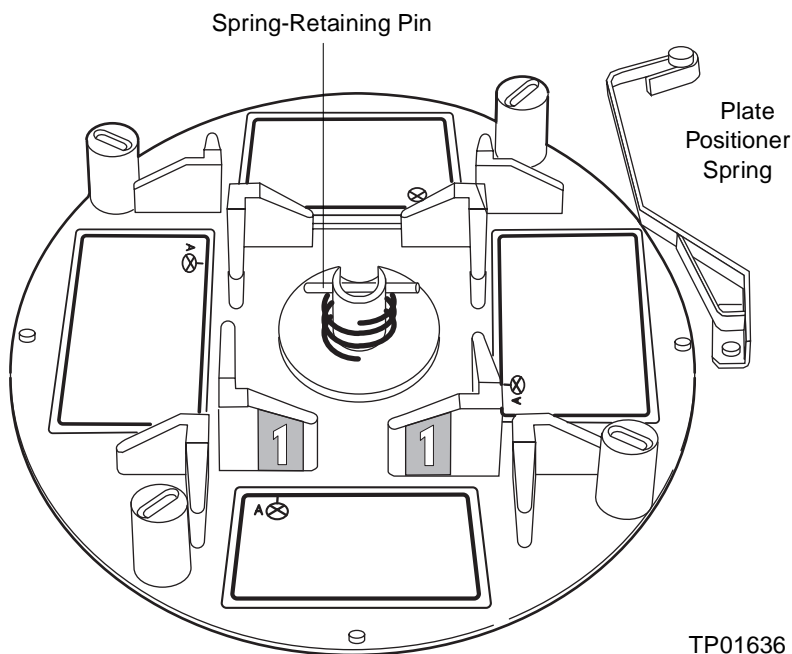
Figure 7-30 Sample Plate Carrier

2. While lifting the front edge of the plate carrier slightly, slide the carrier toward you and remove the carrier.

3. Using a nonabrasive cleaner, clean and then dry the carrier and the carrier floor plate (which remains attached to the floor of the sample compartment).

4. Position the plate carrier so the #1 (blue) position is facing you (see).

5. Reinstall the carrier by sliding the carrier into the sample compartment while pushing down about 1/4 inch on the spring-retaining pin in the center of the carrier.

6. Rotate the carrier a little in both directions to ensure the pin is engaged in the slot in the drive shaft.

7. Using the Allen wrench, reinstall the sample compartment front panel.

8. In the Motors and Valves diagnostics screen, select the **Lockpin** field, press **Enter** to display the drop-down list, then select **Down** from the drop-down list.

9. Press **Exit** to exit from the Motors and Valves screen. Press **Exit** again to return to the Diagnostics screen.

Chapter 8
Diagnostics and Troubleshooting

This chapter describes how to find the source of and fix problems you may encounter with the 2795 Separations Module. This chapter covers:

- Safety and handling
- Using the error log
- Performing diagnostics
- Troubleshooting

Note: *For information about startup diagnostics, see the "Startup Diagnostics" discussion in Chapter 3, Setting Up the 2795 Separations Module.*

Proper Operating Procedures

To keep your 2795 Separations Module running smoothly, follow the operating procedures and guidelines described in Chapter 4, Operating the 2795 Separations Module from the Front Panel, Chapter 5, Creating and Editing Separation Methods and Sample Sets, and Chapter 6, Making Automatic Runs and Setting Up for High-Throughput Operation.

Spare Parts

Refer to Appendix B, Spare Parts, for spare parts information. Parts not included in Appendix B are not recommended for replacement by the customer.

Contacting Waters Technical Service

If you encounter any problems performing diagnostics or troubleshooting the 2795 Separations Module, contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

8.1 Safety and Handling

When you troubleshoot the 2795 Separations Module, keep in mind the following safety considerations:

STOP *Attention: To prevent circuit damage due to static charges, do not touch integrated circuit chips or other components that do not specifically require manual adjustment.*

Caution: To avoid the possibility of electric shock, never disconnect an electrical assembly while power is applied to the 2795 Separations Module. After power is turned off, wait about 10 seconds before you disconnect an assembly.

Caution: To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the 2795 Separations Module. Know the physical and chemical properties of the solvents you use. Refer to the Material Safety Data Sheets for the solvents in use.

Caution: To avoid the possibility of electric shock, do not open the power supply cover. The power supply does not contain user-serviceable parts.

8.2 Using the Error Log

The Error Log records:

- The date and time of each power on and power off
- System errors and warnings generated by the 2795 Separations Module

To access the Error Log:

1. Press the **Log** screen key in the Main screen.

   The Error Log screen (Figure 8-1) displays the most recent 10 messages in chronological order with the most recent message at the bottom of the screen. Use the keypad arrow keys (see Section 3.1.2, Using the Keypad) to view earlier messages. The Error Log saves the most recent 100 messages.
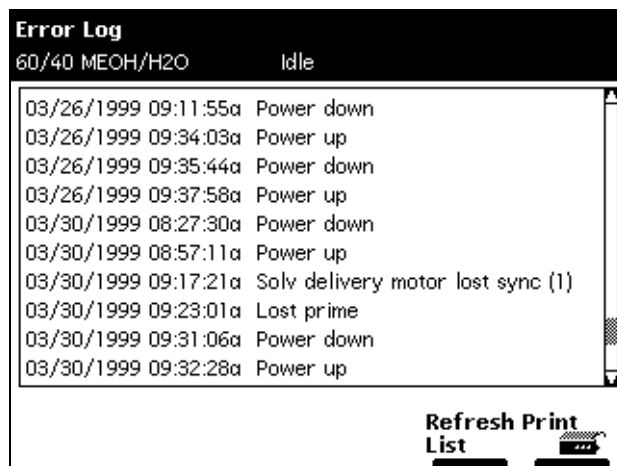
Figure 8-1 Error Log Screen

2. Press the **Refresh List** screen key to display the most recently generated messages.

3. Press the **Print** screen key to copy the error log to a floppy disk, or print the error log to a serial printer.

Note: When you copy the error log to a floppy disk, you must provide a filename.

Table 8-12 provides a list of some of the most commonly encountered 2795 Separations Module error messages.

8.3 Performing Main Diagnostics

To perform user-initiated diagnostics:

1. Press the **Diag** screen key in the Main screen. The Diagnostics screen appears (Figure 8-2) 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 Section 7.1, Maintenance Considerations, the "Recommended Preventive Maintenance Schedule" discussion).

```
Diagnostics
<Default>                    Idle

 Serial Number: [C99SM9000N      ]
 ┌ Transducers ──┐ Lifetime Counters:
 │ System: [0   ] psi │ Eluent delivered = [0.0   ] L
 │ Primary: [0   ] psi │ Injection count =  [1   ]
 └─────────────┘




 Prime    Prime    Service  Sensors,  Other
 SealWsh  NdlWsh   Utilities Motors   Diags
 ▄▄▄▄▄    ▄▄▄▄▄    ▄▄▄▄▄    ▄▄▄▄▄    ▄▄▄▄▄
```

Figure 8-2 Diagnostics Screen

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

Table 8-1 describes the functions of the screen keys in the main Diagnostics screen.

Table 8-1 Diagnostics Screen Keys

Screen Key	Function	Reference
Prime SealWsh	Primes the solvent management system plunger-seal-wash pump	Section 8.3.1, Priming the Plunger-Seal-Wash Pump
Prime Ndl Wash	Primes the sample management system needle wash valve and tubing	Section 8.3.2, Priming the Needle-Wash Pump
Service Utilities	Displays a password-protected list of service diagnostics	Section 8.3.3, Service Utilities Diagnostics
Sensors, Motors	Displays the Sensors Diagnostics screen, with direct access to the Motors and Valves screen	Section 8.3.4, Sensors Diagnostics and Section 8.3.5, Motors and Valves Diagnostics
Other Diags	Displays the Other Diagnostics screen, which lists other hardware-specific tests	Section 8.4, Performing Other Diagnostics

The following sections describe the diagnostics accessed from the Diagnostics screen keys.

Note: Priming the plunger-seal-wash pump and priming the needle-wash pump are procedures, not diagnostics, that you access from the Diagnostics screen.

8.3.1 Priming the Plunger-Seal-Wash Pump

The Prime Plunger-Seal-Wash diagnostic procedure automatically primes the solvent management system plunger-seal-wash pump. Perform this test when:

- There is a lack of flow in the plunger-seal-wash system
- You are changing the seal-wash solvent

For information on performing this procedure, see Section 3.3.4, Priming the Plunger-Seal-Wash Pump.

8.3.2 Priming the Needle-Wash Pump

The Prime Needle Wash Pump diagnostic procedure primes the needle wash system. Perform this procedure when:

- There is a lack of flow in the needle-wash system
- You change the needle-wash solvent

For information on performing this procedure, see Section 3.4.1, Priming the Needle-Wash Pump.

8.3.3 Service Utilities Diagnostics

Service Utilities diagnostics are for use by Waters Field Service Engineers only and are password protected.

8

8.3.4 Sensors Diagnostics

The Sensors diagnostics screen displays the current position of the digital and other sensors for the 2795 Separations Module.

To display the Sensors diagnostics:

1. In the Diagnostics screen, press the **Other Diags** screen key.
2. In the Other Diagnostics screen, press the **Sensors, Motors** screen key. The Sensors Diagnostics screen appears (Figure 8-3) indicating the current position of the:
   - **Inject Valve** – The word "Inject", "Load", or "Prime" is displayed in the pane with sensor codes of 0, 1, 2, and 3. The four sensors form a binary code that identifies the current location of the inject valve. For example, if the sensors are read left to right as Off, Off, Off, On (or 0001), this indicates the Inject Valve is in position 1, the Inject position. A sensors condition exists if no position (0, 1, 2, or 3) for the Inject Valve is indicated (see Table 8-12).
   - **Carrier** – Position, Home, Lockpin
   - **Autosampler** – Needle top, Needle bottom, Syringe empty

In addition, the other sensors indicate:

- **Door Open** – Sample compartment door is open
- **Interlock** – Safety interlock is engaged
- **X home** – Needle is in the X home position
- **Y home** – Needle is in the Y home position
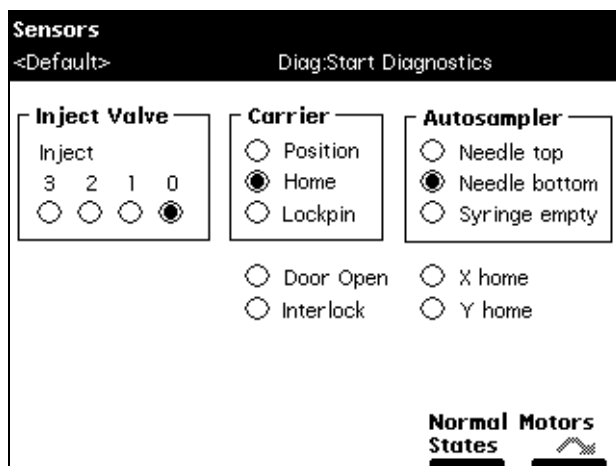
Figure 8-3 Sensors Diagnostics Screen

3. To display the normal (default) states for all three sensors, press the **Normal States** screen key.

   To display the Motors Diagnostics screen, press the **Motors** screen key.

4. Press **Exit** to return to the Other Diagnostics screen.

8.3.5 Motors and Valves Diagnostics

The Motors and Valves diagnostics screen allows you to manually control and check the status of:

- Motors:
  - Needle drive (4 positions)
  - Syringe drive (3 positions)
  - Lockpin (2 positions)
- Solenoid valves:
  - Gradient proportioning valve (GPV)
  - Wash valve (3-way)
  - Purge valve (3-way)

– Waste valve (2-way)

– Bypass valve (2-way)

• Pumps

– Needle wash

– Drain

The Motors and Valves diagnostics screen also displays the system pressure and allows you to change the flow rate without returning to the Configuration screen.

To access the Motors and Valves diagnostics:

1. In the Diagnostics screen, press the **Other Diags** screen key.

2. In the Other Diagnostics screen, press the **Sensors, Motors** screen key.

3. Press the **Motors** screen key to display the Motors and Valves diagnostics screen (Figure 8-4).
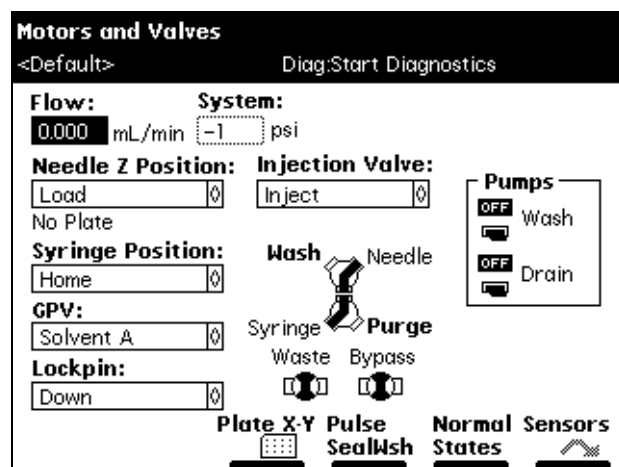
Figure 8-4 Motors and Valves Diagnostics Screen

Controlling Motors

To control the position of the needle or syringe:

1. In the Motors and Valves diagnostics screen, select the motor position parameter (Needle-Z Position or Syringe Position) that you want to change.

2. Press **Enter** to display a drop-down list of parameter values (Table 8-2 and Table 8-3).

3. Select the needle and/or syringe positions, then press **Enter** to save your selection.

Table 8-2 and Table 8-3 describe the position selections available for the needle (injector) and the syringe motors.

Table 8-2 Needle (Injector) Z Motor Position Values

Needle Z Motor Position	Description
Up	Needle is raised to maximum height (home).
Well	Needle tip is at the level of the bottom of the well.
Load	Needle is moved to inject port seat until needle bottom is sensed.
Wash	Needle tip is 1/8-inch above the inject port seat.

Table 8-3 Syringe Motor Position Values

Syringe Motor Position	Description
Empty	Syringe is in the highest position to purge the syringe.
Home	Syringe is in the home (default) position (approximately 20% full).
Full	Syringe is in the lowest permitted position (80% full) to draw in sample.

4. Press the **Normal States** screen key to return the motors and valves to their Idle (default) positions.

Controlling the Injection Valve

To control the position of the injection valve:

1. In the Motors and Valves diagnostics screen, select the **Injection Valve** position parameter.

2. Press **Enter** to display a drop-down list of Injection Valve parameter values.

    Table 8-4 describes the values available in the drop-down list.

3. Select the injection valve position, then press **Enter** to save your selection.

Table 8-4 Injection Valve Position Values

Inject Valve Position	Description
Inject	Inject sample position
Load	Load sample position
Prime	Prime needle wash position

8

4. Press the **Normal States** screen key to return the motors and valves to their Idle (default) positions.

Controlling the Gradient Proportioning Valve

To control the Gradient Proportioning valve (GPV):

1. In the Motors and Valves diagnostics screen, select the **GPV** field.
2. Press **Enter** to display a drop-down list of valve positions.

   Table 8-5 describes the valve positions available in the GPV drop-down list.
3. Select the GPV position, then press **Enter** to save your selection.

Table 8-5 Gradient Proportioning Valve (GPV) Position Parameters

GPV Position	Description
Off	GPV closed (all solvent positions closed)
Solvent A	GPV selecting solvent from reservoir A
Solvent B	GPV selecting solvent from reservoir B
Solvent C	GPV selecting solvent from reservoir C
Solvent D	GPV selecting solvent from reservoir D

4. Press the **Normal States** screen key to return the motors and valves to their Idle (default) positions.

Controlling the Lockpin Position

To control the position of the carrier plate lockpin:

1. In the Motors and Valves diagnostics screen, select the Lockpin position parameter.
2. Press **Enter** to display a drop-down list of lockpin parameter values.
3. Select either **Up** or **Down**, then press **Enter**.
4. Press the **Normal States** screen key to return the motors and valves to their Idle (default) positions.
5. Press the **Sensors** screen key to return to the Sensors Diagnostics screen, then press **Exit** to return to the Other Diagnostics screen.

Pulsing the Seal Wash

To pulse the solvent management system seal wash, press the **PulseSealWsh** screen key in the Motors and Valves diagnostics screen. The solvent management system seal wash pulses once. To prime the seal wash, see Section 8.3.1, Priming the Plunger-Seal-Wash Pump.

8

Controlling the Wash, Purge, Waste, and Bypass Valves

The Wash and Purge valves are 3-way valves that control the flow of solvent to the needle and syringe.

The Waste and Bypass valves are 2-way valves that control the solvent flow to waste, either directly or using the bypass valve (see Figure 1-4).

To change the positions of the wash, purge, waste, and bypass valves:

1. In the Motors and Valves diagnostics screen, use the arrow keys to select the Wash, Purge, Waste, or Bypass valve. A dotted line surrounds the selected valve (Figure 8-5).

2. Use any key to change the position of the valve from open to closed, or vice versa. Press any key again to return the valve to its original position. Figure 8-5 shows the Wash and Purge valves in three different operational positions, and the Waste and Bypass valves open and closed.

3-Way Valves with Flow from Syringe to Needle (Normal Operating Position)

3-Way Valves with Flow from Wash Solvent to Needle (Needle Wash Position)

3-Way Valves with Flow from Purge Solvent to Syringe (Refresh Syringe Position)

Waste Valve Open

Bypass Valve Open
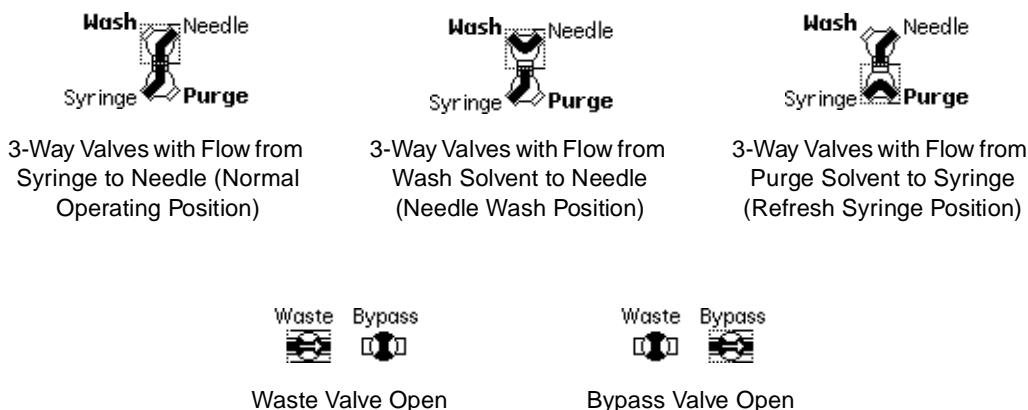
Figure 8-5 2795 Separations Module Valve Positions

8.4 Performing Other Diagnostics

Additional diagnostics tests are available in the Other Diagnostics screen. Some tests in the Other Diagnostics drop-down list are specific to the options installed on the 2795 Separations Module.

To access the Other Diagnostics screen:

1. In the Main screen, press the **Diag** screen key, then press **Other Diags**. The Other Diagnostics screen is displayed (Figure 8-6).
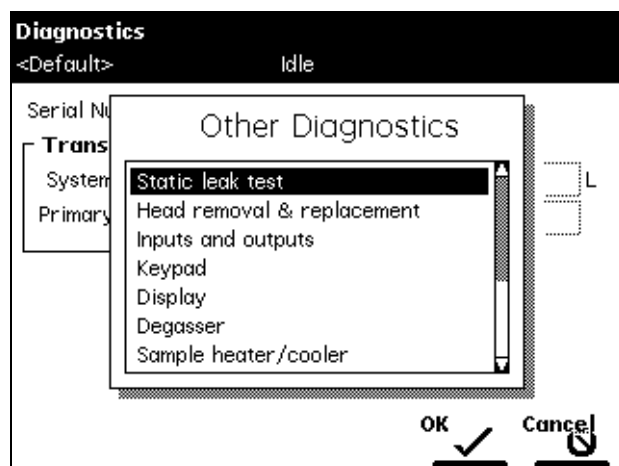
Figure 8-6 Other Diagnostics Screen

Table 8-6 describes the tests available in the Other Diagnostics screen drop-down list.

Table 8-6 Other Diagnostics

Test Name	Function	Reference
Static leak test	Tests for leaks in all the seals and fittings in the high-pressure part of the solvent management system.	Section 8.4.1, Performing the Static Leak Test
Head removal and replacement	Moves either solvent management system plunger into its most forward position to allow for removal of the head and replacement of the plunger and seal-wash assembly.	Section 8.4.2, Performing the Head Removal and Replacement Utility
Inputs and outputs	Displays the current status of the I/O terminals.	Section 8.4.3, Performing the Input and Output Diagnostics
Keypad	Tests the keys on the front panel keypad.	Section 8.4.4, Performing the Keypad Diagnostic
Display	Tests the front panel display function.	Section 8.4.5, Performing the Display Diagnostic
Degasser	Tests the inline degasser module.	Section 8.4.6, Performing the Inline Degasser Diagnostic

Table 8-6 Other Diagnostics *(Continued)*

Test Name	Function	Reference
Sample heater/cooler	Tests the heater/cooler module.	Section 8.4.7, Performing the Sample Heater/Cooler Diagnostic
Defrost sample cooler	Defrosts the heater/cooler module.	Section 8.4.8, Performing the Defrost Sample Heater/Cooler Procedure
Change needle	When you replace the needle assembly, positions the needle properly and rehomes the needle.	Section 8.4.9, Changing the Needle
Column heater	Tests the column heater module.	Section 8.4.10, Performing the Column Heater Diagnostic
Turn off GPV	Closes the gradient proportioning valve (GPV).	Section 8.4.11, Turning Off the Gradient Proportioning Valve
Create "GPV Test" Method	Creates a GPV test separation method, which may be used for operational qualification of the 2795 Separations Module.	Section 8.4.12, Creating a GPV Test Method
Firmware Checksum	Verifies the checksum value to ensure that firmware upgrades were loaded correctly.	Section 8.4.13, Displaying the Firmware Checksum

8.4.1 Performing the Static Leak Test

The Static Leak ("ramp and decay") test:

- Performs a solvent pressure ramp up

- Monitors the pressure decay in the solvent delivery system to determine if the inlet check valves, tubing, fittings, or plunger seals are faulty.

Perform the static leak test whenever you:

- Suspect leakage in the inlet check valves or plunger seals

- Perform maintenance on the solvent management system

- Replace fluidic path fittings

Before You Begin

To ensure a successful Static Leak test, you need to precondition the solvent management system.

To precondition the solvent management system:

1. Ensure that Solvent A is methanol. The 2795 Separations Module diagnostics firmware selects Solvent A for the Static Leak Test. If Solvent A is not methanol, flush Solvent Line A with methanol.

2. Place a restrictor at the inline filter outlet to produce 4000 psi at a flow rate of 1.0 to 1.5 mL/min for 10 minutes. Operate the inline degasser in **Continuous** mode throughout the preconditioning phase.

Required Materials

- Pin plug
- 5/16-inch, open-end wrenches (2)
- 5/8-inch, open-end wrench
- Waste container

Procedure

To perform the Static Leak test:

1. Select **Static leak test** in the Other Diagnostics drop-down list, then press the **OK** screen key. The Static Leak Test dialog box appears (Figure 8-7).
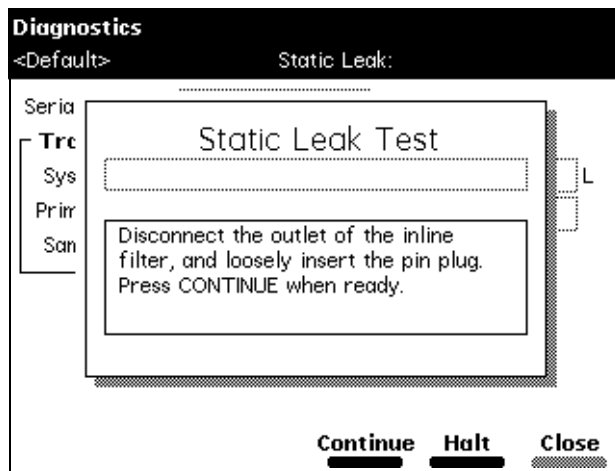
Figure 8-7 Static Leak Test Screen

2. Follow the instructions in the Static Leak Test screen. Disconnect the fluidic line at the outlet of the inline filter and loosely insert the pin plug into the inline filter outlet.

3. Collect the solvent runoff in an appropriate waste container.

4. Press **Continue** after you have inserted the pin plug.

5. Follow the instructions in the subsequent Static Leak Test screens to complete the Static Leak Test.

When the Static Leak Test is finished, the Static Leak Test Results screen appears ().
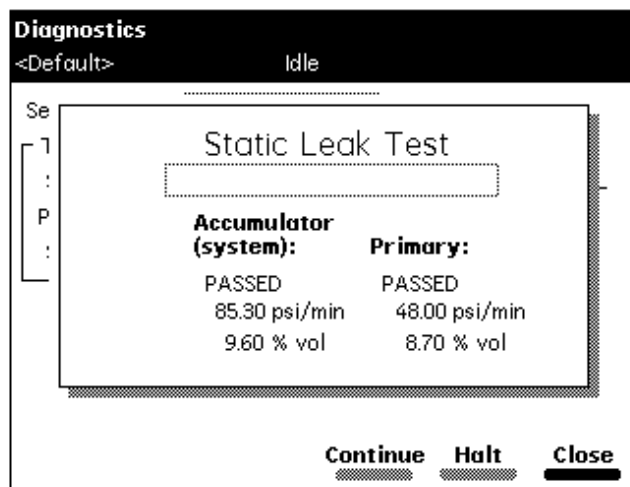
Figure 8-8 Static Leak Test Results Screen

Static Leak Test Failure

If the Static Leak test fails:

- Check for obvious leaks.
- Check that the Prime/Vent valve is closed.
- Check that the degasser is set to **Normal**.
- Check that compression fittings and head nuts are tight.
- Check that you used the appropriate solvent.
- If necessary, check the seal around the plunger and head surface by running the pump at high pressure before running the test. For example, install a restrictor sufficient to achieve 4000 to 4500 psi backpressure at a flow rate of 0.5 mL/min.

Rerun the Static Leak test. If it continues to fail:

1. Set the degasser to **Continuous** mode.

2. Perform a wet prime for 10 minutes at 1.0 mL/min using methanol with a restrictor connected to achieve 4000 to 4500 psi backpressure. Performing the wet prime increases the stroke to its maximum length.

3. After 7 to 8 minutes of wet priming, inspect the fittings for leaks (use thermal paper, which turns dark on contact with methanol).

4. Remove the restrictor, replace it with a pin plug and rerun the Static Leak test.

5. If the Accumulator chamber passes the Static Leak Test but exhibits a high decay rate (>100 psi) and the Primary chamber shows a negative pressure value, reverse the position of the check valves (Accumulator to Primary, Primary to Accumulator), and repeat the test.

8.4.2 Performing the Head Removal and Replacement Utility

Note: *This maintenance procedure is accessed from the Diagnostics screen.*

Use this function to move either of the solvent management system plungers to the most forward position so you can remove and replace a head, seal-wash assembly, plunger seals, or plunger.

For more information, see .

8.4.3 Performing the Input and Output Diagnostics

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Use the Inputs and Outputs diagnostic to view the status of the input and output connections on the I/O connectors of the 2795 Separations Module.

Required Materials

• Jumper wires (2)

Procedure

To perform the Inputs and Outputs diagnostics:

1. Select **Inputs and outputs** in the Other Diagnostics drop-down list, then press **OK**. The I/O Diagnostics screen appears (Figure 8-9).
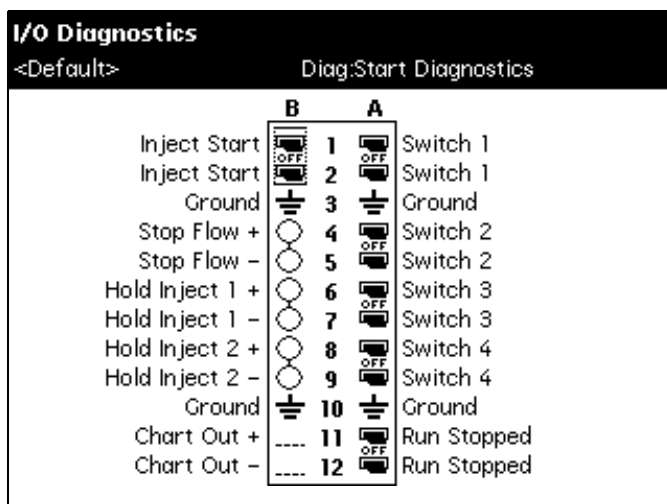
Figure 8-9 I/O Diagnostics Screen

2. Use two jumper wires to connect the two terminals of a paired output switch to the two terminals of one of the inputs. Table 8-7 summarizes the names of the paired switch outputs terminals and the input terminals.

Table 8-7 Connecting I/O Switches and Inputs

Paired Switch Output Terminals	Input Terminals
Switch 1	Stop Flow
Switch 2	Hold Inject 1
Switch 3	Hold Inject 2
Switch 4	
Inject Start	
Run Stopped	

3. In the I/O Diagnostics screen, select the symbol of the paired output switch to which the jumper wires are connected, then press any numeric key to alternate the switch between the On and Off positions.

4. Check that the circle symbols of the input terminals to which the jumper wires are connected change from empty to full as you alternate the switch between the On and Off positions.

5. Perform steps 2, 3, and 4 for each paired output switch that you want to test.

8.4.4 Performing the Keypad Diagnostic

To test the functioning of the keypad:

1. Select **Keypad** in the Other Diagnostics drop-down list, then press **OK**. The Keypad diagnostic screen appears (Figure 8-10).

2. Follow the instructions on the screen to perform the diagnostic.
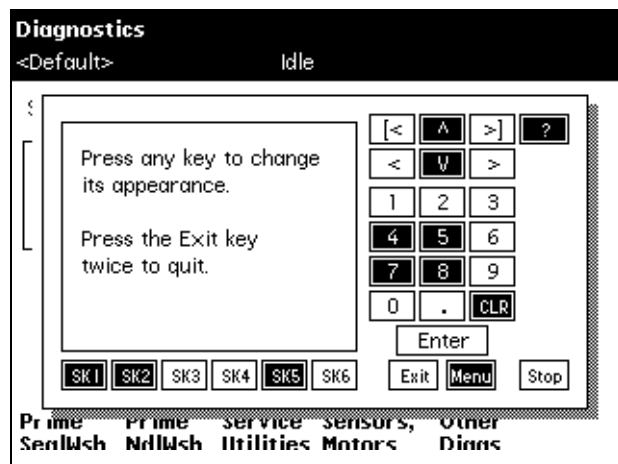
Figure 8-10 Keypad Diagnostic Screen

8.4.5 Performing the Display Diagnostic

Use the Display diagnostic if you experience problems with the display screen on the 2795 Separations Module. The Display diagnostic tests the display screen by projecting test patterns on the screen.

To perform the Display diagnostic:

1. Select **Display** in the Other Tests screen, then press **OK**. The display is tested in two directions, vertical and horizontal.

2. When the Display diagnostic is complete, press **Exit** to return to the Other Diagnostics screen.

8.4.6 Performing the Inline Degasser Diagnostic

Use the In-Line Degasser diagnostic to test the functioning of the inline degasser. This test, which takes approximately 15 min. to complete, checks the rate of loss of vacuum, ramps down the vacuum pressure in the inline degasser, then measures the rate at which the pressure increases.

Note: To monitor the vacuum pressure during the diagnostic, connect a chart recorder to the Chart Out terminals on the back panel of the 2795 Separations Module.

To perform the In-Line Degasser diagnostic:

1. Select **Degasser** in the Other Diagnostics drop-down list, then press **OK**. The In-Line Degasser Diagnostic screen appears (Figure 8-11).

2. Press the **Start** screen key to begin the diagnostic. While the diagnostic is running, the system:
   - Vents the inline degasser chamber to atmospheric pressure
   - Ramps down the vacuum pressure (for approximately 3 min)
   - Allows the vacuum to decay (for approximately 10 min)
   - Evaluates the rate at which the vacuum decays

3. When the diagnostic is complete, the In-Line Degasser Diagnostic screen displays:
   - Pass/Fail test status
   - Change in pressure (psi, kPa, or mbar)
   - Time left (reads "0.00" if the test is idle)
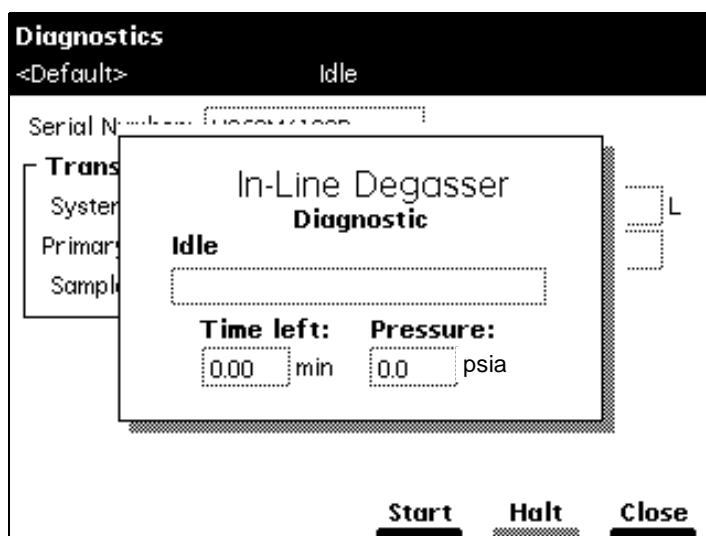
Figure 8-11 Degasser Diagnostic Screen

8.4.7 Performing the Sample Heater/Cooler Diagnostic

Use the Sample Heater/Cooler diagnostic to check the proper functioning of the sample heater/cooler module. This test lowers the temperature in the sample heater/cooler, then measures the rate at which the temperature rises.

Note: To monitor the sample heater/cooler temperature during the test, connect a chart recorder to the Chart Out terminals on the back panel of the 2795 Separations Module.

Before you perform the test:

- Remove all plates from the sample compartment

- Check that the column heater is not in use

- Ensure that the sample compartment starting temperature is between 18 °C and 28 °C

Note: *To ensure the accuracy of this diagnostic, do not open the sample compartment door while the diagnostic is running.*

To perform the Sample Heater/Cooler diagnostic:

1. Select **Sample heater/cooler** in the Other Diagnostics drop-down list, then press **OK**. The Sample Heater/Cooler Diagnostic screen appears (Figure 8-12).
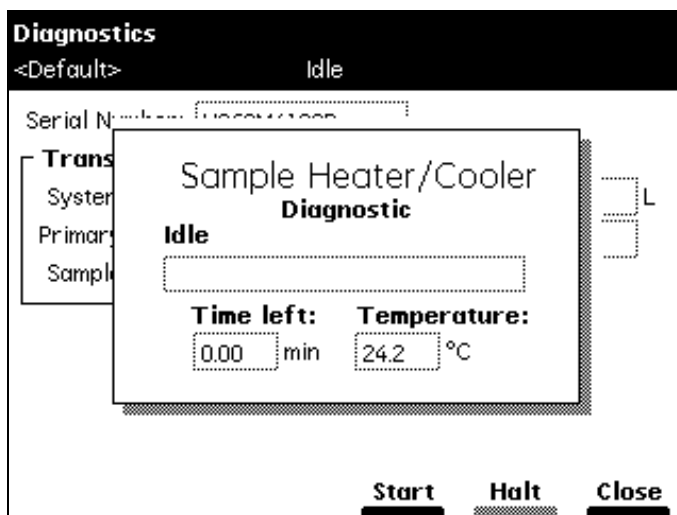
Figure 8-12 Sample Heater/Cooler Test Screen

2. Press **Start** to begin the diagnostic. During the diagnostic, the sample heater/cooler temperature is lowered for approximately 10 min., then is returned to ambient temperature.

When the test is finished, the Sample Heater/Cooler Diagnostic dialog box displays the:

- Pass/Fail test status

- Time left (reads "0.00" if the test is idle)

- Current temperature of the sample compartment

8.4.8 Performing the Defrost Sample Heater/Cooler Procedure

Use the Defrost Sample Heater/Cooler to defrost the Sample Heater/Cooler module and to monitor the temperature change as the module warms up.

Note: To monitor the sample heater/cooler temperature while the procedure is running, connect a chart recorder to the Chart Out terminals on the back panel of the 2795 Separations Module.

Before you perform the procedure:

- Remove all plates from the sample compartment
- Check that the column heater is not in use

To perform the Defrost Sample Heater/Cooler procedure:

1. Select **Defrost** in the Other Diagnostics drop-down list, then press **OK**. The Sample Heater/Cooler Defrost screen appears (Figure 8-13).
2. Press **Start** to begin the procedure.
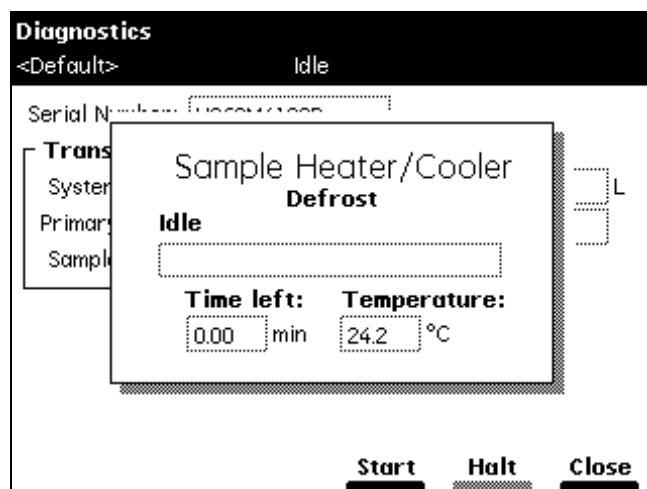
Figure 8-13 Sample Heater/Cooler Defrost Dialog Box

3. Open the sample compartment door when you are prompted. The sample compartment door must remain open during the entire procedure.

   When the procedure is finished, the Sample Heater/Cooler Defrost screen indicates:

   - The procedure has ended
   - Time left (reads "0.00" if the test is idle)
   - Sample heater/cooler temperature
   - A prompt to close the sample compartment door

8.4.9 Changing the Needle

STOP **Attention:** *To prevent damage to the needle during replacement, perform the Change Needle procedure (see Section 7.3.3, Replacing the Needle Assembly) before you remove the needle assembly.*

Note: *This maintenance procedure is accessed from the Diagnostics screen. You can use this procedure to rehome the needle. Follow the procedure in Section 7.3.3, Replacing the Needle Assembly, but do not remove or replace the needle.*

For more information, see Section 7.3.3, Replacing the Needle Assembly.

8.4.10 Performing the Column Heater Diagnostic

The Column Heater diagnostic tests the functioning of the column heater.

Note: *To monitor the column heater temperature during the diagnostic, connect a chart recorder to the Chart Out terminals on the back panel of the 2795 Separations Module.*

Before you perform the Column Heater test, check that:

- The sample heater/cooler is not in use
- The column heater temperature is between 18 °C and 28 °C

Note: *To ensure the accuracy of this test, do not open the column heater door during the test.*

To perform the Column Heater diagnostic:

1. Select **Column heater** in the Other Diagnostics drop-down list, then press **OK**. The Column Heater Diagnostic screen appears (Figure 8-14).
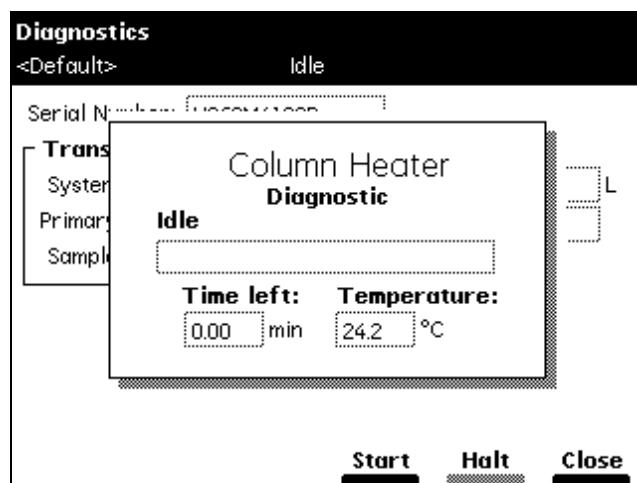
Figure 8-14 Column Heater Diagnostic Screen

2. Press **Start** to begin the test. During the test:

    • You are prompted to close the column heater module door.

    • The column heater temperature is raised (for about 8 minutes).

    • The system measures the increase in temperature.

    When the test is finished, the Column Heater Test screen displays:

    • Pass/Fail test status

    • The increase in temperature

If a column selection valve is installed in the 2795 Separations Module column heater, use the Status Page to control column selection (see Section 4.3, Monitoring System Status).

8.4.11 Turning Off the Gradient Proportioning Valve

Turn off the GPV before you replace an inlet check-valve cartridge (see Section 7.2.5, Replacing an Inlet Check-Valve Cartridge).

8.4.12 Creating a GPV Test Method

Use the Create GPV Test Method function to create a gradient proportioning valve test method that can be used during the operational qualification of the 2795 Separations Module. When you complete an operational qualification, you can delete the GPV Test Method, then rerun the function if you need to requalify the operation of the 2795 Separations Module.

To create a GPV test method, select **Create GPV Test** in the Other Diagnostics drop-down list, then press **OK**. One of the following messages appears:

• The GPV test method is being created

• The GPV test method already exists (Figure 8-15)
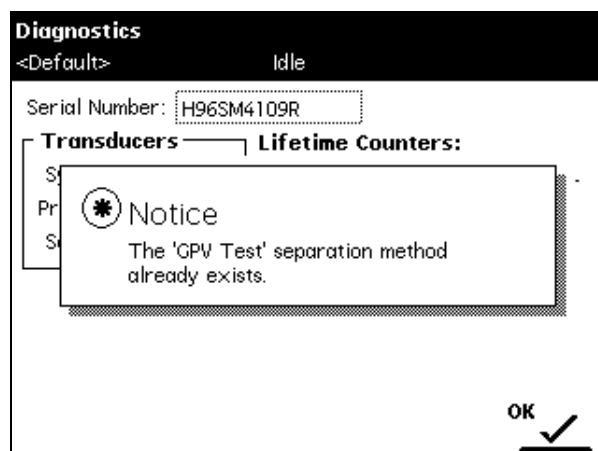
Figure 8-15 GPV Test Separation Method

Note: In the Methods screen (Figure 5-1), use the Delete Methods screen key to delete the GPV test method if you need to requalify your 2795 Separations Module. For more information, refer to Section 5.1.5, Renaming, Viewing, and Deleting Separation Methods.

8.4.13 Displaying the Firmware Checksum

Use the Firmware Checksum utility to test the installation of the firmware by displaying the checksum value.

To display the firmware checksum, select **Firmware checksum** in the Other Diagnostics drop-down list. The checksum and the firmware revision are displayed (Figure 8-16).
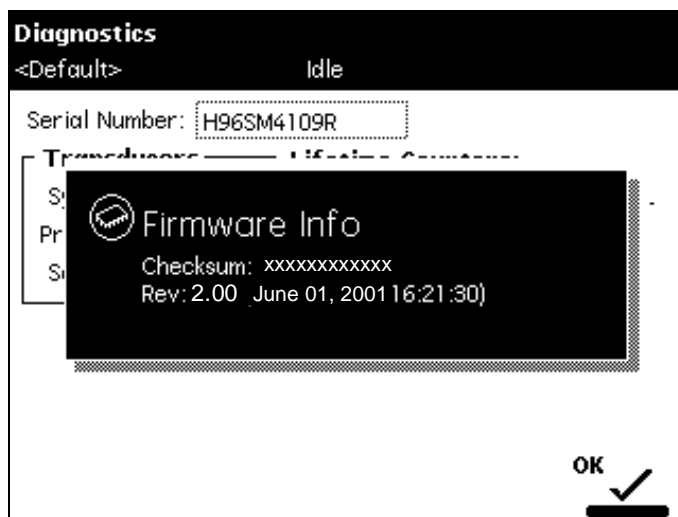
Figure 8-16 Firmware Checksum (Example)

8.5 Troubleshooting

This section covers:

- Section 8.5.1, General Troubleshooting Hints
- Section 8.5.2, Troubleshooting Chromatography Problems
- Section 8.5.3, Troubleshooting Hardware Problems
  – System Troubleshooting
  – Solvent Management System Troubleshooting
  – Sample Management System Troubleshooting
  – Error Messages

Note: Refer to Section 8.1, Safety and Handling, for information on safety and handling considerations while you troubleshoot the 2795 Separations Module.

8.5.1 General Troubleshooting Hints

Basic Troubleshooting Steps

The six basic steps to perform system troubleshooting are:

1. Check the simple things first. Is something obvious causing the problem (for example, is an instrument unplugged or improperly connected)?

2. Compare current system operation with the way the system operated before the problem started. To help you identify normal operating conditions:

   a. Record a map of your LC system (tubing and electrical connections).

   b. Keep a daily log.

   c. Run test chromatograms regularly.

   This step points out the importance of keeping track of system parameters and the results of your chromatography during normal operation. Troubleshooting is easier if you know the conditions when the system is operating correctly.

   For example, if your system usually runs at n psi with a certain method, is the system pressure currently in the same range or drastically higher (possibly caused by a plug) or lower (possibly caused by a leak)? Are pressure fluctuations in the same pressure range as during normal operation?

   When your system is installed, and each time you develop a new method, fill out a Normal Operation Checklist to keep a record of system conditions during normal operation.

3. Identify (in the order listed below) the symptoms that vary from normal system operation:

   a. System pressure (high, low, erratic)

   b. Baseline (fluidic-path-related or detector-electronics-related)

   c. Changes in peak retention time (incorrect or changing over time)

   d. Loss of peak resolution

   e. Abnormal peak shape (smaller than expected, broad, tailing, and so on)

   f. Incorrect qualitative/quantitative results

4. For each isolated symptom, identify a list of suspected causes. For suggested troubleshooting procedures, see:

   - Section 8.5.2, Troubleshooting Chromatography Problems
   - Section 8.5.3, Troubleshooting Hardware Problems

5. Run the performance tests for each chromatographic instrument to determine which instrument is the source of the problem.

6. Refer to the troubleshooting information in the troubleshooting tables of this chapter. The troubleshooting tables are organized according to the parameters in step 3 and allow you to narrow down the possible causes of a symptom and find suggested corrective actions.

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If you determine a problem is related to another system component, refer to the appropriate operators manual.

When to Call Waters Technical Service

You can correct many problems with a Waters 2795 Separations Module. However, if you cannot correct a condition, you can contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only.* Other customers, call your local Waters subsidiary or Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts (U.S.A.) for assistance.

When you call Waters Technical Service, have the following information available:

- Completed Normal Operation Checklist for the method you are using
- Nature of the symptom
- 2795 Separations Module serial number (located behind the syringe access door)
- Detector model number and serial number
- Flow rate
- Operating pressure
- Mobile phase(s) and needle wash and purge solvents
- Detector settings (sensitivity and wavelength)
- Type and serial number of column
- Sample type
- Control mode (Controlled by Millennium[32], Controlled by MassLynx, System Controller, No Interaction, or Operate Gradient by Event In)
- Firmware version and serial number

8.5.2 Troubleshooting Chromatography Problems

Caution: To prevent injury, always observe good laboratory practices when you handle solvents, change tubing, or operate the 2795 Separations Module. Know the physical and chemical properties of the solvents you use. Refer to the Material Safety Data Sheets for the solvents in use.

Table 8-8 provides troubleshooting suggestions for chromatographic problems in the 2795 Separations Module.

Before you use Table 8-8, read Section 8.5.1, General Troubleshooting Hints, and follow the basic troubleshooting steps to isolate the cause of a chromatographic symptom.

Table 8-8 Chromatography Troubleshooting

Symptom	Possible Cause	Corrective Action
Erratic retention times	Air bubble in plunger head	Degas all solvents, prime solvent management system. Check degasser functionality. Turn on bubble detect alarm in the Mobile Phase screen (see Section 6.2.1, Setting Mobile Phase Screen Parameter Values).
	Malfunctioning check valves	Clean/replace inlet check valve cartridges (see Section 7.2.6).
	Leaking plunger seals	Replace seals (see Section 7.2.3).
	Unsuitable separation chemistry	Check mobile phase, column, GPV.
	Clogged solvent filters	Replace filters.
Increased retention times	Incorrect flow rate	Change flow rate.
	Incorrect solvent composition	Change solvent composition, check GPV.
	Column heater module not turned on; temperature low	Turn column heater module on, check temperature.
	Incorrect mobile phase	Use correct mobile phase.
	Column contaminated	Clean/replace column.
	Incorrect column	Use correct column.
	Leak in solvent management system, loss of solvent	Check fittings for leaks. Perform Static Leak Test (see Section 8.4.1, Performing the Static Leak Test).
Decreased retention times	Incorrect flow rate	Change flow rate.
	Incorrect solvent composition	Change composition, check GPV.
	High column temperature	Reduce column temperature.
	Incorrect mobile phase	Use correct mobile phase.
	Column contaminated	Clean/replace column.
	Incorrect column	Use correct column.
	Solvent insufficiently degassed	Degas solvent.

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Table 8-8 Chromatography Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Reproducibility errors	Purge solvent insufficiently degassed	Degas solvent.
	Incorrect chemistry or data processing parameters	Check chemistry/integration.
	Sample management system problem	Troubleshoot sample management system.
	Sample evaporation	Cool sample compartment to minimize evaporation.
Baseline drift, rapid	Column not equilibrated	Equilibrate column.
	Detector not allowed to warm up	Allow detector to warm up until baseline is stable. Time varies with wavelength and sensitivity.
	Solvent contaminated	Use fresh solvent.
	Solvent insufficiently degassed (rapid or slow drift)	Degas solvent. Check inline degasser (see Section 8.4.6, Performing the Inline Degasser Diagnostic).
	Flow fluctuations (rapid or slow drift)	Prime solvent management system, replace plunger seals and check valves (see "Erratic Retention Time" symptom).
	Incorrect wavelength for solvent	Check that solvent does not absorb at the selected wavelength.
Baseline drift, slow	Solvent contaminated	Use fresh solvent.
	Decreased UV lamp energy	Check detector lamp energy using detector diagnostics.
	Ambient temperature fluctuations	Stabilize operating environment temperature to allow full equilibration.
	UV detector flow cell leaking (internal, cross-port)	Check flow cell, tighten connections.
	Dirty flow cell	Clean flow cell.

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Table 8-8 Chromatography Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline noise cycling, short term (30 to 60 sec)	Fluctuating flow rate	Check for leaks and for proper degassing (see "Erratic Retention Times" symptom).
	Solvent not well mixed (short- or long-term cycling)	Stir solvent.
	Radio frequency noise (short- or long-term cycling)	Eliminate interference.
Baseline noise cycling, long-term (approximately 10 min to 1 hour)	Ambient temperature fluctuations	Stabilize ambient temperature.
	Faulty integrator or recorder	Check integrator or recorder for excessive baseline noise.
	UV-absorbing gas, such as O_2 not sufficiently removed from solvent	Change degasser setting from Normal to Continuous and/or reduce the flow rate.

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Table 8-8 Chromatography Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline noise, random	Air in detector	Purge detector to remove air.
	Solvents not properly degassed or sparged	Degas/sparge solvents.
	Bubble(s) present	Re-prime the solvent management system. Remove and vacuum filter solvents. Change degasser mode from Normal to Continuous.
	Solvents contaminated	Use fresh solvent.
	Column contaminated	Clean/replace column.
	Dirty flow cell	Clean flow cell.
	Analog output cable not properly connected between 2795 Separations Module and data system, recorder, or integrator	Properly connect cable.
	System improperly grounded	Plug into outlet on different electrical circuit.
		Use power conditioner.
	Recorder voltage incorrect	Set recorder to correct voltage.
	Unit not cooling properly	Operate unit with covers in place. Check back panel for proper clearance. Perform the Defrost Sample Procedure (see Section 8.4.8, Performing the Defrost Sample Heater/Cooler Procedure).
	Radio frequency noise	Eliminate interference.
	Defective detector	Troubleshoot detector.

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Table 8-8 Chromatography Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Flat baseline, no peaks	No solvent flow	Check flow rate.
	Lamp not on	Use detector diagnostics to check reference/sample energy. Zero energy indicates lamp not on.
		Power on lamp. If this does not resolve the problem, replace the lamp.
	Detector not zeroed	Zero the detector baseline.
	Improper connection between detector and recorder	Check cabling between the detector and the recorder.
	Incorrect wavelength	Check wavelength setting.
	Leak in solvent path	Check fittings.
Flat-topped peaks	Detector not zeroed	Zero the detector baseline.
	Incorrect recorder input voltage	Adjust recorder input voltage, or adjust detector output cable to correct position.
	Sensitivity too high	Select a less sensitive detection range.
	Sample concentration or injection volume exceeds voltage output of detector	Decrease sample concentration or injection volume.

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Table 8-8 Chromatography Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Sensitivity loss	Leak in sample management system	Troubleshoot sample management system.
	Degraded, contaminated, or improperly prepared sample	Use fresh sample.
	Column contaminated	Clean/replace column.
	Loss of column efficiency	Clean/replace column.
	Peaks wider than expected	Troubleshoot sample management system. Check fittings for leaks and proper ferrule installation. Check tubing ID.
	Incorrect filter constant	Set correct filter constant on detector.
	Change in mobile phase composition	Adjust mobile phase pH or ionic composition.
	Leak in flow cell	Tighten cell seal.
	Incorrect flow rate	Change flow rate.
Sample energy decreased, reference energy not decreased	Contaminated mobile phase	Use fresh mobile phase.
	Dirty flow cell	Clean outside of flow cell windows.
		Flush system with water. If necessary, remove the column, then flush the system with a strong solvent to remove particulate or filmy matter from flow cell windows.

8.5.3 Troubleshooting Hardware Problems

This section covers:

- System Troubleshooting
- Solvent Management System Troubleshooting
- Sample Management System Troubleshooting
- Error Messages

Before you use the following troubleshooting tables, read Section 8.5.1, General Troubleshooting Hints, then follow the steps to isolate the cause of a hardware symptom.

System Troubleshooting

Table 8-9 provides troubleshooting suggestions for 2795 Separations Module system problems that are not related to a single module of the system.

Table 8-9 General System Troubleshooting

Symptom	Possible Cause	Corrective Action
Unit does not power on	Power cord not connected	Check power cord.
	No power at outlet	Check line voltage.
	Power supply fuse blown or missing	Replace power supply fuse.
No response from keypad	Keypad cable not connected	Connect keypad cable.
	Keypad broken	Call Waters Technical Service.
Screen is blank	Unit not powered on	Power on unit.
	Startup diagnostics failure	Power off and on again. If failure not corrected, call Waters Technical Service.
Startup diagnostics fail	Internal problem with controller board, solvent management system, sample management system	Power off and on again. If failure not corrected, call Waters Technical Service.
Startup failure with error message Over-pressure: check inline filter	Clogged inline filter	Clean or replace filter element
	Blockage in sample manage-ment waste line	Remove blockage or replace line
	Restriction between solvent and sample management systems	Remove restriction
Front panel firmware "lock up"	Firmware problem or power line problem	Power off and on again. If failure is not corrected, call Waters Technical Service.
Fans not running	Unit not powered on	Power on unit.
	Fan wiring or fan motor problem	Call Waters Technical Service.
	Power supply not working	Call Waters Technical Service.

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Table 8-9 General System Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
2795 Separations Module not fully functional in remote configuration (IEEE-488 remote data system)	Data system not programmed to control all 2795 Separations Module functions 2795 Separations Module not configured for control by Millennium[32] software or MassLynx software	Disconnect the 2795 Separations Module from the remote data system, then run the unit in a stand-alone mode to test functionality. Configure 2795 Separations Module for control by Millennium[32] software or MassLynx software (see Section 3.2.3, Configuring the Operating Mode).

Solvent Management System Troubleshooting

Table 8-10 provides troubleshooting suggestions for solvent management system problems in the 2795 Separations Module.

Table 8-10 Solvent Management System Troubleshooting

Symptom	Possible Cause	Corrective Action
Plunger head leaks solvent	Worn plunger seals	Replace plunger seals (see Section 7.2.2, Replacing the Plunger Seal and Face Seals).
	Worn seal-wash seals	Replace seal-wash seals (see Section 7.2.3, Replacing the Seal-Wash Assembly Seals).
	Loose head	Hand-tighten head nut.
	Loose inlet check valve	Tighten check valve.
	Worn face seals	Replace face seals.
	Seal-wash tubes not properly installed	Install tubes properly.

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Table 8-10 Solvent Management System Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Erratic flow/pressure pulsations	Gas dissolved in mobile phase	Sparge/degas solvents.
	Air bubble in head	Prime solvent management system to remove bubble. Increase sparge rate/degassing time before use. Turn on bubble detect alarm in the Mobile Phase screen (see Section 5.2.1, Setting Mobile Phase Parameter Values).
	Dirty inlet check valves	Clean/replace inlet check valves (see Section 7.2.5, Replacing an Inlet Check-Valve Cartridge).
	Prime/vent valve open or leaking	Close or rebuild prime/vent valve.
	Plugged solvent reservoir diffuser	Clean or replace diffuser.
	Plunger seal leaking	Replace plunger seal assembly (see Section 7.2.2, Replacing the Plunger Seal and Face Seals).

Sample Management System Troubleshooting

Table 8-11 provides troubleshooting suggestions for resolving sample management system problems in the 2795 Separations Module.

Table 8-11 Sample Management System Troubleshooting

Symptom	Possible Cause	Corrective Action
Leak at needle and seat	Damaged or dirty needle or inject port seat	Clean needle with organic solvents, such as acetone or acetonitrile. Or replace needle and inject port seat (see Section 7.3.3, Replacing the Needle Assembly).
Cannot maintain high or low pressure	Leak in the inject valve	Replace or rebuild the inject valve (contact Waters Technical Service).

Table 8-11 Sample Management System Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Reproducibility errors	Syringe contains an air bubble (usually seen at top or bottom of syringe)	Remove air bubble (see Section 7.3.2, Removing and Replacing the Syringe).
	Leaks at fluidic tubing connections	Check for leakage at the connections with a tissue, then tighten connection slightly if the tissue is moistened.
	Inject valve failure	Replace damaged inject valve (contact Waters Technical Service).
	Syringe tip is worn	Replace syringe (see Section 7.3.2, Replacing the Syringe).
Carrier malfunction	Plates not properly positioned	Reposition plates in compartment (see Section 3.4.3, Loading the Sample Plates).
	Plate carrier jammed	Inspect plate positioner spring (see Section 3.4.3, Loading the Sample Plates).
	Plate carrier or sample compartment floor contaminated	Clean sample compartment (see Section 7.3.7, Cleaning the Sample Compartment and Plate Carrier).

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Error Messages

Table 8-12 lists error messages you may encounter while using the 2795 Separations Module and provides possible causes and/or corrective actions. Error messages that are displayed during operation of the 2795 Separations Module are recorded in the error log (Section 8.2, Using the Error Log).

Table 8-12 2795 Separations Module Error Messages

Error Message	Causes/Recovery Actions
General instrument fault	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Initialization fault	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.

Table 8-12 2795 Separations Module Error Messages *(Continued)*

Error Message	Causes/Recovery Actions
General electronics fault	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
System over pressure	Clogged inline filter or column
System under pressure	System leak
Sample temp h/w fault	Reprogram sample temperature. If error persists, contact Waters Technical Service.
Sample temp over limit	Faulty sample heater/cooler. Contact Waters Technical Service.
Lower sample temp	Close sample compartment door. Defrost sample heater/cooler.
Column temp h/w fault	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Column temp over limit	Faulty column heater. Contact Waters Technical Service.
Low column temp	Close column heater door. If error persists, contact Waters Technical Service.
Degasser h/w fault	Faulty solvent degasser. Contact Waters Technical Service.
Degasser pressure fault	Leaking degasser chamber. Replace degasser (contact Waters Technical Service).
Stop Flow key was pressed	Clear alarm and continue operation.
Stop Flow input was received	Check instruments connected to Stop Flow terminals.
Stop Flow request from remote	Check Millennium[32] Chromatography Manager or MassLynx Data System.
User-programmed alert	Clear alarm and continue operation.
Floppy Disk removed while logging	Replace floppy disk in floppy disk drive.
Error writing log to floppy disk	Replace floppy disk in floppy disk drive.
Bubble found on compression	Perform Wet Prime procedure.
Lost prime	Perform Dry Prime procedure followed by Wet Prime.
Primary over pressure	Check connecting tubing between the primary outlet and accumulator inlet solvent valves.

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Table 8-12 2795 Separations Module Error Messages *(Continued)*

Error Message	Causes/Recovery Actions
Accumulator over pressure	Check tubing from the accumulator outlet valve.
Solv delivery motor lost sync	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Solv delivery h/w fault	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Bubble found on intake	Degas solvents thoroughly.
Overpressure: check inline filter message	Check for clogged inline filter and replace.
General solv delivery fault	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Loop too small for sample	Replace sample and/or holding loops with larger sizes (see Table 7-5).
Needle malfunction (top sensor)	Needle failed to detect the Home sensor on returning to UP position. Remove and reinstall needle. Check connections to needle sensor. If error persists, contact Waters Technical Service.
Needle malfunction (bottom sensor)	Needle failed to detect the Bottom sensor on reaching the WELL or LOAD position. Check connections to XYZ mechanism. Remove and reinstall needle. If error persists, contact Waters Technical Service.
Carrier malfunction	Check for obstructions in sample compartment.
Fan failed	Check connections to fans. Replace fans (contact Waters Technical Service).
Syringe movement malfunction	Check connections to syringe drive.
Door is open	Close sample compartment door. Cancel alarm.
Needle is busy	Close sample compartment door.
Needle malfunction	May occur when the needle detects the bottom sensor while moving. Also, may also occur if Home sensor is detected when the needle is not at the HOME position. Check connections to XYZ mechanism. Ensure plate configurations are correct for plate type and well bottom.

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Table 8-12 2795 Separations Module Error Messages *(Continued)*

Error Message	Causes/Recovery Actions
Column heater door open	Close column heater door. Clear alarm.
System transducer range	Power off, then power on the 2795 Separations Module (to zero transducer).
Primary transducer range	Power off, then power on the 2795 Separations Module (to zero transducer).
GPV h/w fault	Check fluidic and electrical connections to the GPV. Replace GPV (contact Waters Technical Service).
Auto Shutdown invoked	Clear alarm. Continue operation.
Degasser high leak rate	Leaking degasser chamber. Replace chamber (contact Waters Technical Service).
Overpressure: check inline filter	Clogged inline filter. Clean or replace.
Injection parameters incorrect	The location field in the injection is incorrect for the plate configuration specified. Reconfigure the plate.
Carrier lockpin movement failed	Lockpin Home sensor not detected at the HOME position. Check connections to the lockpin motor.
Inject valve movement failure	Position sensors do not match the intended position. Check connections to inject valve.
Plate parameters are incorrect	Specified plate dimensions extend beyond the range of needle XY movement. Reconfigure plate.
X Axis malfunction	The X axis Home sensor is not detected at the HOME position. Check connections to the XYZ mechanism.
Y Axis malfunction	The Y axis Home sensor is not detected at the HOME position. Check connection to the XYZ mechanism.
Plate X Axis parameters incorrect	Specified plate dimensions extend beyond the range of X movement. Reconfigure plate.
Plate Y Axis parameters incorrect	Specified plate dimensions extend beyond the range of Y movement. Reconfigure plate.

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Table 8-12 2795 Separations Module Error Messages *(Continued)*

Error Message	Causes/Recovery Actions
Error changing column position	The column-selection valve switch indicates an error in moving to the requested position. Check connection to column heater and column-selection valve.
Plate height error	Measured plate height does not match the selected plate type. Reconfigure plate or change plate or plate type.
Well depth error	Measured depth of well bottom does not match selected plate type. Reconfigure plate or change plate or plate type.
General instrument error	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Absorbance detector fault	Connect detector to IEEE-488 interface.
410 detector fault	Connect 410/2410 RI detector to IEEE-488 interface.
Sample too large for configuration	This alarm is raised whenever an invalid sample size is requested. The factors that limit sample size are loop mode (full or partial) and overfill factor, air gap volumes, syringe size and sample loop size.
Can't understand requested well	Well location from remote data system does not match plate configuration. Check plate configuration. Check remote data system parameters.
Power down	Status message that appears in error log.
Power up	Status message that appears in error log.
Cannot reach minimum pressure	Solvent management system leak or faulty plunger seals. Correct leak and/or replace seals.
Exceeded maximum plunger stroke	Solvent management system leak or faulty plunger seals. Ensure solvent thoroughly degassed. Correct leak and/or replace seals.
Column heater not ambient	Clear alarm. Allow column heater to cool.
Sample heater/cooler running	Clear alarm. Power off sample heater/cooler.
Sample temp not ambient	Clear alarm. Allow sample heater/cooler to cool.
Degasser minimum pressure	Leaking degasser chamber or bad vacuum pump (contact Waters Technical Service).

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Table 8-12 2795 Separations Module Error Messages *(Continued)*

Error Message	Causes/Recovery Actions
Degasser ambient pressure	Clear alarm. Allow degasser to vent.
Procedure interrupted	Clear alarm. Restart procedure.
Parameter range error	Check Help or Operator's Guide for correct range.
Solv delivery internal error	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Injector internal error	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Controller internal error	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Seal wash internal error	Power off, then power on the 2795 Separations Module. If error persists, contact Waters Technical Service.
Default separation method created	This message in the error log indicated that the default method had been corrupted or otherwise lost. However, in situations when a Clear-8 or Clear-0 power-up key sequence is used to erase flash memory, the log entry is normal.
SysPrep requires a degasser	This alarm occurs if the user attempts to run a new SysPrep function that has no Alliance degasser installed (or failed to detect).
Must degas solvents for SysPrep	This alarm occurs when the new System Prep function is invoked with the degasser is Off. The degasser will be turned on automatically.
Top and bottom needle sensors active	This diagnostic alarm occurs in a particular failure mode when both the top and bottom needle sensors fail to activate.
Exclamation point (!) replaces the method or sample set icon	Corrupted or invalid separations methods or sample sets will be identified with an exclamation point (!) in place of either the separations method or sample set icon. Methods marked with an exclamation point are not available for use. See Fatal error.

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Table 8-12 2795 Separations Module Error Messages *(Continued)*

Error Message	Causes/Recovery Actions
Fatal Error (when importing a method or sample set)	Appears when importing an invalid Separations Method or Sample Set created in an firmware version earlier than V1.10, the 2795 Separations Module will display the message, fatal error.
	To recover from this error, remove the floppy disk containing the method or sample set from the disk drive. Power cycle the 2795 Separations Module, and import any valid remaining methods.

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Appendix A
Specifications

This appendix includes the following specifications for the Waters Alliance 2795 XE Separations Module with optional column heater and optional bar code reader:

- Physical
- Environmental
- Electrical
- Solvent management system
- Sample management system
- Instrument control and communication

Table A-1 Physical Specifications

Item	Specification
Height	22.5 in. (57 cm)
Depth	23.5 in. (60 cm)
Width	23 in. (58 cm)
Weight	136 lb. (62 kg)
Wetted Surface Materials	316 stainless steel, UHMWPE, sapphire, ruby, Tefzel® (ETFE), Teflon® (FEP and PTFE), PEEK, Fluoroloy G, Fluoroloy–08R

Table A-2 Environmental Specifications

Item	Specification
Operating Temperature	4 to 40 °C
Relative Humidity	20 to 80%, noncondensing
Acoustic Noise	<65 dB(A)
Solvent Compatibility (see Appendix C, Solvent Considerations)	Solvents consistent with materials of construction. Salts and buffers can reduce seal life, especially at pressures in excess of 3000 psi.

Table A-3 Electrical Specifications

Item	Specification
Line Voltage	85 to 264 Vac
Frequency	47 to 63 Hz

Table A-4 Solvent Management System Specifications

Item	Specification
Number of Solvents	One to four, three with vacuum degas
Solvent Conditioning	Vacuum degas, three solvents plus purge solvent
Typical Operating Flow Rate Range	0.050 to 5.000 mL/min in 0.001-mL/min increments
Programmable Flow Rate Range	0.000 and 0.010 to 10.000 mL/min in 0.001-mL/min increments
Compressibility Compensation	Automatic and continuous
System Delay Volume	<400 µL, independent of backpressure, 1 mL/min
Plunger Seal Wash	Integral, active, programmable
Gradient Profiles	Eleven (11) gradient curves [including linear, step (2 curves), concave (4 curves), and convex (4 curves)]
Dry Prime/Wet Prime	Automatic, front panel control
Flow Ramping	Time (0.01 to 30.00 min in 0.01-min increments) to reach maximum flow rate
Maximum Operating Pressure	5000 psi (345 bar) at 0.010 to 3.000 mL/min; Programmable upper and lower limits
Composition Range	0.0 to 100.0% in 0.1% increments
Composition Accuracy	±0.5% absolute, independent of backpressure (Proportioning Valve Pair Test [degassed methanol:methanol/propylparaben, 2 mL/min, 257 nm])

Table A-4 Solvent Management System Specifications *(Continued)*

Item	Specification
Composition Precision	≤0.15% RSD or ≤0.02 min SD, whichever is greater, based on retention time. (Degassed 60% methanol:40% water Dial-a-Mix, 1 mL/min, six replicates, phenone mix, 257 nm)
Flow Precision	≤0.075% RSD or ≤0.02 min SD, whichever is greater, based on retention time (N = 6) or volumetric measures (0.200 to 5.000 mL/min), isocratic premix
Flow Accuracy	±1% or 10 µL/min, whichever is greater, (0.200 to 5.000 mL/ min), degassed methanol, at 600 psi backpressure

Table A-5 Sample Management System Specifications

Item	Specification
Number of Sample Plates	Total of four plates: • **Microtiter plate** – 96- or 384-well • **Vial plate** – 2.0-mL vial (48) • **Tube plate** – 0.5-mL microcentrifuge tube (48) or 1.5-mL microcentrifuge tube (24) • **Open access plate** – 2.0-mL vial (24 or 48)
Sample Temperature Control	4 to 40 °C, programmable in 1 °C increments
Maximum Sample Capacity	1536 in four 384-well plates
Number of Sample Injections	1 to 99 injections per sample
Sample Delivery Precision	<0.3% RSD, full loop 50 µL. (Default wash/purge conditions, degassed 60% methanol:40% water Dial-a-Mix, 1 mL/min, six replicates, paraben mix, 257 nm)
Sample Volume Linearity	>0.999% correlation, 5 to 20 µL, partial fill in 50-µL loop, 3-µL pre- and post-sample air gaps (Default wash/purge conditions, degassed 60% methanol:40% water Dial-a-Mix, 1 mL/min, six replicates, paraben mix, 257 nm)
Needle Wash Solvents	Two: wash (strong solvent) and purge (sample-compatible) solvent

Table A-5 Sample Management System Specifications *(Continued)*

Item	Specification
Sample Carryover	<0.01% or <2.5 nL (default wash/purge volumes), whichever is greater. (Blank injection, 25 μL, 60% methanol:40% water, after six replicates, 25 μL propylparaben, 1 g/L, 257 nm)

Table A-6 Instrument Control and Communication Specifications

Item	Specification
Parallel Sample Processing	User-programmable next-sample aspiration and loop fill, column equilibration, just-in-time gradient delivery
Advanced Operations	Auto addition, sample pooling, open access, priority samples
Column Heater	20 °C (5 °C above ambient) to 60 °C, in 1 °C increments
Column Selection	3- or 6-column select valve (optional)
IEEE-488 Interface	Control of Waters IEEE-488 equipped detectors; communication with Millennium[32] Chromatography Manager workstations or MicroMass detectors using MassLynx software
RS-232 Interface	Output of ASCII text files to a printer/PC/integrator (Port A)
Floppy Disk Drive	1.44-MB, 3.5-inch floppy disk, for methods transfer and archiving; reportable GLP log; sample list import from ASCII file
Event Inputs	Three TTL or switch closures
Programmable Event Outputs	Six contact closures

Appendix B
Spare Parts

Refer to the tables in this appendix for information on recommended spare parts and options for the 2795 Separations Module. Parts not listed in this appendix are not recommended for customer replacement.

B.1 Solvent Management System Spare Parts

Table B-1 Recommended Spare Parts for the 2795 Separations Module Solvent Management System

Item	Waters Part Number
Check Valve Cartridge Replacement Kit	WAT270941
In-Line Filter Assembly	WAT035190
In-Line Filter Element	WAT088084
Head, Face Seal Replacement Kit	WAT270939
Head, Plunger Seal Replacement Kit	WAT270938
Solvent Bottle Filter	WAT025531
Seal Wash Plunger Seal Replacement Kit	WAT271018
Seal Wash Face Seal Replacement Kit	WAT271017
Plunger	WAT270959
Vacuum Chamber	700000269

B.2 Sample Management System Spare Parts

Table B-2 Recommended Spare Parts for the Sample Management System

Item	Part Number
Syringe: 100-µL 250-µL 500-µL (standard) 1000-µL 2500-µL	 700000564 WAT073109 700000565 700000611 WAT077342
Sample Loop: 5-µL 20-µL 50-µL (standard) 100-µL 500-µL 2000-µL	 700000558 700000559 700000560 700000561 700000562 700000563
Holding Loop: 128-µL 500-µL 3000-µL	 700000555 700000556 700000557
Inject Valve	700000380
Inject Port Seat	700000384
Seal Inject Port Washer	700000385
2795 Separations Module Inject Valve Rebuild Kit	700000411
2795 Separations Module Three-Column Select Valve Rebuild Kit	700000431
2795 Separations Module Six-Column Select Valve Rebuild Kit	700000434
2795 Separations Module Two-Position Regeneration Valve Rebuild Kit	700000424
2795 Separations Module Needle and Housing Assembly	700000388

Table B-3 Recommended Spare Parts for the 2795 Separations Module

Item	Part Number
Cable assembly, AC power	WAT270895
Cable assembly, IEEE-488, 1 m	WAT087198
Detector drip tray assembly	WAT271040
Diffuser assembly, polyethylene	WAT007272
Diffuser assembly, stainless steel (1)	WAT025531

B.3 2795 Separations Module Options

Table B-4 2795 Separations Module Options

Item	Part Number
2795 Column Heater	186000320
2795 Column Heater with Three-Column Selection Valve	186000266
2795 Column Heater with Six-Column Selection Valve	186000306
2795 Column Heater with Column Regeneration Valve	Contact Waters Sales
Waters 2795 Bar Code Reader	176000156

B.4 Recommended Plate Options

STOP **Attention:** *To avoid damage to the needle or XYZ mechanism, do not use vial caps less than 8.8 mm in diameter (OD).*

STOP **Attention:** *To avoid damage to the needle or XYZ mechanism, do not use polyethylene cap mats below 15 °C. If the sample manager is maintained above 15 °C, a polyethylene cap mat for 96 square well plates is acceptable. However, below 15 °C, the mat may become rigid and cause lifting of the plate from the carrier in the inject cycle. Sample stability may also decrease. If the sample manager is maintained from 4 to 15 °C, a PTFE/silicone cap mat is recommended (see Table B-5).*

STOP *Attention: To avoid clogging the inject port and needle, do not use certain adhesive sealing materials to seal vials. You can use thin (household-variety) aluminum foil to seal 96- and 384-well plates. Some heat-sealable foils are also acceptable, such as a heat-sealing apparatus (Marsh Biomedical) and/or the Thermowell™ Sealer for aqueous samples (Corning Costar).*

STOP *Attention: To avoid damaging the 2795 Separations Module, Waters recommends that you consult Table B-5 before operating the 2795 Separations Module. Be sure to read all the recommendations in the footnotes to Table B-5.*

Table B-5 provides important information about Waters-supplied plates and other plates used with the 2795 Separations Module.

! *Caution: Due to variations in dimensions, do not use the Seek Well Bottom feature with thin-bottom glass inserts (such as part number 186000349) or with the two microcentrifuge sample container plates (part numbers 405000560 and 405000772).*

Table B-5 2795 Separations Module Plate Parameters

Waters Part Number	Description	Plate Parameters[a,d]						Comments
		Plate Height	Well Depth[h]	A-1 Offset		Column Space	Row Space	
				Left	Rear			
186000349	Waters 96-well with 700-µL glass inserts	44.1	41.1	14.3[f]	11.3	9	9	2795 Separations Module Startup Kit
WAT058943	Waters 96-well, tall, 350-µL, polypropylene	43.9	13.2	14.1[f]	11.3	9	9	2795 Separations Module Startup Kit
WAT058957	Waters 96-well, tall, 1.0-mL, polypropylene	43.9	23.2	14.1[f]	11.3	9	9	
WAT058958	Waters 96-well, tall, 2.0-mL, polypropylene	43.9	38.7	14.1[f]	11.3	9	9	

Table B-5 2795 Separations Module Plate Parameters *(Continued)*

Waters Part Number	Description	Plate Parameters[a,d]						Comments
		Plate Height	Well Depth[h]	A-1 Offset		Column Space	Row Space	
				Left	Rear			
WAT058959	Waters, sealing cap, piercable, for 96-well tall plates (listed above)[c]	–	–	–	–	–	–	2795 Separations Module Startup Kit (for use with 4 plates above). Also available in pack of 5.
186000857	Waters, PFTE/Silicone sealing cap[j]	–	–	–	–	–	–	
405000562	Waters 48-position 2.0-mL vial holder	25.4	16.8[e]	15	8.3	14	14	2795 Separations Module Startup Kit
186000327	Waters 1.5-mL Max Recovery vial kit with pre-slit septa	–	–	–	–	–	–	2795 Separations Module Startup Kit (contains 100 vials/caps)
186000307	Waters 2-mL glass vial kit with pre-slit septa	–	–	–	–	–	–	100 general purpose 2-mL vials/caps
405000560	Waters 24-position 1.5-mL microcentrifuge tube holder	33.8	28.0[e]	14.2	11.2	19.8	19.8	2795 Separations Module Startup Kit. Cap must be open and inserted in slot.
405000772	Waters 48-position 0.5-mL microcentrifuge tube holder	28.7	23.0[e]	12.0	9.0	14.5	13.5	Cap must be open and inserted in slot

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Waters Part Number	Description	Plate Parameters[a,d]						Comments
		Plate Height	Well Depth[h]	A-1 Offset		Column Space	Row Space	
				Left	Rear			
200000114	Waters Open Access Kit (96 vials) Waters 24-position, 2-mL vial open access plates	20.2[i]	10.2[e]	13.8	12.7	20	20	Contains 4 plates (24-position for 2-mL vials) and 100 2-mL vials with pre-slit septa (186000307)
200000123	Waters High-Capacity Open Access Kit (192 vials) Waters 48-position, 2-mL vial open access plates	25.4	16.8[e]	15	8.3	14	14	Contains 4 plates (48-position for 2-mL vials) and 100 2-mL vials with pre-slit septa (186000307)
–	96-well, 300-µL plate, typical[a]	14.3	10.0	14.1	11.3	9	9	Dimensions vary by manufacturer
–	384-well, 80-µL plate, polystyrene, typical[b,g]	14.4	10.5	12.1	9.0	4.5	4.5	Dimensions vary and are critical. Usable volume typically 80 µL due to needle displacement volume.

a. Check dimensions of all non-Waters plates before entering in plate parameter fields. Use of incorrect dimensions can cause needle damage.

c. Use only sealing caps that are pre-scored or thinned in the needle penetration area.

d. Waters strongly recommends against using adhesively-applied sealing films or foils. The adhesive tends to clog the inject port and needle. Heat-sealable foils are an acceptable alternative.

e. Measured with Waters vials or generic tubes in wells. Confirm tube measurement. Minimum vial cap outside diameter measurement is 8.8 mm.

f. Use Waters piercable sealing cap (Waters part number WAT058959) or Waters PTFE/Silicone Sealing Cap (Waters part number 1860000857) to prevent evaporation or spillage. Also acts as a retainer for glass inserts (Waters part number 186000349).

g. Use care when selecting polypropylene 384-well plates for use, because straightness of rows and columns may be marginal.

h. Do not use thin or flimsy plates or polymerase chain reaction (PCR) tubes. They may jam the XYZ mechanism or be penetrated by the needle.

i. Measure at the "plate height" check point near the number 19 vial location.

j. Ensure that the well contains some air to prevent a vacuum and to ensure sample draw precision.

Appendix C
Solvent Considerations

⚠️ **Caution:** *To avoid chemical hazards, always observe safe laboratory practices when operating your system.*

C.1 Solvent Quality

For best results:

- Use HPLC-grade solvents
- Filter solvents through a 0.45-μm filter
- Degas solvents.

When using unstabilized tetrahydrofuran (THF), ensure that your solvent is fresh. Previously opened bottles of THF contain peroxide contaminants that cause baseline drift.

⚠️ **Caution:** *Tetrahydrofuran (THF) contaminants (peroxides) are potentially explosive if concentrated or taken to dryness.*

C.2 Solvent-System Compatibility

The Waters 2795 Separations Module is constructed of high-quality (316) stainless steel components that, with some minor restrictions, can be used with all solvents. This section lists the solvents that have and have not been approved for use with the 2795 Separations Module.

Solvents to Avoid

You can use any solvent with the 2795 Separations Module. However, long-term *static* exposure to halide salts (for example, fluoride, bromide, chloride, and iodide) causes pitting and corrosion of stainless steel parts. When you use these salts, flush your system

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thoroughly with water if the solvent management system is to be idle for more than two days. Refer to Section 4.2.3, Wet Priming.

Solvents to Use

Materials of construction used in the Separations Module are nonreactive with most acids, bases, salts, and organic solvents.

The solvents listed in Table C-1 through Table C-4 have been approved for use with the Separations Module. These include salts, acids and bases in concentrations up to 1 M (unless otherwise noted), and organic solvents in concentrations of up to 100% (unless otherwise noted). Concentrations higher than those indicated in Table C-1 through Table C-4 can be used in many instances.

For information on the use of a specific solvent or concentration that is not listed in this manual, contact Waters Technical Service.

Table C-1 Aqueous Buffer Components for Use with the 2795 Separations Module

Aqueous Buffer Components			
Acetate	K_2SO_4	Na_2S	Perfluorobutyric acid
Al_2SO_4	$K_3Fe(CN)_6$	Na_2CO_3	NH_4Cl
$Ca(OCl)_2$	$K_4Fe(CN)_6$	Na_2SO_4	Phosphate
$CaCl_2$	KBr	NaCl	Tartrate
Citrate	KCl	Sodium acetate	Trilithium citrate
H_2O_2, up to 10%	$KHCO_3$	NaH_2BO_3	Tris [tris-(hydroxym-ethyl) aminomethane]
HIBA	$KMnO_4$	$NaHCO_3$	4-(2-pyridylazo) resorcinol monoso-dium salt monohydrate
K_2CO_3	KNO_3	$NaHSO_4$	
$K_2Cr_2O_3$	$LiClO_4$	$NaNO_3$	
K_2S	$Na_2B_4O_7$	NaOCl	

Table C-2 Acids for Use with the 2795 Separations Module

Acids		
Acetic acid, glacial	Hydrochloric acid	Perchloric acid
Benzoic acid	Lactic acid	Phosphoric acid
Chromic acid	Methanesulphonic acid	Pyridine-2,6-dicarboxylic acid
Citric acid	Nitric acid, up to 37.5% (6 N)	Sulfuric acid, up to 0.20 M
Formic acid	Octanesulphonic acid	Trifluoroacetic acid (TFA), up to 10%
Glyceric acid	Oxalic acid	

Table C-3 Bases for Use with the 2795 Separations Module

Bases	
Ba(OH)$_2$	NaOH, up to 10 M
KOH	NH$_4$OH, up to 3 M
LiOH	Tetramethylammonium hydroxide pentahydrate

Table C-4 Organic Solvents for Use with the 2795 Separations Module

Organic Solvents			
4-cyanophenol	Chloroform	Ethylene glycol	Methylene chloride
Acetone	Cyclohexane	Formaldehyde	n-Propanol
Acetonitrile	Cyclohexanone	Heptane	Phenol
Amyl acetate	Dibutyl phthalate	Hexane	Tetrahydrofuran (THF)
Benzaldehyde	Dimethyl formamide	iso-Octane	Toluene
Benzene	Dimethyl sulfoxide	iso-Propanol	Waters PIC® Reagents
Benzyl alcohol	Ethanol	Lysine hydrochloride	Xylene
Butanol	Ethyl acetate	Methanol	
Carbon tetrachloride	Ethylene dichloride	Methyl ethyl ketone	

C.3 Solvent Miscibility

Before you change solvents, refer to <u>Table C-5</u> to determine the miscibility of the solvents to be used. When you change solvents, be aware that:

- Changes involving two miscible solvents may be made directly. Changes involving two solvents that are not totally miscible (for example, chloroform and water), require an intermediate solvent (such as methanol).
- Temperature affects solvent miscibility. If you are running a high-temperature application, consider the effect of the temperature on solvent solubility.
- Buffers dissolved in water may precipitate when mixed with organic solvents.

Table C-5 Solvent Miscibility

Polarity Index	Solvent	Viscosity CP, 20 °C	Boiling Point °C (@1 bar atm)	Miscibility Number (M)	λ Cutoff (nm)
−0.3	N-decane	0.92	174.1	29	—
−0.4	Iso-octane	0.50	99.2	29	210
0.0	N-hexane	0.313	68.7	29	—
0.0	Cyclohexane	0.98	80.7	28	210
1.7	Butyl ether	0.70	142.2	26	—
1.8	Triethylamine	0.38	89.5	26	—
2.2	Isopropyl ether	0.33	68.3	—	220
2.3	Toluene	0.59	100.6	23	285
2.4	*p*-xylene	0.70	138.0	24	290
3.0	Benzene	0.65	80.1	21	280
3.3	Benzyl ether	5.33	288.3	—	—
3.4	Methylene chloride	0.44	39.8	20	245
3.7	Ethylene chloride	0.79	83.5	20	—
3.9	Butyl alcohol	3.00	117.7	—	—
3.9	Butanol	3.01	177.7	15	—
4.2	Tetrahydrofuran	0.55	66.0	17	220
4.3	Ethyl acetate	0.47	77.1	19	260

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Table C-5 Solvent Miscibility *(Continued)*

Polarity Index	Solvent	Viscosity CP, 20 °C	Boiling Point °C (@1 bar atm)	Miscibility Number (M)	λ Cutoff (nm)
4.3	1-propanol	2.30	97.2	15	210
4.3	2-propanol	2.35	117.7	15	—
4.4	Methyl acetate	0.45	56.3	15, 17	260
4.5	Methyl ethyl ketone	0.43	80.0	17	330
4.5	Cyclohexanone	2.24	155.7	28	210
4.5	Nitrobenzene	2.03	210.8	14, 20	—
4.6	Benzonitrile	1.22	191.1	15, 19	—
4.8	Dioxane	1.54	101.3	17	220
5.2	Ethanol	1.20	78.3	14	210
5.3	Pyridine	0.94	115.3	16	305
5.3	Nitroethane	0.68	114.0	—	—
5.4	Acetone	0.32	56.3	15, 17	330
5.5	Benzyl alcohol	5.80	205.5	13	—
5.7	Methoxyethanol	1.72	124.6	13	—
6.2	Acetonitrile	0.37	81.6	11, 17	190
6.2	Acetic acid	1.26	117.9	14	—
6.4	Dimethylformamide	0.90	153.0	12	—
6.5	Dimethylsulfoxide	2.24	189.0	9	—
6.6	Methanol	0.60	64.7	12	210
7.3	Formamide	3.76	210.5	3	—
9.0	Water	1.00	100.0	—	—

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How to Use Miscibility Numbers (M-numbers)

Use Miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent (see Table C-5).

To predict the miscibility of two liquids, subtract the smaller M-number value from the larger M-number value.

- If the difference between the two M-numbers is 15 or less, the two liquids are miscible in all proportions at 15 °C.

- A difference of 16 indicates the solvents are miscible at temperatures between 25 and 75 °C, with 50 °C as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible or miscible only above 75 °C.

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents are assigned a dual M-number:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents.
- The second number, always greater than 16, indicates the degree of miscibility with highly hydrophilic solvents.
- A large difference between these two M-numbers indicates a limited range of miscibility.

For example, some fluorocarbons are immiscible with all the standard solvents and have M-numbers of 0 and 32. Two liquids with dual M-numbers are usually miscible with each other.

A liquid is classified in the M-number system by testing for miscibility with a sequence of standard solvents. A correction term of 15 units is then either added or subtracted from the cutoff point for miscibility.

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C.4 Buffered Solvents

When using a buffer, use a good quality reagent and filter it through a 0.45-µm filter.

Do not leave the buffer stored in your HPLC system. Flush all fluidic pathways with HPLC-quality water before shutting down the system and leave distilled water in the system or flush with 90% HPLC-quality water:10% methanol for shutdowns scheduled to be more than one day. Flush using a minimum of 45 mL for inline vacuum degasser-equipped units.

C.5 Solvent Viscosity

Generally, viscosity is not important when you are operating with a single solvent or under low pressure. However, when you run a gradient, the viscosity changes that occur as the solvents are mixed in different proportions can result in pressure changes during the run. For example, a 1:1 mixture of water and methanol produces twice the pressure of either water or methanol alone.

If the extent to which the pressure changes affect the analysis is not known, monitor the pressure during the run using the Chart Out terminal.

C.6 Solvent Degassing

Mobile phase difficulties account for 70 percent or more of all liquid chromatographic problems. Using degassed solvents is important, especially at wavelengths below 220 nm. Degassing provides:

- Stable baselines and enhanced sensitivity
- Reproducible retention times for eluting peaks
- Reproducible injection volumes for quantitation
- Stable pump operation

This section presents information on the solubility of gases, solvent degassing methods, and solvent degassing considerations.

Gas Solubility

The amount of gas dissolved in a given volume of liquid depends on:

- The chemical affinity of the gas for the liquid
- The temperature of the liquid
- The pressure applied to the liquid

Changes in the composition, temperature, or pressure of the mobile phase can lead to outgassing.

Effects of Intermolecular Forces

Nonpolar gases (N_2, O_2, CO_2, He) are more soluble in nonpolar solvents than in polar solvents. Generally, a gas is most soluble in a solvent whose intermolecular attractive forces are similar to those in the gas ("like dissolves like").

Effects of Temperature

Temperature affects the solubility of gases. If the heat of solution is exothermic, the solubility of the gas decreases when you heat the solvent. If the heat of solution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in H_2O decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

Effects of Partial Pressure

The mass of gas dissolved in a given volume of solvent is proportional to the partial pressure of the gas in the vapor phase of the solvent. If you decrease the partial pressure of the gas, the amount of that gas in solution also decreases.

Solvent Degassing Methods

You can degas solvents by either of the following methods:

- Sparging with helium (does not apply to 2795 Separations Module with inline vacuum degasser)
- Vacuum degassing

Note: Sparging is not required when the 2795 Separations Module degasser is used for solvent degassing.

Sparging

Helium sparging gives stable baselines and better detection sensitivity than sonication, and prevents reabsorption of atmospheric gases. Use this method to retard oxidation when you are using THF or other peroxide-forming solvents.

Vacuum Degassing

The longer a solvent is exposed to a vacuum, the more dissolved gases are removed. Two factors affect the amount of time the solvent is exposed to the vacuum:

- **Flow rate** – At low flow rates, most of the dissolved gas is removed as the solvent passes through the vacuum chamber. At higher flow rates, lesser amounts of gas per unit volume of solvent are removed.
- **Surface area of the degassing membrane** – The length of the degassing membrane is fixed in each vacuum chamber. To increase the length of membrane, you can connect two or more vacuum chambers in series.

The degasser for the 2795 Separations Module is factory-installed. Refer to the "PerformancePLUS Degasser" discussion in Section 1.2.

C.7 Wavelength Selection

The tables in this section provide UV cutoff values for:

- Common solvents
- Common mixed mobile phases
- Chromophores

Wavelength Cutoffs for Common Solvents

Table C-6 shows the wavelength cutoff (the wavelength at which the absorbance of the solvent is equal to 1 AU) for some common chromatographic solvents. Operating at a wavelength near or less than the cutoff increases baseline noise due to the absorbance by the solvent.

Table C-6 UV Cutoff Wavelengths for Common Chromatographic Solvents

Solvent	UV Cutoff (nm)	Solvent	UV Cutoff (nm)
1-Nitropropane	380	Ethylene glycol	210
2-Butoxyethanol	220	Iso-octane	215
Acetone	330	Isopropanol	205
Acetonitrile	190	Isopropyl chloride	225
Amyl alcohol	210	Isopropyl ether	220
Amyl chloride	225	Methanol	205
Benzene	280	Methyl acetate	260
Carbon disulfide	380	Methyl ethyl ketone	330
Carbon tetrachloride	265	Methyl isobutyl ketone	334
Chloroform	245	Methylene chloride	233
Cyclohexane	200	*n*-Pentane	190
Cyclopentane	200	*n*-Propanol	210
Diethyl amine	275	*n*-Propyl chloride	225
Dioxane	215	Nitromethane	380
Ethanol	210	Petroleum ether	210
Ethyl acetate	256	Pyridine	330
Ethyl ether	220	Tetrahydrofuran	230
Ethyl sulfide	290	Toluene	285
Ethylene dichloride	230	Xylene	290

Wavelength Cutoffs for Mixed Solvents

Table C-7 provides approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most

commonly used. If you use a different concentration, you can determine approximate absorbance using Beer's Law (absorbance is proportional to concentration).

Table C-7 Wavelength Cutoffs for Different Mobile Phases

Mobile Phase	UV Cutoff (nm)	Mobile Phase	UV Cutoff (nm)
Acetic acid, 1%	230	Sodium chloride, 1 M	207
Ammonium acetate, 10 mM	205	Sodium citrate, 10 mM	225
Ammonium bicarbonate, 10 mM	190	Sodium dodecyl sulfate	190
BRIJ 35, 0.1%	190	Sodium formate, 10 mM	200
CHAPS, 0.1%	215	Triethyl amine, 1%	235
Diammonium phosphate, 50 mM	205	Trifluoracetic acid, 0.1%	190
EDTA, disodium, 1 mM	190	TRIS HCl [*tris*-(hydroxyme-thyl)- aminoethane, hydrochloride], 20 mM, pH 7.0, pH 8.0	202, 212
HEPES (N-2-hydroxyethyl-piperazine-N^1-2-ethane-sulfonic acid), 10 mM, pH 7.6	225	Triton-X™ 100, 0.1%	240
Hydrochloric acid, 0.1%	190	Waters PIC® Reagent A, 1 vial/liter	200
MES [2-(N-morpholine) ethane- sulfonic acid], 10 mM, pH 6.0	215	Waters PIC Reagent B-6, 1 vial/liter	225
Potassium phosphate, monobasic, 10 mM dibasic, 10 mM	190 190	Waters PIC Reagent B-6, low UV, 1 vial/liter	190
Sodium acetate, 10 mM	205	Waters PIC Reagent D-4, 1 vial/liter	190

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Refractive Indices of Common Solvents

Table C-8 lists the refractive indices for some common chromatographic solvents. When using an RI detector, refer to this table to verify that the solvent you intend to use for your

analysis has a refractive index (RI) significantly different from the RIs of the sample components.

Table C-8 Refractive Indices for Common Chromatographic Solvents

Solvent	RI	Solvent	RI
Fluoroalkanes	1.25	Tetrahydrofuran (THF)	1.408
Hexafluoroisopropanol (HFIP)	1.2752	Amyl alcohol	1.410
Methanol	1.329	Diisobutylene	1.411
Water	1.33	n-Decane	1.412
Acetonitrile	1.344	Amyl chloride	1.413
Ethyl ether	1.353	Dioxane	1.422
n-Pentane	1.358	Ethyl bromide	1.424
Acetone	1.359	Methylene chloride	1.424
Ethanol	1.361	Cyclohexane	1.427
Methyl acetate	1.362	Ethylene glycol	1.427
Isopropyl ether	1.368	N,N-Dimethyl formamide (DMF)	1.428
Ethyl acetate	1.370	N,N-Dimethyl acetamide (DMAC)	1.438
1-Pentene	1.371	Ethyl sulfide	1.442
Acetic acid	1.372	Chloroform	1.443
Isopropyl chloride	1.378	Ethylene dichloride	1.445
Isopropanol	1.38	Carbon tetrachloride	1.466
n-Propanol	1.38	Dimethyl sulfoxide (DMSO)	1.477
Methylethylketone	1.381	Toluene	1.496
Diethyl amine	1.387	Xylene	~1.50
n-Propyl chloride	1.389	Benzene	1.501
Methylisobutylketone	1.394	Pyridine	1.510
Nitromethane	1.394	Chlorobenzene	1.525
1-Nitropropane	1.400	o-Chlorophenol	1.547
Isooctane	1.404	Aniline	1.586
Cyclopentane	1.406	Carbon disulfide	1.626

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