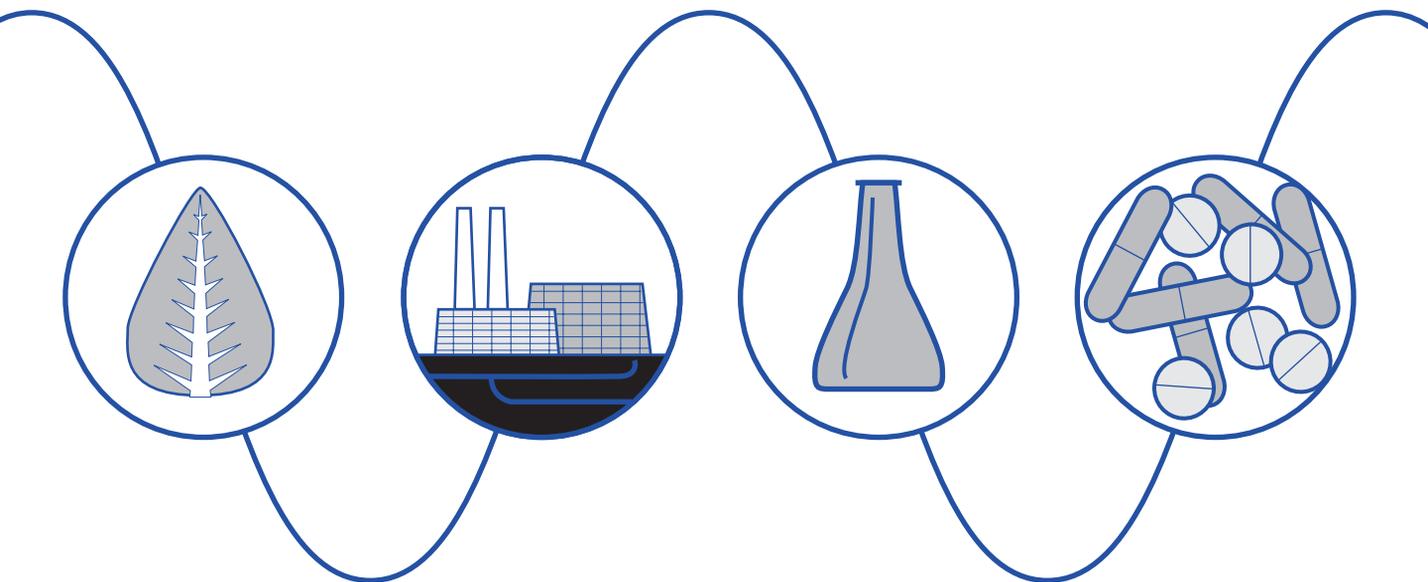


Waters 996 PDA Detector

Operator's Guide



Waters

34 Maple Street
Milford, MA 01757

053021TP, Revision 0

NOTICE

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The quality management system of Waters' chromatography applications software design and manufacturing facility, Milford, Massachusetts, complies with the International Standard ISO 9001 Quality Management and Quality Assurance Standards. Waters' quality management system is periodically audited by the registering body to ensure compliance.



Attention: *This is a highly sensitive instrument. Read this user's manual before using the instrument.*

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 changes. Until you determine the cause of a change, do not rely upon the results of the separations.



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



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.*



Attention: *This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy, and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference, in which case you must correct the interference at your own expense.*

Shielded cables must be used with this unit to ensure compliance with Class A FCC limits.

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.*

Canadian Emissions Notice

This digital apparatus does not exceed the Class A limits for radio noise emissions from digital apparatus set forth in the Radio Interference Regulations of the Canadian Department of Communications.

Le présent appareil numérique n'émet pas de bruits radioélectriques dépassant les limites applicables aux appareils numériques de la classe A prescrites dans les règlements sur le brouillage radioélectrique édictés par le Ministère des Communications du Canada.

Symbols Used on the Waters 996 Photodiode Array Detector

	Direct current
	Alternating current
	Protective conductor terminal
	Frame or chassis terminal
	Caution, risk of electric shock (high voltage)
	Caution or refer to manual
	Caution, hot surface
	Ultraviolet light

Table of Contents

How to Use This Guide.....	10
Chapter 1	
Installation	14
1.1 Installation Site Requirements	14
1.2 Power Connections	15
1.3 Millennium ³² Workstation Connections.....	16
1.3.1 Connecting the IEEE-488 Cable.....	16
1.3.2 Setting the IEEE-488 Address.....	18
1.4 Non-IEEE-488 Communication Connections.....	19
1.4.1 Connecting Analog Output Cables	19
1.4.2 Connecting Event Cables	20
1.5 Fluidic Connections.....	22
1.6 Startup/Shutdown	24
Chapter 2	
Diagnostics and Calibration	27
2.1 Startup Diagnostics.....	27
2.2 User-Initiated Diagnostics	30
2.3 PDA Calibration.....	31

Chapter 3	
Maintenance	33
3.1 Flow Cell Maintenance	33
3.1.1 Flushing the Flow Cell	33
3.1.2 Removing the Flow Cell	34
3.1.3 Disassembling and Cleaning the Flow Cell	36
3.1.4 Installing the Flow Cell Assembly	38
3.2 Replacing the Lamp	39
3.2.1 Checking Lamp Usage	39
3.2.2 Removing the Lamp	41
3.3 Replacing the Fuses	43
Chapter 4	
Principles of the 996 PDA Detector Optics	44
4.1 996 Detector Optics	44
4.2 Resolving Spectral Data	46
4.3 Measuring Light at the Photodiode	47
4.4 Computing Absorbance Data Points	50
4.4.1 Calculating Absorbance	50
4.4.2 Resolution	52
4.4.3 Filtering Data	53
Chapter 5	
Spectral Contrast Theory	54
5.1 Comparing Absorbance Spectra	54
5.2 Representing Spectra as Vectors	55
5.2.1 Vectors Derived from Two Wavelengths	56
5.2.2 Vectors Derived from Multiple Wavelengths	56

5.3 Spectral Contrast Angles	57
5.4 Nonidealities	60
5.4.1 Detector Noise	60
5.4.2 Photometric Error	61
5.4.3 Solvent Changes	61
5.4.4 Threshold Angle.....	61
Appendix A	
Detector Specifications.....	63
Appendix B	
Spare Parts	64
Appendix C	
Warranty Information	65
C.1 Limited Express Warranty.....	65
C.2 Shipments, Damages, Claims, and Returns.....	69
Appendix D	
Mobile Phase Absorbance	70
Index	74

List of Figures

1-1	Waters 996 PDA Detector Dimensions.....	15
1-2	Detector Rear Panel	16
1-3	Example of IEEE-488 Cable Connections	17
1-4	Locating the IEEE-488 Address Switches	18
1-5	Analog Output Terminals	20
1-6	Event Input/Output Terminal Strip.....	22
1-7	Compression Screw Assembly	24
1-8	996 Detector Indicator Lights.....	25
2-1	996 PDA Detector Indicator Lights	28
3-1	Flow Cell Access Door.....	35
3-2	Removing the Flow Cell Assembly	35
3-3	Flow Cell and Fluidic Connections Assemblies	36
3-4	Disassembling the Flow Cell.....	37
3-5	Lamp Access Door	40
3-6	Lamp Usage Indicator.....	40
3-7	Lamp Power Cord and Mounting Screws.....	42
3-8	Fuse Block.....	43
4-1	Optics Assembly Light Path	45
4-2	Benzene Spectrum at 1.2 nm Resolution	47
4-3	Photodiodes Discharged by Light	48
4-4	Absorbance as a Function of Concentration.....	51
5-1	Comparing Spectra of Two Compounds	55
5-2	Plotting Vectors for Two Spectra	56
5-3	Spectra with a Large Spectral Contrast Angle.....	58
5-4	Spectra with a Small Spectral Contrast Angle	59
5-5	Absorbance Spectra of a Compound at Two Concentrations	60
5-6	Effects of pH and Solvent Concentration on the Absorbance Spectrum of <i>p</i> -Aminobenzoic Acid	62

List of Tables

1-1	Site Requirements	14
1-2	Event In (Inject Start) Terminal Specifications on TTL or Switch Closure.....	21
1-3	Event Out Terminal Specifications on Contact Closure	21
2-1	996 Detector Troubleshooting	28
4-1	Optics Assembly Components.....	45
A-1	996 Detector Specifications.....	63
B-1	Spare Parts.....	64
C-1	Warranty Periods.....	68
D-1	Mobile Phase Absorbance Measured Against Air or Water	70

How to Use This Guide

Purpose of This Guide

The *Waters 996 PDA Detector Operator's Guide* describes the procedures for installing, maintaining, and troubleshooting the Waters® 996 PDA Detector. It also describes detector optics and the principles of Spectral Contrast used in the Millennium³² software for analyzing the data from the PDA detector. Also included is information on vector analysis, mobile phase absorbance, specifications, and the warranty.

Audience

This guide is intended for individuals who need to install, operate, maintain, and troubleshoot the Waters 996 PDA Detector. It is also intended for users who need to understand the Spectral Contrast principles underlying the processing of PDA detector data by Millennium³² software.

Structure of This Guide

The *Waters 996 PDA Detector Operator's Guide* is divided into chapters and appendixes. Each chapter and appendix page is marked with a tab and a footer to help you quickly access information.

The following table describes the material covered in each chapter and appendix of this guide.

Chapter/Appendix	Description
Chapter 1, Installation	Describes how to install and set up the 996 detector.
Chapter 2, Diagnostics and Calibration	Describes how to troubleshoot the 996 detector.
Chapter 3, Maintenance	Describes how to replace the flow cell, the lamp, and the fuse.
Chapter 4, Principles of the 996 PDA Detector Optics	Explains the principles involved in resolving spectral data, measuring light at the photodiode, verifying wavelengths, and computing absorbance data.
Chapter 5, Spectral Contrast Theory	Describes the calculations used for Spectral Contrast.

Chapter/Appendix	Description
Appendix A, Detector Specifications	Provides the specifications of the Waters 996 PDA detector.
Appendix B, Spare Parts	Provides a list of recommended and optional spare parts.
Appendix C, Warranty Information	Includes warranty and service information.
Appendix D, Mobile Phase Absorbance	Provides a table of absorbances at several wavelengths for common mobile phases.

Related Documentation

The table below lists other guides related to the *Waters 996 PDA Detector Operator's Guide*.

Title	Description
<i>Millennium³² Online Help</i>	Describes all Millennium ³² windows, menus, menu selections, and dialog boxes. Also includes reference information and procedures for performing all tasks required to use the Millennium ³² software. Included as part of the Millennium ³² software.
<i>Millennium³² Software Getting Started Guide</i>	Provides an introduction to the Millennium ³² System. Describes the basics of how to use Millennium ³² software to acquire data, develop a processing method, review results, and print a report. Also covers basics for managing projects and configuring systems.
<i>Millennium³² PDA Software Getting Started Guide</i>	Describes the basics of how to use Millennium ³² PDA software to develop a PDA processing method and to review PDA results

Title	Description
<i>Millennium³² System Installation/ Configuration Guide</i>	Describes Millennium ³² software installation, including the stand-alone workstation, PowerStation™ system, and client/server system. Discusses how to configure the computer and chromatographic instruments as part of the Millennium ³² system. Also covers the installation, configuration, and use of the busLAC/E card and LAC/E ³² Acquisition Server.
Waters 996 PDA Detector Qualification workbook	Describes qualification procedures for the PDA detector.

Related Adobe™ Acrobat Reader Documentation

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

Printing From This Electronic Document

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

Conventions Used in This Guide

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

- **Purple Text** indicates user action. For example:
Press **0**, then press **Enter** for the remaining fields.
- *Italic* text denotes new or important words, and is also used for emphasis. For example:
An instrument method tells the software how to acquire data.

- [Underlined, Blue Color](#) 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. For example:

Before you replace the detector lamp, check the lamp usage indicator located to the front of the lamp retainer, as described in [Section 3.2.1, Checking Lamp Usage](#).

Notes, Attentions, and Cautions

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

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

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



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

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



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

1 Installation

The Waters® 996 Photodiode Array (PDA) Detector operates in any standard laboratory environment. The detector requires electrical power, sample and waste fluidic lines, and the Millennium®³² Workstation. Optional connections on the detector rear panel allow communication with chart recorders, data integrators, and other instruments that are not compatible with Millennium software control.

1.1 Installation Site Requirements

Install the Waters 996 PDA Detector ([Figure 1-1](#)) at a site that meets the specifications listed in [Table 1-1](#).

Table 1-1 Site Requirements

Factor	Specification
Ambient temperature	4 to 40° C (39 to 104° F)
Relative humidity	20 to 80 percent, noncondensing
Bench space	Width: 11.5 in. (29 cm) Depth: 24 in. (61 cm) Height: 8.125 in. (22 cm)
Bench support	Capable of supporting 31.5 pounds (14.3 kg)
Clearance	At least 4 in. (10 cm) on the back and left sides for ventilation

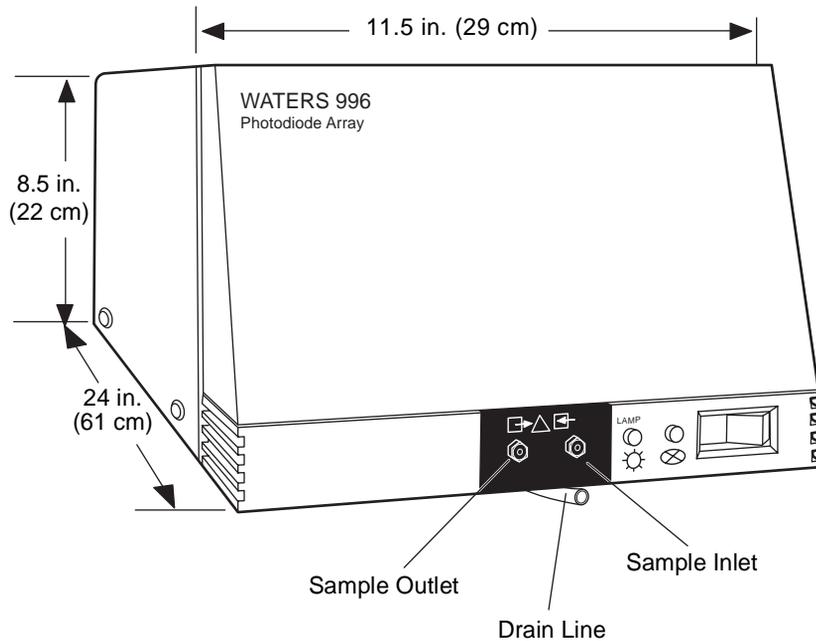


Figure 1-1 Waters 996 PDA Detector Dimensions

1.2 Power Connections

Ensure that power connections for the 996 PDA Detector are made according to the procedures that follow.

Operating Voltage

The 996 PDA Detector has a universal input power supply that requires no voltage adjustment. The electrical power requirements for the Waters 996 PDA Detector are:

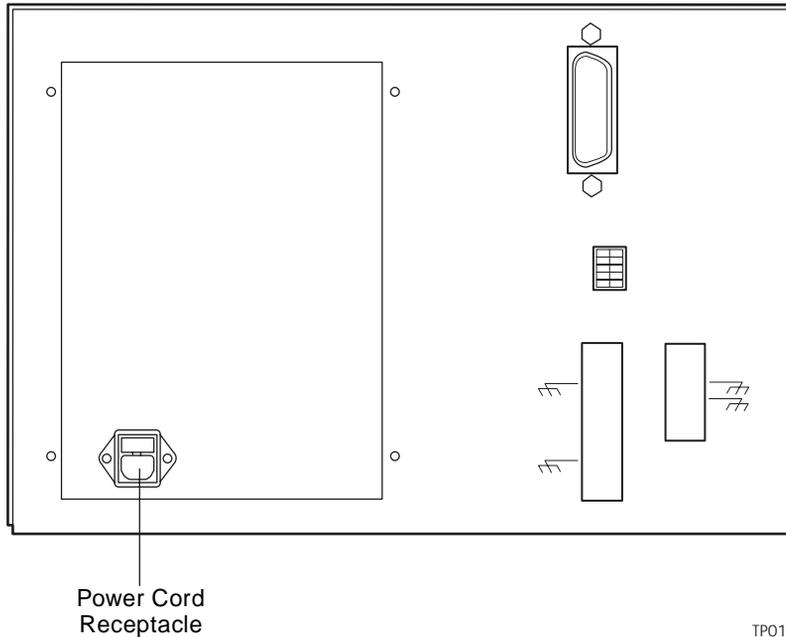
- **Voltage range:** 95 to 240 Vac ($\pm 10\%$)
- **Frequency range:** 50 to 60 Hz (± 3 Hz)

Fuses

The Waters 996 PDA Detector is shipped with fuses rated for North American operation. If you operate the Waters 996 PDA Detector in another location, install the IEC-rated fuses (supplied in the Waters 996 Detector Startup Kit) in the fuse holder in the rear of the detector (refer to [Section 3.3, Replacing the Fuses](#)).

Connecting the Power Cord

Connect one end of the 996 detector power cord to the rear panel power receptacle ([Figure 1-2](#)) and the other end to a power outlet.



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Figure 1-2 Detector Rear Panel

1.3 Millennium³² Workstation Connections

The 996 detector requires signal connections to the Millennium³² Workstation over the IEEE-488 bus. All detector control and data acquisition communications take place over the IEEE-488 bus.

Note: If an inject start signal is not available over the IEEE-488 bus, you must provide a signal at the Event In terminals on the 996 detector rear panel (see [Section 1.4.2, Connecting Event Cables](#)).

1.3.1 Connecting the IEEE-488 Cable

Note: If the 996 detector is to be rack-mounted or stacked on other instruments, use the right-angle adaptor included in the Startup Kit when you make the IEEE-488 connection.

To connect the 996 detector to a Millennium³² Workstation:

1. Connect one end of the IEEE-488 cable to the IEEE-488 receptacle on the rear panel of the 996 detector. Connect the other end of the cable (stackable connector for daisy-chaining additional instruments) to the IEEE-488 connector on any of the other instruments in your chromatographic system ([Figure 1-3](#)).

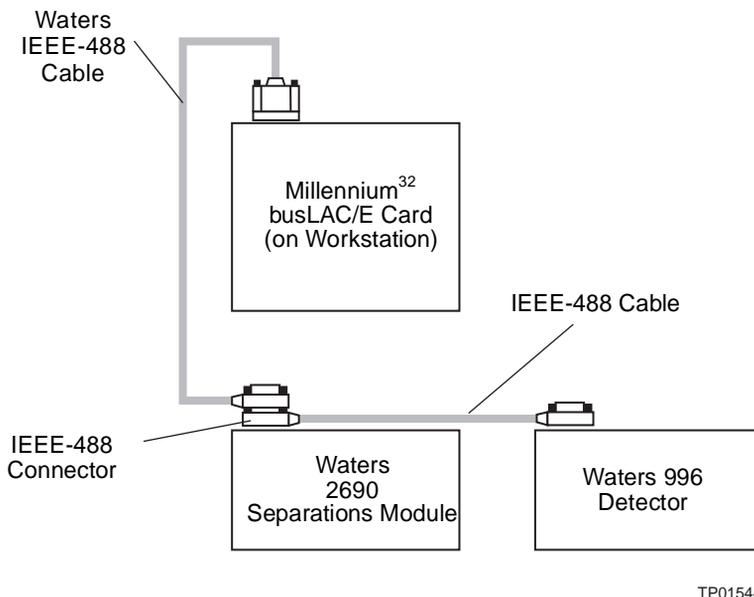


Figure 1-3 Example of IEEE-488 Cable Connections

Note: The order in which you connect IEEE-488 devices to the busLAC/E card on the workstation is not important. For example, you can connect the 2690 separations module before or after the 996 detector.

2. Use another IEEE-488 cable to connect to the stackable connector on the first instrument and the IEEE-488 connector on another instrument.
3. Repeat step 2 for each IEEE-488 instrument in your chromatographic system, up to a maximum of 14 IEEE-488 instruments.

Note: Keep in mind cable-length limitations when you set up your system. For a list of IEEE-488 interface guidelines, refer to the Millennium³² System Installation/Configuration Guide, Section 2.3.1, Connecting IEEE-488 Devices.

4. Ensure that all IEEE-488 cable screws are fastened finger-tight.

1.3.2 Setting the IEEE-488 Address

To set the IEEE-488 address for the 996 detector:

1. Use a small screwdriver (or similar device) to set the DIP switches on the detector rear panel ([Figure 1-4](#)) to the IEEE-488 address of the 996 detector. The address must be a number from 2 to 29 and must be unique within your network.

Refer to the *Millennium³² System Installation/Configuration Guide*, Section 2.3.1, Connecting IEEE-488 Devices, for the correct IEEE-488 DIP switch settings.

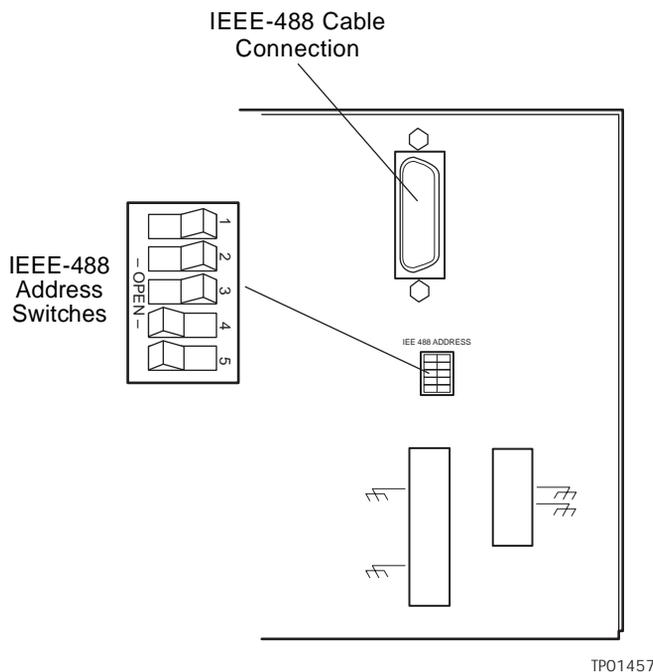


Figure 1-4 Locating the IEEE-488 Address Switches

2. To instruct the 996 detector to accept the new IEEE-488 address, power off, then power on the detector (see [Section 1.6, Startup/Shutdown](#)).

1.4 Non-IEEE-488 Communication Connections

Non-IEEE-488 communication connections on the 996 detector include:

- **Analog Outputs** – The 996 detector provides two, unattenuated, 1 volt-per absorbance-unit analog output channels to integrators, chart recorders, or other components.
- **Event Inputs and Outputs** – The 996 detector sends and receives contact closure signals to and from other instruments.

You make all non-IEEE-488 communication cable connections to the 996 detector at the rear panel (see [Figure 1-2](#)).



Attention: *To meet the regulatory requirements of immunity from external electrical disturbances that may affect the performance of this instrument, do not use cables longer than 9.8 feet (3 meters) when you make connections to the screw-type barrier terminal strips. In addition, ensure that you always connect the shield of each cable to chassis ground at one instrument only.*

1.4.1 Connecting Analog Output Cables

The values of the analog output signals generated by the 996 detector are specified by parameter values set from the Millennium³² Workstation. For details, refer to the “Waters 996 Detector Properties” topic in the Millennium³² Online Help Find tab.

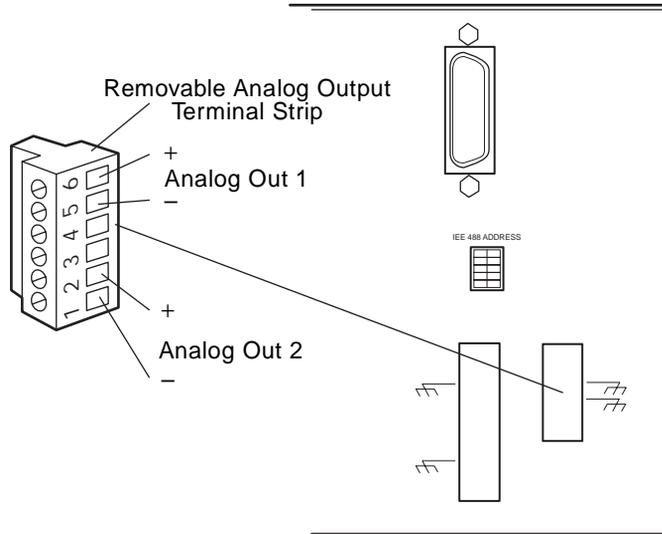
Required Materials

- One small, flat-blade screwdriver
- One electrical insulation stripping tool
- Analog signal cables (from Startup Kit)

Procedure

To connect the Waters 996 detector to a device that receives analog output signals from the 996 detector

1. Pull off the Analog output terminal strip from the 996 detector rear panel ([Figure 1-5](#)). This step simplifies the following steps.



TPO1456

Figure 1-5 Analog Output Terminals

2. Insert the bare wires at one end of an analog signal cable into the positive (+) and negative (–) terminals of Analog Out 1 (see [Figure 1-5](#)). Tighten the two screws to secure the + and – wires.
3. Connect the other end of the analog signal cable to the appropriate analog input terminal on the external device, being sure to maintain negative-to-negative and positive-to-positive continuity.
4. Reinstall the Analog Output strip.

1.4.2 Connecting Event Cables

The 996 detector has four terminal strip connections for contact closure signals:

- Two input (inject start) signal terminals
- Two output (programmable event table) signal terminals

If an inject start signal is not available over the IEEE-488 bus, you must provide a signal at an Event In terminal on the 996 detector rear panel. Manual injectors such as the Rheodyne 7725i provide a cable that connects the injector to an Event In terminal on the 996 detector rear panel.

The values of the event output signals generated by the 996 detector are specified by parameter values set from the Millennium³² Workstation. For details, refer to the “Waters 996 Detector Properties” topic in the *Millennium³² Online Help* Find tab.

Electrical Specifications

Before you connect an external device to an event input or output terminal, refer to the electrical specifications in [Table 1-2](#) and [Table 1-3](#).

Table 1-2 Event In (Inject Start) Terminal Specifications on TTL or Switch Closure

Parameter	Specification
Low trigger	<1.8 V
High trigger	>3.0 V
Protected to	±30 V
Minimum pulse width	30 msec
Maximum current	5 mA

Table 1-3 Event Out Terminal Specifications on Contact Closure

Parameter	Specification
Maximum power	10 W
Maximum current	0.5 A at 20 V
Maximum voltage	24 V RMS



Attention: To avoid damage to the 996 detector electronics, be sure you make the proper electrical connections as outlined in this section.

Required Materials

- Small flat-blade screwdriver
- One electrical insulation stripping tool
- Event signal cables (in Startup Kit)

Making Event Input/Output Connections

To connect the 996 detector to an external event input or output device:

1. Pull off the Event Input/Output terminal strip from the rear panel ([Figure 1-6](#)). This simplifies the following steps.

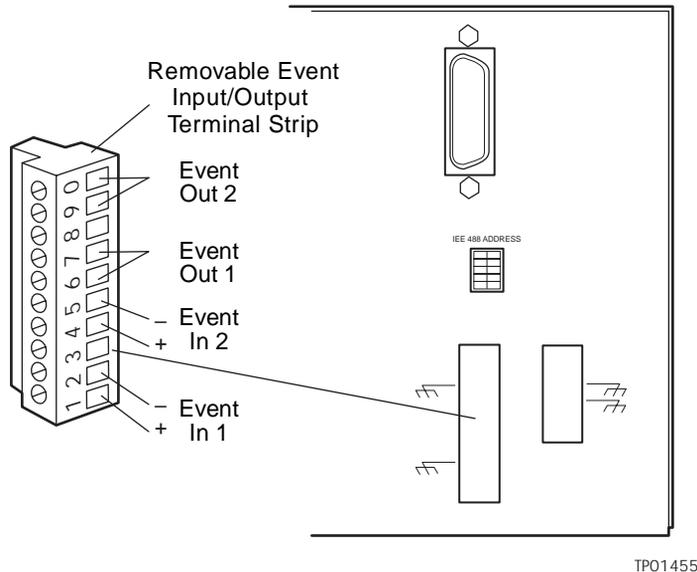


Figure 1-6 Event Input/Output Terminal Strip

2. Insert the bare wires at one end of the event signal cable into the positive (+) and negative (-) slots of the appropriate event input or output terminal (see [Figure 1-6](#)). Tighten the two screws to secure the + and - wires.
3. Connect the other end of the event signal cable to the appropriate event input or event output terminal on the external device.
4. Reinstall the Event Input/Output strip.

1.5 Fluidic Connections



Caution: To avoid chemical hazards, always observe safe laboratory practices when handling solvents. Refer to the Material Safety Data Sheets for the solvents in use.

Required Materials

- 5/16-inch open-end wrench
- 0.009-inch (0.23 mm) I.D. stainless steel tubing (in Startup Kit)
- Stainless steel tubing cutter or scribing file
- Pliers, plastic-covered, or with cloth
- Compression screw assemblies, three

Procedure

To make fluidic connections to the 996 detector:

1. Measure the lengths of tubing needed to connect:

- The column outlet to the 996 detector inlet.

Note: *Be sure that you keep the length of this tubing as short as possible to prevent band broadening.*

- The 996 detector outlet to a waste collection bottle.

Note: *Ensure the length of this tubing is at least 1 to 2 feet (30 to 60 cm) to prevent air bubbles from forming in the flow cell.*

2. Cut the two lengths of tubing as follows:

- Use a Waters 1/16-inch stainless steel tubing cutter or a file with a cutting edge to scribe the circumference of the tubing at the desired break point.
- Grasp the tubing on both sides of the scribed mark with cloth- or plastic-covered pliers (to prevent marring the surface), then gently work the tubing back and forth until it separates.
- File the tubing ends smooth and straight to minimize dead volume and band broadening.

3. Assemble a compression fitting (as shown in [Figure 1-7](#)) at both ends of the column outlet line and at one end of the detector outlet line.

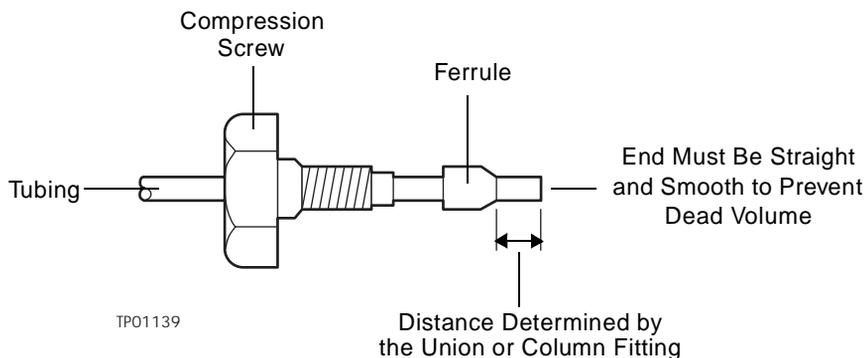


Figure 1-7 Compression Screw Assembly

4. Bottom one end of the column outlet tubing in the fitting seat of the column outlet, then tighten the compression screw about 3/4-turn past finger-tight (using the 5/16-inch open-end wrench).
5. Bottom the other end of the tubing in the fitting seat of the detector inlet, then tighten the compression screw as in step 4.
6. Bottom one end of the detector outlet tubing with the compression fitting in the fitting seat of the detector outlet, then tighten the compression screw about 3/4-turn past finger-tight. Insert the other end of the tubing in the waste container.



Attention: To avoid damage to the flow cell, avoid pressures approaching the maximum pressure for the analytical flow cell, 1000 psi (70 kg/cm²).

1.6 Startup/Shutdown

Follow the procedures in this section to ensure reliable detector performance.

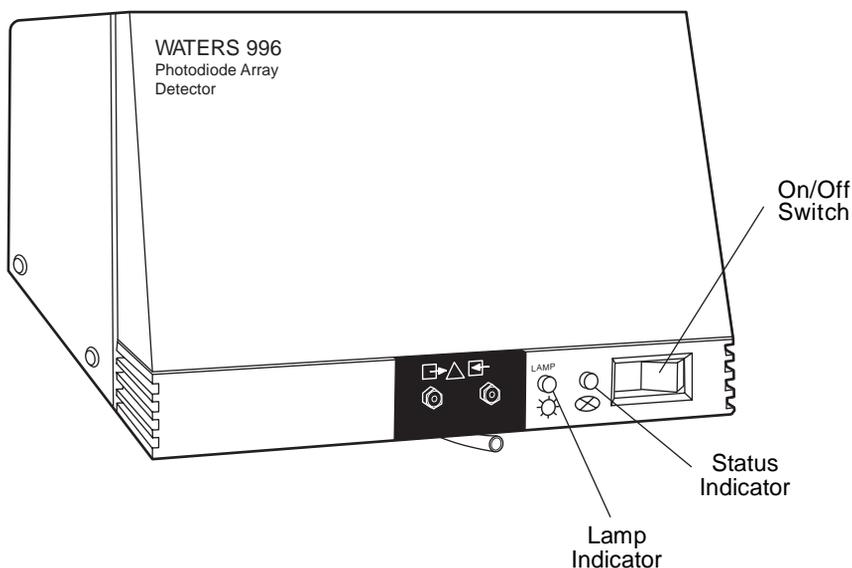
Startup

To start up the 996 detector:

1. In your instrument method, set the solvent delivery system or pump to deliver 1 mL/min of degassed mobile phase. For details, refer to the “Waters 2690 Separations Module Properties” or the “Waters 600 Properties” topic in the *Millennium*³² *Online Help* Find tab.

Note: Use only thoroughly degassed HPLC-grade solvents. Gas in the mobile phase may form bubbles in the flow cell and cause the detector to fail the Reference Energy diagnostic.

2. Flush the detector for 10 minutes or until no bubbles appear in the outlet line.
3. Press the 0/1 (Off/On) switch on the front panel of the detector ([Figure 1-8](#)) to the 1 (On) position.
4. Observe the Lamp and Status indicator lights on the front panel of the detector ([Figure 1-8](#)).
 - If both lights remain illuminated, the detector passed the internal diagnostics.
 - If either indicator light blinks or is off, refer to the troubleshooting tables in [Chapter 2, Diagnostics and Calibration](#).



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Figure 1-8 996 Detector Indicator Lights

5. Wait 1 hour for the deuterium lamp to stabilize before you attempt to acquire data at low absorbances.

Shutdown

To shut down the 996 detector:

1. If the mobile phase contains buffers, set the solvent delivery system or pump to deliver 1 mL/min of HPLC-grade water for 10 minutes. Otherwise, set the solvent delivery system or pump to deliver 1 mL/min of degassed methanol for 10 minutes.
2. Press the 0/1 (Off/On) Switch on the front panel of the detector to the 0 (Off) position.

2

Diagnostics and Calibration

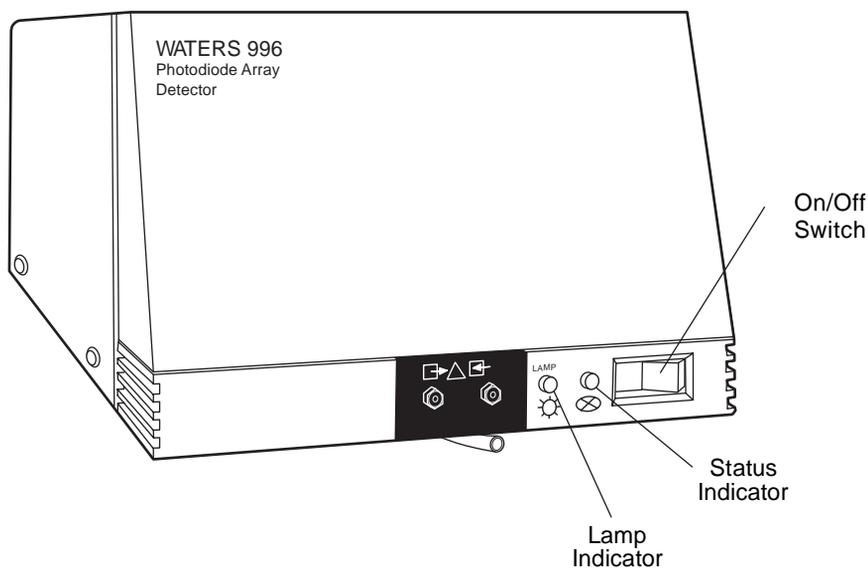
The Waters 996 Photodiode Array Detector automatically runs a series of internal diagnostics upon start up. The indicator lights on the front of the detector and messages at the Millennium³² Workstation show the results of the start up internal diagnostics ([Figure 2-1](#)).

If you need to determine the cause of a problem during operation of the detector, you can run the same internal diagnostics from the Millennium³² Workstation. Additional information about the performance of the detector is also available through the PDA Calibration window, accessed from QuickSet in the Millennium³² software.

If you encounter a problem that you cannot troubleshoot (see [Section 2.1, Startup Diagnostics](#)), contact Waters Technical Service at (800) 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

2.1 Startup Diagnostics

Refer to [Table 2-1](#) to troubleshoot problems encountered during startup diagnostics and during detector operation.



TP01460

Figure 2-1 996 PDA Detector Indicator Lights

Table 2-1 996 Detector Troubleshooting

Symptom	Possible Cause	Corrective Action
Status light off	No power.	1. Check line cord connections. 2. Check outlet for power.
	Blown fuse.	Replace fuse (see Section 3.3, Replacing the Fuses).
	Detector is still performing diagnostics	Wait for diagnostics to end.
Status light blinks and lamp light off	Failed startup diagnostics.	1. Check that lamp door is secure. 2. Replace lamp. If replacing the lamp fails to correct the problem, contact Waters Technical Service.

Table 2-1 996 Detector Troubleshooting (Continued)

Symptom	Possible Cause	Corrective Action
Status light blinks and lamp light on	Failed startup diagnostics. Insufficient energy reaching photodiode array because of air bubble or dirty flow cell can cause shutter diagnostic to fail.	Flush the flow cell (see Section 3.1.1, Flushing the Flow Cell). Flush the flow cell (see Section 3.1.1, Flushing the Flow Cell). To prevent air bubbles from forming, check that there is a 1- to 2-foot (30- to 60-cm) length of 0.009-inch (0.23-mm) I.D. tubing connected to the detector waste outlet.
	Weak lamp.	Replace lamp (see Section 3.2, Replacing the Lamp).
Shutter failure message.	Shutter failure.	Run the Shutter diagnostic. For details, refer to the “PDA Diagnostics Window” topic in the <i>Millennium³² Online Help</i> Find tab.
Detector not responding to Millennium ³² Workstation	Detector not connected to busLAC/E or to LAC/E ³² acquisition server in the Millennium ³² Workstation	Check IEEE-488 cable connections, tighten connectors.
	Incorrect IEEE-488 address.	<ol style="list-style-type: none"> 1. Ensure that the 996 detector IEEE-488 address is unique and within the range 2 to 29 (see the <i>Millennium³² System Installation/Configuration Guide</i>). 2. Rescan the IEEE-488 bus. For details, see the “Scanning the bus/LAC/E Card for Serial Instruments” topic in the <i>Millennium³² Online Help</i> Find tab.

Table 2-1 996 Detector Troubleshooting (Continued)

Symptom	Possible Cause	Corrective Action
Change in reference spectrum	Mobile phase contains gas or is contaminated.	Prepare fresh mobile phase and degas thoroughly.
	Air bubbles trapped in flow cell.	Flush the flow cell, or apply slight backpressure on the detector waste outlet. To prevent air bubbles, check that there is a 1- to 2-foot (30- to 60-cm) length of 0.009-inch (0.23-mm) I.D. tubing connected to the detector waste outlet.
Solvent in drain line	Leak from flow cell gasket.	Rebuild flow cell with a new gasket (see Section 3.1.3, Disassembling and Cleaning the Flow Cell).
	Leak from flow cell fittings.	Check fittings for overtightening or undertightening, and replace fittings if necessary.

2.2 User-Initiated Diagnostics

There are two types of user-initiated PDA diagnostic tests:

- **Internal Tests** – Tests run by the instrument firmware that help you determine the source of a malfunction. These tests do not require connections to external devices.
- **Interactive Tests** – Tests that check analog output and event input/output signal communications between the detector and connected external devices. These tests require connections to pump flow and/or test equipment.

Note: You cannot perform diagnostics on a 996 detector while it is acquiring data.

Note: The system administrator can restrict access to the 996 detector diagnostics by disabling user access to Quick Set. For details, refer to the “User Type Properties Dialog Box” topic in the Millennium³² Online Help Find tab.

You can run all user-initiated diagnostics from QuickSet in the Millennium³² software. For more information on QuickSet and PDA diagnostics, refer to the “PDA Diagnostics Window” topic in the *Millennium³² Online Help* Find tab.

If you encounter a problem that you cannot troubleshoot (see [Section 2.1, Startup Diagnostics](#)), contact Waters Technical Service at (800) 252-4752, *U.S. and Canadian customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters for assistance at 1-508-478-2000 (U.S.).

2.3 PDA Calibration

You can adjust, or calibrate, the 996 detector to ensure that wavelength readings are accurate. Recalibrate the 996 detector *only* if the Wavelength Accuracy diagnostic (in the Internal Diagnostics tests) fails.

You calibrate the 996 detector using the PDA Calibration window, which you access from QuickSet and which allows you to:

- View the effects of exposure time on photodiode saturation for a given wavelength range.
- Verify the wavelength location of the deuterium spectrum Balmer lines (486.0 nm and 656.1 nm).
- Recalibrate to set the 486-nm peak at the proper wavelength.
- Ensure precise data for library matching.

Note: *The system administrator can restrict access to the PDA Calibration window. Recalibrating the wavelength requires that spectral libraries be reentered.*

Required Materials

- HPLC-grade methanol
- HPLC-grade water

Preparing for Calibration

Note: *Ensure that the flow cell is clean before you check calibration. (See [Section 3.1.1, Flushing the Flow Cell](#).)*

To prepare for calibration:

1. Set the pump to deliver 1 mL/min of degassed methanol for 10 minutes. If methanol is not miscible with the previous solvent, flush with a miscible solvent before switching to methanol.
2. If you have been using buffers, flush with HPLC-quality water at 1 mL/min for 10 minutes, then switch to methanol for 10 minutes.

Note: *Ensure that the solvent is miscible with the previous mobile phase.*

For information on performing calibration, refer to the “PDA Calibration Window” topic in the *Millennium³² Online Help* Find tab.

3

Maintenance

This chapter covers maintenance of the Waters 996 Photodiode Array Detector flow cell, lamp, and fuse.



Caution: To avoid the possibility of electric shock, do not remove the 996 detector power supply covers. The power supply does not contain user-serviceable components.

3.1 Flow Cell Maintenance

The flow cell requires maintenance when:

- The reference spectrum changes.
- The Lamp diagnostic (in the Millennium³² PDA Diagnostics window) fails, and the lamp status light is on (see [Table 2-1](#)).
- The 996 detector causes high backpressure.

Note: Conditions other than a dirty flow cell may cause decreased lamp intensity. For more information, refer to [Chapter 2, Diagnostics and Calibration](#).

Flow cell maintenance consists of:

- Flushing the flow cell
- Removing the flow cell
- Cleaning the flow cell
- Installing the flow cell assembly

3.1.1 Flushing the Flow Cell

Required Materials

- HPLC grade water
- HPLC grade methanol

If the flow cell requires cleaning, the first cleaning method to try is flushing the flow cell with solvent.

To flush the flow cell:

1. Select a solvent compatible with the samples and mobile phases that you have been using. If you have been using buffers, flush with HPLC-grade water for 10 minutes at 1 mL/min, then switch to a low-surface-tension solvent such as methanol.



Attention: *Ensure that the solvent is miscible with the previous mobile phase.*

2. Set pump flow to **1** mL/min, then run the pump 10 minutes.
3. Test the lamp energy by performing the Lamp diagnostic test. For details, refer to the “PDA Diagnostics Window” topic in the *Millennium³² Online Help* Find tab.

If the lamp diagnostic fails and the lamp has not been used more than 1000 hours, disassemble the flow cell and clean the flow cell components using the procedure described in [Section 3.1.2, Removing the Flow Cell](#).

3.1.2 Removing the Flow Cell

Note: *You do not need to shut down the 996 detector to remove and replace the flow cell.*

Required Materials

- 5/16-inch open-end wrench
- Phillips screwdriver

Procedure

To remove the 996 detector flow cell:

1. Set the flow to **0.0** mL/min.
2. Power off the solvent delivery system or pump.



Caution: *To avoid the possibility of leaking mobile phase, do not disconnect the inlet or outlet fluidic lines while there is pressure in the chromatographic system. Always vent your system before disconnecting fluidic lines.*

3. Use the 5/16-inch wrench to disconnect the fluidic lines at the front of the detector.
4. Lift up the 996 detector front cover and pull the front cover from the detector chassis.
5. Open the flow cell access door by pulling the black thumbtab, then pull the door gently toward you ([Figure 3-1](#)).

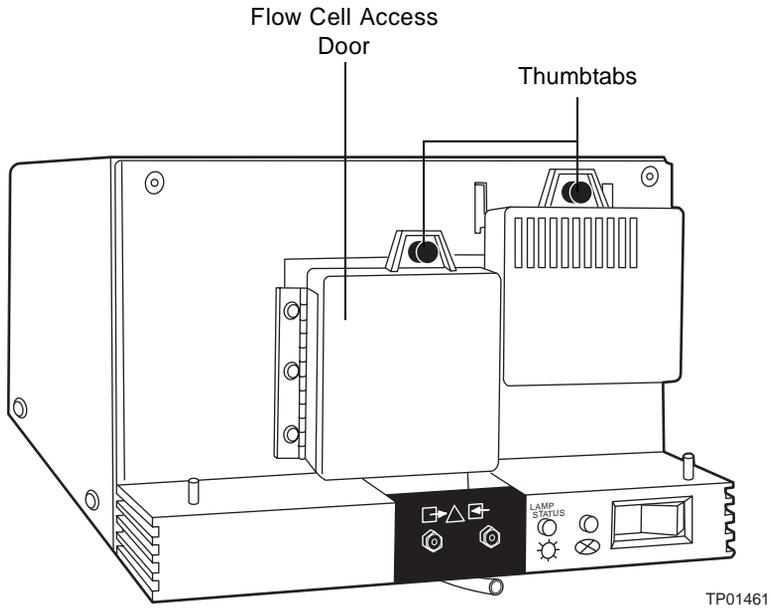


Figure 3-1 Flow Cell Access Door

6. Use the Phillips screwdriver to loosen the two thumbscrews that hold the flow cell assembly to the optics bench and the thumbscrew that secures the bracket holding the fluidic connections, then detach the bracket ([Figure 3-2](#)).

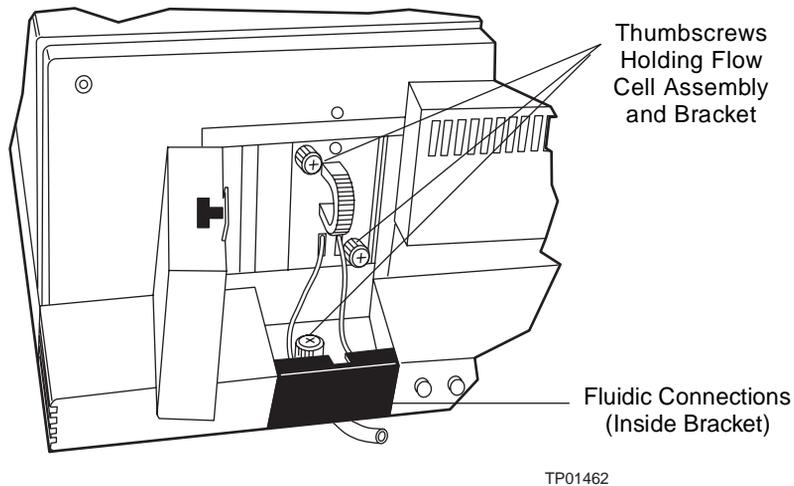


Figure 3-2 Removing the Flow Cell Assembly

7. Pull the flow cell assembly and fluidic connection bracket gently toward you to remove it from the detector ([Figure 3-3](#)).

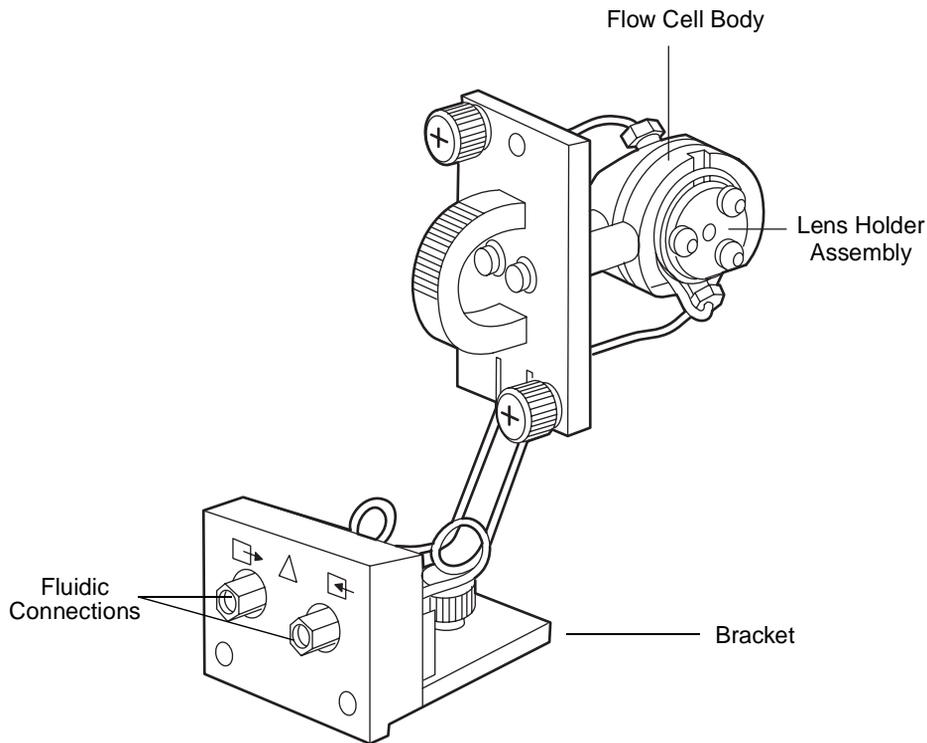


Figure 3-3 Flow Cell and Fluidic Connections Assemblies

3.1.3 Disassembling and Cleaning the Flow Cell

The lens surface finish and the alignment of the lenses are critical to the performance of the 996 detector. Be careful not to touch or damage the lenses and the lens holders.



Attention: To prevent contamination, use powder-free gloves when disassembling, inspecting, cleaning, or replacing parts within the flow cell or when removing or replacing the flow cell within its assembly.



Attention: To prevent contamination, use powder-free gloves when disassembling, inspecting, cleaning, or replacing parts within the flow cell or when removing or replacing the flow cell within its subassembly.

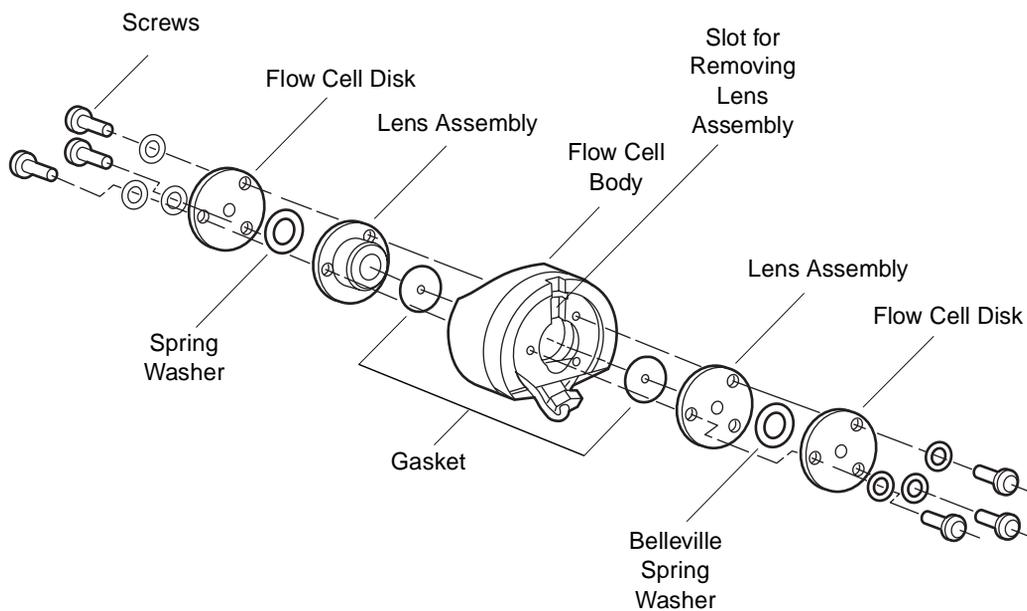
Required Materials

- TORX T10 screwdriver
- Small, flat-blade screwdriver
- Lens tissue or nonparticulating swab
- HPLC-grade methanol
- Belleville spring washer
- Flow cell gasket

Procedure

To disassemble and clean the flow cell (and lenses):

1. Use the TORX T10 screwdriver to remove the three screws that secure one of the lens holder assemblies ([Figure 3-4](#)).



TP01464

Figure 3-4 Disassembling the Flow Cell

2. Use the small, flat-blade screwdriver to gently pry the lens assembly from the flow cell body at the slots.



Attention: Solvents other than methanol may damage a disassembled flow cell. In normal use, the gasket protects the lens holder from solvents.

3. Use a lens tissue or a nonparticulating swab to wipe the lens with methanol.
4. Remove and discard the gasket.
5. Repeat steps 1 through 4 to remove, disassemble, and clean the other lens holder assembly.
6. Use methanol and a nonparticulating swab to clean the flow cell body.

Reassembling the Flow Cell

To reassemble the flow cell (see [Figure 3-4](#)):

1. Insert a replacement gasket into one side of the flow cell body.
2. Align the screw holes of the lens assembly with the holes in the flow cell body.
3. Place the new Belleville spring washers (with the concave side facing out) onto the lens assembly.
4. Place the flow cell disk over the lens assembly.
5. Insert the three screws using the TORX T10 driver to gradually tighten each screw, alternating between the screws in a clockwise pattern. Tighten until the screws meet the flow cell disk, then tighten each screw 1/4-turn. If a torque screwdriver is available, tighten the screws to 16 in-oz (0.113 N-m).



Attention: Be careful not to overtighten the screws.

6. Repeat steps 1 through 5 to reassemble the other side of the flow cell.

3.1.4 Installing the Flow Cell Assembly



Attention: The alignment of the flow cell in the optics bench is critical to detector operation. Be careful not to damage the flow cell body.

To install the flow cell assembly:

1. While you hold the flow cell assembly in a vertical orientation (see [Figure 3-3](#)), insert the assembly into the optics bench. Note that the flow cell is self-aligning and uses the guide pins on the optics bench.
2. Gently push the front of the assembly until it seats on the front alignment pins.
3. Hand-tighten the thumbscrews.
4. Secure the fluidic connection bracket.
5. Reconnect the fluidic lines.

6. Replace the front cover.
7. Flush the flow cell (refer to [Section 3.1.1, Flushing the Flow Cell](#)).

3.2 Replacing the Lamp

Replace the lamp in the 996 detector when either of the following conditions exists:

- Intensity is low enough that sensitivity is not sufficient for your method.
- The sampling rate requires an exposure time shorter than the minimum exposure time you can set with the current lamp.

Before you replace the detector lamp, check the lamp usage indicator located to the front of the lamp retainer, as described in [Section 3.2.1, Checking Lamp Usage](#).

3.2.1 Checking Lamp Usage

The Waters 996 detector lamp is designed to provide adequate energy for more than 1000 hours of operation. You can monitor lamp usage by checking the lamp usage indicator, a mercury column with a scale of 0 to 10, where 10 represents 1000 hours. As the lamp ages, the bubble in the mercury column moves toward the 10.



Caution: To avoid electrical hazards and exposure to UV light, turn off the power and disconnect the power cord before you begin this procedure. Note that the lamp and housing are extremely hot. To avoid the possibility of contacting hot surfaces, allow the lamp to cool for 15 minutes before you handle the lamp assembly or surfaces close to the lamp.

Procedure

To inspect the lamp usage indicator:

1. Power off the 996 detector, remove the power cord, and allow the lamp to cool for at least 15 minutes.
2. Lift up the front panel cover and pull it away from the chassis.
3. Open the lamp access door by pulling the thumbtab, then pulling the door toward you ([Figure 3-5](#)).

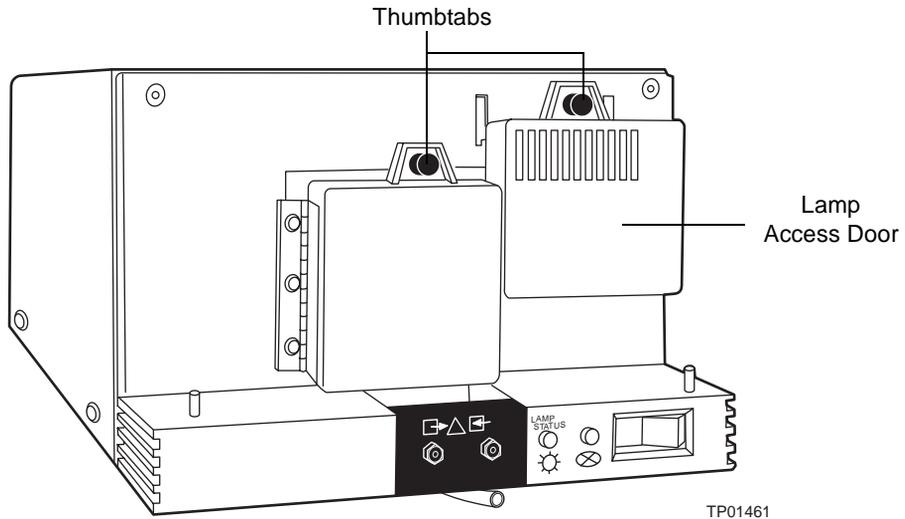


Figure 3-5 Lamp Access Door

4. Examine the lamp usage indicator ([Figure 3-6](#)).

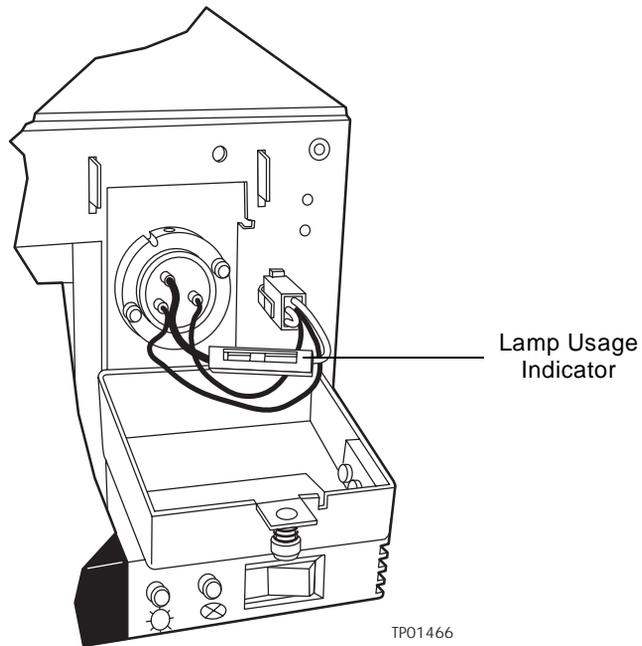


Figure 3-6 Lamp Usage Indicator

Note: If lamp intensity is low, but the lamp has not been used for 1000 hours, you may be able to increase lamp intensity by cleaning the flow cell (see [Section 3.1, Flow Cell Maintenance](#)).

Absorbance by the mobile phase also affects the apparent lamp intensity. For example, acetonitrile is more transparent than methanol at wavelengths under 220 nm.

3.2.2 Removing the Lamp



Attention: Do not touch the lamp glass while unpacking or inserting the lamp. Touching lamp glass damages the lamp and reduces life expectancy.



Attention: To prevent contamination, use powder-free gloves when removing or replacing the lamp.

Required Materials

TORX T20 screwdriver



Caution: To avoid electrical hazards when you perform the following procedure, power off the 996 detector and disconnect the power cord.

Procedure

To replace the lamp in the 996 detector:

1. Power off the 996 detector, remove the power cord, and allow the lamp to cool for at least 15 minutes.



Caution: To avoid the possibility of contacting hot surfaces, wait at least 15 minutes after powering off the detector before you handle the lamp.

2. Lift up the front panel cover and pull it away from the chassis.
3. Open the lamp access door by pulling the thumbtab, then pull the door toward you (see [Figure 3-5](#)).
4. Disconnect the lamp power connector ([Figure 3-7](#)).

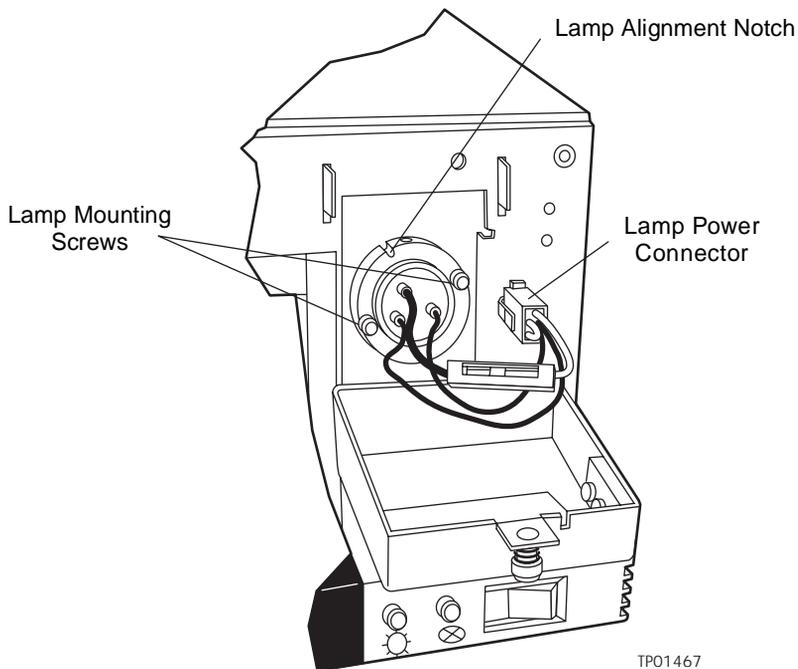


Figure 3-7 Lamp Power Cord and Mounting Screws

5. Use the TORX T20 screwdriver to unscrew the two T20 lamp mounting screws.
6. Grip the metal base of the lamp, pull the lamp out, and set it aside.
7. Carefully unpack the replacement lamp.
8. While wearing powder-free gloves and holding the lamp by its base, orient the lamp so that the notch in the base aligns with the positioning pin in the optics bench.
9. Insert the lamp and secure it with the two T20 screws. Make sure that the lamp base is flush against the lamp housing.
10. Reconnect the lamp power connector (see [Figure 3-7](#)).
11. Close the lamp door and secure it with the thumbtab.
12. Install the front panel cover.
13. Reconnect the power cord and power on the 996 detector.

3.3 Replacing the Fuses

Replace the fuses under the conditions indicated in the troubleshooting table (see [Section 2.1, Startup Diagnostics](#)). The 996 detector requires two 4 A, 250 V fuses (5 mm × 20 mm).



Caution: To avoid electrical hazards, power off the 996 detector and disconnect the power cord before you perform the following procedure.

Procedure

To replace the two fuses in the 996 detector:

1. Power off the 996 detector and remove the power cord.
2. Locate the fuse block above the power cord plug ([Figure 3-8](#)) on the rear panel.

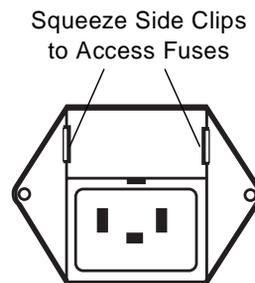


Figure 3-8 Fuse Block

3. Squeeze the two side clips on the fuse block while you pull out the block.
4. Remove the fuses from the block, then install the new fuses.
5. Orient the fuse block with the small tab pointing down, then push in the block until the side clips engage.
6. Connect the power cord, then power on the 996 detector.

4

Principles of the 996 PDA Detector Optics

To use the Millennium³² PDA software effectively, you must be familiar with the principles of operation of the optics and electronics of the Waters 996 PDA Detector.

4.1 996 Detector Optics

The 996 detector is an ultraviolet/visible light (UV/Vis) spectrophotometer with:

- 512 diodes
- Optical resolution of 1.2 nm per diode
- Operating wavelength range from 190 nm to 800 nm

The light path through the optics assembly of the 996 detector is shown in [Figure 4-1](#).

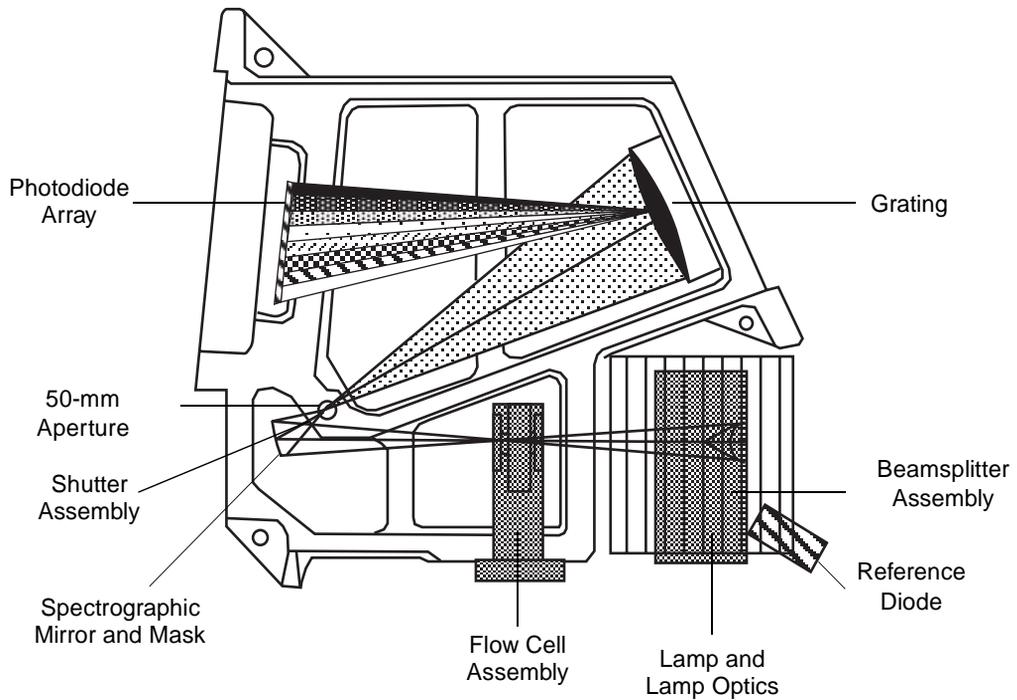


Figure 4-1 Optics Assembly Light Path

[Table 4-1](#) describes the optics assembly components in the 996 detector.

Table 4-1 Optics Assembly Components

Component	Function
Lamp and lamp optics	Focuses light from the deuterium source lamp through a beamsplitter to the flow cell.
Beamsplitter and reference diode	Reflects part of the light back to a reference diode, which measures the intensity of the light emitted by the lamp. The detector uses this measurement to keep the lamp output constant.

Table 4-1 Optics Assembly Components (Continued)

Component	Function
Flow cell assembly	Houses the segment of the flow path (containing eluent and sample) through which the polychromatic light beam passes. This arrangement of optical components, with the flow cell positioned between the lamp and the grating, is commonly called reversed optics.
Spectrograph mirror and mask	The mirror focuses light transmitted through the flow cell onto the aperture at the entrance to the spectrographic portion of the optics. The mirror mask defines the beam of light focused on the spectrograph mirror.
Aperture	Controls wavelength resolution and intensity of light striking the photodiodes. The width of the aperture is 50 μm .
Shutter assembly	Prevents light from reaching the photodiode array except during sampling and calibration. For details on the dark current, see Section 4.4.1, Calculating Absorbance .
Grating	Disperses the light into bands of wavelengths and focuses those wavelength bands onto the plane of the photodiode array.
Second-order filter	Reduces the contribution of second-order reflection of UV light (less than 350 nm) to the light intensity observed at visible wavelengths (greater than 350 nm).
Photodiode array	An array of 512 diodes arranged linearly. The diode width and spacing provide a single wavelength resolution of 1.2 nm.

4.2 Resolving Spectral Data

The ability to distinguish similar spectra depends on photodiode spacing and the bandwidth of the light striking the photodiode. The bandwidth of the light striking the photodiodes depends on the aperture width.

The aperture width determines:

- Attainable wavelength bandwidth at the photodiode array
- Intensity of the light reaching the photodiode array (optical throughput)

The aperture creates a narrow beam that reflects from the grating to the photodiode array. The wavelength that strikes a particular diode depends on the angle of reflection from the grating.

[Figure 4-2](#) shows an absorbance spectrum of benzene obtained from the 996 detector using the standard 50- μm aperture. In this spectrum, the wavelength resolution is sufficient to resolve five principal benzene absorption peaks.

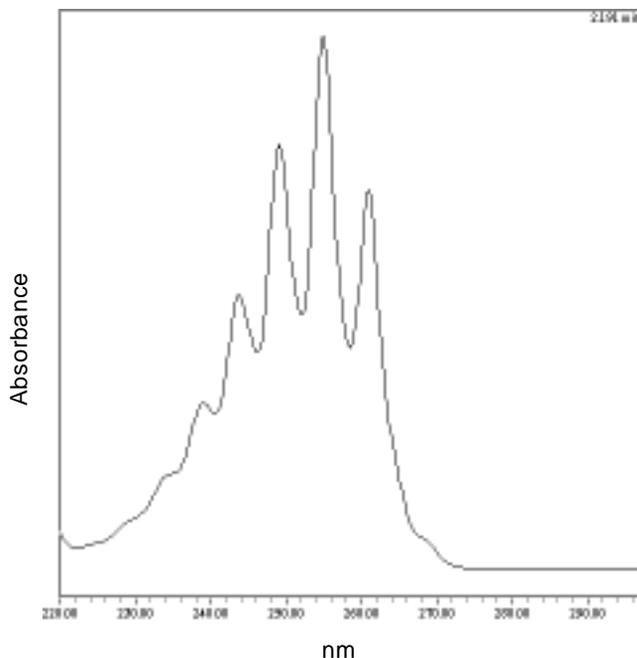


Figure 4-2 Benzene Spectrum at 1.2 nm Resolution

4.3 Measuring Light at the Photodiode

The Waters 996 Photodiode Array Detector measures the amount of light striking the photodiode array to determine the absorbance of the sample in the flow cell.

The array consists of 512 photodiodes arranged in a row. Each photodiode acts as a capacitor by holding a fixed amount of charge.

Light striking a photodiode discharges the diode ([Figure 4-3](#)). The magnitude of the discharge depends on the amount of light striking the photodiode.

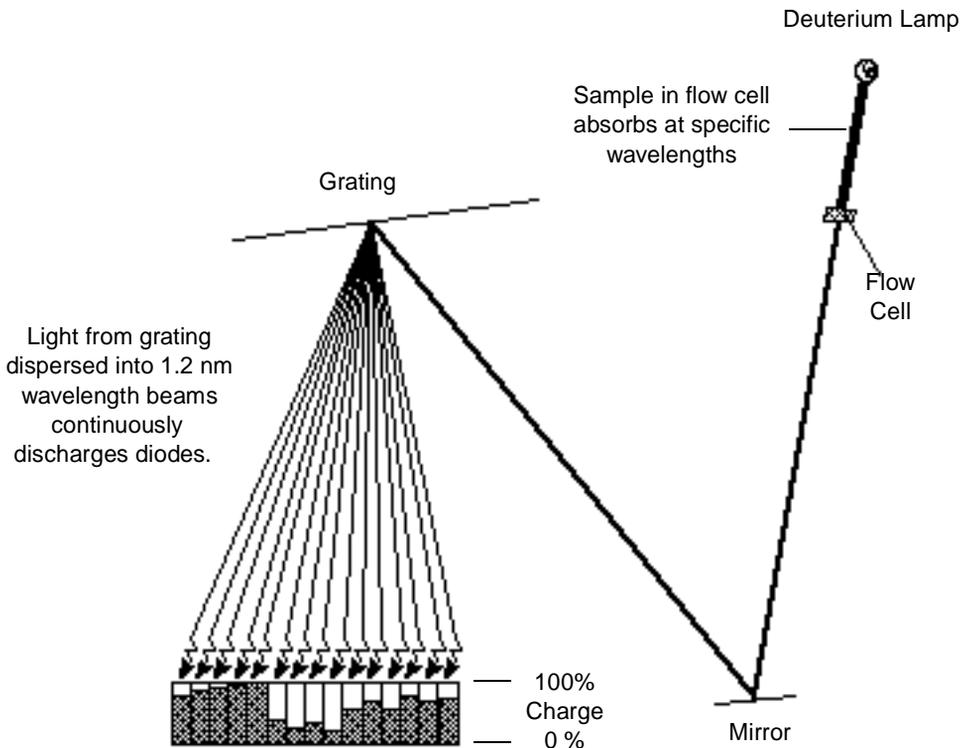


Figure 4-3 Photodiodes Discharged by Light

The 996 detector measures the amount of current required to recharge each photodiode. The current is proportional to the amount of light transmitted through the flow cell over the interval specified by the diode exposure time.

Exposure Time

The 996 detector recharges each diode and reads the recharging current one diode at a time. The interval between two readings of an individual diode is the exposure time. The 996 detector requires 11 msec to sequentially read all of the diodes in the array. The minimum exposure time is 11 msec. You can set exposure time from 11 to 500 msec.

For example, if an exposure time is set to 50 milliseconds, the Waters 996 detector:

1. Recharges diode 1 and reads the current required to recharge diode 1.
2. Recharges diode 2 and reads the current required to recharge diode 2.
3. Sequentially recharges and reads the current required to recharge all the remaining 510 photodiodes.

4. After all of the diodes have been recharged and read (11 msec), the detector waits 39 msec before beginning the recharge-and-reading sequence with diode 1.

You set the exposure time parameter in the General tab of the 996 PDA Instrument Method Editor. You can specify either Auto Exposure or Exposure Time. For details, refer to the “Waters 996 PDA Detector Properties” topic in the *Millennium³² Online Help* Find tab.

Using the Auto Exposure Parameter

The Auto Exposure time parameter allows the 996 detector optics to calculate the optimum exposure time needed to recharge the diodes based on lamp energy, lamp spectrum, mobile phase absorbance, and the chosen wavelength range. To minimize detector noise, Auto Exposure adjusts the exposure time to 80 to 90 percent of full scale.

The Auto Exposure time setting ensures that the photodiodes are:

- Not saturating due to overexposure
- Operating above the range of normal, dark current discharge

With auto exposure enabled, the 996 detector:

- Calculates exposure time at the start of a run based on maximum light intensity within the wavelength range
- Limits the exposure so that no diode within the given wavelength range is discharged more than 80%
- Provides proper settings for signal-to-noise and dynamic range for each run

The Auto Exposure time setting may not support certain sampling rates or wavelength ranges required for your analysis. If this is the case, you can set the exposure time manually to adjust the exposure time from experiment to experiment.

Using the Exposure Time Parameter

The Exposure Time parameter enables you to manually set the length of time the photodiodes are exposed to light before they are read. The supported range is 11 to 500 msec.

Be aware that increasing the Exposure Time parameter has the potential to saturate the photodiodes. A longer exposure time may cause the 996 detector to lose the signal at certain wavelengths because of diode saturation. When specifying the Exposure Time, select a value that provides settings for an optimum signal-to-noise ratio over the wavelength range of your analysis (see [“Optimizing the Signal-to-Noise Ratio”](#) below).

Optimizing the Signal-to-Noise Ratio

To optimize signal-to-noise ratios, choose an acquisition wavelength range that includes only the wavelengths of interest and over which the mobile phase absorbs minimally (see [Appendix D, Mobile Phase Absorbance](#)).

4.4 Computing Absorbance Data Points

The 996 detector calculates absorbance values before transmitting the data to the Millennium³² database. To calculate absorbance, the 996 detector:

- Computes the absorbance at each diode using the dark current and reference spectrum (see [Section 4.4.1, Calculating Absorbance](#)).
- Averages the absorbances at a particular wavelength as specified in the spectra per second sample rate and reports the average as a single data point (see [Section 4.4.2, Resolution](#)).
- Can apply a filter that acts like an analog filter (see [Section 4.4.3, Filtering Data](#)).

4.4.1 Calculating Absorbance

The 996 detector computes absorbance by subtracting the dark current and reference spectrum from the acquired spectrum. Absorbance is based on the principles of Beer's Law.

Beer's Law

The relationship between the quantity of light of a particular wavelength arriving at the photodiode and the concentration of the sample passing through the flow cell is described by the Beer-Lambert Law (commonly called Beer's Law). Beer's Law is expressed as

$$A = \epsilon lc$$

Where:

A = absorbance

ϵ = molar absorptivity

l = path length (1.0 cm in the 996 detector normal flow cell)

c = molar concentration

Beer's Law applies only to well-equilibrated dilute solutions. It assumes that the refractive index of the sample remains constant, that the light is monochromatic, and that no stray light reaches the detector element. As concentration increases, the chemical and instrumental requirements of Beer's law may be violated, resulting in a deviation from (absorbance versus concentration) linearity (Figure 4-4). The absorbance of mobile phase can reduce the linear range by the amounts shown in [Appendix D, Mobile Phase Absorbance](#).

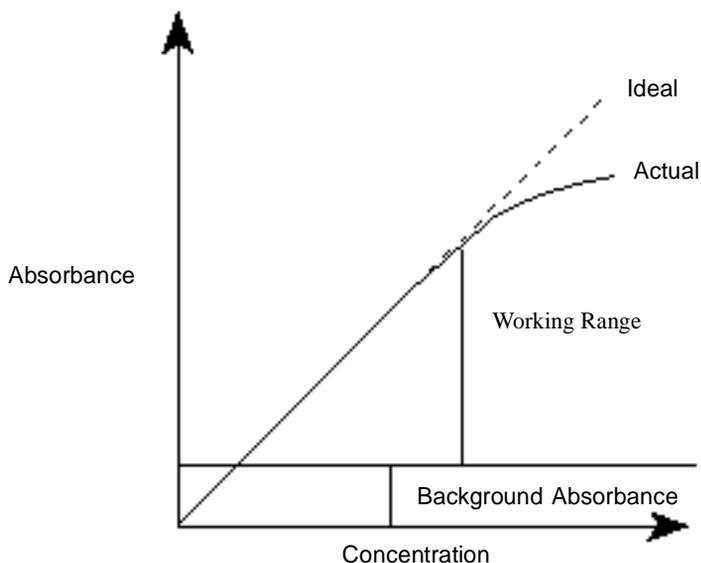


Figure 4-4 Absorbance as a Function of Concentration

Dark Current

Photodiodes lose charge over time even when they are not exposed to light. The amount of charge lost is called *dark current*.

At the start of a chromatographic run, the 996 detector closes the shutter to take a dark current reading for each diode. The shutter closes after the exposure time is calculated and stays closed for the same interval as the exposure time.

The detector subtracts the dark current values from the current values recorded during absorbance measurements for both the sample and the reference spectra.

Reference Spectrum

Immediately after the dark current measurement and before any components are eluted, the 996 detector records a reference spectrum. The reference spectrum is a measure of lamp intensity and mobile phase absorbance over the interval specified in the exposure time taken with the shutter open.

Note: For best results, the reference spectrum should be representative of the initial mobile phase.

Note: For extremely long exposure times, the dark current and reference spectrum readings may take several minutes to finish.

Absorbance

The 996 detector calculates the absorbance for each diode at the end of each exposure time using the following equation:

$$Absorbance = \log \left[\frac{(S_n - D_n)}{(R_n - D_n)} \right]$$

where:

S = Signal obtained during sample analysis

D = Signal obtained during the dark test

R = Signal obtained from the reference spectrum

n = Diode number

4.4.2 Resolution

The data reported by the 996 detector to the Millennium³² database can be the average of a number of data points. After calculating absorbance, the detector averages absorbance values based on:

- Spectral resolution
- Sample rate

Averaging Spectral Data Based on Resolution

Spectral resolution (or bandwidth) is the wavelength interval (in nanometers) between data points in an acquired spectrum. The minimal resolution of the 996 detector is 1.2 nm. Spectral resolution with the 996 detector is always a multiple of 1.2 nm. For example, the 996 detector averages three diodes for each reported wavelength when the spectral resolution is set in the Millennium³² software to 3.6 nm.

Averaging Chromatographic Data Based On Sample Rate

Sample rate is the number of data points per second reported to the Millennium³² database. The number of times the photodiodes are read during the sample rate interval is dependent on the exposure time.

For example, if exposure time is 25 msec, and sample rate is 1 second, then readings per data point are

$$\frac{1000 \text{ msec}}{25 \text{ msec}} = 40$$

The readings are averaged and reported as a single data point.

Combining Spectral Resolution and Sample Rate

Spectral resolution and sample rate have opposite effects on noise and spectral detail. Increasing the value of the spectral resolution parameter and decreasing the number of spectra per second decrease the size of the data file.

Note: The data storage rate is based on wavelength range, spectral resolution, and sample rate, which are set in the General tab of the 996 PDA Instrument Method Editor. For details, refer to the “Waters 996 PDA Detector Properties” topic in the Millennium³² Online Help Find tab.

4.4.3 Filtering Data

Use the Channel 1 tab of the 996 PDA Instrument Method Editor (for details, refer to the “Waters 996 Detector Properties” topic in the Millennium³² Online Help Find tab) to apply an optional noise filter (the Filter Response parameter) to the data sent to the Millennium³² software database. A noise filter of 1 second is the default value, which provides a good signal-to-noise ratio for most chromatographic separations.

Note the following with regard to filtering data:

- The noise filter is similar in function to an analog RC filter.
- The filter calculates a data point that is a modified rolling average for a wavelength over a number of readings.
- The filter values are comparable to the effects of a 1-, 2-, or 3-second RC filter.

5

Spectral Contrast Theory

This chapter explains the theory behind the Spectral Contrast technique, which is used to compare UV/Vis absorbance spectra collected by the 996 detector. Spectral Contrast makes use of the fact that different compounds have differently shaped absorbance spectra. This chapter describes how Spectral Contrast represents absorbance spectra as vectors. When applied to the UV/Vis absorbance data collected by the 996 detector, the Spectral Contrast technique determines whether differences between spectra are due to the presence of multiple compounds in the same peaks (coelution) or due to nonideal conditions such as noise, photometric error, or solvent effects.

5.1 Comparing Absorbance Spectra

The shape of an absorbance spectrum is determined by the relative absorbance at different wavelengths. The shape of a compound's absorbance spectrum is a characteristic of that compound at the solvent and pH conditions under which the absorbance spectrum is measured.

[Figure 5-1](#) shows the absorbance spectra for the two compounds, A and B. The ratio of the absorbance at 245 nm to the absorbance at 257 nm is approximately 2.2 for compound A and 0.7 for compound B.

The absorbance ratios of two wavelength pairs is a limited spectral comparison. For more information, you need to compare the absorbance ratios of multiple wavelength pairs.

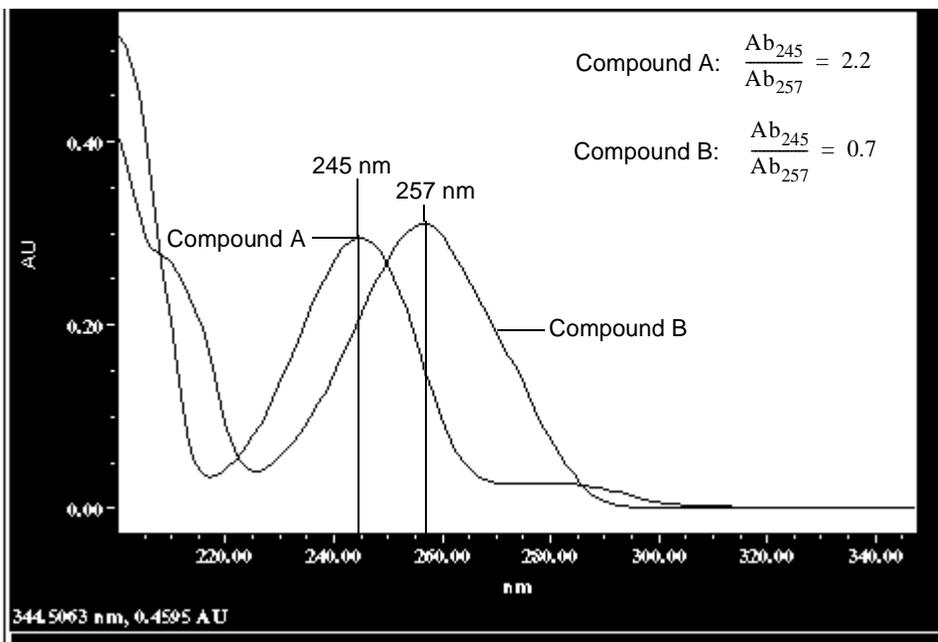


Figure 5-1 Comparing Spectra of Two Compounds

5.2 Representing Spectra as Vectors

The Spectral Contrast technique uses vectors to quantify differences in the shapes of spectra. Spectral Contrast converts baseline-corrected spectra to vectors and then compares the vectors. Spectral vectors have two properties:

- **Length** – Proportional to analyte concentration.
- **Direction** – Determined by the relative absorbance of the analyte at all wavelengths (its absorbance spectrum). Direction is independent of concentration.

Vector direction contributes to the identification of a compound, since the direction is a function of the absorbance spectrum of the compound. The ability of spectral vectors to differentiate compounds depends on the resolution of spectral features. As both wavelength range and spectral resolution increase, the precision of a spectral vector for the resultant spectrum increases. A vector derived from the Waters 996 PDA Detector can include absorbances in any range between 190 nm and 800 nm with a spectral resolution of 1.2 nm.

5.2.1 Vectors Derived from Two Wavelengths

The Spectral Contrast algorithm uses vectors to characterize spectra ([Figure 5-2](#)). To understand the vector principle, consider two vectors ([Figure 5-2](#)) based on the spectra depicted in [Figure 5-1](#).

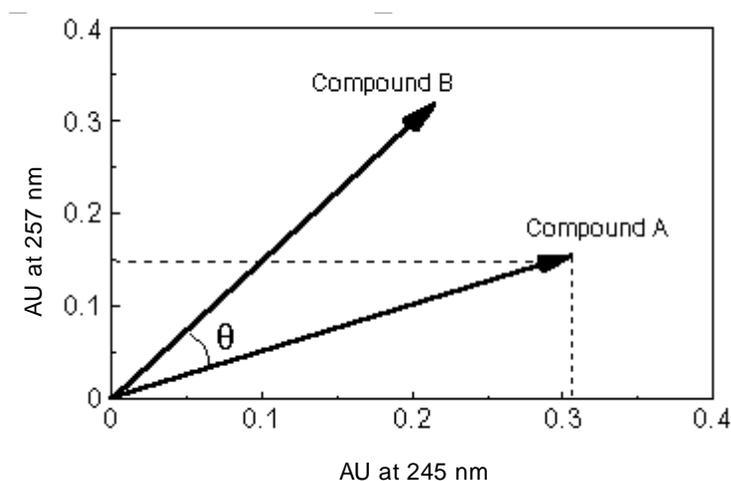


Figure 5-2 Plotting Vectors for Two Spectra

The axes in [Figure 5-2](#) are in absorbance units at the two wavelengths used to calculate the absorbance ratio shown in [Figure 5-1](#). The head of the vector for Compound A is at the intersection of the absorbance values (for Compound A) at the two wavelengths represented by each axis. The other vector is similarly derived for the spectrum of Compound B.

The vector for Compound B points in a direction different from that of the vector for Compound A. The difference in direction, which reflects the difference in the absorbance ratios of the two compounds at wavelengths 245 nm and 257 nm, is called the Spectral Contrast Angle. A Spectral Contrast angle (e.g., θ in [Figure 5-2](#)) greater than zero indicates a shape difference between spectra (see [Section 5.3, Spectral Contrast Angles](#)). The length of the vector is proportional to the concentration.

5.2.2 Vectors Derived from Multiple Wavelengths

When absorbance ratios are limited to two wavelengths, the chance that two different spectra will have the same absorbance ratio is much greater than if comparison is made using absorbance ratios at many wavelengths. Therefore, the Spectral Contrast technique uses absorbances from multiple wavelengths to form a vector in an n -dimensional vector space, where n is the number of wavelengths from the spectrum.

To compare two spectra, the Spectral Contrast technique forms a vector for each spectrum in an n -dimensional space. The two spectral vectors are compared mathematically to compute the angle between the two vectors.

Just as in the 2-wavelength comparison, a Spectral Contrast angle of zero in n -dimensional space means that all ratios of absorbances at corresponding wavelengths match. Conversely, if any comparison of ratios does not match, then the corresponding vectors point in different directions.

5.3 Spectral Contrast Angles

Spectra that have the same shape have vectors that point in the same direction. Spectra that have different shapes have vectors that point in different directions. The angle between the two vectors of any two spectra, the Spectral Contrast angle, quantifies the magnitude of the shape difference between the spectra. The *Spectral Contrast angle* is the difference in direction between the spectral vectors of two spectra.

A Spectral Contrast angle can vary from 0 degrees to 90 degrees. A Spectral Contrast angle near 0 degrees indicates little shape difference between the compared spectra. Matching a spectrum to itself produces a Spectral Contrast angle of exactly 0 degrees. The maximum Spectral Contrast angle, 90 degrees, indicates that the two spectra do not overlap at any wavelength.

To illustrate the relationship between Spectral Contrast angle and spectral shape differences, consider the pairs of spectra shown in [Figure 5-3](#), [Figure 5-4](#), and [Figure 5-5](#).

Spectra with Different Shapes

In [Figure 5-3](#), the absorbance spectra of two compounds, A and B, are distinctly different, and therefore, have a large Spectral Contrast angle (62.3°).

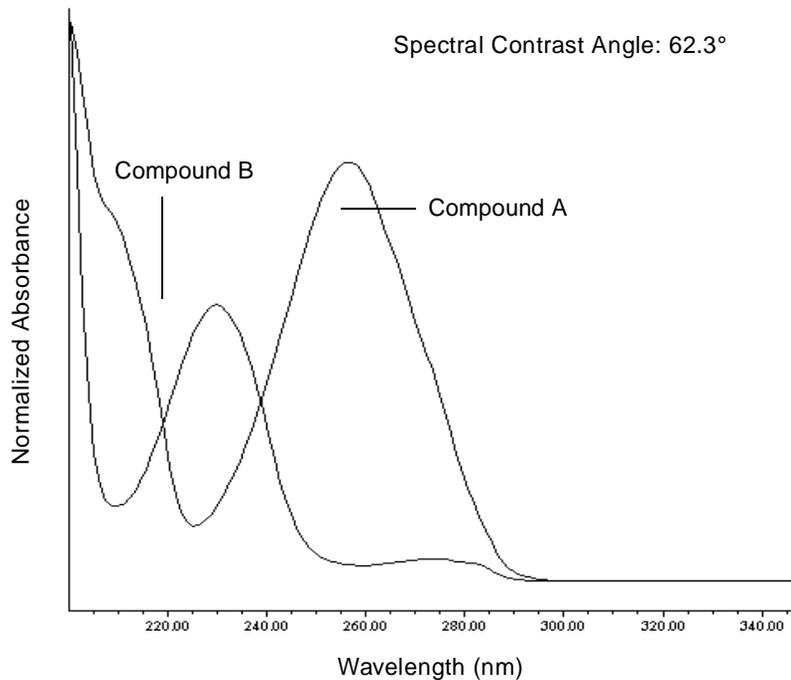


Figure 5-3 Spectra with a Large Spectral Contrast Angle

Spectra with Similar Shapes

In [Figure 5-4](#), the absorbance spectra of two compounds, A and B, are similar, and therefore, have a small Spectral Contrast angle (3.0°).

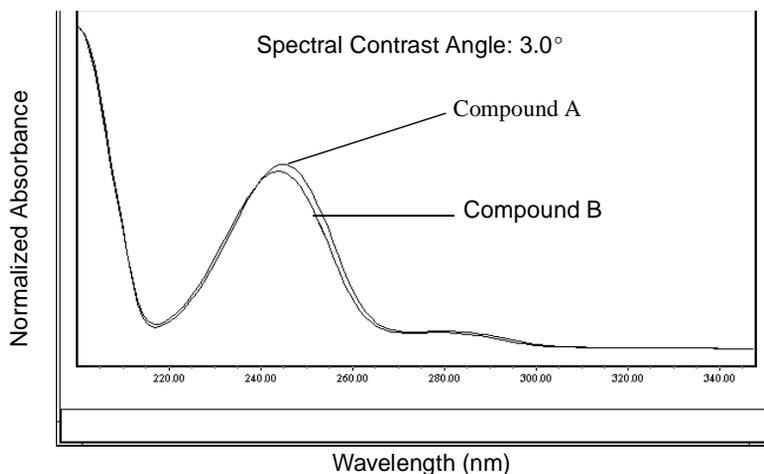


Figure 5-4 Spectra with a Small Spectral Contrast Angle

Differences Between Spectra of the Same Compound

Small but significant differences between absorbance spectra can occur because of factors other than those due to the absorbance properties of different compounds. For example, multiple spectra of the *same* compound may exhibit slight differences because of detector noise, photometric error, high sample concentration, or variations in solvent conditions. The spectra in [Figure 5-5](#), for example, show how instrument noise can affect the shape of an absorbance spectrum of one compound. This effect is most likely to occur at low concentrations where the signal-to-noise ratio is low. Note that the Spectral Contrast angle between these absorbance spectra of the same compound is 3.4° .

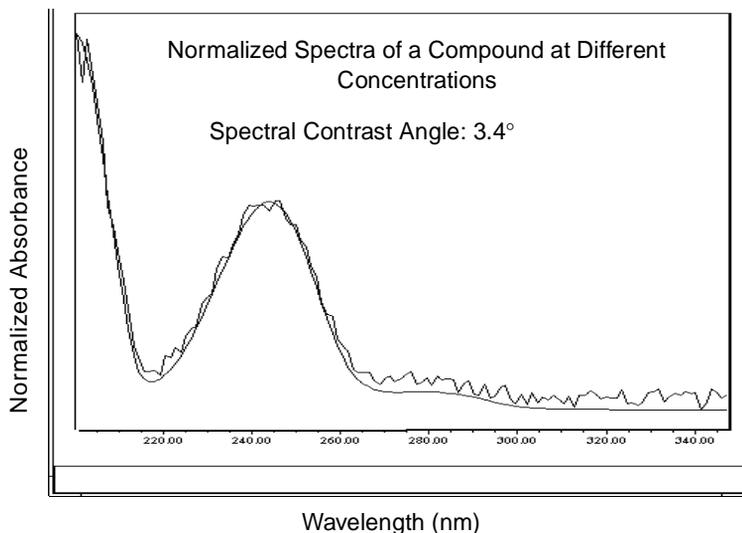


Figure 5-5 Absorbance Spectra of a Compound at Two Concentrations

5.4 Nonidealities

Shape differences between absorbance spectra can be caused by one or more of the following nonideal phenomena:

- Detector noise
- Photometric error caused by high sample concentration
- Variation in solvent composition

These sources of spectral variation can cause chemically pure, baseline-resolved peaks to exhibit a small level of spectral inhomogeneity. You can assess the significance of spectral inhomogeneity by comparing a Spectral Contrast angle to a *Threshold angle* (see Section 5.4.4).

5.4.1 Detector Noise

Statistical and thermal variations add electrical noise to the absorbance measurements made by the 996 detector. The noise manifests itself as fluctuations in the baseline, known as *baseline noise*. The magnitude of any absorbance differences caused by statistical and thermal variations can be predicted from the instrument noise in the baseline region of a chromatogram.

5.4.2 Photometric Error

At high absorbances (generally greater than 1 AU), a combination of effects can produce slight departures (about 1%) from Beer's Law due to photometric error. Although photometric errors at this level may have a negligible effect on quantitation, they can be a significant source of spectral inhomogeneity. To minimize the effects of photometric error for all Spectral Contrast operations, the maximum spectral absorbance of a compound should be less than 1 AU. Keep in mind that the absorbance of the mobile phase reduces the working linear dynamic range by the amount of mobile phase absorbance at each wavelength. For examples of mobile phase absorbance, see [Appendix D, Mobile Phase Absorbance](#).

Note: For more information about the effects of the photometric error curve, refer to *Principles of Instrumental Analysis, third edition, by Douglas A. Skoog, Saunders College Publishing, 1985, pp 168–172.*

5.4.3 Solvent Changes

As long as solvent concentration and composition do not change (isocratic operation), the background absorbance, if any, by the solvent remains constant. A change, however, in solvent pH or composition, such as occurs in gradient operation, can affect the intrinsic spectral shape of a compound, as shown in [Figure 5-6](#).

5.4.4 Threshold Angle

In addition to computing Spectral Contrast angles, the Spectral Contrast technique also computes a Threshold angle. The Threshold angle is the maximum Spectral Contrast angle between spectra that can be attributed to nonideal phenomena.

Comparison of a Spectral Contrast angle to its Threshold angle can assist in determining if the shape difference between spectra is genuine, that is, generated by mixtures that are dissimilar. In general, a Spectral Contrast angle less than its Threshold angle indicates that shape differences can be attributed to nonideal phenomena alone, and that there is no evidence for genuine differences between the spectra. A Spectral Contrast angle greater than its Threshold angle indicates that the shape differences are due to genuine differences between the spectra.

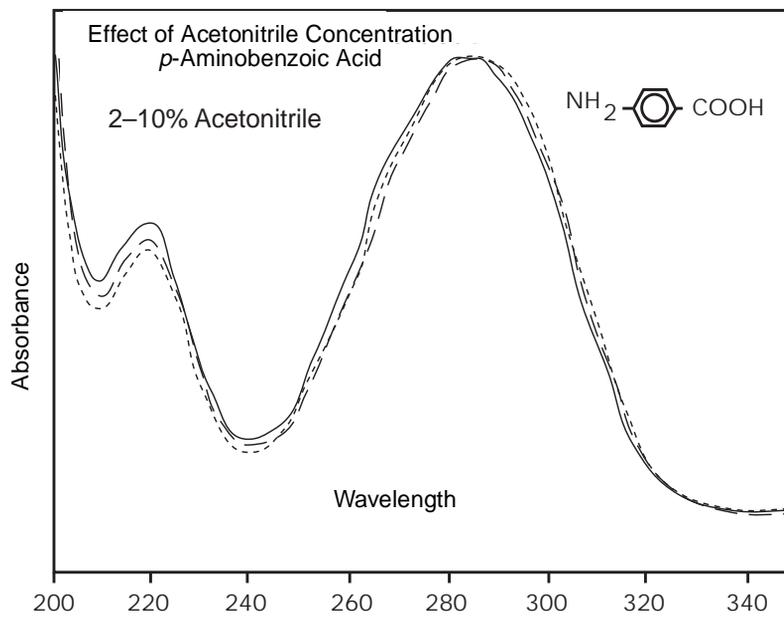
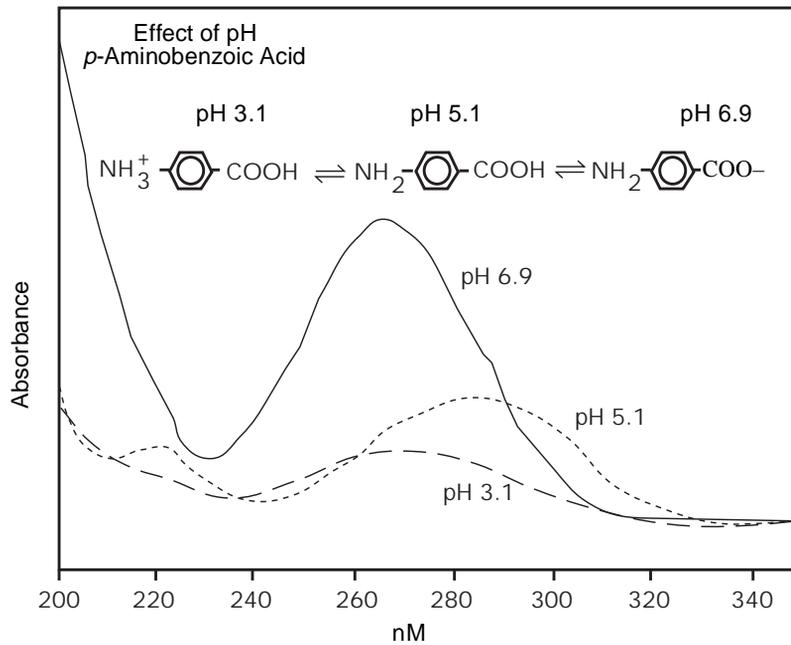


Figure 5-6 Effects of pH and Solvent Concentration on the Absorbance Spectrum of *p*-Aminobenzoic Acid

Appendix A

Detector Specifications

[Table A-1](#) lists the Waters 996 PDA Detector specifications.

Table A-1 996 Detector Specifications

Item	Specification
Dimensions	11.5 × 22 inches (29 × 56 cm)
Weight	31 lbs (14.3 kg)
Wavelength range	190 to 800 nm
Wavelength accuracy	±1 nm
Linearity range ¹	5% at 2.0 AU, propylparaben, at 258 nm
Spectral resolution	1.2 nm
Baseline noise	±1.5 × 10 ⁻⁵ AU peak-to-peak, dry, at 254 nm
Drift	1 × 10 ⁻³ AU/hour at 254 nm (after warmup)
Flow cells	Pathlength (mm): Tubing (I.D.)
Standard	10 0.009 in
Semi-preparative	3 0.040 in
Microbore	3 0.005 in
Inert	10 0.010 in

¹ Per ASTM 685-79

Appendix B

Spare Parts

The spare parts listed in [Table B-1](#) are those parts recommended for customer installation. Damage incurred by performing unauthorized work on your 996 detector may invalidate certain warranties.

Table B-1 Spare Parts

Item	Part Number
Flow cell, standard	WAT057919
Flow cell, semi-preparative	WAT057463
Flow cell, microbore	WAT057462
Flow cell, inert	WAT057461
Gasket, flow cell (2)	WAT057924
Belleville washer (2)	WAT057925
Lens mount and lens (2)	WAT057923
Semi-prep lens kit	WAT057968
Deuterium lamp	WAT057760
Fuse, 4 A (5 × 20 mm)	WAT057337
Waters Erbium Perchlorate Wavelength Accuracy Solution	WAT042885
Waters Absorbance Detector Linearity Solution	WAT042881
Waters 996 PDA Detector Qualification Workbooks	WAT509-01



Appendix C

Warranty Information

This appendix includes information on:

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

C.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 996 PDA Detector 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 996 PDA Detector [other than Third-Party Components (non-Waters named)], will not deviate materially from the Specifications for such detectors. 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 996 PDA Detector that results from (a) use of the Waters 996 PDA Detector for any purpose other than general purpose use and specifically excluding use of the Waters 996 PDA Detector in clinical diagnostic procedures, or use of the Waters 996 PDA Detector 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 996 PDA Detector 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 996 PDA Detector, (g) any malfunction of any information system or instrument with which the Waters 996 PDA Detector may be connected, or (h) failure to establish or maintain the operating environment for the Waters 996 PDA Detector in accordance with the operator's manual.

Exclusive Remedy

In the event of any failure of the Waters 996 PDA Detector 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 996 PDA Detector, or refund of all amounts theretofore paid by Customer to Waters Corporation for the Waters 996 PDA Detector.

Disclaimers

THE LIMITED WARRANTY SET FORTH HEREIN IS EXCLUSIVE AND IN LIEU OF, 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 996 PDA DETECTOR 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 996 PDA DETECTOR AND ITS MANUFACTURE, WATERS CORPORATION CANNOT AND DOES NOT WARRANT THAT THE OPERATION OF THE WATERS 996 PDA DETECTOR WILL BE WITHOUT DEFECT. CUSTOMER EXPRESSLY ACKNOWLEDGES THAT CUSTOMER IS SOLELY RESPONSIBLE FOR USE OF THE WATERS 996 PDA DETECTOR IN CLINICAL DIAGNOSTIC PROCEDURES OR FOR INVESTIGATIONAL USE WITH OR WITHOUT CONFIRMATION OF DIAGNOSIS BY ANOTHER MEDICALLY ESTABLISHED DIAGNOSTIC PRODUCT OR PROCEDURE.

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 (8 AM to 5 PM, EST, 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 operating supplies.

Repair or replacement is not made:

- For expendable items such as gaskets, windows, lenses, and fuses, 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

The warranty period begins when the product is installed or, in the case of a customer installation, 15 days after shipment from Waters.

In no case does the warranty period extend beyond 15 months from date of shipment. If an item is replaced during its warranty period, the replacement part is warranted for the balance of the original warranty period. [Table C-1](#) summarizes the warranty periods for applicable components.

Table C-1 Warranty Periods

Item	Warranty
Waters 996 PDA Detector	1 year
Deuterium lamp	1000 hours
Gaskets Windows Lenses Fuses Plunger seals Tubing and fittings	Not warranted items

Lamp Replacement Warranty

The Waters 996 PDA Detector deuterium lamp is warranted to light and pass powerup verification tests for 1000 hours.

C.2 Shipments, Damages, Claims, and Returns

Shipments

As all shipments are made Free On Board (FOB) shipping point, we suggest insurance be authorized on all shipments. Instruments and major components are packed and shipped via surface, unless otherwise required. Supplies and/or replacement parts are packed and shipped via United Parcel Service (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 in transit. Unpack shipment promptly after receipt as there may be concealed damage even though no evidence of it is apparent. When concealed damage is discovered, cease further unpacking of the unit involved and request immediate inspection by local agent or carrier and secure written report of his findings to support claim. This request must be made within 15 days of 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 number (RMA).

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 and authorization. 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.

Appendix D

Mobile Phase Absorbance

This appendix provides a list of the absorbances at several wavelengths for commonly used mobile phases. Choose your mobile phase carefully to reduce baseline noise.

The best mobile phase for your application is one that is transparent at the chosen detection wavelengths. Such a mobile phase ensures that any absorbance is due only to the sample. Absorbance by the mobile phase also reduces the linear dynamic range of the detector by the amount of absorbance that is autozeroed out. Wavelength, pH and concentration of the mobile phase will affect its absorbance. Examples of several mobile phases are provided below.

Table D-1 Mobile Phase Absorbance Measured Against Air or Water

	Absorbance at Specified Wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Solvents										
Acetonitrile	0.05	0.03	0.02	0.01	0.01	<0.01	—	—	—	—
Methanol (not degassed)	2.06	1.00	0.53	0.37	0.24	0.11	0.05	0.02	<0.01	—
Methanol (degassed)	1.91	0.76	0.35	0.21	0.15	0.06	0.02	<0.01	—	—
Isopropanol	1.80	0.68	0.34	0.24	0.19	0.08	0.04	0.03	0.02	0.02
Unstablized Tetrahydrofuran (THF, fresh)	2.44	2.57	2.31	1.80	1.54	0.94	0.42	0.21	0.09	0.05
Unstablized Tetrahydrofuran (THF, old)	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	>2.5	2.5	1.45

Table D-1 Mobile Phase Absorbance Measured Against Air or Water (Continued)

	Absorbance at Specified Wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Acids and Bases										
Acetic acid, 1%	2.61	2.63	2.61	2.43	2.17	0.87	0.14	0.01	<0.01	—
Hydrochloric acid, 0.1%	0.11	0.02	<0.01	—	—	—	—	—	—	—
Phosphoric acid, 0.1%	<0.01	—	—	—	—	—	—	—	—	—
Trifluoroacetic acid	1.20	0.78	0.54	0.34	0.22	0.06	<0.02	<0.01	—	—
Diammonium phosphate, 50 mM	1.85	0.67	0.15	0.02	<0.01	—	—	—	—	—
Triethylamine, 1%	2.33	2.42	2.50	2.45	2.37	1.96	0.50	0.12	0.04	<0.01
Buffers and Salts										
Ammonium acetate, 10 mM	1.88	0.94	0.53	0.29	0.15	0.02	<0.01	—	—	—
Ammonium bicarbonate, 10 mM	0.41	0.10	0.01	<0.01	—	—	—	—	—	—
EDTA, disodium, 1 mM	0.11	0.07	0.06	0.04	0.03	0.03	0.02	0.02	0.02	0.02
HEPES, 10 mM, pH 7.6	2.45	2.50	2.37	2.08	1.50	0.29	0.03	<0.01	—	—
MES, 10 mM, pH 6.0	2.42	2.38	1.89	0.90	0.45	0.06	<0.01	—	—	—

Table D-1 Mobile Phase Absorbance Measured Against Air or Water (Continued)

	Absorbance at Specified Wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Potassium phosphate, monobasic (KH_2PO_4), 10 mM	0.03	<0.01	—	—	—	—	—	—	—	—
Potassium phosphate, dibasic, (K_2HPO_4), 10 mM	0.53	0.16	0.05	0.01	<0.01	—	—	—	—	—
Sodium acetate, 10 mM	1.85	0.96	0.52	0.30	0.15	0.03	<0.01	—	—	—
Sodium chloride, 1 M	2.00	1.67	0.40	0.10	<0.01	—	—	—	—	—
Sodium citrate, 10 mM	2.48	2.84	2.31	2.02	1.49	0.54	0.12	0.03	0.02	0.01
Sodium formate, 10 mM	1.00	0.73	0.53	0.33	0.20	0.03	<0.01	—	—	—
Sodium phosphate, 100 mM, pH 6.8	1.99	0.75	0.19	0.06	0.02	0.01	0.01	0.01	0.01	<0.01
Tris HCl, 20 mM, pH 7.0	1.40	0.77	0.28	0.10	0.04	<0.01	—	—	—	—
Tris HCl, 20 mM, pH 8.0	1.80	1.90	1.11	0.43	0.13	<0.01	—	—	—	—

D

Table D-1 Mobile Phase Absorbance Measured Against Air or Water (Continued)

	Absorbance at Specified Wavelength (nm)									
	200	205	210	215	220	230	240	250	260	280
Waters PIC Reagents										
PIC A, 1 vial/L	0.67	0.29	0.13	0.05	0.03	0.02	0.02	0.02	0.02	<0.01
PIC B6, 1 vial/L	2.46	2.50	2.42	2.25	1.83	0.63	0.07	<0.01	—	—
PIC B6, low UV, 1 vial/L	0.01	<0.01	—	—	—	—	—	—	—	—
PIC D4, 1 vial/L	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.02	0.02	0.01
Detergents										
BRIJ 35, 1%	0.06	0.03	0.02	0.02	0.02	0.01	<0.01	—	—	—
CHAPS, 0.1%	2.40	2.32	1.48	0.80	0.40	0.08	0.04	0.02	0.02	0.01
SDS, 0.1%	0.02	0.01	<0.01	—	—	—	—	—	—	—
Triton™ X-100, 0.1%	2.48	2.50	2.43	2.42	2.37	2.37	0.50	0.25	0.67	1.42
Tween™ 20, 0.1%	0.21	0.14	0.11	0.10	0.09	0.06	0.05	0.04	0.04	0.03



Index

A

- Absorbance
 - maximum [61](#)
 - mobile phase [70](#)
 - photometric error [61](#)
 - solvent change effects [62](#)
 - Waters 996 calculations [50](#), [52](#)
- Acquisition
 - Auto Exposure parameter [49](#)
 - Exposure Time parameter [49](#)
- Address, setting [18](#)
- Analog output specifications [19](#), [21](#)
- Aperture width [47](#)
- Auto Exposure parameter [49](#)

B

- Beer's law [50](#), [61](#)

C

- Calibration [31](#)
- Claims, warranty [69](#)
- Column, connecting [23](#)
- Compression fittings [23](#)
- Connections
 - column [23](#)
 - events [22](#)
 - fluidic [23](#)
 - non-IEEE-488 [19](#)
 - rear panel [16](#)
 - terminal strip [22](#)
- Contact closures [19](#)
- Contacting Waters Technical Service [27](#), [31](#)

D

- Damage, warranty [69](#)
- Dark current [51](#)
- Data acquisition
 - Auto Exposure parameter [49](#)
 - Exposure Time parameter [49](#)
- Derived vectors [56](#), [56](#)
- Diagnostics [30](#)
- DIP switch, setting [18](#)

E

- Electrical connections [15](#)
- Events
 - connections [19](#), [22](#), [22](#)
 - electrical specifications [21](#)
 - terminal strip connections [20](#)
- Exception to service warranty [67](#)
- Exclusive remedy [66](#)
- Exposure Time parameter [49](#)

F

- Fittings [23](#), [23](#), [23](#)
- Flow cell
 - access [35](#)
 - cleaning [37](#)
 - exploded view [37](#)
 - flushing [33](#)
 - maintenance [33](#)
 - removing [34](#)
- Fluid
 - connecting lines [23](#)
 - fittings [23](#)

Fuses
IEC-rated [15](#)
maintenance [43](#)
replacement [43](#)

I

IEC-rated fuses [15](#)
IEEE-488 address, setting [18](#)
Inputs [19](#), [21](#), [22](#), [22](#)
Installation
electrical [15](#)
fluidic [23](#)
site selection [14](#), [14](#)
Instrument method
Auto Exposure parameter [49](#)
Exposure Time parameter [49](#)

L

Lamp
hardware theory [45](#)
replacement [39](#), [41](#)
replacement warranty [68](#)
usage indicator [39](#)
Liability [66](#)
Limited express warranty [65](#)

M

Maintenance
flow cell [33](#)
fuse [43](#)
lamp [39](#)
PDA detector [33-43](#)
Match Angle, photometric error effects [61](#)
Maximum absorbance [61](#)
Millennium³² Chromatography Manager,
connections [16](#)

Mobile phase
absorbances [70](#)
wavelengths [70](#)

N

Network address [18](#)
Noise effects [60](#)
Nonidealities [60](#)
Non-IEEE-488 connections [19](#)

O

Outputs [19](#), [21](#), [22](#), [22](#)

P

Parts, spare [64](#)
Photodiode array [47](#)
Photometric error [61](#), [61](#)
Power connections [15](#)
Purity Angle, photometric error effects [61](#)

R

Rear panel connections [16](#)
Reference spectrum [52](#)
Returns, warranty [69](#)

S

Service
exceptions to warranty [67](#)
warranty [67](#)
Setting
DIP switch [18](#)
IEEE-488 address [18](#)
Shipments [69](#)

- Shutdown, procedure [26](#)
- Solvent Angle, photometric error effects [61](#)
- Solvent changes [61](#)
- Spare parts [64](#)
- Specifications
 - analog output [19](#), [21](#)
 - event inputs [21](#)
 - event outputs [21](#)
 - Waters 996 [63](#)
- Spectra
 - derived vectors [56](#), [56](#)
 - spectral shape differences [60](#)
 - vectors [55](#)
- Spectral Contrast
 - derived vectors [56](#), [56](#)
 - spectral shape differences [60](#)
 - theory [54–62](#)
 - vectors [55](#)
- Spectral resolution [47](#)
- Spectrum match, spectral shape
 - differences [60](#)
- Start up, procedure [24](#)

T

- Terminal strip
 - connections [20](#), [22](#)
 - diagram [22](#)
- Threshold angle [60](#)
- Troubleshooting [27–32](#)
- Tubing, cutting [23](#)

V

- Vectors
 - derived from multiple wavelengths [56](#)
 - derived from two wavelengths [56](#)
 - spectra, representing [55](#)
 - spectral contrast [55](#)

W

- Warranty
 - claims [69](#)
 - damages [69](#)
 - disclaimers [66](#)
 - exclusions [65](#)
 - information [65–68](#)
 - lamp replacement [68](#)
 - limited [65](#)
 - period [68](#)
 - returns [69](#)
 - service [67](#)
 - service exceptions [67](#)
- Waters 996
 - absorbance calculations [50](#), [52](#)
 - aperture width [47](#)
 - dark current [51](#)
 - detector optics, overview [44–46](#)
 - hardware theory [44–53](#)
 - photodiode array overview [47](#)
 - reference spectrum [52](#)
 - spare parts [64](#)
 - specifications [63](#)
 - spectral resolution [47](#)
- Waters liability [66](#)
- Waters Technical Service, contacting [27](#), [31](#)
- Wavelength
 - accuracy [31](#)
 - derived vectors [56](#), [56](#)
 - mobile phase absorbances [70](#)