Waters 1500 Series HPLC Pump

Installation and Maintenance Guide





34 Maple Street Milford, MA 01757



The information in this document is subject to change without notice and should not be construed as a commitment by Waters Corporation. Waters Corporation assumes no responsibility for any errors that may appear in this document. This manual is believed to be complete and accurate at the time of publication. In no event shall Waters Corporation be liable for incidental or consequential damages in connection with, or arising from, the use of this manual.

© 2000 WATERS CORPORATION. PRINTED IN THE UNITED STATES OF AMERICA. ALL RIGHTS RESERVED. THIS BOOK OR PARTS THEREOF MAY NOT BE REPRODUCED IN ANY FORM WITHOUT THE WRITTEN PERMISSION OF THE PUBLISHER.

Breeze and LAC/E are trademarks, and PIC and Waters are registered trademarks of Waters Corporation.

Microsoft, Windows, and Windows NT are registered trademarks of Microsoft Corporation.

All other trademarks are the sole property of their respective owners.



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.



Attention: 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 take steps to determine the reason for the change. Until you determine the cause for the change, do not rely on the results of the separations.



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



Caution: For continued protection against fire hazard, replace fuses with those of the same type and rating.



Caution: To avoid possible electric shock, do not remove the pump cover. The interior of the pump does not contain user-serviceable parts.



Caution: Before you remove any fluidic connection on the pump, position the eluent reservoir below the height of the pump inlet manifold to prevent gravity flow of eluent out through disassembled fluidic connections.



Caution: To avoid high temperature hazards, always allow adequate time to cool the system before you perform maintenance or troubleshooting. Wear protective clothing whenever you open the sample compartment or the analysis compartment.

Symbols Used on the Waters 1500 Series HPLC Pumps

÷

\sim	Alternating current
	Protective conductor terminal
	Hot surfaces or hot temperatures
1	Caution, risk of electric shock (high voltage)
Â	Caution or refer to manual

Intended Use

The Waters[®] 1500 Series HPLC Pumps can be used for in-vitro diagnostic testing to analyze many compounds, including diagnostic indicators and therapeutically monitored compounds. When developing 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.

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. Concentration range should cover the entire range of quality control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples. 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 form day to day and run to run. Data collected when quality control samples are out of range may not be valid. Do not report this data before ensuring that chromatographic system performance is acceptable.

Table of Contents

List of	Figures	10
List of	Tables	11
How to	Use This Guide	12
Chapte	er 1	
Overvi	ew of the Waters 1500 Series HPLC Pump	15
1.1	Fluid-Handling Components	16
1.2	Electronic Components	20
1.3	1500 Series Pump Control	21
Chapte	er 2	
Installi	ng a Waters 1500 Series HPLC Pump	22
2.1	Site Requirements	22
2.2	Unpacking the Pump	24
2.3	Making Electrical Connections	24
	2.3.1 Connecting the Power Supply	25
	2.3.2 Setting the IEEE-488 Address for the Pump.	25
	2.3.3 Making the IEEE-488 Connections	27
	2.3.4 Connecting the Inject Start Signal	
	(1500 Series Manual Injector)	29
2.4	Making Fluidic Connections	33
	2.4.1 Making Eluent Supply Connections	33
	2.4.2 Installing Different Mixer Configurations	35

	2.4.3 To Install an Optional 1500 Series	
	Manual Injector	39
	2.4.4 Installing an Optional 1500 Series	
	Column Heater	39
	2.4.5 Connecting the Pump Outlet	40
	2.4.6 Connecting Fluid Waste Lines	41
Chapte	er 3	
Prepar	ing for Operation	43
3.1	Powering On the Pump	43
3.2	Preparing the Pump	44
	3.2.1 Priming and Purging the Pump	45
	3.2.2 Purging the Injector and Detector	46
	3.2.3 Equilibrating the System	46
3.3	Powering Off the Pump	47
Chapte	er 4	
Mainta	ining the Waters 1500 Series HPLC Pump	48
4.1	Maintenance Considerations	48
4.2	Diagnostic Tests	49
	4.2.1 Retention Time Stability Test	49
	4.2.2 Ramp-and-Decay Test	49
4.3	Replacing and Cleaning Plunger Seals and Plungers.	51
	4.3.1 Preparing for Plunger Seal Replacement	51
	4.3.2 Cleaning and Replacing the Plungers	54
4.4	Replacing Check Valves	59
4.5	Replacing a Draw-Off Valve	61

Replacing Fuses	. 64
ter 5 Joshooting	66
Troublookasting Dump Brobloms	67
	. 07
Identifying and Correcting Noises	. 73
B Identifying Chromatographic Problems	. 74
ndix A	
fications	. 78
ndix B	
Parts and Accessories	. 81
ndix C	
nt Considerations	. 85
I Introduction	. 85
2 Solvent Compatibility	. 86
3 Solvent Miscibility	. 88
Buffered Solvents	. 92
5 Head Height	. 92
Solvent Viscosity	. 92
7 Mobile Phase Solvent Degassing	. 92
C.7.1 Gas Solubility	. 93
C.7.2 Eluent Degassing Methods	. 93
3 Wavelength Selection	. 95
	 Replacing Fuses

List of Figures

Waters 1525 HPLC Pump with Optional Column Heater	. 16
Fluid-Handling Components in the Waters 1525 Pump	. 17
Rear Electronic Components of a Waters 1500 Series Pump	. 20
Dimensions of a 1500 Series Pump	. 23
IEEE-488 Connection Example	. 28
Inject Start Connection to a 2487 Detector	. 31
Inject Start Connection to a 2410 Detector	. 32
Reverse Ferrule and Compression Screw Assembly	. 34
1525 Pump Showing Different Mixer Configurations	. 39
Unscrewing the Pump Head Mounting Bolts	. 53
Plunger Seal	. 53
Exposed Head Support Assembly	. 55
Removing the Plunger Assembly	. 56
Disassembled Plunger	. 57
Inserting the Plunger	. 58
Disconnecting the Outlet Tubing	. 60
Outlet Check Valve Assembly	. 61
Draw-Off Valve/Inlet Manifold Assembly	. 63
Rear Panel Fuse Holder	. 64
	Waters 1525 HPLC Pump with Optional Column Heater Fluid-Handling Components in the Waters 1525 Pump Rear Electronic Components of a Waters 1500 Series Pump Dimensions of a 1500 Series Pump IEEE-488 Connection Example Inject Start Connection to a 2487 Detector Inject Start Connection to a 2410 Detector Reverse Ferrule and Compression Screw Assembly 1525 Pump Showing Different Mixer Configurations Unscrewing the Pump Head Mounting Bolts Plunger Seal Exposed Head Support Assembly Disassembled Plunger Disconnecting the Outlet Tubing Outlet Check Valve Assembly Draw-Off Valve/Inlet Manifold Assembly Rear Panel Fuse Holder

List of Tables

1-1	Fluid-Handling Components in the Waters 1525 Pump	18
1-2	Electronic Components in a 1500 Series Pump	21
2-1	Installation Site Requirements	22
2-2	IEEE-488 DIP Switch Settings	25
2-3	I/O Terminal Block Connections	29
3-1	Pump Preparation Recommendations	44
5-1	Determining Pump Malfunctions	67
5-2	Identifying Noises	73
5-3	Correcting Chromatographic Problems	74
A-1	Physical Specifications for 1500 Series Pump	78
A-2	Physical Specifications for Column Heater	78
A-3	Environmental Specifications	79
A-4	Electrical Specifications	79
A-5	Performance Specifications	79
A-6	Instrument Control and Communication Specifications	80
B-1	Spare Parts	81
B-2	Accessories	82
B-3	Maintenance and Operation Kits	83
C-1	Aqueous Buffers for Use with the 1500 Series HPLC Pump	87
C-2	Acids for Use with the 1500 Series HPLC Pump	87
C-3	Bases for Use with the 1500 Series HPLC Pump	88
C-4	Organic Solvents for Use with the 1500 Series HPLC Pump.	88
C-5	Solvent Miscibility	89

C-6	UV Cutoff Wavelengths for Common Chromatographic	
	Solvents	95
C-7	Wavelength Cutoffs for Different Mobile Phases	97
C-8	Refractive Indices for Common Chromatographic Solvents	98
D-1	Waters 1500 Series HPLC Pump Warranty Periods	103

How to Use This Guide

Purpose

The Waters 1500 Series HPLC Pump Installation and Maintenance Guide describes the procedures for unpacking, installing, operating, maintaining, and troubleshooting the Waters 1515 Isocratic and 1525 Binary HPLC Pumps. It also includes appendixes for specifications, spare parts, eluent considerations, validation support, and warranty information.

Audience

This guide is intended for use by individuals who need to install, maintain, and/or troubleshoot the Waters 1515 Isocratic and 1525 Binary HPLC Pumps.You should be familiar with HPLC terms, practices, and basic HPLC system operations such as making fluidic connections.

Structure

The Waters 1500 Series HPLC Pump Installation and Maintenance Guide has five chapters and four appendixes. Each page is marked with a tab and a footer to help you access information.

The table below describes the material covered in this book.

Chapter/Appendix	Description
Chapter 1, Overview of the Waters 1500 Series HPLC Pump	Describes the Waters 1500 Series HPLC Pumps, including features and options.
<u>Chapter 2, Installing a</u> <u>Waters 1500 Series HPLC</u> <u>Pump</u>	Tells how to unpack and install the Waters 1500 Series HPLC Pumps.
Chapter 3, Preparing for Operation	Describes how to power on the pump, prime and purge the pump, purge the injector and detector, equilibrate the system, and power off the pump.

Chapter/Appendix	Description
Chapter 4, Maintaining the Waters 1500 Series HPLC Pump	Covers routine maintenance, diagnostics, and troubleshooting procedures.
<u>Chapter 5,</u> <u>Troubleshooting</u>	Explains how to diagnose and resolve problems with the pump.
Appendix A, Specifications	Provides the specifications of the Waters 1500 Series HPLC Pumps.
Appendix B, Spare Parts and Accessories	Lists the recommended and optional spare parts.
Appendix C, Solvent Considerations	Provides information about properly preparing and using solvents.
Appendix D, Warranty Information	Includes warranty and service information.

Conventions Used in this Guide

This guide uses the following conventions to make text easier to understand:

• Courier text indicates system output. For example:

Hi Press means that the pump high pressure limit has been exceeded.

• Bold text indicates user action. For example:

Press Menu to store the parameter.

• *Italic* text denotes new or important words, and is also used for emphasis. For example:

Before operation, always check for eluent miscibility.

 <u>Underlined</u>, <u>Blue Color</u> text indicates hypertext cross-references to a specific chapter, section, subsection, or sidehead. Clicking this topic using the hand symbol automatically brings you to this topic within the electronic document. Right-clicking and selecting **Go Back** from the popup context menu brings you back to the originating topic.

Notes, Attentions, and Cautions

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

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

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



Attention: To avoid having eluent leak from a pump outlet, position each eluent reservoir below its corresponding pump inlet until the outlet is connected to the system.

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



Caution: To avoid possible electric shock, do not remove the pump cover. The interior of the pump does not contain user-serviceable parts.



Caution: To avoid high temperature hazards, always allow adequate time to cool the system before you perform maintenance or troubleshooting. Wear protective clothing whenever you open the sample compartment or the analysis compartment.



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

1 Overview of the Waters 1500 Series HPLC Pump

This chapter describes the Waters[®] 1515 and 1525 HPLC Pumps, discusses fluid-handling and electronic components, and talks about controlling the pumps.

The Waters 1500 Series HPLC Pumps combine the most important aspects of eluent delivery for HPLC: high precision, reliability, smooth eluent flow, and proven performance. Both pumps perform their intended functions equally well:

- The 1515 Isocratic HPLC Pump is designed for precise isocratic analyses.
- The 1525 Binary HPLC Pump achieves reproducible, binary gradient delivery with exceptionally smooth concurrent-stream blending.

Both pumps are controlled by the Waters BreezeTM HPLC system software. The microprocessor-controlled stepper motor and noncircular gears of each 1500 Series Pump ensures smooth and precise flow regardless of back pressure, flow-rate setting, or eluent compressibility.

Figure 1-1 shows a Waters 1525 Binary HPLC Pump with an optional 1500 Series Column Heater.





1.1 Fluid-Handling Components

Before you install a Waters 1500 Series Pump, familiarize yourself with its fluid-handling components. Figure 1-2 identifies the fluid-handling components of a Waters 1525 Binary HPLC Pump.



Figure 1-2 Fluid-Handling Components in the Waters 1525 Pump

<u>Table 1-1</u>describes the functions of the fluid-handling components.

Table 1-1 Fluid-Handling Components in the Waters 1525 Pum p

Component	Description
Gradient mixer	Combines eluents together and helps to increase homogeneity. Also adds a desired delay volume to the system.
Draw-off valve	Enables you to attach a syringe and draw eluent through the eluent reservoir line into the pump for priming.
Reference valve	Directs flow from the pumps to waste for purging, or through the injector, the column, and the rest of the system.
Reference valve waste line	Routes flow from the reference valve to the waste container.
Drip tray	Catches fluid leaks.
Drip tray waste exit	Drains leaks to the waste container.
Seal wash holes (not visible in <u>Figure 1-2</u>)	Allow you to flush and clean the plunger seals.
Pump head assembly	Draws in and delivers eluent.
Pulse dampeners	Dampens operating pressure fluctuations. It is located on the left side of the unit under the mixer and behind the shroud.
Inlet manifold	Provides the connection for eluent inlet tubing and routes eluent to the inlet check valve on each pump head.
Pressure transducer	Senses operating pressure and converts values to electronic signals for monitoring.
Pump outlet	Routes eluent to the injector, column, and detector.
	Caution: To prevent eluent from leaking out of the pump outlet, make sure you position the eluent reservoir below the pump inlet manifold before you disconnect the pump outlet fitting.

Table 1-1 Fluid-Handling Components in the Waters 1525 Pump (Continued)

Component	Description
Optional 1500 Series Manual Injector	Enables a manual sample injection. Generates an inject start to the detector.
Manual injector valve waste lines	Drains fluid overflow from the syringe to the waste container.
Inlet and outlet check valve assemblies	Maintain flow direction by opening in one direction only.
Plunger indicator rods	Show the position of each pump head plunger.
Optional 1500 Series Column Heater (not shown)	Maintains elevated column temperature to facilitate better method reproducibility.

1.2 Electronic Components

Before you install the Waters 1500 Series Pump, familiarize yourself with its electronic components as illustrated in Figure 1-3.



Figure 1-3 Rear Electronic Components of a Waters 1500 Series Pump

<u>Table 1-2</u> describes the functions of the electronic components in a 1500 Series Pump.

Table 1-2 Electronic Components in a 1500 Series Pump

Component	Description
Cooling fan vent	Exhausts air for cooling internal electronics.
I/O terminal block	Provides input and output contact closures for connection to external devices.
Power entry module	Provides receptacles for power cord connection and fuses.
IEEE-488 connector	Connects the pump to a PCI busLAC/E TM card in the Breeze workstation.
IEEE-488 address switches	Sets the IEEE-488 address for the pump.
Power switch	Powers the pump on and off.
Fuse holder	Contains power fuses.

1.3 1500 Series Pump Control

The Waters Breeze software allows you to control and monitor Waters 1500 Series HPLC Pumps in isocratic or binary applications. Using Breeze software, you can:

- · Set all pump control parameters and operating ranges
- Define binary gradient conditions for a run (1525 HPLC Pump only)
- Prime and purge the pump fluidics

2 Installing a Waters 1500 Series HPLC Pump

This chapter lists the site requirements for a Waters[®] 1500 Series HPLC Pump, describes how to unpack the pump, and make electrical and fluidic connections.

2.1 Site Requirements

Install the 1500 Series HPLC Pump at a site that meets the specifications indicated in <u>Table 2-1</u>.

Factor	Requirement
Temperature	4 to 40 °C (39 to 104 °F)
Relative humidity	20 to 80%, noncondensing
Bench space	Width: 17 in. (43 cm) including bottle holder [24" (61 cm) with 1500 Series Column Heater] Depth: 24 in. (61 cm) Height: 17 in. (43 cm)
Vibration	Negligible
Clearance	At least 6 in. (15 cm) at rear for ventilation and cable connections
Static electricity	Negligible
Input voltage and frequency	Grounded ac, 120/240 Vac, 50/60 Hz, single phase

Table 2-1 Installation Site Requirement s

Table 2-1 Installation Site Requirements (Continued)

Factor	Requirement
Electromagnetic fields	No nearby source of electromagnetic noise, such as arcing relays or electric motors
Power requirement	200 VA for pump 150 VA for column heater

Figure 2-1 shows the dimensions of a 1500 Series Pump.



Attention: To avoid overheating and to provide clearance for cable connections, make sure there is at least 6 inches (15 cm) of clearance at the rear of the 1500 Series Pump.



Figure 2-1 Dimensions of a 1500 Series Pump

2.2 Unpacking the Pump

A Waters 1500 Series HPLC Pump is shipped in one carton that contains the following items:

- Waters 1515 Isocratic or 1525 Binary HPLC Pump
- Startup Kit
- Bottle Holder
- Certificate of Structural Validation
- Waters 1500 Series HPLC Pump Installation and Maintenance Guide

To unpack a 1500 Series Pump:

- 1. Open the carton and remove the Startup Kit and other items from the top of the carton.
- 2. Using both hands, lift the pump (and its foam packing material) out of the carton.
- 3. Carefully set the pump down, then remove the foam packing material from both ends of the pump.
- 4. Check the contents of the Startup Kit against the Startup Kit parts list to confirm that all items are included.
- 5. Verify that the serial number on the inside left frame of the pump matches the serial number on the Certificate of Structural Validation. Keep the Certificate of Structural Validation with this guide for future reference.
- Inspect all items for damage. Immediately report any shipping damage to both the shipping company and your Waters representative. Contact Waters Technical Service at (800) 252-4752, U.S. and Canadian customers only. Other customers, call your local Waters subsidiary, or call Waters corporate headquarters at (508) 478-2000 (U.S.A.). Refer to <u>Appendix D, Warranty Information</u>, for complete information on reporting shipping damages and submitting claims.

2.3 Making Electrical Connections

For proper operation, the 1500 Series HPLC Pump requires:

- A grounded ac power supply with no abrupt voltage fluctuations
- A unique IEEE-488 address
- An IEEE-488 connection to a BreezeTM workstation and other IEEE-488 devices
- An Inject Start output connection if an optional manual injector is installed

For proper connection, the 1500 Series HPLC Pump requires the following materials:

- 2-meter IEEE-488 cable with busLAC/E[™] connector (supplied with the Breeze workstation)
- Power cord (Startup Kit)
- Flat-blade screwdriver small (required when connecting the Inject Start signal)

2.3.1 Connecting the Power Supply

The 1500 Series HPLC Pumps automatically adjust for ac input voltage.

To make the power supply connection:

- 1. Insert the 120 V or 240 V power cord into the power connector on the rear of the pump.
- 2. Plug the other end of the power cord into a grounded power outlet.

2.3.2 Setting the IEEE-488 Address for the Pump

You must set a unique address for the 1500 Series HPLC Pump (and for each device connected to the Breeze Workstation on the IEEE-488 bus). Valid IEEE-488 instrument addresses are 2 through 29, and are set using the DIP switches on the rear panel of the pump.

To set the IEEE-488 address:

- 1. Ensure that power to the pump is off.
- 2. Set the DIP switches on the rear panel of the pump to a unique IEEE-488 address. Refer to <u>Table 2-2</u> for DIP switch settings for valid IEEE-488 addresses.

Table 2-2 IEEE-488 DIP Switch Settin gs

IEEE-488 Address	DIP Switch Settings*				
	16	8	4	2	1
2	OFF	OFF	OFF	ON	OFF
3	OFF	OFF	OFF	ON	ON
4	OFF	OFF	ON	OFF	OFF
5	OFF	OFF	ON	OFF	ON
6	OFF	OFF	ON	ON	OFF

IEEE-488 Address	DIP Switch Settings*				
	16	8	4	2	1
7	OFF	OFF	ON	ON	ON
8	OFF	ON	OFF	OFF	OFF
9	OFF	ON	OFF	OFF	ON
10	OFF	ON	OFF	ON	OFF
11	OFF	ON	OFF	ON	ON
12	OFF	ON	ON	OFF	OFF
13	OFF	ON	ON	OFF	ON
14	OFF	ON	ON	ON	OFF
15	OFF	ON	ON	ON	ON
16	ON	OFF	OFF	OFF	OFF
17	ON	OFF	OFF	OFF	ON
18	ON	OFF	OFF	ON	OFF
19	ON	OFF	OFF	ON	ON
20	ON	OFF	ON	OFF	OFF
21	ON	OFF	ON	OFF	ON
22	ON	OFF	ON	ON	OFF
23	ON	OFF	ON	ON	ON
24	ON	ON	OFF	OFF	OFF
25	ON	ON	OFF	OFF	ON
26	ON	ON	OFF	ON	OFF

Table 2-2 IEEE-488 DIP Switch Settings (Continued)

IEEE-488 Address	DIP Switch Settings*				
	16	8	4	2	1
27	ON	ON	OFF	ON	ON
28	ON	ON	ON	OFF	OFF
29	ON	ON	ON	OFF	ON

Table 2-2 IEEE-488 DIP Switch Settings (Continued)

^{*} ON = 1 and OFF = 0.

2.3.3 Making the IEEE-488 Connections

The 1500 Series Pump, other chromatography devices in the Breeze system such as the column heater, autosampler, detector, and the Breeze workstation interconnect using IEEE-488 cables. Most chromatography devices are shipped with a 1-meter cable with dual-receptacle connectors at each end. The Breeze workstation comes with a 2-meter cable with a dual-receptacle connector at one end and a single-receptacle (busLAC/E) connector at the other end.

Before you connect the pump, refer to the IEEE-488 guidelines below.

IEEE-488 Guidelines

Follow these guidelines when you install and use your system:

- Always keep all devices powered on while your system is in use.
- While a system is active on the IEEE-488 bus, do not power on or off any device on the bus.
- The maximum number of devices that can be connected together to form one interface system is 15 (14 instruments plus the PCI busLAC/E card).
- The maximum total cable length to connect the devices and the PCI busLAC/E card in one interface system is 2 meters (6.5 feet) times the number of devices, or 20 meters (65 feet), whichever is *smaller*.
- The maximum cable length between two devices is 4 meters (13 feet).
- The minimum cable length between two devices is 1 meter (3.25 feet).



Attention: Cable lengths greater than the maximum values or less than the minimum values can cause IEEE-488 communication failures. Use the minimal cable lengths to ensure proper signal transmission.

Connecting IEEE-488 Devices

Note: The steps in this procedure assume that you have not electronically connected any of the other Breeze system IEEE-488 devices (the autosampler or detector, for example) to the Breeze workstation. If you have already connected other devices to the Breeze workstation, connect the 1500 Series Pump to the existing chain of devices (see Figure 2-2). The order in which you connect IEEE-488 devices is not important.

To make the IEEE-488 connections for the Breeze system:

1. Connect the single-receptacle end of the 2-meter IEEE-488 cable to the PCI busLAC/E card on the Breeze workstation. See <u>Figure 2-2</u>.



Figure 2-2 IEEE-488 Connection Example

- 2. Connect the dual-receptacle end of the 2-meter IEEE-488 cable to the IEEE-488 port on one of the IEEE-488 devices (pump, column heater, autosampler, or detector).
- 3. Connect one end of a 1-meter IEEE-488 cable (with dual-receptacle connectors at each end) to the cable receptacle on the first device. Connect the other end of the cable to the IEEE-488 port on the next device.
- 4. Repeat step 3 for each additional IEEE-488 device.
- 5. Make sure that all IEEE-488 cable connector screws are finger-tight.

2.3.4 Connecting the Inject Start Signal (1500 Series Manual Injector)

For 1500 Series Pumps with an optional 1500 Series Manual Injector, you need to make an Inject Start output signal connection from the I/O terminal block on the 1500 Series Pump to the appropriate inputs on the Waters 2487 or 2410 Detector.

You need the two-wire cable from the manual injector and a small flat-blade screwdriver for this procedure.

To make the Inject Start signal connection from the pump to the detector:

- 1. Remove the terminal block from the back of the 1500 Series Pump.
- 2. Insert the red stripped wire into position 1 of the terminal block, then tighten the screw to secure the wire.
- 3. Insert the black stripped wire into position 2 of the terminal block, then tighten the screw to secure the wire.
- 4. Firmly push the terminal into the back of the pump.
- 5. Remove the terminal block from Connector A on the back of the 2487 (Figure 2-3) or 2410 detector (Figure 2-4).
- 6. Insert the red stripped wire into position 1 of the terminal block as indicated in <u>Table 2-3</u> (Inject Start + on the 2487 or Chart Mark + on the 2410), then tighten the screw to secure the wire.
- Insert the black stripped wire into position 2 of the terminal block (Inject Start on the 2487 or Chart Mark – on the 2410), then tighten the screw to secure the wire.

Note: To connect the Inject Start pump signal to other detector inputs (for example, to the Autozero inputs), refer to the appropriate detector operator's guide for details.

8. Firmly push the terminal block into the back of the detector.

Table 2-3 I/OTerminal Block Connection s

Function	Pin Number	Direction
Inject Start +	1	Output or Input
Inject Start –	2	

Function	Pin Number	Direction	
Stop Flow +	3	Input	
Stop Flow –	4		
Event Relay 1A	5	Output	
Event Relay 1B	6		
Event Relay 2A	7	Output	
Event Relay 2B	8		
Event Relay 3A	9	Output	
Event Relay 3B	10		
Event Relay 4A	11	Output	
Event Relay 4B	12		

Table 2-3 I/OTerminal Block Connections (Continued)

<u>Figure 2-3</u> shows the Inject Start connection from the I/O terminal block on a 1500 Series Pump to Connector A on a Waters 2487 Detector.



Figure 2-3 Inject Start Connection to a 2487 Detector

Note: The 1500 Series Manual Injector must be in the **LOAD** position when you start the Breeze software. The busLAC/E card may not boot if the injector is left in the **INJECT** position.

Note: When you use the 1500 Series Manual Injector in conjunction with compositional gradients, you must turn the injector back to the **LOAD** position before the end of the run. Leaving the injector in the **INJECT** position automatically triggers the gradient table for subsequent injections, but the start of data acquisition does not occur.

<u>Figure 2-4</u> shows the Inject Start connection from the I/O terminal block on a 1500 Series Pump to Connector B on a Waters 2410 Detector.



Figure 2-4 Inject Start Connection to a 2410 Detector

Note: If you are using the 2410 detector with the 1500 Series Manual Injector, the 2410 does not start acquisition until you turn the injector back to the **LOAD** position. When injecting, make sure that you keep the injector in the **INJECT** position for a minimum of two loop volumes before turning back to the **LOAD** position to begin data acquisition.

2.4 Making Fluidic Connections

This section describes how to make fluidic connections to the Waters 1500 Series Pump:

- · Making eluent supply connections
- Installing different mixer configurations
- · Installing an optional manual injector
- · Installing an optional column heater
- · Making the outlet connection
- · Connecting fluid waste lines

2.4.1 Making Eluent Supply Connections

Follow the instructions in this section to connect a 1500 Series Pump inlet to an eluent reservoir.

Note: If you are using a Waters in-line degasser, refer to the degasser operator's guide for details on connecting the degasser to the reservoir and pump.

Required Materials

Before you make eluent supply and needle wash connections, please obtain the following necessary items:

- Tefzel[®] ferrule and compression screw (Startup Kit) one set per pump
- 1/8-inch OD ETFE tubing (P/N WAT270714)
- ETFE tubing (Startup Kit) (P/N WAT024036)
- Reservoir containing filtered, degassed eluent one per pump
- Bottle holder
- Bottle caps (1 L) (Startup Kit) (P/NWAT062479)
- Stainless steel solvent filter (Startup Kit) one per pump (P/N WAT025531)
- Plastic tubing cutter (not included) (P/N WAT031795) or razor blade
- Inlet tubing labels (not included with the 1515 Pump) (P/Ns WAT087186[A] and WAT087187[B])

Installing the Bottle Holder

To install the bottle holder:

- 1. Carefully position the rack along the left side of the pump so the two cutouts at the bottom inside of the rack rest on their corresponding positioning screws on the pump.
- 2. Holding the rack flush against the surface of the pump, finger-tighten the captive screw to secure the rack.

Connecting to the Pump Inlet

To connect the eluent tubing to a 1500 Series Pump inlet:

 Measure the length of 1/8-inch ETFE tubing required to connect an eluent reservoir (mounted in the bottle holder) to an inlet manifold on the pump. If you are using an in-line degasser, refer to the degasser operator's guide for details on connecting to the reservoir and pump.

Note: It may be necessary to substantially increase reservoir height if you use high-viscosity eluents (18 to 24 inches above the inlet manifold is not uncommon).

- 2. Insert the ETFE tubing into the 1/8-inch diameter hole of the tubing cutter, making sure that the tubing that extends from the metal side of the cutter is the correct length.
- 3. Insert the razor blade into the cutter and press down to cut the tubing. Make sure the cut end is straight and free from burrs.
- Slide a compression screw over one end of the tubing, followed by a ferrule with its tapered end facing away from the tubing end, and its wide end flush with the tubing end, as shown in <u>Figure 2-5</u>.



Figure 2-5 Reverse Ferrule and Compression Screw Assembly

5. Firmly seat the tubing end into the inlet manifold on the pump, then finger-tighten the compression screw



Attention: To avoid damaging the ferrule, do not overtighten the compression screw.

6. If you have a 1525 Binary HPLC Pump, repeat steps 1 through 5 for the second pump.

Connecting to an Eluent Reservoir

Attention: To avoid having eluent leak from a pump outlet, position each eluent reservoir below its corresponding pump inlet until the outlet is connected to the system.

To connect the eluent tubing to an eluent reservoir:

- 1. Slide inlet tubing label onto appropriate tube (see Figure 1-2).
- 2. Insert the free end of the 1/8-inch ETFE inlet tubing into the cap of an eluent reservoir.
- 3. Slide one of the pieces of tubing (P/N WAT024036) over the end of the 1/8-inch ETFE tubing for about 3/4 of an inch (1.9 cm).
- 4. Insert the stainless tubing fitting on the solvent filter into the open end of this tubing.
- 5. Install the cap onto the eluent reservoir and push the tubing through the cap until the filter reaches the bottom of the reservoir.
- 6. If you have a 1525 Binary HPLC Pump, repeat steps 1 through 5 for the second pump.

If the 1500 Series Pump is to be connected to a Waters 717plus Autosampler, you can use one position of the bottle holder to hold a bottle that contains the needle wash solvent.

2.4.2 Installing Different Mixer Configurations

The Waters[®] 1500 Series HPLC Pumps can be used with various configurations of eluent mixers. This section describes these configurations and how to install each one.
1525 Binary HPLC Pump

The 1525 Pump is shipped with one standard GM150 gradient mixer (P/N WAT055847), mounted on the upper-left side of the chassis face (shown as configuration 1 at the top of Figure 2-6). If you require an additional GM150 mixer, see configuration 2 in Figure 2-6. If you want to replace the standard GM150 mixer with a large-volume gradient mixer, see configuration 3 in Figure 2-6.

1515 Isocratic HPLC Pump

Since most isocratic separations do not need a mixer, the 1515 pump does not include one. If you need mixing capability, you may separately purchase any of the three configurations in <u>Figure 2-6</u>.

Installing Mixers

There are four pairs of mounting holes on the upper-left side of the chassis face. For the following configurations, these hole pairs are numbered from 1 to 4 top to bottom. Refer to Figure 1-2 or Figure 2-6 to check all fluidic connections.

Single GM150 Gradient Mixer

This mixer is standard on the 1525 Pump. To install it on the 1515 Pump:

- 1. Acquire a mixer (P/N WAT055847).
- 2. Remove the mounting screws and mixer clamps from the upper-left corner of the chassis face.
- 3. Orient the mixer between the clamps as shown in configuration 1 of Figure 2-6 using hole pairs 1 and 3.
- 4. Firmly tighten the mounting screws.
- 5. Cut an appropriate length of 0.009-inch tubing (P/NWAT026973 in the Startup Kit) and attach one end to the pressure transducer outlet and the other end to the mixer inlet using a compression screw and ferrule on each end.
- Cut another length of 0.009-inch tubing. Connect one end to the mixer outlet and the other end to the reference valve inlet using a compression screw and ferrule on each end.

Dual GM150 Gradient Mixers

To use this configuration for a 1515 Pump, you must:

- Acquire two mixers (P/N WAT055847).
- Remove the mounting screws and the mixer clamps from their positions on the upper-left corner of the chassis face.

To install the dual GM150 gradient mixers in the 1515 Pump:

- 1. Put the mixers inside the mounting clamps as shown in configuration 2 of Figure 2-6 using hole pairs 2 and 4.
- 2. Firmly tighten the mounting screws.
- 3. Cut an appropriate length of 0.009-inch tubing (P/NWAT026973 in the Startup Kit) and attach one end to the pressure transducer outlet and the other end to the bottom mixer inlet using a compression screw and ferrule on each end.
- 4. Cut one 3 3/4-inch minimum length of 0.009-inch tubing (P/N WAT026973 in the Startup Kit) and bend it to form a U-tube, and place a compression screw and ferrule on each end.
- 5. Connect the outlet of the bottom mixer to the inlet of the top mixer with the U-tube.
- 6. Cut another length of 0.009-inch tubing. Connect one end to the top mixer outlet and the other end to the reference valve inlet using a compression screw and ferrule on each end.

To use this configuration with a 1525 Pump, you must:

- Acquire one additional mixer (P/NWAT055847).
- Loosen the compression screws on the inlet and outlet tubing of the existing GM150 mixer.
- Move the tubing enough to allow repositioning of the mounting clamps.
- Loosen the compression screw on the outlet tubing.
- Remove the mounting screws, the single GM150 gradient mixer, and mounting clamps.

To install the dual GM150 gradient mixers in the 1525 Pump:

- 1. Put the mixers inside the mounting clamps as shown in configuration 2 of Figure 2-6 using hole pairs 2 and 4.
- 2. Firmly tighten the mounting screws.
- 3. Reattach the pressure transducer outlet tubing to the inlet of the bottom mixer.
- 4. Connect the outlet of the bottom mixer to the inlet of the top mixer with the U-tube (P/N 430000405) (from the 1525 Pump Startup Kit).
- 5. Reconnect the outlet of the top mixer to the reference valve inlet tubing.

Large-Volume Gradient Mixer

To use this configuration for a 1515 Pump, you must:

• Acquire a large-volume gradient mixer (P/N WAT051518).

• Remove the existing tubing that connects the pressure transducer outlet to the GM150 mixer inlet and place aside.

Note: The existing tubing that connects the pressure transducer outlet to the GM150 mixer inlet may be used as the inlet tubing to the large-volume gradient mixer. However, the existing tubing that connects the GM150 mixer outlet to the reference valve is too short to be used.

- Remove the existing tubing that connects the GM150 mixer outlet to the reference valve and place aside.
- Remove the mounting screws and the mixer clamps from their positions on the upper-left corner of the chassis face.

To use this configuration with a 1525 Pump, you must:

- Acquire a large-volume gradient mixer (P/N WAT051518).
- Remove the mounting screws, the single standard GM150 gradient mixer, and mounting clamps.
- If you intend to use the GM150 mixer in the future, label the mixer outlet tube and store it with the GM150 mixer.

To install the large-volume gradient mixer in either a 1525 or 1515:

- 1. Put the mixer inside the mounting clamps as shown in configuration 3 of <u>Figure 2-6</u> using hole pairs 1 and 4.
- 2. Firmly tighten the mounting screws.
- 3. Cut an appropriate length of 0.009-inch tubing (P/NWAT026973) from the startup kit and attach one end to the pressure transducer outlet and the other end to the mixer inlet using a compression screw and ferrule on each end.
- 4. Cut another length of 0.009-inch tubing. Connect one end to the mixer outlet and the other end to the reference valve inlet using a compression screw and ferrule on each end.



Figure 2-6 1525 Pump Showing Different Mixer Configurations

2.4.3 To Install an Optional 1500 Series Manual Injector

Please refer to the instructions and startup list included with the 1500 Series Manual Injector.

2.4.4 Installing an Optional 1500 Series Column Heater

The column heater is mounted on the right side panel with three screws. Connectors on the rear panel provide power and control of the column heater.

To install the column heater:

1. Remove the three screws from the right side panel of the pump.

- 2. Insert the screws through the standoffs (included with the Column Heater Startup Kit) so that the flat side of the standoffs are flush with the right side panel.
- 3. Mount the column heater on the three screws on the right side panel.
- 4. Insert one end of the power cable to the power connector on the rear of the column heater and the other end into the power source outlet.
- 5. Connect the IEEE signal cable to the IEEE connector on the rear of the column heater. The other end of this cable must be connected to your Breeze workstation for software control.
- 6. See <u>Section 2.3.2</u>, <u>Setting the IEEE-488 Address for the Pump</u> for setting up an IEEE-488 device.

2.4.5 Connecting the Pump Outlet



Caution: To avoid having eluent leak from a pump outlet, position each eluent reservoir below its corresponding pump inlet until the outlet is connected to the system.

Follow the instructions in this section to connect the pump outlet to the system. The instructions describe how to:

- Connect to a Waters 717plus Autosampler
- Connect an optional 1500 Series Manual Injector to the column or to an optional column heater

Required Materials

Before you connect the pump outlet, please obtain the open-end wrench that is included in the Startup Kit.

Connecting to a Waters 717plus Autosampler

To connect the pump to a 717plus Autosampler:

- 1. Route the blue line from the Waters 717plus Autosampler under the column heater (if you have installed one) and into the pump through the notch in the right front portion of the drip tray. At the pump, connect the blue line to the output of the reference valve. If necessary, refer to the *Waters 717plus Autosampler Operator's Manual* for more details about autosampler fluidic connections.
- 2. Connect the red line from the autosampler to the column. If you are using a column heater, route the red line so that it enters the column heater from the bottom of the door.

2.4.6 Connecting Fluid Waste Lines

Follow the instructions in this section to connect the fluid waste lines. The instructions describe how to:

- · Connect reference valve and optional manual injector waste lines
- · Connect the drip tray waste line

Required Materials

Before you connect the waste lines, please obtain the following necessary items:

- Tubing (Startup Kit) (P/N WAT076775)
- Teflon tubing (Startup Kit) (P/N WAT050693)
- Convoluted ETFE tubing (Startup Kit) (P/N WAT241095)
- Plastic tubing cutter (not included) (P/N WAT031795) or razor blade

Connecting Pump Waste Lines

To connect fluid waste lines:

- 1. Obtain a piece of convoluted tubing (P/N WAT241095) from the Startup Kit and pull it to stretch it apart.
- 2. Take a piece of smooth tubing (P/N WAT050693) and feed it through the convoluted tubing until ends of both pieces are flush with one another. This dual tubing will prevent the waste line from developing kinks.
- 3. Mount this piece of dual tubing in parallel with the drip tray (as shown in Figure 1-2) and secure it in the provided spring clips.
- 4. Insert the reference valve waste line and the optional manual injector waste lines together in the top end of the dual tubing.
- 5. Insert the other end of the dual tubing in a properly situated waste container.

Connecting the Drip Tray Waste Line

To connect the drip tray to waste:

- 1. Attach tubing (P/N WAT076775) to the drip tray waste exit (shown in Figure 1-2).
- 2. Insert the other end of the tubing in a waste container.

Connecting an Optional 1500 Series Manual Injector to the Column or to an Optional 1500 Column Heater

Note: Make sure you have already installed the manual injector as described in the instructions included with the optional manual injector:

- 1. Purge the injector and detector (see <u>Section 3.2.2</u>, <u>Purging the Injector and</u> <u>Detector</u>).
- 2. Connect the free end of the manual injector outlet tubing to the column or column heater.

3 Preparing for Operation

This chapter describes how to do the following:

- 1. Power on a 1500 Series Pump
- 2. Prime and purge a 1500 Series Pump
- 3. Purge the injector and detector
- 4. Equilibrate the Breeze system
- 5. Power off a 1500 Series Pump

3.1 Powering On the Pump

Note: Power on the pump and other chromatographic system devices before you power on the Breeze[™] workstation.

Make sure the pump is installed according to the instructions in Chapter 2, Installing a Waters 1500 Series HPLC Pump.

To power on the pump, find the power switch on the left side panel and set it to the 1 (ON) position.

3.2 Preparing the Pump

Overview

The procedures to prepare your pump for operation depend on the status of your chromatography system, as indicated in <u>Table 3-1</u>. Use this table as a quick reference guide for determining when to perform certain preparation procedures.

Table 3-1 Pump Preparation Recommendatio	reparation Recommendations
--	----------------------------

Status	Prime ¹	Purge [*]	Equilibrate [*]
Initiating operation	√	√	√
Changing eluent	1	1	√
Adding fresh eluent		√	√
Changing columns			√
System has been idle for some time	√	√	✓

¹ Priming, purging, and equilibrating procedures are fully described in this section.

Eluent Guidelines

When you prepare a 1500 Series Pump for operation, follow these guidelines:

- Use methanol to prime a new pump, or one that has been stored for an extended period.
- Use an intermediate solvent when you change between eluents that are not miscible.
- Use only HPLC-grade eluents to obtain accurate, reproducible chromatography.
- To prevent air from being drawn into the pump, keep the eluent level in the reservoir at least four inches (10 cm) higher than the inlet manifold. Do not place eluent reservoirs on top of a Waters[®] 1500 Series Pump.
- · Use only filtered, degassed eluents.
- After use with a buffer, purge the pump with HPLC-grade water.
- When possible, use eluents of similar viscosity when you run a gradient.

- Know the properties of your eluents; for example, cyclohexane solidifies at 3.5 K psi.
- Consider column chemistry. If the column can be damaged by an intermediate or new eluent, remove the column from the system and substitute a union. Refer to the care and use guide for your column.
- Dedicate an eluent supply tube and filter assembly for each eluent. If this is not possible, purge the tube and filter of any remaining eluent before you use a new eluent.
- To prevent cross contamination of eluents, dedicate a set of glassware for preparing, storing and supplying each eluent. Once a container has been used for an eluent, do not use it for any other eluent unless it has been thoroughly cleaned.

Caution: To avoid chemical hazards, observe safe laboratory practices when you handle eluents. Refer to the Material Safety Data Sheets for the eluents you use. For more information on eluent properties, see <u>Appendix C, Solvent Considerations</u>.

3.2.1 Priming and Purging the Pump

Priming and purging the pump is necessary to ensure proper pump operation when:

- · You start the pump for the first time
- · You change eluent
- The system has been idle for some time

Required Material

The only material you need is the priming syringe (P/N WAT010337) that is included in the Startup Kit.

Priming and Purging

To prime and purge the pump:

- 1. Power on all Breeze system instruments and any peripheral devices, such as a printer.
- 2. Power on the Breeze system computer and log on to Windows NT®.
- 3. From the Start menu, select **Programs**, **Breeze**, then **Breeze** (again). The Breeze main window appears.
- 4. Close the Waters Breeze Help window unless it is needed.
- 5. Wait for bus LAC/E to boot.
- 6. In the Acquisition bar, click

(Prime/Purge wizard).

- 7. Select Prime/Purge Pumps, then click Next.
- 8. Follow the wizard instructions.

3.2.2 Purging the Injector and Detector

Note: The Purge Detector function is available only for Waters 2410 Detectors.

Purging ensures that all eluent in the fluidic path is replaced with new eluent before you run samples. Purge when:

- · You start the pump for the first time
- You change eluent or add fresh eluent
- The system has been left idle for some time

Before Purging

- Make sure the eluent is filtered and degassed.
- Prime and purge the pump with fresh eluent as described in <u>Section 3.2.1,</u> <u>Priming and Purging the Pump</u>.

Note: When you change between two eluents that are not totally miscible, perform the steps in this procedure with an intermediate eluent before purging with the final eluent. See Appendix C, Eluent Considerations, for more information.

Purging

STOP

Attention: To avoid damaging the column, make sure any changes to conditions that affect the column are gradual such as flow or pressure.

To purge the injector and the 2410 Detector:

- 1. Run the Breeze Purge/Prime wizard.
- 2. Follow the on-screen instructions.

3.2.3 Equilibrating the System

Equilibrate your HPLC system when one or more of the following conditions occur:

- · You start your system for the first time
- After you change eluents or add fresh eluent
- After you change columns
- After you leave the system idle for more than one day

Before Equilibrating

- Make sure the eluent is filtered and degassed.
- Prime and purge the pump as described in <u>Section 3.2.1, Priming and Purging the</u> <u>Pump</u>.
- Allow the eluent and the system to reach proper temperature before pump operation.

Equilibrating

To equilibrate the system:

- 1. Make sure all Breeze system instruments and any peripheral devices, such as a printer are powered on.
- 2. Make sure all the Breeze system computer is powered on and you are logged on to Windows NT.
- 3. From the Start menu, select **Programs**, **Breeze**, then **Breeze** (again). The Breeze Main window appears.
- 4. In the Acquisition bar, click



5. Select an equilibration method and click Equilibrate.

For more information about selecting an equilibration method, refer to *Waters Breeze Help*.

3.3 Powering Off the Pump

To power off the pump:

- If you have been using buffers, purge the buffer from the pump and other HPLC system components with HPLC-grade water. If the pump will be idle for more than one day, purge with a methanol/water solution to prevent the growth of microorganisms.
- 2. If the pump is running, powering off the pump will stop flow immediately. Since abrupt flow changes may damage columns, it is advisable to ramp flow to zero with a Breeze shutdown method, which is created by the user.
- 3. Turn the power switch to the **O** (OFF) position.

4 Maintaining the Waters 1500 Series HPLC Pump

This chapter provides important safety and handling considerations for the Waters[®]1500 Series Pump, describes how to run two diagnostic tests and set pump calibration parameters and tells how to replace pump components.

4.1 Maintenance Considerations

Safety and Handling

When you perform maintenance procedures on your 1500 Series Pump, keep the following safety considerations in mind.

Caution: To prevent injury, always observe good laboratory practices when you handle eluents, change tubing, or operate the 1500 Series HPLC Pump. Know the physical and chemical properties of the eluents. Refer to the Material Safety Data Sheets for the eluents in use.



Caution: To avoid the possible eye injury or cuts, handle the plunger with care. Wear safety glasses and use the plunger insertion tool. Be aware that the pieces of a broken plunger are very sharp.



Caution: To avoid possible electric shock, do not remove the cover. The interior of the pump does not contain user-serviceable parts.

Proper Operating Procedures

To keep your 1500 Series Pump running smoothly, follow the operating procedures and guidelines in <u>Section 3.2, Preparing the Pump</u>.

Spare Parts

Refer to <u>Appendix B, Spare Parts and Accessories</u>, 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 replacing parts in the 1500 Series Pump, 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 at (508) 478-2000 (U.S.A.).

4.2 Diagnostic Tests

Regular diagnostic tests can help you to track system performance and prevent or identify potential problems before they interfere with operation. Two tests are:

- · Retention time stability test
- Ramp-and-decay test

4.2.1 Retention Time Stability Test

Observing retention time stability during system performance monitoring tests is useful for determining the performance of your HPLC system and its components, including the pump. Erratic or changing retention times could be a result of dirty or malfunctioning check valves, worn plungers or plunger seals, air bubbles in the lines, incorrectly set flow rate, leaks, or other pump-related problems. Be aware that other factors, such as system and column equilibration, column age, operating temperature, and so on, can also affect retention time stability.

4.2.2 Ramp-and-Decay Test

Use the ramp-and-decay test to monitor check valve performance.

Required Materials

In preparation for the ramp-and-decay test, you need the following items:

- Three compression plugs (P/N WAT025566) (Startup Kit)
- Tissues
- 5/16-inch open-ended wrench (Startup Kit)
- HPLC-grade methanol

Monitor Check Valve Performance

To perform the ramp-and-decay test:

- 1. Disconnect the pump outlet tube to the system at the reference valve and install a compression plug in its place.
- 2. Purge pump A with 100% methanol. If your eluent is not miscible with methanol, use an intermediate eluent. Refer to <u>Section 3.2.1</u>, <u>Priming and Purging the Pump</u>.
- 3. For the 1525 Pump, disconnect pump B from the tee by removing the outlet tube of the pulse dampener and install a compression plug in its place.
- 4. Disconnect the left pump head outlet tube from the draw-off valve and install a compression plug in its place.
- 5. Place tissues under the disconnected pump head outlet tube and the disconnected pulse dampener tube to catch drips.
- 6. Ensure that the draw-off valves are closed and the reference valve is closed to waste.
- 7. Set the following pump parameter values:
 - Flow parameter: 0.3 mL/min
 - High pressure limit: 6000 psi (41,370 kPa, 401 bar)
- 8. With the 100% methanol reservoir still connected to the pump inlet, click **Go** on the Breeze Acquisition pane to start the pump. Observe pump pressure on the Acquisition pane:
 - If the check valves are operating properly, pressure rises with each plunger stroke on the connected head, then holds as the plunger recedes.
 - If there is a bad inlet check valve, pressure may stop at a certain point, or may not rise at all.
 - If there is a bad outlet check valve, pressure may increase, then immediately decrease as the plunger recedes.
 - If the pressure does not rise to the high-pressure limit, try repriming the pump or increasing the Flow parameter setting.
- 9. Allow the pump to reach the high-pressure limit. The pump should stop flow automatically.
- After two minutes, record the pressure (P1). One minute later, record the pressure again (P2). Calculate head pressure decay with the formula (P1 P2)/P1. Verify that head pressure decay is 0.15 or less. If the pressure decay for the pump head is greater than 0.15, you may have bad check valves. Remove and clean or replace the inlet and outlet check valves (see <u>Section 4.4, Replacing Check Valves</u>).

4

- 11. Relieve head pressure by slowly opening the reference valve.
- 12. Disconnect the right pump head outlet tubing from the draw-off valve. Transfer the compression plug from the left port of the draw-off valve to the right port of the draw-off valve. Reconnect the left pump head outlet tubing to the left port of the draw-off valve.
- 13. Repeat steps 5 through 12 to test the left pump head.
- 14. Remove the compression plugs from the draw-off valve and the tee. Reconnect the right pump head outlet tubing. Reconnect the tubing from the pulse dampener.
- 15. For the 1525, repeat steps 3 through 14 on pump B. In step 3, disconnect pump A from the tee.
- 16. When you finish, purge the pump with eluent and close the refence valve.
- 17. Remove the compression plug from the reference valve and reconnect the outlet tube to the system.

4.3 Replacing and Cleaning Plunger Seals and Plungers

This section describes how you:

- · Remove and install plunger seals for replacement
- Remove and install plungers for cleaning and replacement

The plungers in a Waters 1500 Series Pump are ultrasmooth, chemically inert sapphire rods. Salt crystals that precipitate out from the eluent can form on the plunger and cause wear on the plunger seals and on the plunger itself. The result is a slow leak and a very slight cyclic pressure fluctuation, with a possible increase in retention time.

For continued high performance of the 1500 Series Pumps:

- Replace the plunger seals twice yearly, or as needed
- Clean and inspect the plunger every six months (or sooner if using abrasive eluents)
- Replace the plunger once each year, or as needed

4.3.1 Preparing for Plunger Seal Replacement

Required Materials

Before replace the plunger seal, you must obtain the following items:

• 5/16-inch open-end wrench (Startup Kit)

- 5/32-inch Allen wrench (Startup Kit)
- Priming syringe (Startup Kit)
- Fitting plug (Startup Kit)
- Replacement seal kit and seal insertion tool (Startup Kit) (refer to <u>Appendix B</u>, <u>Spare Parts and Accessories</u>, for part numbers)
- HPLC-grade methanol

Removing the Pump Head

To gain access to a plunger seal, you must first remove the pump head.

To remove a pump head:

1. Purge the pump with methanol (see <u>Section 3.2.1, Priming and Purging the Pump</u>). If methanol is not miscible with your current eluent, use an intermediate eluent.



Attention: Before you continue with this procedure, lower the eluent reservoir to eliminate gravity flow of eluent.

- 2. Disconnect the eluent line from the inlet manifold on the pump.
- 3. Disconnect the outlet tubing from the bottom of the draw-off valve and install a fitting plug in the outlet. Tighten the plug with the 5/16-inch open-end wrench.
- 4. Insert the priming syringe into the luer fitting at the center of the draw-off valve handle, then turn the handle counterclockwise about 1/2 turn to open the valve.
- 5. Use the syringe to withdraw all methanol.
- 6. In the Breeze Acquisition pane, set the Flow parameter to **0.3** mL/min, then click **Go** to run the pump.
- 7. When the indicator rod fully retracts into the pump head, click the **Stop Flow** button to turn off the pump. This ensures that the weight of the pump head does not rest on the plunger while you remove the head.
- 8. Use the 5/16-inch open-end wrench to remove the inlet and outlet tubing from the check valves on the pump.

 Use the 5/32-inch Allen wrench to remove the two pump head assembly mounting bolts. Loosen the bolts 1/2-turn at a time for the first two turns (Figure 4-1).





10. Carefully slide the pump head assembly off the pump.

Replacing the Plunger Seal

To replace the plunger seal:

1. Use the seal insertion tool or other nonmetalic tool to pry the seal out of the pump head (see Figure 4-2).



Figure 4-2 Plunger Seal

2. Place the new plunger seal onto the seal insertion tool.

- 3. Moisten the seal with methanol.
- 4. Use the seal insertion tool to firmly seat the seal in the pump head.
- 5. Remoisten the seal and plunger with methanol.
- 6. Slide the pump head assembly into position over the plunger and reinstall the two pump head mounting bolts. Alternately tighten each bolt, using half turns to evenly align the assembly.
- 7. In the Breeze Acquisition pane, set the Flow parameter to **0.3** mL/min, then click **Go** to start the pump. Verify that the indicator rod moves freely.
- 8. Click the **Stop Flow** button to stop the pump.
- 9. Reconnect the inlet and outlet tubing to the pump head.
- 10. Reconnect the eluent line to the pump inlet manifold.
- 11. Remove the fitting plug from the draw-off valve outlet, then reconnect to the outlet tube.
- 12. Prime the pump as described in <u>Section 3.2.1, Priming and Purging the Pump</u>. If you notice leaks, verify pump head and plunger seal installation.

4.3.2 Cleaning and Replacing the Plungers

The plungers are sapphire rods that require careful handling. Although cleaning the plungers is not difficult, it is important to follow these instructions carefully to avoid damaging the plungers. Assemble all materials and read the procedure thoroughly before you begin.

Required Materials

Before you clean and replace a plunger, you need the following items:

- 5/16-inch open-end wrench (Startup Kit)
- 5/32-inch Allen wrench (Startup Kit)
- 9/64-inch Allen wrench (Startup Kit)
- Snap-ring pliers (P/N WAT025263)
- Plunger insertion tool (P/N WAT011042)
- Sonicator
- HPLC-grade water
- HPLC-grade methanol
- Replacement plunger
- Replacement plunger seals

Removing the Plunger

Caution: To avoid possible eye injury or cuts, handle the plunger with care. Wear safety glasses and use the plunger insertion tool. Pieces of a broken plunger are very sharp.

To remove the plunger from the pump:

- 1. Remove the pump head as described in <u>Section 4.3.1, Preparing for Plunger Seal</u> <u>Replacement</u>.
- Remove the four head support screws with the 9/64-inch Allen wrench (see Figure 4-3), then carefully slide the head support assembly and the indicator rod off the pump. Set the head support assembly on the benchtop.



Figure 4-3 Exposed Head Support Assembly

- In the Breeze Acquisition pane, set the Flow parameter to 0.3 mL/min, then click Go to run the pump. When the plunger is fully extended, click the Stop Flow button to stop the pump.
- 4. Use the snap-ring pliers to remove the snap-ring that holds the plunger in place.

5. Remove the plunger assembly and set it aside (see Figure 4-4).



TP01725

Figure 4-4 Removing the Plunger Assembly

Cleaning the Plunger

Caution: To avoid possible cuts or eye injury, handle the plunger with care. Use the plunger insertion tool and wear safety glasses. Be aware that the pieces of a broken plunger are very sharp.

Clean the plunger by sonicating it in 50:50 methanol/water for a few minutes. The parts of the plunger assembly are shown in <u>Figure 4-5</u>.



TP01413

Figure 4-5 Disassembled Plunger

Inspecting the Plunger

Caution: To avoid possible cuts or eye injury, handle the plunger with care. Use the plunger insertion tool and wear safety glasses. Be aware that the pieces of a broken plunger are very sharp.

After you clean the plunger, inspect it for damage by holding it under a bright white light and looking down its length for nicks and scratches. It is easier to see scratches under a bright light than to feel them with your fingers.

If the plunger is not scratched or otherwise damaged, reassemble the plunger with new seals. If the plunger is damaged, replace the plunger and the seals.

Replacing the Plunger

Caution: To avoid possible eye injury or cuts, handle the plunger with care. Wear safety glasses and use the plunger insertion tool. Be aware that the pieces of a broken plunger are very sharp.

Whether you are reinstalling a plunger after cleaning and inspecting it, or replacing a damaged plunger with a new one, use the following procedure:

- 1. Reassemble the components of the plunger assembly as shown in <u>Figure 4-5</u>. Make sure the brass seat is located at the bottom of the piston.
- 2. Use the plunger insertion tool and snap-ring pliers to insert the plunger assembly into the pump (see <u>Figure 4-6</u>).



Figure 4-6 Inserting the Plunger

- In the Breeze Acquisition pane, set the Flow parameter to 0.3 mL/min, then click Go to run the pump. When the plunger is fully retracted, click the Stop Flow button to stop the pump.
- 4. With the indicator rod hole oriented to the upper right, reinstall the head support assembly. Alternately tighten the four screws. Do not overtighten.

- 5. Wet the plunger seal and plunger with methanol.
- 6. Gently slide the pump head onto the plunger and alternately tighten the two bolts. Check for even head alignment by observing the gap between the pump head and the pump head support assembly.
- 7. In the Breeze Acquisition pane, set the Flow parameter to 0.3 mL/min, then click Go to run the pump. Pull out and release the indicator rod. If the rod does not snap back easily, the head is misaligned. Stop the pump, loosen the pump head, then repeat steps 6 and 7.
- 8. Click the **Stop Flow** button to stop the pump.
- 9. Reconnect the inlet and outlet tubing to the pump head.
- 10. Reconnect the eluent line to the pump inlet manifold.
- 11. Remove the fitting plug from the draw-off outlet, then reposition the eluent reservoir.
- 12. Prime the pump as described in <u>Section 3.2.1, Priming and Purging the Pump</u>. If you notice leaks, verify pump head and plunger seal installation as described in <u>Section 4.3.1, Preparing for Plunger Seal Replacement</u>.

4.4 Replacing Check Valves

This section describes how you replace inlet and outlet check valves

Required Materials

Before you replace a check valve, you need the following items:

- 5/16-inch open-end wrench (Startup Kit)
- Priming syringe (Startup Kit)
- Fitting plug (Startup Kit)
- · Replacement check valve cartridges
- HPLC-grade methanol
- Forceps

Replacing the Check Valve

To replace a check valve:

1. Purge the pump with methanol as described in <u>Section 3.2.1, Priming and Purging</u> <u>the Pump</u>. If methanol is not miscible with your current eluent, use an intermediate eluent.



Attention: Before you continue with this procedure, lower the eluent reservoir to eliminate gravity flow of eluent.

- 2. Disconnect the eluent supply line from the inlet manifold on the pump.
- 3. Use the 1/2-inch open-end wrench to remove the check valve housing from the inlet or outlet side of the pump head (see <u>Figure 4-7</u>).



TP01733

Figure 4-7 Disconnecting the Outlet Tubing

4. Using forceps, remove the cartridge from the housing and replace it with the new cartridge (see Figure 4-8).



TP01732

Figure 4-8 Outlet Check Valve Assembly

Note: The arrow printed on the check valve cartridge indicates the direction in which it will allow liquid to flow. Therefore, its direction is critical for proper operation. For both inlet and outlet check valve assemblies, the arrow should point upward when installed in the pump head.

Note: To help keep the cartridge seated within the housing, moisten it with methanol during installation.

- 5. With the check valve held upright to prevent the cartridge from falling out, hand-tighten the check valve into the pump head. Tighten the check valve another 1/2-turn with the 1/2-inch open-end wrench.
- 6. Reconnect the eluent supply line to the inlet manifold on the pump.
- Prime the pump as described in <u>Section 3.2.1, Priming and Purging the Pump</u>. Check for leaks.

4.5 Replacing a Draw-Off Valve

Replace a draw-off valve if you notice that the valve leaks even after you tighten the knob.

Required Materials

Before you replace a draw-off valve, you need the following items:

• 5/16-inch open-end wrench (Startup Kit)

- 7/64-inch Allen wrench (Startup Kit)
- Priming syringe (Startup Kit)
- Phillips screwdriver
- Replacement draw-off valve
- HPLC-grade methanol

Removing the Draw-Off Valve

To remove the draw-off valve:

1. Purge the pump with methanol as described in <u>Section 3.2.1, Priming and Purging</u> <u>the Pump</u>. If methanol is not miscible with your current eluent, use an intermediate eluent.



- 2. Disconnect the eluent supply line from the inlet manifold on the pump.
- 3. Insert the priming syringe into the luer fitting at the center of the draw-off valve handle, then turn the handle counterclockwise about 1/2 turn to open the valve.
- 4. Use the syringe to withdraw all the methanol.
- 5. Use the 5/16-inch open-end wrench to disconnect the three stainless steel compression screws from the draw-off valve (see Figure 4-9).



Figure 4-9 Draw-Off Valve/Inlet Manifold Assembly

- Use the 5/16-inch open-end wrench to disconnect the two stainless steel compression screws from the inlet manifold (these screws connect the inlet manifold to the inlet check valves). See <u>Figure 4-9</u>.
- Use the 7/64-inch Allen wrench to remove the two Allen screws that hold the draw-off valve assembly and bracket to the pump. Carefully remove the assembly.
- 8. Remove the two Phillips screws that hold the draw-off valve to the bracket.

Installing a Draw-Off Valve

To install the draw-off valve:

- 1. Attach the new draw-off valve to the bracket using the two Phillips screws. Make sure the new valve is oriented on the bracket as shown in Figure 4-9.
- 2. Reposition the draw-off valve/inlet manifold assembly onto the pump, being careful not to pinch the stainless tubing behind the bracket. When correctly positioned, the stainless tubing extends through the cutouts on the sides of the bracket.
- 3. Secure the bracket to the pump with the two Allen screws.
- 4. Reconnect the inlet tubing to the inlet manifold.
- 5. Reconnect the outlet tubing to the new draw-off valve.

6. Reposition the eluent reservoir, then prime the pump (see <u>Section 3.2.1, Priming</u> <u>and Purging the Pump</u>). Check for leaks.

4.6 Replacing Fuses

The fuse holder is located on the rear panel of the pump (Figure 4-10). The 1500 Series Pump is shipped with two factory-installed 3.15 A fuses.



Figure 4-10 Rear Panel Fuse Holder

Required Materials

Before you replace any fuses, you need the following items:

- Small flat-blade screwdriver
- Replacement fuses



Caution: To avoid possible electrical shock, power down and unplug the instrument before checking the fuses. For continued protection against fire hazard, replace fuses only with those of the same type and rating.

Identifying Faulty Fuses

Suspect a faulty fuse if:

- The pump fails to power up.
- The fan does not turn on.

Replacing the Rear Panel Fuses

To replace a blown or faulty fuse:

- 1. Power off the pump, then remove the power cord from its connector.
- 2. Use the small flat-blade screwdriver to remove the fuse holder located just above the power cord connector.
- 3. Remove and discard the old fuses.



Caution: For continued protection against fire hazard, replace fuses with those of the appropriate type and rating.

- 4. Insert the new fuses into the fuse holder.
- 5. Insert the fuse holder into the receptacle and gently push until it locks into position.

5 Troubleshooting

This chapter describes the following:

- Troubleshooting pump problems based on error messages and malfunctions
- · Identifying and correcting noises
- · Identifying pump-related chromatographic problems

Caution: To prevent injury, always observe good laboratory practices when you troubleshoot the 1500 Series HPLC Pump.

Contacting Waters Technical Service

If you encounter problems troubleshooting the 1500 Series Pump that you cannot resolve, 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 at (508) 478-2000 (U.S.A.).

5.1 Troubleshooting Pump Problems

<u>Table 5-1</u> is a guide to troubleshooting pump problems. It lists pump error messages and pump-related symptoms and their possible causes, and suggests corrective actions.

Symptom	Possible Cause	Corrective Action
High Pressure	Pump flow rate set too high	Set to correct flow rate.
	High pressure limit set too low	Set high pressure limit to the correct value.
	Blocked column	Clean the column according to the column care and use manual.
	Blocked mixer	Backflush or disassemble the mixer.
	Viscous eluents in use	Check eluent viscosity (the observed pressure may be normal for the column/eluent blend). If necessary, change to a less viscous eluent.
	Defective pressure transducer	Contact Waters Technical Service.
	Blocked outlet tubing or fluid path (in pump outlet, detector, column, or injector)	Locate the source of blockage. Clean or replace tubing according to the appropriate operator's manual.
	Ambient temperature has changed	Stabilize the operating temperature.

Table 5-1 Determining Pump Malfunctions (Continued)

Symptom	Possible Cause	Corrective Action
Low Pressure	Pump flow rate set too low	Set to the correct flow rate.
	Low pressure limit set too high	Set low pressure limit to correct value.
	Eluent reservoir empty	Fill the reservoir.
	Air in eluent lines	Prime the pump (see <u>Section 3.2.1,</u> <u>Priming and Purging the Pump</u>).
	Incorrect eluent used	Change to the correct eluent.
	Visible leaks	Carefully tighten any loose fittings.
	Ambient temperature has changed	Stabilize the operating temperature.
Pump does not run	Pump not connected to power source	Ensure power cable is properly connected to power source and pump.
	No power at outlet	Check the outlet by connecting to another electrical unit known to be working. If that unit does not work, relocate the pump to a functioning electrical outlet.
	Blown fuse	Replace the fuses.

	Table 5-1	Determining	Pump	Malfunctions	(Continued)
--	-----------	-------------	------	--------------	------------	---

Symptom	Possible Cause	Corrective Action
Pump not delivering eluent	Draw-off valve open or leaking	Close the draw-off valve. If eluent still leaks, replace the valve (see <u>Section 4.5, Replacing a Draw-Off</u> <u>Valve</u>).
	Pump low-pressure limit set higher than operating pressure	Set to the correct low-pressure limit.
	Flow rate set to zero	Set the desired pump flow rate.
	Pressure transducer out of adjustment or defective	Contact Waters Technical Service.
	Pump not primed	Prime the pump (see <u>Section 3.2.1,</u> <u>Priming and Purging the Pump</u>).

Table 5-1 Determining Pump Malfunctio	ns (Continued)
---------------------------------------	----------------

Symptom	Symptom Possible Cause Corre	
Pump not delivering eluent (continued)	Immiscible eluents in pump head	Purge pump with appropriate eluents. Verify miscibility of eluents being used and change to more miscible eluents.
	Dirty or malfunctioning inlet or outlet check valve	Replace the check valves (see <u>Section 4.4, Replacing Check</u> <u>Valves</u>).
	Damaged plunger seal (indicated by eluent leaking from behind the pump head or by salt crystal build-up around the back of the pump head)	Verify if both pump heads can maintain pressure as outlined in <u>Section 4.2.2, Ramp-and-Decay</u> <u>Test</u> . If a head is leaking, replace the plunger seal (see <u>Section 4.3,</u> <u>Replacing and Cleaning Plunger</u> <u>Seals and Plungers</u>).
	 Pump cavitation due to one of the following: Eluent reservoirs positioned at or below the height of the pump Loose, bent, or blocked inlet tubing Improperly degassed eluent Dirty eluent reservoir inlet filter Volatile eluents in pump head Tubing ID too small 	 Perform the following corrective action: Raise eluent reservoirs above the pump. Check tubing. Tighten, straighten, or replace tubing. Degas. Replace filter. Prime pump (see <u>Section 3.2.1.</u> Priming and Purging the Pump). Use correct tubing.
	Ruptured high-pressure noise filter	Contact Waters Technical Service.
	Defective pump motor	Contact Waters Technical Service.
	Defective circuit board	Contact Waters Technical Service.

Symptom	Possible Cause	Corrective Action
Leak from pump head	Worn pump plunger seals	Replace defective plunger seals (see <u>Section 4.3.2, Cleaning and</u> <u>Replacing the Plungers</u>).
	Worn plunger	Replace the plunger (see <u>Section 4.3.2, Cleaning and</u> <u>Replacing the Plungers</u>).
	Loose pump head	Tighten the two pump head screws. Ensure that both screws are tightened equally, otherwise seal wear may result. Do not overtighten.
	Loose inlet or outlet check valve	Tighten the loose check valve(s). Do not overtighten.
		Check fittings and ferrules for wear. Replace if necessary.
Leak from draw-off valve	Draw-off valve open or broken	Close the draw-off valve. If leak continues, replace the valve (see <u>Section 4.5, Replacing a Draw-Off</u> <u>Valve</u>).
Erratic flow rate/pump pulsations	Pump not primed	Prime the pump. If you are using a volatile eluent (such as hexane), prime the pump with a miscible, less volatile eluent such as THF or methanol. Ensure that the column is disconnected to avoid disrupting equilibrium.

Table 5-1 Determining Pump Malfunctions (Continued)
Symptom	Possible Cause	Corrective Action
Erratic flow rate/pump pulsations (continued)	Reservoir low or out of eluent	Refill reservoir (filter and degas eluent).
	Air bubble in pump head	Prime pump to remove bubble. Ensure there are no air bubbles in the inlet lines. Degas eluents.
	Dirty or malfunctioning check valve(s)	Replace the check valves (see <u>Section 4.4, Replacing Check</u> <u>Valves</u>).
	Inlet filter or inlet lines blocked	Check lines for blockages. Replace the inlet filter frit.
	Pump plunger seal leaking (under pump head)	Replace pump plunger seal (see <u>Section 4.3, Replacing and</u> <u>Cleaning Plunger Seals and</u> <u>Plungers</u>).
	Worn pump plunger	Replace the plunger (see Section 4.3, Replacing and Cleaning Plunger Seals and Plungers).
	Immiscible eluents in pump head	Refer to the Corrective Action under the "Pump not delivering eluent" symptom listed above.
	Pump cavitation	Refer to the Corrective Action(s) under the "Pump not delivering eluent" symptom listed above.
	Pump electronics failure	Contact Waters Technical Service.
High system pressure due to pump	Pump Flow parameter set too high	Set the correct flow parameter value.
	Pressure transducer out of adjustment or defective	Contact Waters Technical Service.

5.2 Identifying and Correcting Noises

<u>Table 5-2</u> is a guide to troubleshooting and correcting noises in the Waters 1500 Series Pump.

Table 5-2	Identifying	Noise s
-----------	-------------	---------

Symptom	Possible Cause	Corrective Action
Click or loud snap	Binding plunger seal	If click does not stop and cannot be isolated to one pump head, replace the plunger seals, one at a time (see <u>Section 4.3, Replacing and Cleaning</u> <u>Plunger Seals and Plungers</u>).
	Worn indicator rod spring	Replace the spring.
Squeak	Plunger seals dry	Wet plunger with appropriate eluent through pump head access holes.
	Binding plunger seal	Replace plunger seal assembly (see <u>Section 4.3, Replacing and Cleaning</u> <u>Plunger Seals and Plungers</u>).
	Improper plunger seal	Install correct pump plunger seal (see Section 4.3, Replacing and Cleaning Plunger Seals and Plungers).
	Binding piston indicator rod	Replace indicator rod.

5.3 Identifying Chromatographic Problems

<u>Table 5-3</u> is a guide to troubleshooting and correcting chromatographic problems.

Table 5-3 Correcting Chromatographic Problem s

Symptom	Possible Cause	Corrective Action
Erratic retention times	Air bubble in pump head	Degas all eluents and prime pump (see <u>Section 3.2.1,</u> <u>Priming and Purging the</u> <u>Pump</u>).
	Malfunctioning check valves	Replace pump check valves (see <u>Section 4.4, Replacing</u> <u>Check Valves</u>).
	Leaking pump seals	Replace pump seals (see <u>Section 4.3, Replacing and</u> <u>Cleaning Plunger Seals and</u> <u>Plungers</u>).
	Leaks around fittings	Tighten fittings
	Separation chemistry	Check eluent and column.
	Clogged eluent filters	Replace filters.

Symptom	Possible Cause	Corrective Action
Increased retention	Incorrect flow rate	Change flow rate.
umes	Incorrect eluent composition	Change eluent composition.
	Column heater not turned on	Turn column heater on.
	Incorrect eluent	Use correct eluent.
	Column contaminated	Clean or replace column.
	Incorrect column	Use correct column.
	Air bubble in pump head	Degas all eluents and prime pump (see <u>Section 3.2.1,</u> <u>Priming and Purging the</u> <u>Pump</u>).
	Clogged eluent filter	Replace filter.
Reduced retention times	Incorrect flow rate	Change flow rate.
	Incorrect eluent composition	Change composition.
	High column temperature	Reduce column temperature.
	Incorrect eluent	Use correct eluent.
	Column contaminated	Clean or replace column.
	Incorrect column	Use correct column.
	Eluent not properly degassed	Degas eluent.
Reproducibility errors	Eluent not properly degassed	Degas eluent.
	Incorrect chemistry/integration	Check chemistry/integration.

Table 5-3 Correcting Chromatographic Problems (Continued)

Symptom	Possible Cause	Corrective Action
Baseline drift, rapid	Column not equilibrated	Equilibrate column.
	Eluent contaminated	Use fresh eluent.
	Eluent not properly degassed (rapid or slow drift)	Degas eluent.
	Flow fluctuations (rapid or slow drift)	Fix pump problems, replace pump seals and check valves.
Baseline drift, slow	Eluent contaminated	Use fresh eluent.
	Ambient temperature fluctuations	Stabilize operating environment temperature enough to allow full equilibration.
Baseline noise cycling, short-term (30 to 60 sec)	Fluctuating flow rate	Degas eluent. Try repriming the pump(s). If necessary, check flow accuracy of the pump(s).
	Inadequate eluent blending	Install a mixer as shown in Section 2.4.2, Installing Different Mixer Configurations.
	Power source (short- or long- term cycling)	Disconnect other instruments on the power line; try a different wall outlet; have line voltage checked; use power conditioner.
	Radio frequency noise (short- or long-term cycling)	Eliminate interference.
Baseline noise cycling, long-term (for 1 hour)	Ambient temperature fluctuations	Stabilize ambient temperature.
	Integrator or recorder faulty	Check integrator or recorder for excessive baseline noise.

Table 5-3 Correcting Chromatographic Problems (Continued)

Symptom	Possible Cause	Corrective Action
Baseline noise, random	Eluents not properly degassed	Degas eluents.
	Flow erratic, pump not primed	Prime the pump (see <u>Section 3.2.1, Priming and</u> <u>Purging the Pump</u>).
		Check for air in the pump, failing seals.
	Eluents contaminated	Use fresh eluent.
	Column contaminated	Clean/replace column.
	System improperly grounded	Plug into outlet on different electrical circuit.
		Use power conditioner.
	Recorder voltage incorrect	Set recorder to correct voltage.
	Radio frequency noise	Eliminate interference.
Straight baseline, no peaks	No pump flow	Set pump flow rate.
	Leak in eluent path	Repair the leak.
Sensitivity loss	Degraded, contaminated, or improperly prepared sample	Use fresh sample.
	Column contaminated	Clean or replace column.
	Loss of column efficiency	Clean or replace column.
	Change in eluent composition	Correct eluent pH or ionic composition.
	Incorrect flow rate	Change flow rate.

Table 5-3 Correcting Chromatographic Problems (Continued)

5

Appendix A Specifications

This appendix provides the following types of specifications for the Waters[®] 1500 Series Pumps:

- Physical
- Environmental
- Electrical
- Performance
- Instrument control and communication

Table A-1 Physical Specifications for 1500 Series Pump

Item	Specification
Height	17.0 in (43 cm)
Depth	24.0 in (61.0 cm)
Width	12.0 in (30.5 cm) {without bottle holder}
Weight (1525)	60.0 lb (27.2 kg)
Weight (1515)	45.0 lb (20.4 kg)
Wetted Surface Material	316 stainless steel, sapphire, reinforced fluorocarbon polymer seals, carbon-reinforced Tefzel®

Table A-2 Physical Specifications for Column Heater

ltem	Specification
Height	17.0 in (43 cm)
Depth	14.0 in (35.5 cm)
Width	6.0 in (15.2 cm)
Weight	13 lb (5.9 kg)

Table A-3 Environmental Specifications

Item	Specification
Operating Temperature	4 to 40 °C (39 to 104°F)
Humidity	20 to 80%, non condensing
Audible Noise	<70 dBA at operator position
Solvent Compatibility	Solvents consistent with materials of construction. Salts and buffers can reduce seal life, especially at pressures in excess of 3000 psi.

Table A-4 Electrical Specifications

Item	Specification
Power Requirements (Pump)	200 VA
Power Requirements (Column Heater)	150 VA
Line Voltage	120/240 Vac
Frequency	50/60 Hz, single phase
Fuses	3.15 A, 250 V, two

Table A-5 Performance Specifications

Item	Specification
Programmable Flow Rate Range 100-µL heads	0.00 to 10.00 mL/min, in 0.01-mL/min increments
Maximum Operating Pressure	6000 psi (41,370 kPa, 401 bars); programmable upper and lower limits

Table A-5 Performance Specifications (Continued)

Item	Specification
Pressure Ripple (one pump)	≤2.0% at 1 mL/min, degassed methanol, at 1000 psi backpressure
Flow Precision	≤0.1% RSD or 2 seconds SD, six replicates based on retention time or volumetric measures at 1 mL/min, 1000 to 2000 psi backpressure, PQ test (each pump individually)
Flow Accuracy	±1.0% of setting at 1 mL/min, or 30 μL/min, whichever is larger, using degassed methanol at 1000 to 2000 psi backpressure (each pump individually)
Gradient Accuracy (1525)	±0.5% of setting at 1 mL/min and 1000 psi backpressure (Methanol: methanol with 5.6 mg/L propyl paraben) with one GM-150 mixer
Gradient Precision (1525)	<0.5% RSD at 1 mL/min and 1000 psi backpressure (Methanol: methanol with 5.6 mg/L propyl paraben) with one GM-150 mixer

Table A-6 Instrument Control and Communication Specifications

Item	Specification
Inject	Voltage Range: 0 to 30 V Current: 200 mA
Stop Flow Input	0 to 30 V
Contact Closure Outputs	Voltage Range: ± 30 V Current: 500 mA
External Control	IEEE-488

Appendix B Spare Parts and Accessories

This appendix describes the spare parts, accessories, and maintenance and operation kits for the Waters[®] 1500 Series HPLC Pumps.

The spare parts in <u>Table B-1</u> are recommended for customer installation. Any parts not listed in this table may require installation by a trained Waters service representative.

Item	Quantity	Part Number
Check Valve Assembly	2	70000254
Pump Plunger	2	WAS207069
Indicator Rod Assembly	1	WAT069506
Pump Head Support Assembly	2	WAS207112
Draw-Off Valve Assembly	1	WAS207085
Inlet Tubing Assembly (LH)	1	WAS207081
Inlet Tubing Assembly (RH)	1	WAS207082
Outlet Tubing Assembly (LH)	1	WAS207083
Outlet Tubing Assembly (RH)	1	WAS207084
Lower Drip Tray	1	700001091
Injecter Valve Drip Tray	1	700001092
Tube Assembly, Tee - Transducer	1	700001093
Tube Assembly, Transducer - Ref. Valve	1	700001094

Table B-1 Spare Parts

Table B-1 Spare Parts (Continued)

Item	Quantity	Part Number
Tube Assembly, Ref. Valve - Waste	1	700001095
Tube Assembly, Transducer - Mixer	1	700001162
Tube Assembly, Mixer - Ref. Valve	1	700001163
Tube Assembly, Mixer - Mixer	1	700001164
Tube Assembly, Ref. Valve - Manual Inj.	1	700001165
Tube Assembly, Manual Inj Column	1	700001166
Tube Assembly, Manual Inj Waste	1	700001169
Fuses, F 3.15 A/250 V (20 mm)	2	WAT163-16

Use the accessories in <u>Table B-2</u> to optimize the Waters 1500 Series Pump for your application and to simplify common procedures.

Table B-2 Accessories

Item	Quantity	Part Number
1515 PM Kit	1	201000113
1525 PM Kit	1	210000114
Manual Injector Rebuild Kit	1	PSL637799
Plunger Wash Kit	1	WAT031870
Replacement Pump Head Assembly	1	WAS207006
Extended Flow Range Option Kit	1	WAT207119
Extended Flow Pump Head Assembly	2	WAT060303
Extended Flow Plunger Seal	2	WAT026644

Table B-2 Accessories (Continued)

Item	Quantity	Part Number
Extended Flow Outlet Check Valve Assembly	2	WAT025216
Eluent Reservoir Filter	1	WAT025531
Syringe, 10-mL, Polypropylene	1	WAT010337
#316 Stainless Steel Tubing:		
$1/16$ -inch OD \times 0.009-inch ID, 10 feet	1	WAT026973
(3 m)	1	WAT026804
1/16-inch OD × 0.020-inch ID, 10 feet (3 m)		
ETFE Tubing:		
1/8-inch OD $ imes$ 0.062 ID, 25 feet (7.7 m)	1	WAT026808
0.80-inch OD \times 0.058 ID, 25 feet (7.7 m)	1	WAT026974
Manual Inject Valve Kit	1	186000872
Gradient Mixer	1	WAT055847
Large Volume Gradient Mixer	1	WAT051518
1500 Column Heater	1	186001500

The kits in <u>Table B-3</u> contain the parts you need for common maintenance operations.

Item	Quantity	Part Number
Solvent Clarification Kit:		
110 V/60 Hz	1	WAT085113
220 V/50 Hz	1	WAT085122
Seal Replacement Kit (Clear-100 [™] ; general-purpose seals)	1	WAT022934

Table B-3 Maintenance and Operation Kits

Table B-3 Maintenance and Operation Kits (Continued)

Item	Quantity	Part Number
Seal Replacement Kit (Clear-100 [™] ; general-purpose seals)	4	WAT022946
Seal Replacement Kit (black, general-purpose seals)	1	WAT026613
Seal Replacement Kit (black, general-purpose seals)	4	WAT038423
Seal Replacement Kit (for use with aqueous applications)	2	WAT025296
Seal Replacement Kit (for use with aqueous applications)	4	WAT025297
Seal Replacement Kit with springs (for use with buffer applications)	2	WAT069581
Seal Insertion Tool	1	WAT076765
Compression Screw/Ferrule Kit	5/pkg	WAT025604
Plunger Insertion Tool	1	WAT011042
Snap Ring Pliers	1	WAT025263

Appendix C Solvent Considerations



Caution: To avoid chemical hazards, always observe safe laboratory practices when operating your system.

C.1 Introduction

Clean Solvents

Clean solvents provide:

- Reproducible results
- · Operation with minimal instrument maintenance

A dirty solvent can cause:

- · Baseline noise and drift
- Blockage of the solvent filters with particulate matter

Solvent Quality

Use HPLC-grade solvents to ensure the best possible results. Filter solvents through 0.45-µm filters before use. Solvents distilled in glass generally maintain their purity from lot to lot; use them to ensure the best possible results.

Preparation Checklist

The following solvent preparation guidelines help to ensure stable baselines and good resolution:

- Filter solvents with a 0.45-µm filter.
- Degas and/or sparge the solvent.
- Stir the solvent.
- Keep in a place free from drafts and shock.

Water

Use water only from a high-quality water purification system. If the water system does not provide filtered water, filter it through a 0.45-µm membrane filter before use.

Buffers

When you use buffers, dissolve salts first, adjust the pH, then filter to remove insoluble material.

Tetrahydrofuran (THF)

When using unstabilized THF, ensure that your solvent is fresh. Previously opened bottles of THF contain peroxide contaminants, which cause baseline drift.



Caution: THF contaminants (peroxides) are potentially explosive if concentrated or taken to dryness.

C.2 Solvent Compatibility

The Waters 1500 Series HPLC Pump 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 1500 Series HPLC Pump.

Solvents to Avoid

You can use any solvent with the 1500 Series HPLC Pump. However, long-term *static* exposure to halide salts (for example, fluoride, bromide, chloride, and iodide) will cause pitting and corrosion of stainless steel parts. When using these salts, flush your system thoroughly with water if the pump will be idle for more than two days.

Solvents to Use

Materials of construction used in the 1500 Series HPLC Pump are nonreactive with most acids, bases, salts, and organic solvents.

The solvents listed in <u>Table C-1</u> through <u>Table C-4</u> have been approved for use with the 1500 Series HPLC Pump. 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). Higher concentrations can be used in many instances.

Information on the use of a specific solvent or concentration that is not listed in this manual can be obtained by contacting Waters.

Aqueous Buffers			
Acetate	K ₂ SO ₄	Na ₂ S	Perfluorobutyric acid
Al ₂ SO ₄	K ₃ Fe(CN) ₆	Na ₂ CO ₃	NH ₄ CI
Ca(OCI) ₂	K ₄ Fe(CN) ₆	Na ₂ SO ₄	Phosphate
CaCl ₂	KBr	NaCl	Tartrate
Citrate	КСІ	Sodium acetate	Trilithium citrate
H_2O_2 up to 10%	KHCO3	NaH ₂ BO ₃	Tris
HIBA	KMnO ₄	NaHCO ₃	4-(2-pyridylazo) resorcinol monosodium salt monohydrate
K ₂ CO ₃	KNO ₃	NaHSO ₄	
K ₂ Cr ₂ O ₃	LiClO ₄	NaNO ₃	
K ₂ S	Na ₂ B ₄ O ₇	NaOCI	

Table C-1 Aqueous Buffers for Use with the 1500 Series HPLC Pump

Table C-2 Acids for Use with the 1500 Series HPLC Pump

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 1500 Series HPLC Pump

Bases		
Ba(OH) ₂	NaOH, up to 10 M	
КОН	NH_4OH , up to 3 M	
LiOH	Tetramethylammoniu m hydroxide pentahydrate	

Table C-4 Organic Solvents for Use with the 1500 Series HPLC Pump

Organic Solvents					
4-cyanophenol	Chloroform	Ethylene glycol	Methylene chloride		
Acetone	Cyclohexane	Formaldehyde	n-Propanol		
Acetonitrile	Cyclohexanone	Heptane	Phenol		
Amyl acetate	Dibutyl phathalate	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, from chloroform to 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 higher temperature on solvent solubility.
- Buffers dissolved in water may precipitate when mixed with organic solvents.

When you switch from a strong buffer to an organic solvent, flush the buffer out of the system with distilled water before you add the organic solvent.

Polarity Index	Solvent	Viscosity CP, 20 °C	Boiling Point °C (@1 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	<i>P</i> -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	—	—

Table C-5 Solvent Miscibilit y

Polarity Index	Solvent	Viscosity CP, 20 °C	Boiling Point °C (@1 atm)	Miscibility Number (M)	λ Cutoff (nm)
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
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	210
6.2	Acetic acid	1.26	117.9	14	
6.4	Dimethylformamide	0.90	153.0	12	

Table C-5 Solvent Miscibility (Continued)

Table C-5 Solvent Miscibility (Continued)

Polarity Index	Solvent	Viscosity CP, 20 °C	Boiling Point °C (@1 atm)	Miscibility Number (M)	λ Cutoff (nm)
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		

How to Use Miscibility Numbers (M-Numbers)

Use Miscibility numbers (M-numbers) to predict the miscibility of a liquid with a standard solvent (see <u>Table C-5</u>).

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 a critical solution temperature between 25and 75 °C, with 5 0°C as the optimal temperature.
- If the difference is 17 or greater, the liquids are immiscible or their critical solution temperature is above 75 °C.

Some solvents prove immiscible with solvents at both ends of the lipophilicity scale. These solvents receive a dual M-number:

- The first number, always lower than 16, indicates the degree of miscibility with highly lipophilic solvents.
- The second number applies to the opposite end of the scale. A large difference between these two 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.

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 the system after use. Flush all fluidic pathways with HPLC-quality water before shutting down the system and leave water in the system (flush with 90 percent HPLC-quality water:10 percent methanol for shutdowns scheduled to be more than one day). Flush using a minimum of 15 mL.

C.5 Head Height

Position the solvent reservoirs in the bottle holder supplied with the 1500 Series HPLC Pump (with adequate spill protection).

C.6 Solvent Viscosity

Generally, viscosity is not important when you are operating with a single solvent or under low pressure. However when you are running 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.

C.7 Mobile Phase Solvent Degassing

Mobile phase difficulties account for the majority 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.

C.7.1 Gas Solubility

Only a finite amount of gas can be dissolved in a given volume of liquid. This amount 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.

C.7.2 Eluent Degassing Methods

You can degas eluents using any of the following methods:

- · In-line degassing
- Heating
- Vacuum sonication

Vacuum Degassing

An in-line vacuum degasser operates on the principle of Henry's Law to remove dissolved gases from the solvent. According to Henry's Law, the mole fraction of a gas dissolved in a liquid is proportional to the partial pressure of that gas in the vapor phase above the liquid. If the partial pressure of a gas on the surface of the liquid is reduced, for example, by evacuation, then a proportional amount of that gas comes out of solution.

Note: Vacuum degassing may change the composition of mixed solvents.

In-Line Degassing

In-line degassing removes gases from the eluent as it passes through a gas-permeable membrane enclosed in a vacuum chamber. The vacuum in the chamber accelerates the rate at which the dissolved gas diffuses through the gas-permeable membrane. This method provides an automatic, continuous method of removing dissolved gasses, and allows for quick eluent changeover. Waters® makes two in-line degassers: part numbers WAT079700 (2 Eluent) and WAT079800 (4 Eluent). Contact Waters for details.

Using an In-Line Degasser

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.

Heating

Heating to remove dissolved gas is usually not effective unless you boil the eluent, and this is

not practical or safe for mixed, flammable, or volatile eluents. However, raising the temperature even slightly raises the partial pressure of the eluent, and thereby reduces the rate of resolubilization of a gas.

Vacuum Sonication

Sonication in combination with a vacuum degasses eluents very quickly. This technique does not change the composition of mixed eluents appreciably.

Caution: Apply vacuum only to suitable vessels. The brown gallon bottles in which eluent is shipped are not designed for vacuum degassing. There is a high risk of implosion if these bottles are used for vacuum degassing.

Conclusions

With any of the above techniques, the eluent reequilibrates to air saturation in 12 to 24 hours, depending on the eluent.

Degassing by vacuum or sonication or both is often performed for improved pump performance in multipump gradient applications.

C.8 Wavelength Selection

The tables in this section provide UV cutoff values for:

- Common solvents
- · Common mixed mobile phases
- Chromophores

UV Cutoffs for Common Solvents

<u>Table C-6</u> shows the UV 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 below the cutoff increases baseline noise due to the absorbance of 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

Solvent	UV Cutoff (nm)	Solvent	UV Cutoff (nm)
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	<i>n</i> -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

Table C-6 UV Cutoff Wavelengths for Common Chromatographic Solvents (Continued)

Mixed Mobile Phases

<u>Table C-7</u> provides approximate wavelength cutoffs for some other solvents, buffers, detergents, and mobile phases. The solvent concentrations represented are those most commonly used. If you want to use a different concentration, you can determine approximate absorbance using Beer's Law, since absorbance is proportional to concentration.

Table C-7	Wavelength	Cutoffs for	Different	Mobile	Phase s
-----------	------------	-------------	-----------	--------	---------

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 HCI, 20 mM, pH 7.0, pH 8.0	202, 212
HEPES, 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, 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

Refractive Indices of Common Solvents

<u>Table C-8</u> lists the refractive indices for some common chromatographic solvents. Use 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.

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	<i>n</i> -Decane	1.412
Acetonitrile	1.344	Amyl chloride	1.413
Ethyl ether	1.353	Dioxane	1.422
<i>n</i> -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	<i>N,N-</i> Dimethyl Formamide (DMF)	1.428
Ethyl acetate	1.370	<i>N,N-</i> 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

Table C-8 Refractive Indices for Common Chromatographic Solvents

Table C-8	Refractive	Indices for	Common	Chromatographic	: Solvents	(Continued))

Solvent	RI	Solvent	RI
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

Appendix D Warranty Information

This appendix includes information on:

- · Limited express warranty
- · Shipments, damages, claims, and returns

D.1 Limited Express Warranty

Waters Corporation provides this limited express warranty (the Warranty) to protect customers from nonconformity in the product workmanship and materials. The Warranty covers all new products manufactured by Waters.

Waters warrants that all products that it sells are of good quality and workmanship. The products are fit for their intended purpose(s) when used strictly in accordance with Waters' instructions for use during the applicable warranty period.

Limited Warranty

WATERS Corporation warrants that the Waters 1500 Series HPLC Pump is a Class I medical device under 21 CFR 862.2260, as now in effect, and is for general purpose use and is not for use in clinical diagnostic procedures, and that during the Warranty period, the performance of all components of the Waters 1500 Series HPLC Pump [other than Third-Party Components], will not deviate materially from the Specifications for such Waters 1500 Series HPLC Pump. Warranties, if any, that may be applicable to Third-Party Components shall be provided by the respective manufacturers or suppliers of such Third-Party Components, and WATERS Corporation shall use reasonable efforts to assist Customer in securing the benefits of any such warranties.

Exclusions

The foregoing warranty does not apply to any material deviation from the Specifications by any component of the Waters 1500 Series HPLC Pump that results from (a) use of the Waters 1500 Series HPLC Pump for any purpose other than general purpose use and specifically excluding use of the Waters 1500 Series HPLC Pump in clinical diagnostic procedures, or use of the Waters 1500 Series HPLC Pump for investigational use with or without confirmation of diagnosis by another, medically established diagnostic product or

procedure, (b) errors or defects in any Third-Party Component, (c) modification of the Waters 1500 Series HPLC Pump by anyone other than WATERS Corporation, (d) failure by Customer to install any Standard Enhancement in accordance with an update procedure, release of firmware or any operating system release, (e) any willful or negligent action or omission of Customer, (f) any misuse or incorrect use of the Waters 1500 Series HPLC Pump, (g) any malfunction of any information system or instrument with which the Waters 1500 Series HPLC Pump may be connected, or (h) failure to establish or maintain the operating environment for the Waters 1500 Series HPLC Pump in accordance with the Operator's Manual.

Exclusive Remedy

In the event of any failure of the Waters 1500 Series HPLC Pump to perform, in any material respect, in accordance with the warranty set forth herein, the only liability of WATERS Corporation to Customer, and Customer's sole and exclusive remedy, shall be the use, by WATERS Corporation, of commercially reasonable efforts to correct for such deviations, in WATERS Corporation's sole discretion, replacement of the purchased Waters 1500 Series HPLC Pump, or refund of all amounts theretofore paid by Customer to WATERS Corporation for the Waters 1500 Series HPLC Pump.

Disclaimers

THE LIMITED WARRANTY SET FORTH HEREIN IS EXCLUSIVE AND IN LIEUOF, AND CUSTOMER HEREBY WAIVES, ALL OTHER REPRESENTATIONS, WARRANTIES AND GUARANTEES, EXPRESS OR IMPLIED, INCLUDING WITHOUT LIMITATION ANY IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS OF THE WATERS 1500 SERIES HPLC PUMP FOR A PARTICULAR PURPOSE, INCLUDING FITNESS FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE, OR NONINFRINGEMENT, AND ANY WARRANTIES ARISING OUT OF COURSE OF DEALING OR COURSE OF PERFORMANCE. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT BECAUSE OF THE COMPLEX NATURE OF THE WATERS 1500 SERIES HPLC PUMP AND ITS MANUFACTURE, WATERS CORPORATION CANNOT AND DOES NOT WARRANT THAT THE [OPERATION/COMPOSITION*] OF THEWATERS 1500 SERIES HPLC PUMP WILL BE [WITHOUT INTERRUPTION OR ERROR-FREE OR WITHOUT DEFECT*]. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT CUSTOMER IS SOLELY RESPONSIBLE FOR USE OF THEWATERS 1500 SERIES HPLC PUMP IN CLINCIAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE.

*Changes based on product

Warranty Service

Warranty service is performed at no charge and at Waters' option in one of three ways:

- A service representative is dispatched to the customer facility.
- The product is repaired at a Waters repair facility.
- Replacement parts with appropriate installation instructions are sent to the customer.

Nonconforming products or parts are repaired, replaced with new or like-new parts, or refunded in the amount of the purchase price, when the product is returned. Warranty service is performed only if the customer notifies Waters during the applicable warranty period.

Unless otherwise agreed at the time of sale, warranty service is not provided by dispatching a service representative when the equipment has been removed from the initial installation location to a new location outside the home country of the selling company.

Warranty service is provided during business hours (8AM to 5 PM, Monday through Friday). Service is not available when Waters offices are closed in observance of legal holidays.

Warranty Service Exceptions

Warranty service is not performed on:

- Any product or part that has been repaired by others, improperly installed, altered, or damaged in any way.
- Products or parts identified prior to sale as not manufactured by Waters. In such cases, the warranty of the original manufacturer applies.
- Products that malfunction because the customer has failed to perform maintenance, calibration checks, or observe good operating procedures.
- Products that malfunction due to the use of unapproved parts and supplies.

Repair or replacement is not made:

- For expendable items such as filament devices, panel lights, fuses, batteries, and seals, if such items were operable at the time of initial use.
- Because of decomposition due to chemical action.
- For used equipment.
- Because of poor facilities, operating conditions, or utilities.

Warranty Period

This instrument is warranted against defects in workmanship and materials for a period of twelve months (the "Warranty Period"), excluding assemblies, modules, serviceable parts, and components that may have different warranty periods. Refer to <u>Table D-1</u> below for the warranty periods of such assemblies, modules, serviceable parts, and components.

The Warranty Period commences at the date of product shipment. The Warranty Period may be extended for such time, not to exceed one month, required to deliver and install the product at the customer's site. In no case does the Warranty Period extend beyond **13 months** from date of shipment. If an item is repaired or replaced during the Warranty Period, the replacement part or repair is warranted for the balance of the original warranty period.

<u>Table D-1</u> summarizes the warranty periods for the Waters 1500 Series HPLC Pump and its components.

Component	Warranty Period
Waters 1500 Series HPLC Pump, excluding the components listed below	1 year
Normal Wear and Maintenance Parts in Performance Maintenance Kit*	90 days
Expendables, Consumables, and Operating Supplies • Fuses	Warranted to function properly when delivered
 Tubing and fittings 	

Table D-1 Waters 1500 Series HPLC Pump Warranty Periods

*Instrument improvements may result in changes to the contents of this kit.

Shipments

Since all shipments are made free on board (FOB) shipping point, Waters suggests that you authorize insurance on all shipments. Instruments and major components are shipped by surface, unless otherwise required. Supplies and/or replacement parts are shipped via UPS, UPS Blue, air parcel post, or parcel post unless otherwise requested.

Damages

The Interstate Commerce Commission has held that carriers are as responsible for concealed damage as for visible damage that occurs in transit. Unpack shipment promptly after receipt as there may be concealed damage. When concealed damage is discovered, cease further unpacking of the unit involved and request immediate inspection by the local agent or carrier. Secure a written report of the inspector's findings to support the claim. The request for inspection must be made within 15 days of shipment receipt. Otherwise, the claim will not be honored by the carrier. Do not return damaged goods to the factory without first securing an inspection report and contacting Waters for a return merchandise authorization (RMA) number.

Claims

After a damage inspection report is secured, Waters cooperates fully in supplying replacements and handling of a claim, which may be initiated by either party.

Returns

No returns may be made without prior notification of, and authorization from, Waters. If for any reason it is necessary to return material to Waters, please contact Waters Customer Service or your nearest Waters subsidiary or representative for a return merchandise authorization (RMA) number and forwarding address.

Index

A

Accessories <u>81</u> Addresses DIP switch settings <u>25</u> setting IEEE-488 <u>25</u> Air bubbles <u>72</u>

B

Buffered solvents92Buffers44, 56, 86

C

Cable lengths 27 Cavitation in pump head 70, 72 Check valve description 19 loose 71 malfunctioning 70 performance 49 removing 60 replacing 59 Chromatographic problems 74 Cleaning plungers 56 Components electronic 20 fluid-handling 16 Connecting eluent reservoir 33 power supply 25 pump intlet 34 Connections electrical 24 fluidic 33 guidelines 27

Contacting Waters Technical Service <u>49</u> Contamination, eluent <u>45</u> Cooling fan <u>21</u> Cyclic pressure difference <u>51</u>

D

Damage, reporting 24 Degassing 70, 92–95 benefits 92, 94 considerations 94 in-line 94 Devices, setting IEEE-488 addresses 25 Diagnostic tests ramp-and-decay 49 retention time stability 49 DIP switches 25 Draw-off valve leaking 69, 71 replacing 61

E

Electrical connections 24 IEEE-488 27 IEEE-488 address 25 inject start signal 29 power supply 25 Electronics components 20 Eluent contamination 45 Eluent filter 45, 70, 72 Eluent level. See Head height Eluent reservoir connecting 35 positioning 35 Eluents

buffered <u>89</u> considerations <u>85</u> guidelines <u>44</u> heating <u>93</u> viscosity <u>44</u>, <u>92</u> volatile <u>70</u> Equilibrating the system <u>46</u>

F

Faulty fuses 65 Ferrule 71 Filters eluent 45, 70, 72 high-pressure noise 70 Flow direction 19 pulsations 71, 72 Flow rate 69 erratic <u>71</u>, <u>72</u> Fluid-handling components 16 Fluidic connections 33 Flushing. See Purging Fuse faulty 65 holder 64 replacing 64

G

Gas solubility <u>93</u> Guidelines connection <u>27</u> eluents <u>44</u>, <u>85</u>

Η

Head height <u>44</u>, <u>70</u> Heating eluents <u>94</u> High-pressure noise filter <u>18</u>, <u>70</u> High-pressure shutdown <u>50</u>

|

Identifying faulty fuses 65 **IEEE-488** address 25 guidelines 27 setting device addresses 25 Immiscible eluents 70 Indicator rod 19, 59 Inject start signal, connecting 29 Inlet check valve description 19 location 19 Inlet manifold 18 In-line degassing 94 Installation site requirements 22 Interface guidelines 27

L

Leaking draw-off valves <u>69, 71</u> plunger seals <u>72</u> Leaks <u>71</u>

M

Maintenance and operation kits <u>83</u> Maintenance considerations <u>48</u> Methanol <u>71</u> Miscibility numbers <u>89</u> Miscibility of eluents <u>70</u> Miscibility of solvents <u>88</u> M-numbers. *See* Miscibility numbers Mobile phase compatibility <u>86</u> degassing <u>92</u> miscibility <u>88</u> viscosity considerations <u>92</u> Mobile phase. *See* Eluents Modes of operation <u>21</u> Monitoring system performance <u>49</u>

Ν

Noises, troubleshooting 73

0

Operating guidelines <u>44</u> modes <u>21</u> Outlet check valve description <u>19</u> location <u>19</u>

Ρ

Plunger insertion tool 54, 58 Plunger seals 70 leaking 72 replacing 51 squeaking 73 worn 71 Plungers

cleaning 56 inspecting 57 replacing 58 worn 71 Power entry module 21 Power supply, connecting 25 Power switch 21 Powering off the pump 47 Powering on the pump 43 Pressure transducer 18, 69, 72 Pressure, relieving 51 Priming the pump 45, 69, 71 Pulsations in eluent delivery 71, 72 Pump head alignment 59 description 18 replacing 59 Pump head assembly, removing 53 Pump head support assembly, replacing 58 Pump outlet connecting 40 18 description Purging detector injector pump 45

R

Ramp-and-decay test <u>49</u> Rear panel fuses <u>64</u> Refractive indices <u>98</u> Relieving system pressure <u>51</u> Removing check valve <u>60</u> pump head assembly <u>52</u>
Replacing draw-off valve <u>61</u> fuses <u>64</u> inlet check valve <u>51</u> plunger seals <u>51</u> plungers <u>54</u> Reservoirs, positioning <u>92</u> Retention time increased <u>51</u> observing stability <u>49</u> Returning damaged shipments <u>104</u>

S

Safety considerations 48 Salt crystals 51, 70 Seal wash holes 18 Shipping damage, reporting 24 Site requirements 22 Solvent buffered solvents 92 compatibility 86 general considerations 85 guidelines 85 miscibility 88 refractive indices 98 UV cutoff 95 viscosity considerations 92 Solvent reservoirs. See Reservoirs Solvents. See Eluents Sonication 95 Spare parts 81 Specifications 78 System performance, monitoring 49 System pressure, relieving 51

T

Tests ramp-and-decay <u>49</u> Retention time stability test <u>49</u> THF (tetrahydrofuran) <u>71, 86</u> Troubleshooting chromatographic problems <u>74</u> noises <u>73</u> Tubing <u>33, 33, 41</u> eluent supply <u>45</u> making connections <u>33</u> problems with <u>70</u>

U

Unpacking the pump 24

V

Vacuum sonication95Viscosity, eluent44, 92Volatile eluents70, 71

W

Warranty information100Waters Technical Service, contacting49Wavelength selection95