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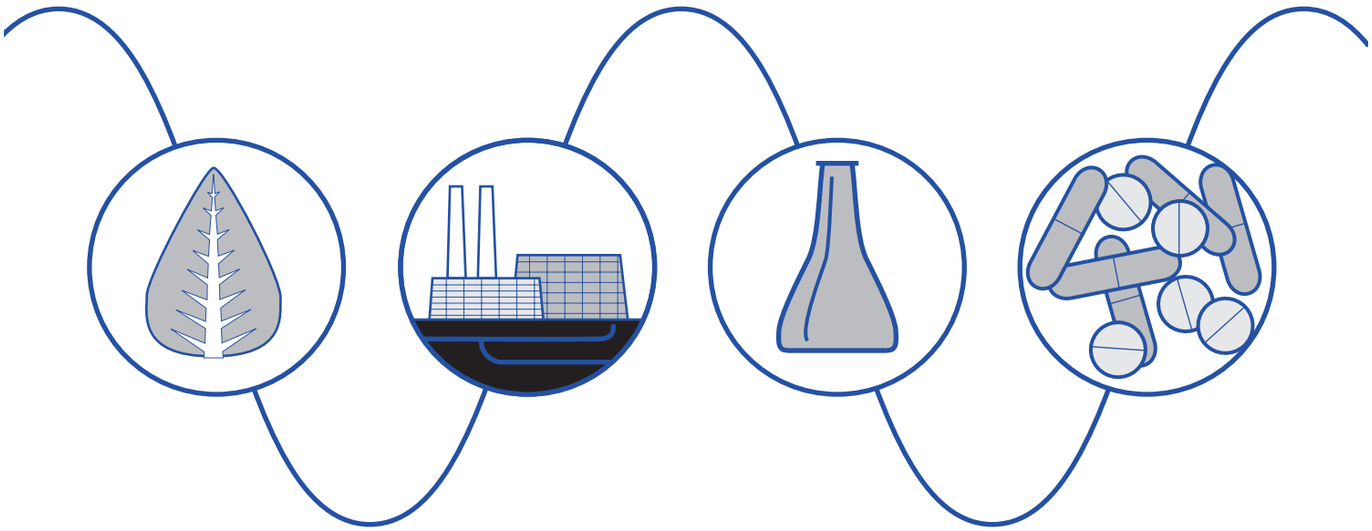
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Waters 486 Tunable Absorbance Detector Operator's Manual



Waters

34 Maple Street
Milford, MA 01757

049758TP, Revision 1

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Attention: *The Waters™ 486 Tunable Absorbance Detector can be used for IN VITRO diagnostic applications. This is a highly sensitive instrument. Read the accompanying operator's manual before using.*

When using 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.



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 the Class A FCC limits.



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



Caution: *To avoid the possibility of electric shock, power off the 486 detector and disconnect the power cord before you service the instrument.*



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 you always connect the shield of the cable to chassis ground at one instrument only.*

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.

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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 Rear Panel of the 486 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 or high temperature

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How to Use This Guide

Purpose of this guide

The *Waters 486 Tunable Absorbance Detector Operator's Manual* details the procedures for unpacking, installing, maintaining, and troubleshooting the 486 detector. It also includes an appendix describing warranty and service information, including spare parts.

Audience

This manual is intended for use by anyone interested in installing, maintaining, and troubleshooting the Waters 486 Tunable Absorbance Detector.

Structure of this guide

The *Waters 486 Tunable Absorbance Detector Operator's Manual* is divided into chapters. Each chapter page is marked with a tab and a footer facilitating access to information within the chapter.

The table below defines each chapter in this manual.

Title	Description
Chapter 1, Introduction and Theory of Operation	Describes principles of operation and solvent considerations for the Waters 486 Detector.
Chapter 2, Installation and Instrument Verification	Describes how to install, verify, and connect instruments to the Waters 486 Detector.
Chapter 3, Making Electrical Connections	Describes how to connect other Waters instruments to the Waters 486 Detector.
Chapter 4, Using the Waters 486 Detector	Describes the features of the Waters 486 Detector front panel.
Chapter 5, Maintenance Procedures	Describes how to clean and replace various parts of the Waters 486 Detector.
Chapter 6, Error Codes and Diagnostics	Describes the error messages and diagnostics used to troubleshoot and configure the Waters 486 Detector.

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Title	Description
Chapter 7, Troubleshooting	Describes troubleshooting methods for the Waters 486 Detector. Contains troubleshooting flow charts and symptom-cause-solution tables.
Appendix A, Specifications	Contains operational, voltage, and optional flow cell specifications for Waters 486 Detector.
Appendix B, Spare Parts	Provides a list of recommended and optional spare parts for the Waters 486 Detector.
Appendix C, Warranty/Serial Number Information	Includes warranty and service information.

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 beginning the procedures in this section, connect the 10-mV output to a recorder of 10-mV output. Refer to Section 3.3.2, Chart Recorder Connections.

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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.*



Caution: *To avoid the possibility of electrical shock and possible injury, always turn off the detector and unplug the power cord before performing maintenance procedures.*



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

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1 Introduction and Theory of Operation

This chapter provides physical and functional descriptions of the Waters 486 Tunable Absorbance Detector hardware.

Refer to Appendix A, Specifications, for the Waters 486 Tunable Absorbance Detector specifications.

1.1 Waters 486 Detector Description

Figure 1-1 illustrates the Waters 486 Detector hardware.

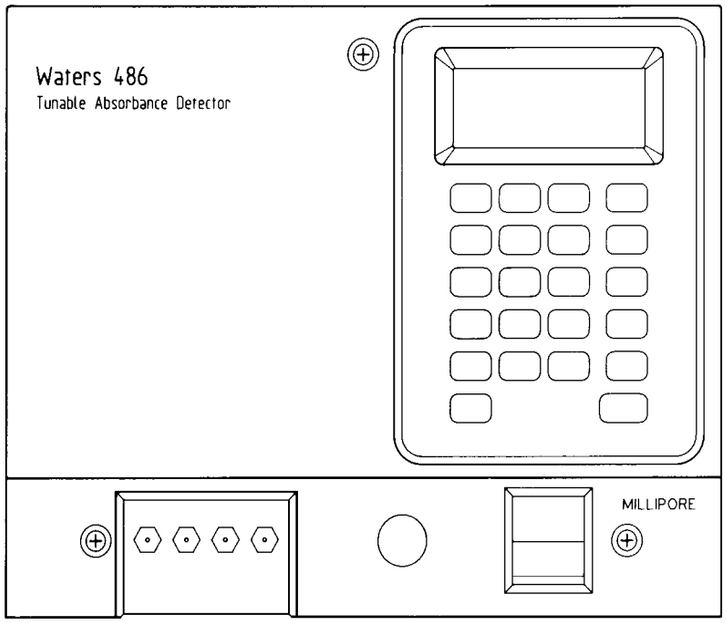


Figure 1-1 Waters 486 Tunable Absorbance Detector

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1

The Waters 486 Tunable Absorbance Detector is a single-channel, tunable, ultraviolet/visible (UV/VIS) detector designed for high-performance liquid chromatography (HPLC) applications.

The Waters 486 Detector can operate either as a stand-alone unit (with a chart recorder) or as part of a chromatography system with other Waters system modules.

The Waters 486 Detector can be configured with the following Waters systems:

- Millennium™ Chromatography Manager
- Waters 860 Networking Computer System
- Waters 600E Multisolvent Delivery System
- Waters 745/745B or 746 Integrators

Wavelength range and sensitivity

The Waters 486 Detector provides a usable light intensity over the wavelength range 190 to 600 nanometers (nm), although its primary use is in the ultraviolet range between 190 and 380 nm. The maximum sensitivity of the instrument is 0.001 absorbance units full scale (AUFS).

Features

The Waters 486 Detector offers the following features:

- Easy to use operator interface
- Self-calibration to ensure wavelength accuracy
- Battery backup to retain parameter settings when the detector is powered down or during power interruptions
- A deuterium arc lamp
- The patented Taper-Cell® flow cell design, which reduces interference caused by refractive index effects.
- Optional cells:
  - Non-metallic
  - Semi-prep
  - Microbore
  - High-pressure (optimized for operation in LC/MS) Lamp shut-off to lengthen usable lamp life
- User-selectable chart mark on IV integrator output (standard on 10 mV)
- Wavelength scanning to determine optimal wavelength
- Diagnostic routines to verify proper functioning
- A modular design for easy servicing

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1.2 Principles Of Operation

To effectively use the Waters 486 Tunable Absorbance Detector, you should be familiar with the optic and electronic design and principles of operation.

This section describes:

- Waters 486 Detector optics
- Waters 486 Detector electronics
- Wavelength calibration
- Taper-Cell flow cell
- Noise filter time constant

Figure 1-2 illustrates the Waters 486 Detector functions.

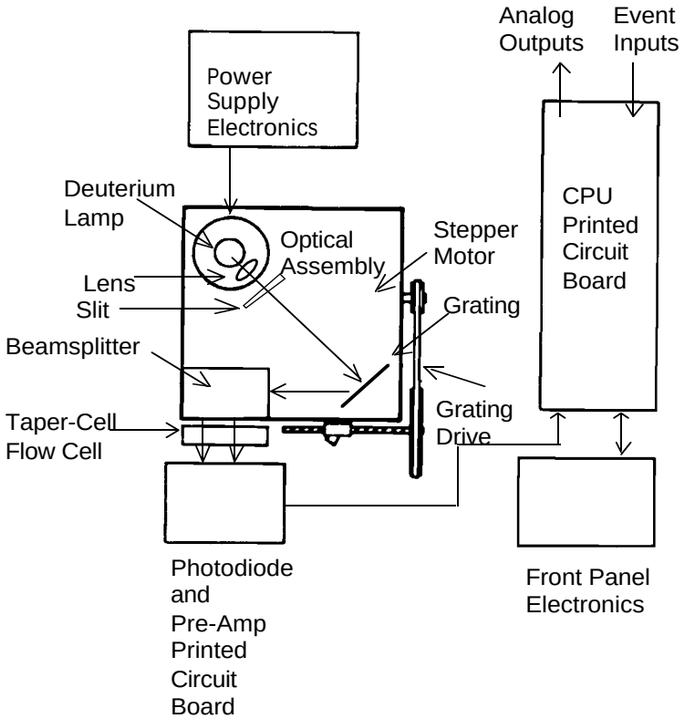


Figure 1-2 Waters 486 Detector Functional Diagram

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1.2.1 Waters 486 Detector Optics

The 486 Detector optics include the following:

- Deuterium lamp
- Illumination lens
- Aperture slit
- Diffraction grating
- Beamsplitter
- Flow cell, with sample and reference sides
- Dual photodiode

Optics assembly light path

The Waters 486 Detector light path is as follows:

1. Light from the deuterium lamp passes through the lens, and the slit then focuses on the grating.
2. When the wavelength setting on the front panel is adjusted, the Waters 486 Detector rotates the grating using the stepping motor and the grating drive, ensuring that light of only the desired wavelength projects onto the beam splitter.
3. The beam splitter divides the light into two beams: a sample beam and a reference beam.
4. The dual light beams pass through the sample and reference sides of the flow cell to dual photodiodes where they are converted into analog currents.
5. The pre-amp board converts the analog currents from the photodiodes to digital signals for processing by the central processing unit (CPU) and output to a chart recorder, integrator, or computer.

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Figure 1-3 illustrates the Waters 486 Detector optics assembly light path and components.

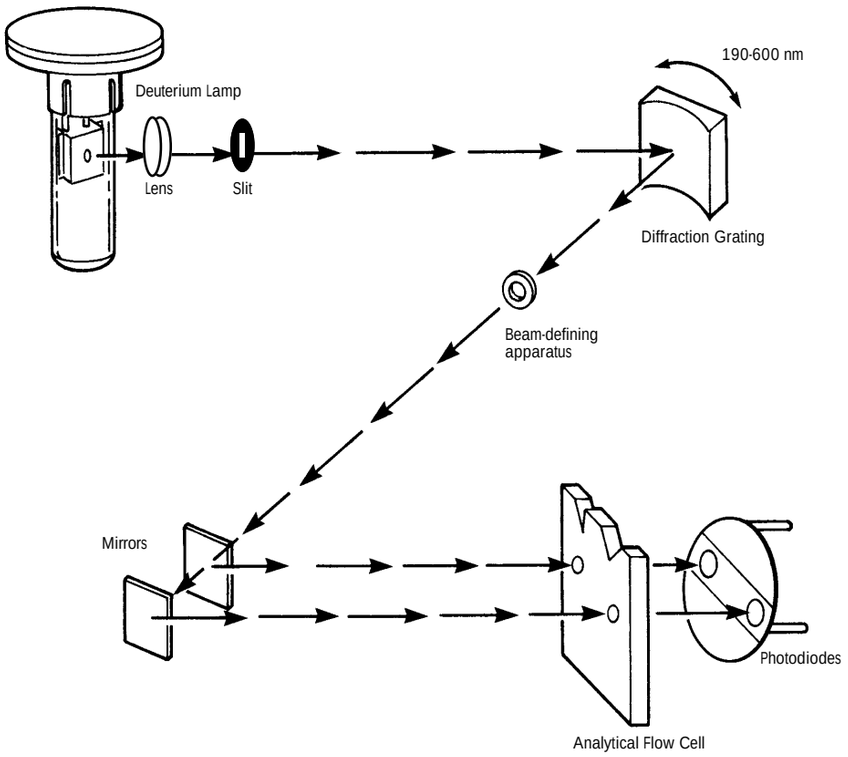


Figure 1-3 Waters 486 Detector Optics Assembly

1.2.2 Light Path Wavelength Calibration

During wavelength calibration, the Waters 486 Detector locates and references the 656 nm peak in the deuterium lamp emission spectrum to ensure that the wavelength calibration is accurate.

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Wavelength calibration is performed in two ways:

- Automatically on powerup
- On demand when using Diagnostic 4

When the Waters 486 Detector is run continuously, it is prudent to perform wavelength calibration weekly.

Calibration error codes

Calibration error codes are generated when:

- The maximum reference light beam energy is too low
- The slope change at the 656 nm emission peak is not found or if the peak is too small

These errors generally occur because deuterium lamp intensity has deteriorated significantly, or foreign matter is interfering with the light path. Suggestions for correcting calibration errors are listed in Chapter 6, Error Codes and Diagnostics.

1.2.3 Waters Taper-Cell

The Waters Taper-Cell, part of the Waters 486 Detector, eliminates some common absorbance detection problems.

Refractive index changes

A classical problem in absorbance detection is distinguishing between genuine absorbance and changes in energy due to refractive index (RI) phenomena. RI changes can occur due to the characteristics of solvent flow and composition.

Refractive index changes in the solvent cause light rays to bend within the flow cell, creating absorbance errors (Figure 1-4).

Gradient changes

Gradient elution can cause other changes to occur, such as refraction or reflection at the junction of the window and the cell. The light beam may also show sensitivity to variations in flow rate.

As a result, absorption errors occur, even when there is no analyte present.

True Absorption with Taper-Cell

The patented analytical Taper-Cell corrects problems associated with some refractive index phenomena.

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The Taper-Cell design ensures that all light entering the flow cell passes through the cell if no true absorption takes place. The cell walls diverge at an angle greater than the most extreme refraction angle, eliminating signal loss related to cell wall absorption (see Figure 1-4).

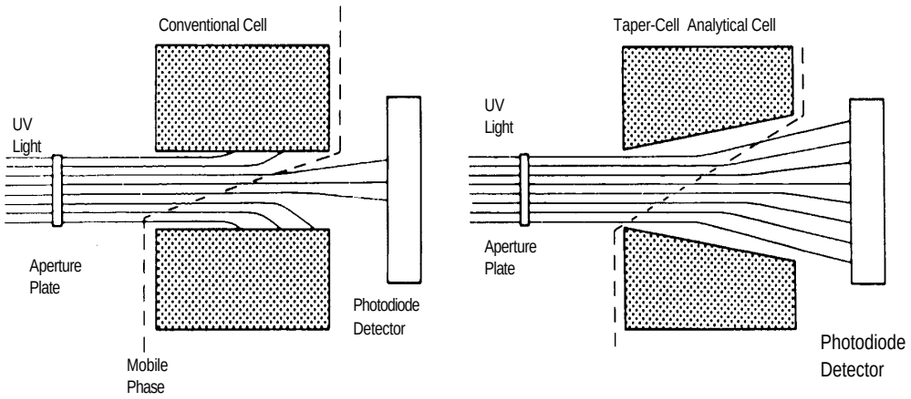


Figure 1-4 Comparison of Flow Cell Characteristics

Path length

The standard analytical, nonmetallic, and mass spectrometer cells have a path length of 10 mm. The semi-prep and microbore cell path length is 3 mm.

Hamming filter

The Waters 486 Detector uses a Hamming filter to minimize noise. The performance of the filter depends on the filter constant that you select. The filter constant adjusts the response time to achieve optimal signal-to-noise ratio.

Filter constants

Low filter constant settings:

- Produce narrow peaks with minimal peak distortion and time delay
- Remove less baseline noise
- Make very small peaks harder to discriminate from baseline noise

Higher filter constant settings:

- Shorten and broaden peaks
- Greatly decrease baseline noise

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The default time constant (value for the Filter parameter) of 1.0 second is satisfactory for most applications.

Calculating the filter constant

To calculate an appropriate time (filter) constant for special applications, use the formula:

$$TC = 0.2 \times PW$$

where:
TC = time constant (filter) setting

PW = peak width at half height of the narrowest peak

Figure 1-5 shows the relationship between increased time constant and response times.

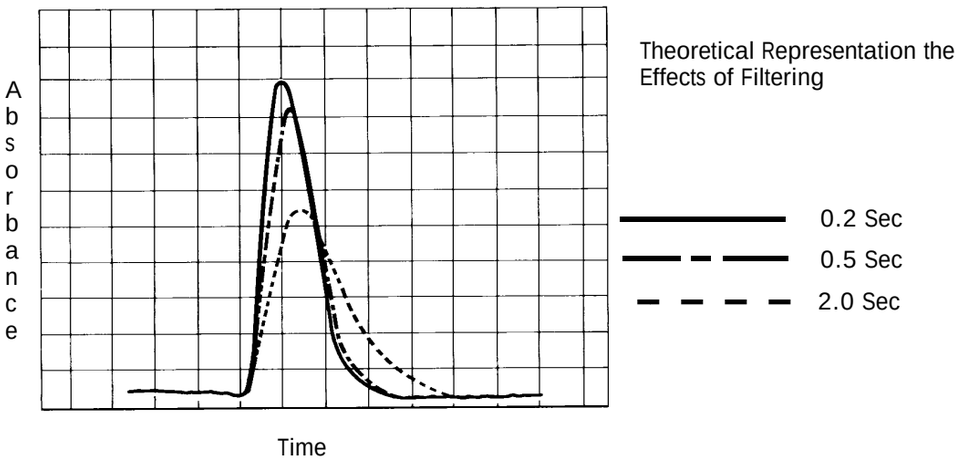


Figure 1-5 Time Constant (Filter Setting) Comparison

Note: *Although the peak shape shows some distortion and the signal output is delayed with different time constants, the peak area remains the same.*

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1.2.4 Waters 486 Detector Electronics

The Waters 486 Detector combines digital-based electronics, with inter-connecting printed circuits (PCs).

Electronics hardware

The 486 Detector consists of the following electronics:

- **Preamplifier** - Handles the interface of analog input signals from the optics to the microprocessor for further signal conditioning. Houses the photodiode and the log amplifier circuits.
- **Microprocessor Board** - Receives inputs from the preamplifier board, keyboard, and external events.
- **IEEE-488 Interface Board** - IEEE communication allows the Waters 486 to communicate with other Waters modules that have the IEEE-488 interface capability and the appropriate software.
- **Keypad** - Allows the user to program, calibrate and troubleshoot the Waters 486 detector.
- **Lamp Power Supply Board** - Controls the lamp power-up sequence and operation. Also acts as the DC power distributor for the system.
- **Low Voltage Power Supply Board** - Provides voltage for the analog and digital circuitry.

Electronics software

The 486 Detector software controls the following:

- Sensitivity and filter settings
- Wavelength selection
- Auto Zero
- Chart Polarity
- Chart (Inject) Mark
- IEEE-488 Interface
- Lamp On/Off Control
- Diagnostics
- Wavelength Scan

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1.3 Principles Of Absorbance Detection

When light is transmitted through a mobile phase containing a light-absorbing compound, the compound absorbs some of the light. The amount of light absorbed, expressed in absorbance units (A) is:

- Proportional to the concentration (C) of the compound in solution.
- Dependent on the wavelength of the light and the absorption spectrum of the component, which is unique to that compound (molar absorptivity, ε).
- Dependent on the distance that the light travels through the solution (path length, l).

Beer's Law

The relationship between absorbance and concentration is the concentration of the sample through which the light passes as described by the Lambert-Beer Law (commonly referred to as Beer's Law).

$$A = \varepsilon lc$$

For well-equilibrated dilute solutions, the detector response is linear with increasing concentration (Figure 1-6), assuming that the refractive index of the sample remains constant, the light is monochromatic, and minimal stray light reaches the detector.

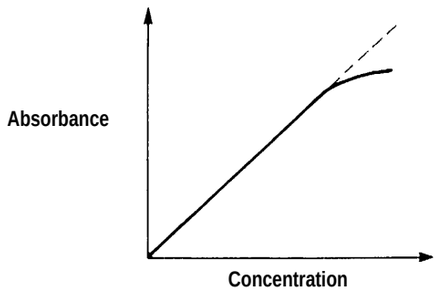


Figure 1-6 Absorbance versus Concentration

As concentration increases, the chemical and instrumental requirements of Beer's law may be violated, resulting in a deviation from linearity.

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Each compound has a unique absorption spectrum, absorbing strongly at some wavelengths and weakly or not at all at others. Figure 1-7 shows how absorbance changes as wavelength changes for a hypothetical compound.

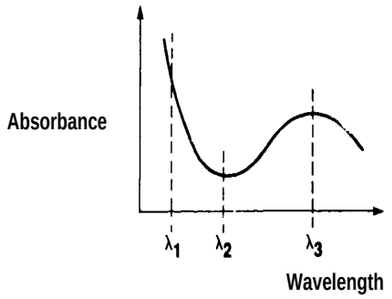


Figure 1-7 Absorbance Spectrum Example

Wavelength selection consideration

The maximum linear dynamic range between absorbance and concentration occurs at wavelengths where the sample shows the minimal amount of absorbance change with wavelength (for example, between λ_2 and λ_3).

Wavelength λ_2 yields good linear dynamic range but low sensitivity. Wavelength λ_3 produces both good linearity and high sensitivity. Wavelength λ_1 shows the highest sensitivity, but the linear dynamic range is not as good as in the other two cases.

Section 1.4.6, Wavelength Selection for Chromophore Detection Detection, contains a table representing chromophores. Refer to the table for help in determining λ_{max} for a particular sample matrix.

Where deviations in linear dynamic range between absorption and concentration occur, accurate quantification of a sample is still possible. To do so requires a nonlinear calibration model (quadratic, cubic, point-to-point) created from a series of samples with known concentrations.

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1.4 Solvent Considerations



Caution: *Observe safe laboratory practices when handling solvents. Refer to the Material Safety Data Sheets shipped with solvents.*

This section presents helpful information on choosing a solvent. Selection topics include:

- UV cutoff ranges for solvents and mixed mobile phases
- Solvent degassing information

Selecting a mobile phase

Selecting the correct mobile phase for your analysis is very important. An ideal mobile phase is transparent at the selected wavelength, generates a baseline with minimal noise, and provides suitable separation characteristics for the application.

Solvent purity

Clean the solvents to ensure:

- Reproducible results
- Operation with minimal instrument maintenance

Dirty solvents can cause:

- Baseline noise and drift
- Blocked solvent inlet filter
- Plugged column

Guidelines

Use HPLC-grade solvents to ensure the best results. Filter solvents through 0.45 µm membrane filters before use. These filters are found in the solvent Clarification Kit (see Appendix B, Spare Parts for part numbers).

When using water, use a high-quality water source Milli-Q® water or equivalent. If your water system does not provide filtered water, filter through a 0.45 µm membrane filter before use.

When using buffers, dissolve salts, adjust the PH, then filter to remove any insoluble material.

Note: *When using unstabilized tetrahydrofuran (THF), ensure that it is fresh. Previously opened bottles of THF contain peroxide contaminants that show strong UV absorption; these contaminants interfere with UV detection and increase UV background with time.*

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1.4.1 Solvent UV Cutoff Ranges

The UV wavelength at which the absorbance of an solvent is one is the UV cutoff of the solvent.

Table 1-1 summarizes the UV cutoff for some common chromatographic solvents. Operating at a wavelength near or below the cutoff will increase baseline noise, decrease the linear dynamic range and may result in a loss of response, (peak height or area may decrease).

Table 1-1 UV Cutoffs for Chromatographic solvents

Solvent	UV Cutoff (nm)	Solvent	UV Cutoff (nm)
N-Pentane	190	Methyl isobutyl ketone	334
Iso-octane	215	Tetrahydrofuran	230
Petroleum ether	210	Ethylene dichloride	230
Cyclohexane	200	Methyl ethyl ketone	330
Carbon tetrachloride	265	Dioxane	215
Amyl chloride	225	Ethyl acetate	256
Xylene	290	Methyl acetate	260
Isopropyl ether	220	Amyl alcohol	210
Isopropyl chloride	225	Diethyl amine	275
Toluene	285	Nitromethane	380
Cyclopentane	200	1-Nitromethane	380
Carbon disulfide	380	Acetone	330
Amyl chloride	225	Ethyl acetate	256
Xylene	290	Methyl acetate	260
n-Propyl chloride	225	Acetonitrile	190
Benzene	280	Pyridine	330

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Table 1-1 UV Cutoffs for Chromatographic solvents *(Continued)*

Solvent	UV Cutoff (nm)	Solvent	UV Cutoff (nm)
Ethyl ether	220	2-Butoxyethanol	220
Ethyl sulfide	290	Isopropanol	205
Chloroform	245	n-Propanol	210
Methylene chloride	233	Ethanol	210
Methanol	205	Ethylene glycol	210

Table 1-2 contains approximate wavelength cutoffs for other solvents, and mobile phase components.

Table 1-2 UV Cutoffs for Common Mobile Phase Components

Mobile Phase	UV Cutoff (nm)	Mobile Phase	UV Cutoff (nm)
Acetic acid, 1%	230	Hydrochloric acid, 0.1%	190
Trifluoroacetic acid, 0.1%	205	Diammonium phosphate, 50 mM	205
Triethylamine, 1%	235	Waters PIC® Reagent A, 1 vial/L	< 200
PIC Reagent B-6, 1 vial/liter	225	PIC Reagent B-6, low UV, 1 vial/L	190
PIC Reagent D-4, 1 vial/liter	190	Ammonium acetate, 10 mM	205
Ammonium bicarbonate, 10 mM	190	EDTA, disodium, 1 mM	190
HEPES, 10 mM; pH 7.6	225	MES, 10 mM, pH 6.0	225

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Table 1-2 UV Cutoffs for Common Mobile Phase Components *(Continued)*

Mobile Phase	UV Cutoff (nm)	Mobile Phase	UV Cutoff (nm)
Potassium phosphate, monobasic, 10 mM; dibasic, 10 mM	190	Sodium acetate, 10 mM	205
Sodium chloride, 1 M	208	Sodium citrate, 10 mM	225
Sodium formate, 10 mM	200	Tris HCl, 20 mM, pH 7.0; pH 8.0	204 212
BRIJ 35, 0.1%		CHAPS, 0.1%	215
Sodium dodecyl sulfate, 5 mM		Triton-X™ 100, 0.1%	240

1.4.2 Solvent Degassing

Using degassed solvents is important, especially at wavelengths below 220 nm. Degassing provides:

- Stable baselines and enhanced sensitivity
- Reproducible retention times for eluting peaks
- Stable pump operation

1.4.3 Theory of Solvent Degassing

This section presents the theory involved in solvent degassing, some solvent degassing methods, and solvent degassing considerations.

Only a finite amount of gas can dissolve in a given volume of liquid under specific conditions. This amount depends on the temperature of the liquid, the pressure applied to the liquid, and the chemical affinity of the gas for the liquid.

Effects of intermolecular forces

Generally, a gas is most soluble in a solvent where the intermolecular attractive forces between molecules of the solvent are similar to those of the gas. (“like dissolves like”) If the main attractive forces are the Van der Waals forces, the gas is more soluble in this solvent than in one with dipole forces or hydrogen bonding. Thus, larger amounts of hydrogen, nitrogen, oxygen, or helium dissolve in alkanes and alcohol than in water.

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Effects of temperature

Temperature affects the solubility of gases in two ways:

- The higher the boiling point of a solvent, the more soluble a gas is a given solvent.
- The Increasing the temperature of the gas-liquid solution affects the percentage of gas in solution.

If the heat of solution results in:

- An exothermic reaction, the percentage of gas in solution decreases.
- An endothermic reaction, the percentage of gas in solution increases.

For example, the solubility of helium in water decreases with an increase in temperature, however, its solubility of helium in water decreases with an increase in temperature, yet the solubility of helium 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 the pressure of the gas decreases, the amount of that gas in solution also decreases.

Effects of dissolved oxygen

Dissolved oxygen affects UV/VIS detector performance in several different ways.

Oxygen dissolved in solvents may form a UV-absorbing complex. The amount of complex formed varies with the solvent. The effect is particularly evident in the solvents methanol and tetrahydrofuran (THF). Dissolved oxygen has a minimal affect on the absolute responsivity of a UV detector, however; it can cause:

- Increased noise due to less light reaching detector
- Baseline drift
- Increased temperature response
- Reduced linear dynamic range

Changes in the characteristics of a solvent may adversely affect the precision and accuracy of the solvent delivery pump. This may cause variations in component retention time and peak height or area.

The best solution does not result in an endothermic or an exothermic reaction. Dissolving the solute either consumes heat, an endothermic reaction, or liberates heat.

- For an endothermic reaction, increasing the temperature will cause the concentration of dissolved gas to increase.
- For an exothermic reaction, increasing temperature will decrease the concentration of dissolved gas.

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1.4.4 Solvent Degassing Methods

There are four main methods of degassing solvent:

- Sparging with a less soluble gas
- Heating
- Reducing pressure through vacuuming
- Sonication

These methods may be used individually or combined:

Sparging

Sparging, or bubbling through solvent, displaces other gases from the solution while saturating the solvent with helium. Sparging decreases background absorbance in UV detectors, and improves pump performance. Gas sparging brings the solvent to a state of equilibrium that can be maintained by keeping either a blanket of sparge gas over the solvent, or by maintaining a constant, low-rate dispersion of the gas through the solvent.

Heat

Heat raises the vapor pressure of the solvent at its surface. As the partial pressure of the solvent is raised, the partial pressure of the ambient gas decreases proportionately, thus preventing further absorption of the gas. This effect occurs with most gasses in water, but not necessarily with organic solvents.

Vacuum

A vacuum reduces the pressure on the surface of the solvent. Since the mass of gas in solution is proportional to the partial pressure of the gas at the surface of the solvent, the mass of gas in solution decreases as the pressure decreases.

Sonication

Sonication causes the submicron-sized bubbles of gas to aggregate. As the gas aggregates, the bubbles become large enough to float out of the solvent and dissipate. Sonication alone degasses a gallon of solvent in approximately 20 minutes. However, sonication does not remove as much oxygen as sparging.

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1.4.5 Solvent Degassing Considerations

When selecting a degassing method for your application, consider the following:

- Helium sparging provides stable baselines and prevents reabsorption of atmospheric gasses.
- To degas a solvent using heat, you must boil the solvent. Lower levels of heat will reduce the rate of dissolution of the gas.



Caution: *It is dangerous to apply vacuum to the brown gallon bottles in which solvent is shipped. There is a high risk of implosion under these conditions.*

- Vacuum alone is too slow to be an acceptable means of degassing solvent. However, a 0.5-micron sintered glass Millipore™ vacuum filter with just under 300 mm of vacuum, can filter and degas a gallon of solvent in approximately eight minutes. However, vacuum degassing may change the composition of mixed solvents.
- Sonication plus vacuum can degass one liter of solvent in minutes. This technique is less likely to change the composition of mixed solvents because the solvents are held under the vacuum for only a short time.

1.4.6 Wavelength Selection for Chromophore Detection

Certain functional groups found in most compounds absorb light selectively. These groups, known as chromophores, and their behavior can be used to categorize the detection of sample molecules.

Table 1-3 lists some common chromophores, and their detection wavelengths (λ_{max}), as well as the molar absorptivity (ε_{max}) of each group. Use this information as a guide to select the optimal operating wavelength for a particular analysis. Because of the diversity possible within a given sample, scanning over a range of wavelengths may be necessary to determine the best wavelength for a particular analysis.

Table 1-3 Electronic Absorption Bands of Representative Chromophores[1]

Chromophore	Chemical Configuration	λmax (nm)	$\in$ max (L/m/cm)	λmax (nm)	$\in$ max (L/m/cm)
Ether	—O—	185	1000		
Thioether	—S—	194	4600	215	1600
Amine	—NH_2	195	2800		

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Table 1-3 Electronic Absorption Bands of Representative Chromophores[1] *(Continued)*

Chromophore	Chemical Configuration	λmax (nm)	$\in$max (L/m/cm)	λmax (nm)	$\in$max (L/m/cm)
Thiol	—SH	195	1400		
Disulfide	—S—S—	194	5500	255	400
Bromide	—Br	208	300		
Iodide	—I	260	400		
Nitrile	—C≡N	160	—		
Acetylide	—C≡C—	175-180	6000		
Sulfone	—SO_2 —	180	—		
Oxime	—NOH	190	5000		
Azido	>C=N—	190	5000		
Ethylene	—C=C—	190	8000		
Ketone	>C=O	195	1000	270-285	18-30
Thioketone	>C=S	205	strong		
Esters	—COOR	205	50		
Aldehyde	—CHO	210	strong	280-300	11-18
Carboxyl	—COOH	200-210	50-70		
Sulfoxide	>S—O	210	1500		
Nitro	—NO_2	210	strong		
Nitrile	—ONO	220-230	1000-2000	300-400	10
Azo	—N=N—	285-400	3-25		
Nitroso	—N=O	302	100		

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Table 1-3 Electronic Absorption Bands of Representative Chromophores[1] *(Continued)*

Chromophore	Chemical Configuration	λmax (nm)	$\in$ max (L/m/cm)	λmax (nm)	$\in$ max (L/m/cm)
Nitrate	—ONO_2	270 (shoulder)	12		
	—$(C=C)_2$— (acyclic)	210-230	21,000		
	—$(C=C)_3$—	260	35,000		
	—$(C=C)_4$—	300	52,000		
	—$(C=C)_5$—	330	118,000		
	—$(C=C)_2$— (alicyclic)	230-260	3000-8000		
	C=C—C≡C	219	6,500		
	C=C—C=N	220	23,000		
	C=C—C=O	210-250	10,000-20,000		
	C=C—NO_2	229	9,500		

[1]Willard, H. H. and others. *Instrumental Methods of Analysis*, 6th ed. Litton Educational Publishing, Inc., 1981.

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2 Installation and Instrument Verification

After installing the Waters 486 Tunable Absorbance Detector, you should verify its functions and keep the verified chart recording on file. Verification ensures proper operation of the detector optics and electronics.

Figure 2-1 illustrates the size and the physical characteristics of the Waters 486 Detector.

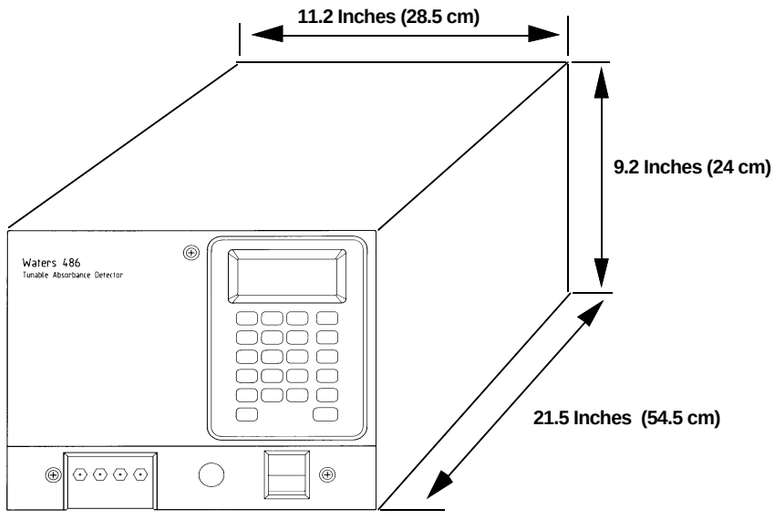


Figure 2-1 Waters 486 Tunable Absorbance Detector

2.1 Site Selection and Power Requirements

Site selection requirements

Install the Waters 486 Detector in an area where:

- Temperature is maintained at 15 to 32°C. Avoid direct sunlight, heat registers, and air-conditioning vents.

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- Relative humidity is 20 to 80 percent, non-condensing.
- Surface is level, to allow proper function of the drip tray, which diverts solvent leaks from the flow cell or front fluid connections.
- There is a 5-inch clearance at the back of the unit and a two-inch clearance on each side, to provide adequate air circulation.

Power requirements

The Waters 486 Detector requires:

- Grounded alternating current (AC) power source.
- Minimal power transients and fluctuations.
- Line voltage of 100 to 120 Vac nominal or 220 to 240 Vac nominal. Power consumption is 100 volt amps (VA).

The Waters 486 Detector can be adapted to operate in nominal voltage ranges as indicated in Table 2-1

Table 2-1 Voltage and Fuse Requirements

Nominal Voltage	Voltage Range	Fuse Required
100 Vac, 50/60 Hz	85 to 110	MDL 1.5A, SLO-BLO, 1 1/4-inch long F 1.25A/250V, FAST BLO, 20 mm long
120 Vac, 50/60 Hz	102 to 132	MDC 1.5 A, SLO-BLO, 1 1/4-inch long F 1.25A/250V, FAST BLO, 20 mm long
220 Vac, 50/60 Hz	187 to 242	MDC .75 A, SLO-BLO, 1 1/4-inch long F .63A/250V, FAST BLO, 20 mm long
240 Vac, 50/60 Hz	204 to 264	MDC .75 A, SLO-BLO, 1 1/4-inch long F .63A/250V, FAST BLO, 20 mm long

Line frequency ranges are 47 to 53 Hz at a nominal setting of 50 Hz, and 57 to 63 Hz at a nominal setting of 60 Hz.

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Power cords for use in North America

Use the appropriate power cord for the selected voltage, as shown in Table 2-2.

Table 2-2 North American Power Plugs

Voltage	Power Cord Part Number
100, 120 V	Waters 97194
220, 240 V	Waters 49760

Power cords for International use

If a power plug other than the one supplied is needed for use in the laboratory, consult the color codes (Table 2-3) and choose an appropriate replacement. Be sure to observe all applicable local regulations.

Table 2-3 International Power Plugs

International	USA	Connection
Blue	White	Neutral
Green/Yellow	Green	Ground (Earth)
Brown	Black	Hot

2.2 Unpacking and Inspecting

List of components

Contents of the shipping carton include:

- Waters 486 Tunable Absorbance Detector
- Power cord for 110V operation
- Startup Kit
- *Waters 486 Tunable Absorbance Detector Operator's Manual*

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Unpacking

Find the packing list. As you unpack, check the packing list against the contents of the shipping carton.

Save the shipping carton for future transport or shipment.

Reporting damage

Inspect all items for damage. Immediately report any damage to both the shipping carrier and to Waters Technical Service. You can contact Waters Technical Service at (800) 252-4752, *Canadian and U.S. customers only.* Other customers, call your local Waters subsidiary or your local Waters Technical Service representative, or call Waters corporate headquarters for assistance at (508) 478-2000 (U.S.).

If any items are damaged, use the shipping container for subsequent claim purposes.

See Appendix C, Warranty/Serial Number Information, for information on returning a damaged shipment.

2.3 Voltage Selection and Fuse Installation

The Waters 486 Detector voltage selector card is accessible from the fuse holder module, located to the right of the AC receptacle on the rear panel (see Figure 1-2).



Caution: *To avoid the possibility of electrical shock, be sure the detector is off and unplugged before performing these procedures.*

Voltage selection

To select the voltage, you must first:

1. Remove the fuse holder module by inserting a screwdriver as shown in (Figure 2-2).

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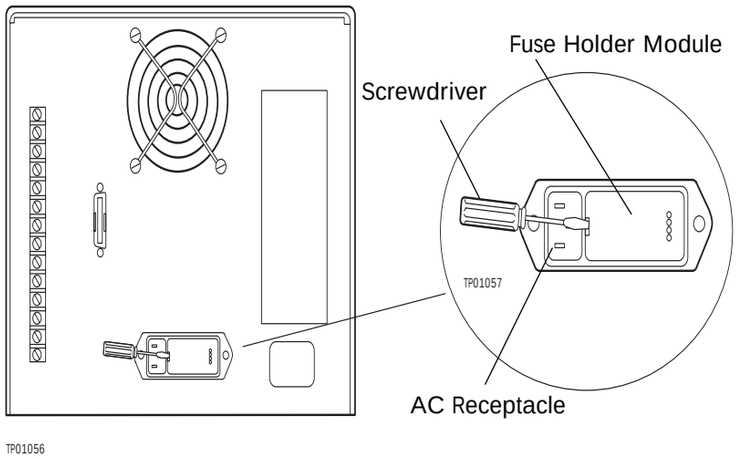


Figure 2-2 Voltage Selection

2. Remove the voltage selector card and set it to the desired voltage.
3. Reinstall the voltage selector card and the fuse holder module.

Fuse installation

You must install a fuse before operating the detector. The Waters 486 Detector is shipped without fuses. All the required fuses are found in the Startup Kit.

To install a fuse:

1. Remove the fuse holder module by inserting a screwdriver as shown in Figure 2-3.

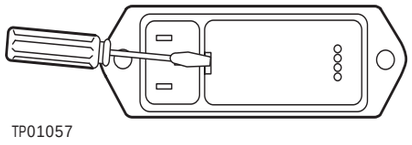


Figure 2-3 Fuse Installation

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2. Select the appropriate fuse for your site requirements from Table 2-4.
3. Install the fuse, and reinstall the fuse holder module.

Table 2-4 Voltage and Fuse Requirements

Nominal Voltage	Voltage Range	Required Fuse
100 Vac, 50/60 Hz	85 to 110	MDC 1.5 A, 1 1/4 inches long F 1.25 A/250 V, 20 mm long
120 Vac, 50/60 Hz	102 to 132	MDC 1.5 A, 1 1/4 inches long F 1.25A/250 V, 20 mm long
220 Vac, 50/60 Hz	187 to 242	MDC .75 A, 1 1/4-inch long F .63 A/250 V, 20 mm long
240 Vac, 50/60 Hz	204 to 264	MDC .75 A, 1 1/4-inch long F .63A/250V, 20 mm long

2.4 Instrument Verification

After you have installed the Waters 486 Tunable Absorbance Detector, verify that the detector is operating properly by performing the procedures provided in this section.

Verification procedures and assumptions

This section provides instructions for the following:

- Recording sample and reference energy
- Noise and drift tests
- Peak response test
- Diagnostics

If these procedures are successful, the Waters 486 Detector optics and electronics are functioning properly.

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Before you begin

Before beginning the procedures in this section, connect the 10-mV output to a recorder of 10-mV output. Refer to Section 3.3.2, Chart Recorder Connections. Set the chart speed to 5 cm/min.

Powerup and calibration

Turn on the Waters 486 Detector. The front panel displays:

- **486** and the software revision number
- **486** and **CAL** (calibrating)

The calibration process takes approximately three minutes. When calibration is successful, the front panel displays the default wavelength (254) and current absorbance reading.

If calibration fails, note the error code to determine the corrective action. Refer to Chapter 6, Error Codes and Diagnostics.

Reference scan

To monitor lamp aging (decreased lamp output energy), record the initial reference scan to compare against future scans.

1. Connect a chart recorder to the Reference Energy Out terminal on the rear panel of the Waters 486 Detector.
2. Set the wavelength to 190.
3. Start the chart recorder.
4. Set the wavelength to 600.
5. Record sample and reference beam energies for future reference (Section 2.4.1, Recording Sample and Reference Beam Energies).

Flushing the flow cell

Before performing the following procedures, flush the flow cell with approximately 30 to 60 mL of HPLC-grade methanol at a flow rate of 1 mL/min to clean the cell.

Verify test failures

If a test fails, repeat the test.

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2.4.1 Recording Sample and Reference Beam Energies

Purpose

You need to record the sample and reference beam energies to determine baseline values for future reference.

Use the *baseline values* when troubleshooting the 486 Detector to determine whether the:

- Solvent is dirty
- Flow cell is dirty
- Lamp needs to be replaced

Recording procedure

Set the wavelength to 230 nm by pressing the λ key and entering **230**. Wait 10 seconds, then press the **Display** key to sequence through the sample and reference energy values. Record the values for further reference.

2.4.2 Noise and Drift Tests

Purpose

The noise and drift test is performed to ensure that the baseline is stable over a period of time. The ambient temperature must be constant before and during the test.

Either of the following methods may be used:

Dry - No solvent flowing, checks only the electronics

Wet - Solvent flowing, checks solvent and flow cell

Note: *Before beginning the wet test, be sure to make the appropriate fluid connections. Refer to Section 2.5, Making Fluid Connections.*

Note: *Before beginning the dry test procedures, connect the 10 mV absorbance output to a 10 mV recorder. Refer to Section 3.3.2, Chart Recorder Connections.*

Before you begin

Set the chart speed to 5 cm/min.

Dry test procedure

1. Set the sensitivity level for the Waters 486 Detector by pressing the **AUFS** key on the front panel. When the sensitivity LCD appears, enter **.001**.
2. Set the wavelength to **230, 254,** or **280** nm.

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3. Set the Hamming filter to 1.0 seconds.
4. Record the baseline for 10 minutes.
5. Determine the noise level by measuring the height of the largest noise peak on the recorder printout.
6. Determine the drift level by drawing the mean baseline through the baseline trace on the recorder printout.

Result

The test has completed successfully when the noise level is less than or equal to 2 percent, peak to peak, and the drift is less than or equal to 10 percent per hour after sufficient warm-up (about 1 hour).

Wet test procedure

After Successful completion of the dry test, proceed with the wet test as follows:

Before performing the wet test, verify that the solvent has been thoroughly degassed. Refer to Section 1.4.2, Solvent Degassing.

1. Without a column attached, connect approximately two feet of 0.009-inch ID tubing from the injector to the inlet port of the detector.
2. Connect a second length of 0.009-inch tubing from the outlet port of the detector.
3. Set the pump flow to 1.0 mL/min. Make sure the pump flow is not pulsing.
4. Using the AUFS, wavelength, and filter settings from the dry test, record the baseline for 10 minutes.
5. Measure the noise and drift as described in step 5 and step 6 of the dry test procedure.

Before you begin

The test has completed successfully when:

- Noise level is less than or equal to 2 percent, peak to peak.
- Drift level is less than or equal to 10 percent, per hour after warm

2.4.3 Peak Response Test

Note: *Make the fluid connections described in Section 2.5, Making Fluid Connections, before beginning the Peak Response test.*

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Purpose

The Peak Response test (optional) is used to check the level of peak responses.

Procedure

To check peak response levels:

1. Set the sensitivity to **2.0** AU.
2. Set the wavelength to **254** nm.
3. Set the pump flow rate to **1.0** mL/min.
4. Inject **1** µL acetone.

Result

When the test is successful, the peak height is equal to or larger than 75 percent of full scale. Refer to Section 4.5, Making a Test Run, for the complete procedure.

Perform the user diagnostic tests (in this section) to verify operation of the Waters 486 Detector electronics. Refer to Chapter 6, Error Codes and Diagnostics, for additional information on using the diagnostics.

The diagnostics described in this section check:

- Absorbance signal
- Output signal
- Auto Zero
- Front panel and keys
- Peak response

Performing diagnostics

To perform diagnostics:

1. Press the **Diag** key.

   The detector displays ddd on the lower display, indicating diagnostic mode. Refer to Chapter 6, Error Codes and Diagnostics, for a list of the diagnostic error codes.

2. To access a particular diagnostic, enter the number code of the diagnostic as indicated in the following procedures, and press **Enter.**

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2.4.4 Absorbance Signal Test

Purpose

The Absorbance Signal test sets the absorbance signal at a given voltage output. You can then verify that the voltage selected through the software is being sent through the output terminal on the rear panel.

Note: *Do not perform an auto zero test before performing an absorbance signal test if you have a 486 version 1.0. If an auto zero was performed during a wavelength change or a manual auto zero, be sure to power down and restart the Waters 486 Detector.*

Procedure

To perform the absorbance test:

1. Connect the 10mV recorder to the 10mV absorbance output.
2. Set the detector sensitivity to 0.002 AUFS.
3. Select **Diagnostic 5,** Fix Absorbance Input.
4. Press **Enter** twice to advance to the 0.001 AU value.
5. With the 0.001 AU setting displayed, record the baseline for 10 minutes.
6. After the 10-minute period, press **Enter** several times to exit the diagnostic.

Results

When the test is successful:

- The baseline is at 50 percent of full scale
- The noise level peak is less than or equal to 1 percent during the run.

Purpose

The Output Test ensures that voltages (selected in volts) are properly communicated through the 1-V output terminal to the recording device on the detector.

Procedure

To perform the output test:

1. Connect the 10mV recorder to the 1V absorbance output.
2. Select **Diagnostic 6,** Fix Integrator Output.
3. Press **Enter** to advance through the voltage selections and exit from the diagnostic.

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Result

When the test is successful, the recording device shows an appropriate change in signal for each voltage selected.

2.4.5 Output Test (Millivolts)

Purpose

The Output test checks that the voltages selected (in millivolts) are properly communicated through the 10mV output terminal to the recording device on the Waters 486 Detector.

Procedure

To perform the output test:

1. Connect the 10-mV recorder to the 10-mV absorbance output.
2. Enter **Diagnostic 7, Fix Recorder Output.**
3. Press **Enter** to advance through the voltage selections and exit from the diagnostic.

Result

When the test is successful:

The recording device shows a change that corresponds to the selected value in the voltage selection.Auto Zero Test

2.4.6 Auto Zero Test

Purpose

The **Auto Zero** test checks the amount of offset correction (- 0.3 to +0.9 AU) applied by the Auto Zero function.

Procedure

To perform the Auto Zero Test:

1. Press the **Auto Zero** key on the front panel.
2. Select **Diagnostic 8, Display Auto Zero**. Note the offset value that displays.
3. Select **Diagnostic 9, Cancel Auto Zero** to return the offset value to zero.
4. Press the **Auto Zero** key again to reset the absorbance to zero.

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Result

If there is any baseline deviation, auto zero returns the baseline to zero. Diagnostic 8 displays the AU value by which the baseline was corrected. A large offset may indicate dirty solvent. Diagnostic 9 removes the correction.

2.4.7 Front Panel and Keypad Test

Purpose

The front panel and keypad test checks that all indicators, displays, and keys function properly during operation.

Procedure

To perform the front panel and keypad test:

1. Select **Diagnostic 10, Front Panel Display Test**, to check indicators and displays. Each display element glows as the test sequences. When complete, the system exits the diagnostic program.
2. Select **Diagnostic 11, Keypad Test**, to check keypad functions.
3. Press each key to verify that the appropriate character or indicator appears.

Result

If there are no indicators lit, or if an inappropriate character displays when a key is pressed, there may be a problem with the front panel or microprocessor board.

2.4.8 Peak Response

Purpose

This test generates a test peak that is used to check the microprocessor board communication between the Waters 486 Detector and the recording device connected to the detector.

Procedure

To perform the Peak Response Test:

1. Enter **Diagnostic 14, Test Peak**.
2. Press **Enter** to start the test peak generation. The absorbance value on the front panel changes as the peak plots.
3. Compare the peak height to full scale.

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Result

A Gaussian peak 1 AU full scale is generated. If you repeat the test peak procedure again, there should be less than 1 percent error between peaks. If the peaks are integrated, each peak should have the same area value.

2.5 Making Fluid Connections

Caution: *Observe safe laboratory practices when handling solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

You need to make the following connections on your detector unit:

- Column connections
- Drip tray connections

Before completing the column connections, perform the verification procedures described in Section 2.4, Instrument Verification.

Fluid connections to the Waters 486 Detector are located on the solvent manifold of the detector, on the left side of the front panel, as shown in Figure 2-4 below:

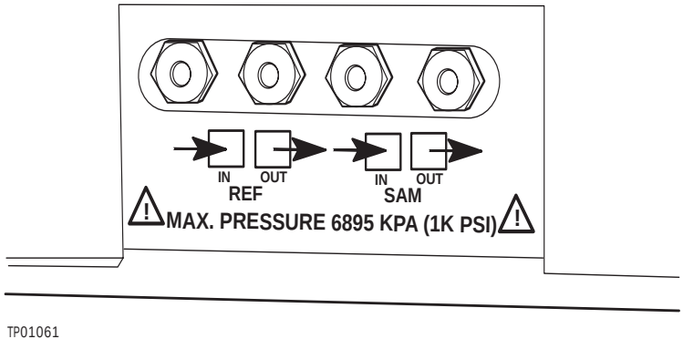


Figure 2-4 Waters 486 Detector Fluid Connections

Preparation

The following materials are needed to connect a column:

- Compression fittings and ferrules
- 5/16-inch open-end wrench

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- 1/16-inch stainless steel tubing, .009-inch ID
- Waters 1/16-inch stainless steel tubing cutter or file with cutting edge

Connecting a column

To connect a column:

1. Measure the minimum lengths of tubing needed to connect the:
   - Inlet on the lower left of the front panel to the outlet of the column
   - Column outlet to a waste collection bottle
2. Cut the two lengths of tubing:
   - 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-covered pliers (to prevent marring the surface) and gently work the tubing back and forth until it separates.
   - File the ends smooth and straight for maximum column efficiency.
3. Assemble the outlet connection from the column to the Waters 486 Detector inlet on the front panel. Slide the compression fitting over the tube followed by the ferrule, large end over the taper first (see Figure 2-5).

Figure 2-5 illustrates compression screw connections.

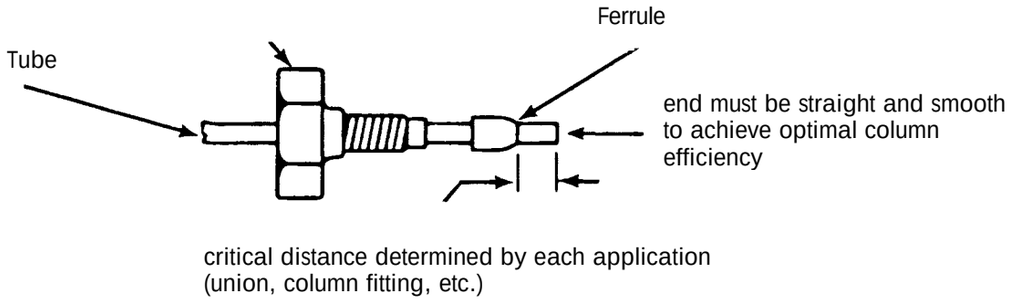


Figure 2-5 Compression Screw Assembly

4. Bottom the tube in the fitting seat to determine the distance between the end of the tube and the ferrule.
5. Seat the ferrule by tightening 3/4 turn past finger tight with a 5/16 inch open-end wrench.

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6. Repeat steps 3 through 5 to connect the detector outlet to a waste collection bottle.



Attention: *The maximum pressure for the Waters 486 Detector analytical flow cell is 1000 psi. The flow cell, beam splitter, and grating may be damaged if the pressure is exceeded.*

Drip tray connection

The detector contains a drip tray underneath the flow cell to divert solvent that may leak from the cell or fluid connections to the front of the unit. Connect 1/8-inch Teflon® tubing from the teflon elbow fitting located underneath the solvent manifold to a waste container. Be sure to make the connection to the waste container a level lower than that of the instrument to allow flow.

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3 Making Electrical Connections

This chapter describes the procedures you follow when making IEEE-488 or screw terminal connections from the Waters 486 Detector to other Waters devices.

IEEE-488 Connection Devices:

- Waters Millennium Chromatography Manager (BUS LAC/E card)
- Waters ExpertEase™ 845/860 Data System (LAC/E™ module)

Screw Terminal Connection Devices: (non-IEEE-488 connections)

- Waters 600 Series Pumps
- Waters 745/745B/746 Data Module
- Waters 700 Series Autosampler (717 plus, 717, 715, 712)
- Waters U6K Injector
- Waters Chart Recorder

3.1 Waters 486 Detector Rear Panel

The Waters 486 Detector connects to other Waters components through rear panel electrical connections (see Figure 3-1 and Figure 3-2).

Rear panel connections enable the following signals:

- **Analog output** - Transmits1V, 10mV, and reference energy signals to an external device
- **External trigger input** - Accepted from an external device, including Chart Mark, Auto Zero, and Lamp On/Off
- **IEEE-488 Interface Bus** - Transmitted/received through an IEEE-488 cable

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Figure 3-1 illustrates the detector rear panel connections.

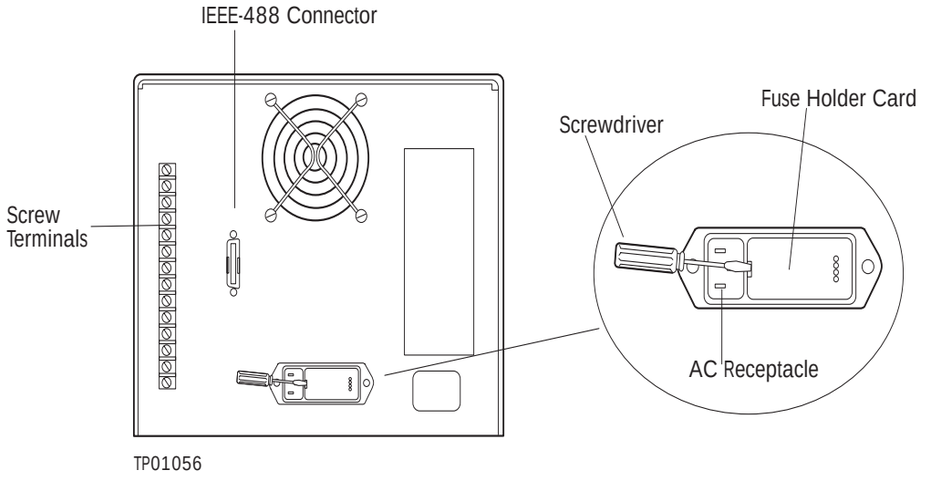


Figure 3-1 Waters 486 Detector Rear Panel Connectors

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Figure 3-2 illustrates the Waters 486 Detector rear panel screw terminal connections.

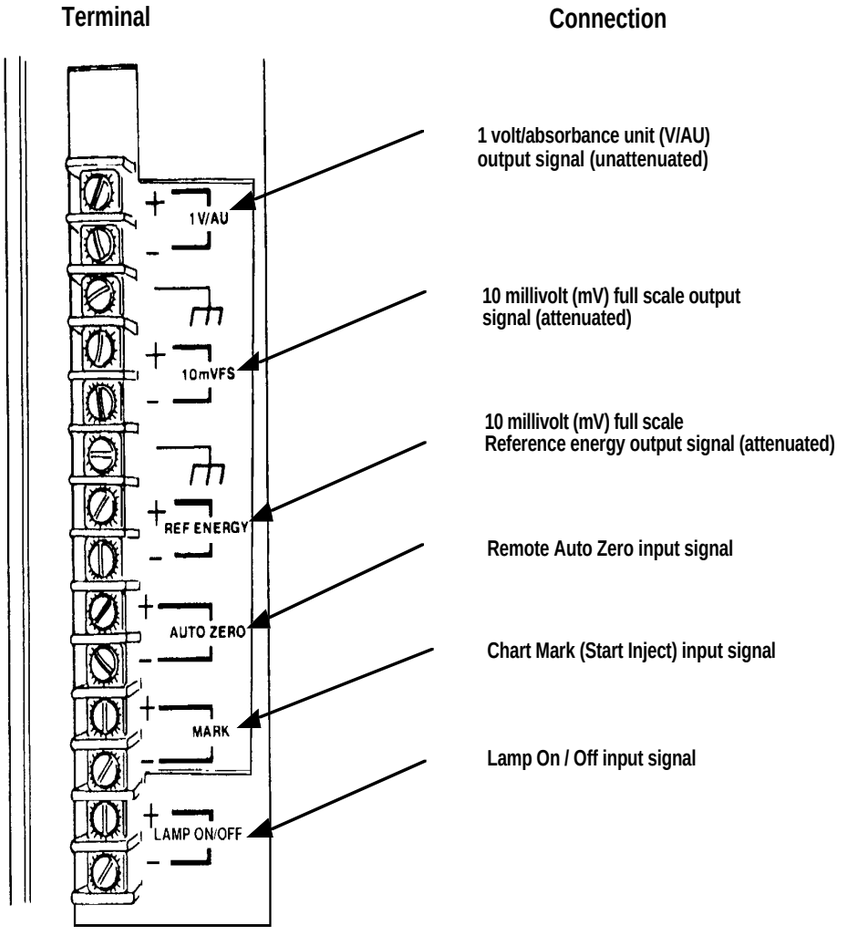


Figure 3-2 Rear Panel Screw Terminal Connections

Functions of the rear panel electrical connections are described in Table 3-1.

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Table 3-1 Waters 486 Detector Rear Panel Connections

Connection	Function
IEEE-488	Enables interface connection from the Waters 486 detector to the IEEE-488 communication bus on other Waters data products and instrumentation.
1 V/AU	Sends a 0 to 2V full scale signal that corresponds to the actual absorbance (0 to 2 AU) that is not affected by the AUFS setting to a digital integrator.
10 mVFS	Sends a 0 to ±10-mV full scale signal corresponding to the AUFS setting on the Waters 486 detector to a 10-mV recorder.
Reference Energy	Sends a 0 to 10-mV full scale signal corresponding to the lamp energy in the reference cell.
Auto Zero	Accepts a switch closure signal sent from the injector or 600 Series Controller at injection to automatically zero the detector.
Mark	Accepts a switch closure from the injector or 600 Series Controller providing a 1-mV pulse on the 10-mV analog output terminals. A 10 percent deflection in the baseline marks the injection. You can select a chart mark deflection on the 1V output by using Diagnostic 25.
Lamp On/Off	Allows you to program the lamp to turn on or off automatically through a contact or switch closure. Programmable switch closures are available on several Waters instruments.

For details on making IEEE-488 interface connections refer to Section 3.2, Making IEEE-488 Interface Connections.

For details on making screw terminal connections with external devices refer to Section 3.3, Making Screw Terminal Connections.

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Attaching the AC power cord

To attach the AC power cord to the Waters 486 detector:

1. Insert the D-shaped connector end of the power cord into the AC power receptacle on the Waters 486 detector rear panel (see Figure 3-1).
2. Insert the other end of the power cord into the wall outlet. Refer to Section 2.3, Voltage Selection and Fuse Installation for more information on operating voltages.

3.2 Making IEEE-488 Interface Connections

This section describes the procedures you use to make IEEE-488 interface connections This section describes:

- Making IEEE-488 connections with data systems
- Making IEEE-488 connections with 600 Series PowerLine Controllers
- Setting IEEE-488 addresses

3.2.1 Making IEEE-488 Connections with Data Systems

When controlling the Waters 486 detector from a Waters data system (Millennium Chromatography Manager or Waters 845/860 workstation), use the IEEE-488 interface to receive information from the data system. The data system operates as the system controller on the IEEE-488 interface.

To connect IEEE-488 cables to a Waters data system:

1. Connect the single receptacle end of the IEEE-488 cable (supplied with either the Waters 486 detector or the Waters data system) to your data system:
   - **Millennium Chromatography Manager** - Attach the cable to the Bus LAC/E™ (Laboratory Acquisition and Control/Environment) card (Figure 3-3)
   - **Waters 845/860 Workstation** - Attach the cable to the LAC/E module (Figure 3-4)

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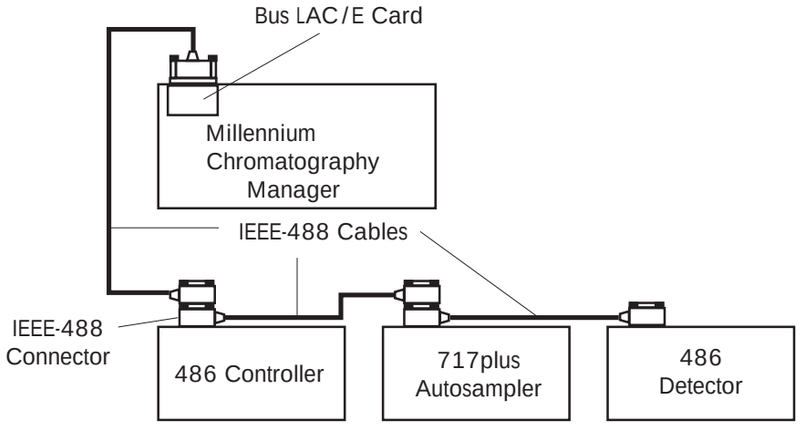


Figure 3-3 IEEE-488 Connections in a Millennium System

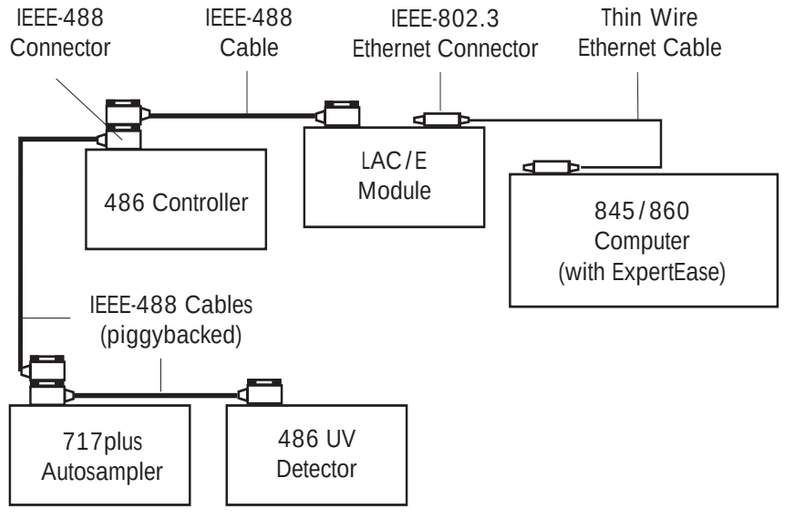


Figure 3-4 IEEE-488 Connections in Waters 845/860 System

Connect the other end of the cable (stackable connector for daisy-chaining additional instruments) to the IEEE-488 connector on the Waters 486 detector rear panel.

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2. If you are using the Waters 486 detector as part of a multicomponent configuration (as illustrated in Figure 3-3 and Figure 3-4), connect a second IEEE-488 cable to the “stackable” connector on the detector. Connect the other end of the cable to the IEEE-488 port on the next component.
3. Repeat steps 2 and 3 for each additional component.

   Note: *The maximum total IEEE-488 cable length in a system is 65 feet (20 meters). The maximum recommended cable length between two IEEE-488 devices is 13 feet (4 meters). Longer total cable lengths can cause intermittent IEEE-488 communication failures.*

4. Ensure all IEEE-488 cable screws are fastened finger-tight.
5. Set a unique IEEE-488 address between 2 and 29 for each device connected on the IEEE-488 bus. Refer to Section 3.2.3, Setting IEEE-488 Addresses.

3.2.2 Making IEEE-488 Connections with 600 Series PowerLine Controllers

If you are controlling the Waters 486 detector from a 600 Series PowerLine Controller, use the IEEE-488 interface to communicate with the detector (PowerLine controller manages IEEE-488 interface functions). Set up the 600 Series Controller as a PowerLine Controller.

To set up the 600 Series Controller as a PowerLine Controller, refer to the Waters 600E Multisolvent Delivery System User's Guide, Section 4.1, Setting Up the 600 as a PowerLine Controller.

To connect IEEE-488 cables:

1. Connect the single-receptacle end of the IEEE-488 cable (supplied with the 600 series system) to the IEEE-488 connector on the 600 controller rear panel (Figure 3-5).
2. Connect the other end of the cable (“stackable” connector for daisy-chaining additional instruments) to the IEEE-488 connector on the Waters 486 detector.

If using a multicomponent configuration (as illustrated in Figure 3-3 and Figure 3-4), connect a second IEEE-488 cable to the “stackable” connector on the PowerLine instrument. Connect the other end of the cable to the IEEE-488 port on the next PowerLine component.

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Figure 3-5 illustrates IEEE-488 cable connection.

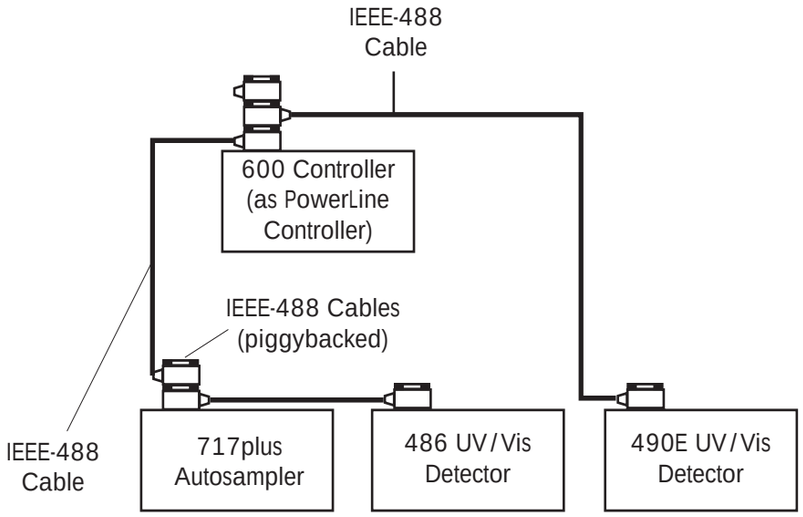


Figure 3-5 PowerLine Controller IEEE-488 Connections

3. Repeat steps 2 and 3 for each additional PowerLine component.

   Note: *The maximum total cable length between IEEE-488 devices is 65 feet (20 meters). The maximum recommended cable length between two IEEE-488 devices is 13 feet (4 meters). Longer total cable lengths can cause intermittent IEEE-488 communication failures.*

4. Ensure all IEEE-488 cable screws are fastened finger-tight.
5. Set a unique IEEE-488 address between 2 and 29 for each device connected to the IEEE bus, (Section 3.2.3, Setting IEEE-488 Addresses).

3.2.3 Setting IEEE-488 Addresses

You must set a unique IEEE-488 address between 2 and 29 for the Waters 486 detector to be recognized on the IEEE-488 interface (Bus LAC/E card or LAC/E module).

When using the Waters 486 detector with a data system Millennium Chromatography Manager, Waters 845/860 workstation, or Waters 600 Series PowerLine Controller, you set the IEEE-488 address for the detector from the front panel of the Waters 486 detector.

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Procedure

To set or change an IEEE-488 address:

1. Select **Diagnostic 13** and press **Enter** on the front panel. The current IEEE-488 address will appear.
2. Type the desired IEEE-488 address and press **Enter**.
3. Power the unit down, then power up again to store the new settings.

Setting 486 and 490E IEEE-488 addresses

If you are connecting a Waters 486 detector and a 490E detector to a PowerLine controller, the address of the 486 detector must be lower than that of the 490E.

3.3 Making Screw Terminal Connections

The 486 detector screw terminals enable the detector to receive signals from or generate signals to external devices (for example, a Waters 600 Series pump, a non-IEEE-488 autosampler, or a Waters 746 Data Module).

Depending on the external devices within your particular system, you use the appropriate screw terminals listed earlier in Table 3-1 (and the procedures in the remainder of this section) to connect the device to the 486 detector.



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 you always connect the shield of the cable to chassis ground at one instrument only.*

When not using the Waters 486 detector as an IEEE-488 device, signals to and from the detector are made through screw terminal connections. When using the 486 detector as an IEEE-488 device, signals which are not IEEE-488 supported can only be transmitted through screw terminals.

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This section describes how to make 486 detector screw terminal connections to the following external devices:

- Waters 745/745B/746 Data Module
- Chart Recorder
- Waters 600 Series Pumps
- Waters 700 Series Autosamplers
- Waters U6K Injector

3.3.1 Waters 745/745B/746 Data Module Connections

Analog output signal connection

To send analog output signals from the Waters 486 detector to the Waters 745/745B/746 data module, make the cable connections indicated in the table below. If the cable has a shield, connect it to chassis ground at one end only (Figure 3-6).

486 1V/AU Terminals	745/745B/746 Terminals
Red (+) Black (-)	Red output connector (+) Black output connector (-)

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Figure 3-6 illustrates the connections between the detector and the 745/745B/746 for detector output signals.

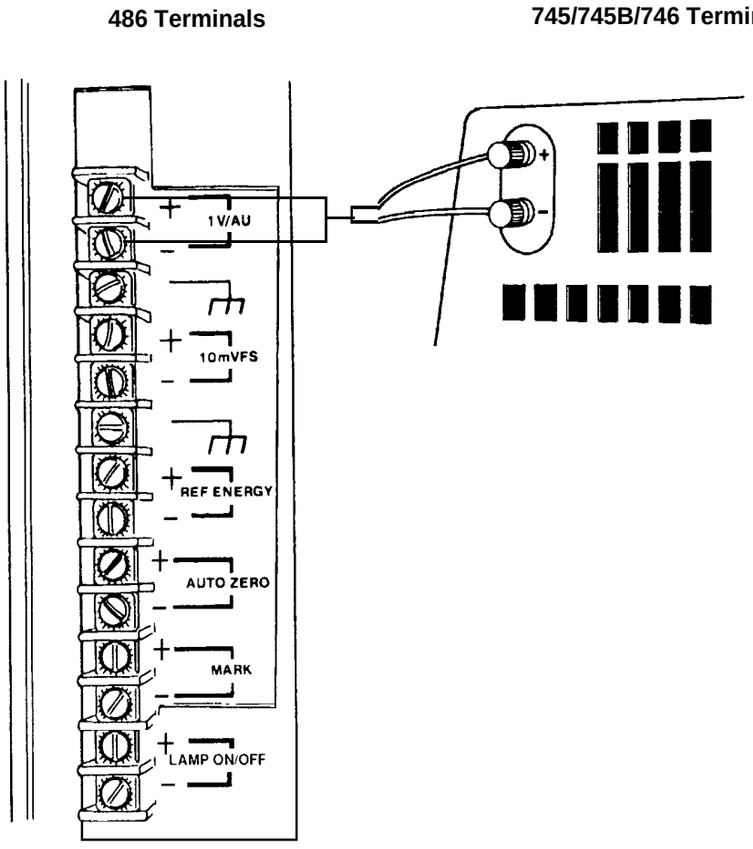


Figure 3-6 Waters 745/745B/746 Data Module Connections

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3.3.2 Chart Recorder Connections

10 mV analog output signal connection

To send analog output signals from the Waters 486 detector to a chart recorder, use the recorder cable to make the connections indicated in the table below:

486 10 mV FS Terminals	Chart Recorder Terminals
Red (+) Black (-)	Pen 1(+) Pen 1(-)

Connect the cable shield to chassis ground at one end.

Reference energy signal connection

To monitor the reference beam energy of the detector, use a second cable to connect the reference energy output signal to the second channel of the recorder (Figure 3-7) or second recorder, as indicated in the table below:

486 Ref Energy Terminals	Chart Recorder Terminals
Red (+) Black (-)	Pen 2 (+) Pen 2 (-)

Connect the cable shield to chassis ground at one end.

Chart mark signal connection

To record a chart mark at the beginning of each run, connect the 486 detector Mark terminal to an inject start signal terminal from a data system, 600 Series PowerLine controller, or injector.

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Figure 3-7 illustrates connections between the Waters 486 detector and a recorder for 10 mV output and reference energy signals.

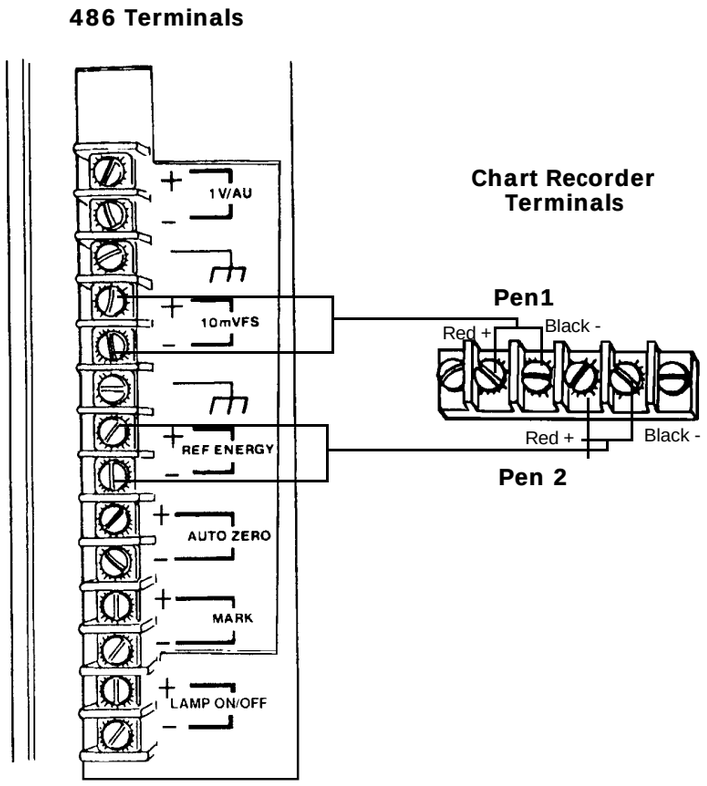


Figure 3-7 Chart Recorder Connections

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3.3.3 Waters 600 Series System Connections

The Waters 486 detector is an IEEE-488 supported instrument, however; the following signals are transmitted over the screw terminal connections to the Waters 600 Series Controller rather than over the IEEE-488 bus:

- Chart mark
- Auto Zero
- Lamp On/Off

The Waters 600 Series System can be configured with a manual injector (part of the slide-out drawer or rack) or with a stand-alone autosampler. In both cases, an external trigger signal is sent to the detector from:

- The 600 series system when a manual injector is used.
- The 700 series autosampler, when a stand-alone autosampler is used.

Chart mark connections

To configure the 486 detector to receive chart mark (inject start) signals from a 600 Series Controller (Figure 3-8), make the signal cable connections indicated in the table below:

486 Mark Terminals	600 Series Controller Terminals
Red (+) Black (-)	INJ GND (Do not use chassis ground connection)

Auto Zero connections

To configure the 486 detector to receive remote Auto Zero signals from a 600 Series Controller (see Figure 3-8), make the signal cable connections indicated in the table below:

486 Ref Energy Terminals	600 Series Controller Terminal
Red (+) Black (-)	INJ GND (Do not use chassis ground connection)

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Lamp On/Off connections

To configure the 486 detector to receive the Lamp On/Off signals from the 600 Series Controller, (see Figure 3-8), make the signal cable connections indicated in the table below:

486 Lamp On/Off Terminals	600 Series Controller Terminals
Red (+) Black (-)	INJ GND (Do not use chassis ground connection)

Figure 3-8 illustrates the Auto Zero, Chart Mark, and Lamp On/Off connections from the Waters 486 Detector to the Waters 600 Series Controller.

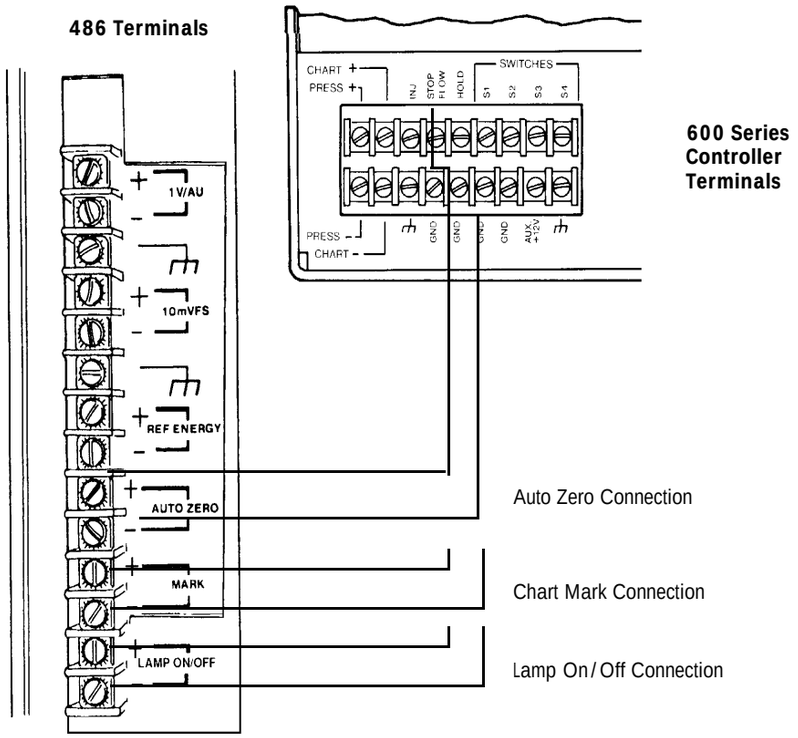


Figure 3-8 Waters 600 Series Controller Connections

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3.3.4 Waters 700 Series Autosampler Connections

The Waters 486 detector accepts the following external trigger signals from a Waters 700 Series Autosampler:

- Chart mark (inject start) signal from a contact closure signal with each injection.
- Remote auto zero signal that automatically zeroes the detector each time the Waters 700 Series Autosampler makes an injection.

Note: *If you connect the Waters 700 Series Autosampler to more than one instrument, avoid connecting more than one set of cables to each set of terminal connections.*

Chart mark signal connection

To configure the Waters 486 detector to receive chart mark signals from the Waters 700 Series Autosampler, make the cable connections as indicated in the table below (Figure 3-9). If the cable has a shield, connect it to the chassis ground at one end only.

Autosampler	Autosampler Connections	486 Mark Terminals
717 plus, 717, or 715	Inject Start	(+) and (-)
700 or 712	Integ Start (or Chart Mark)	(+) and (-)
Non-Waters	NA	(+) and (-)

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Figure 3-9 illustrates the connections between the Waters 486 detector and the Waters 700 Series Autosampler for chart mark signals.

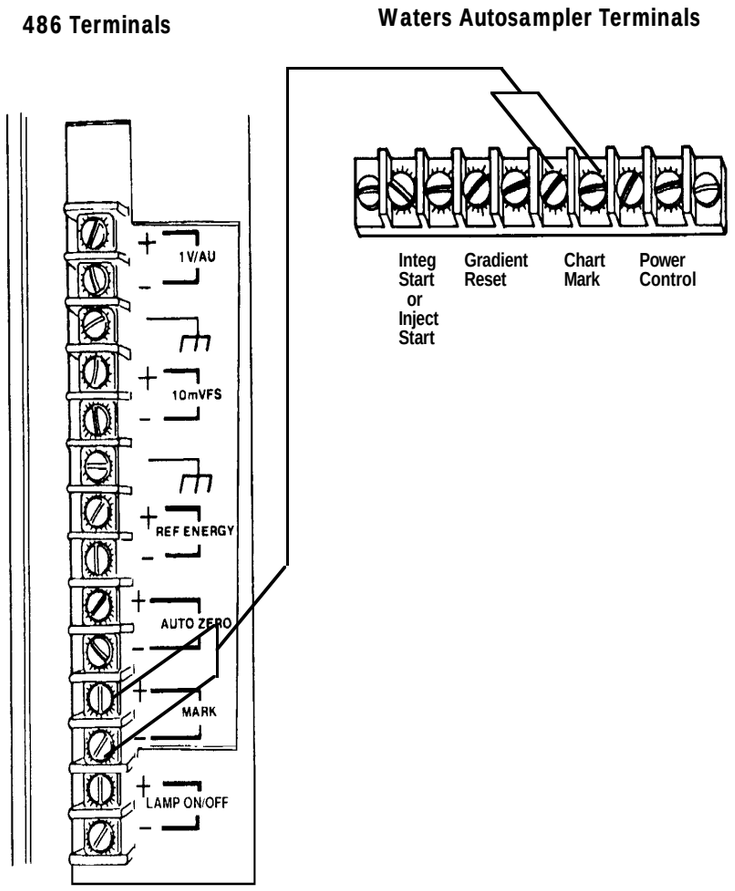


Figure 3-9 Waters 700 Series Autosampler Chart Mark Connections

Note: *The Waters 486 detector can be zeroed by the Waters 700 Series Autosampler each time an injection is made.*

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Remote Auto Zero connection

To configure the detector to receive remote auto zero signals with each Waters 700 Series Autosampler, (Figure 3-10) make the cable connections indicated in the table below:

Autosampler	Autosampler Connection	486 Auto Zero Terminal
717 plus, 717, or 715	Inject Start	(+) and (-)
700 or 712	Integ Start (or Chart Mark)	(+) and (-)
Non-Waters	NA	(+) and (-)

If the cable has a shield, connect it to a chassis ground terminal at one end only.

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Figure 3-10 illustrates connections between the Waters 486 detector and the Waters 700 Series Autosampler for Remote Auto Zero signals.

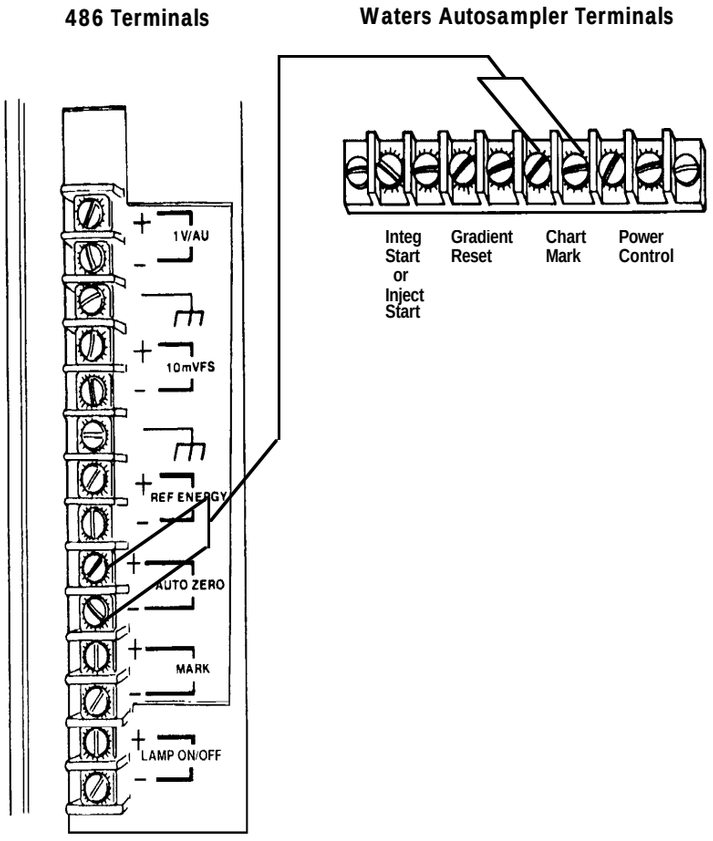


Figure 3-10 Waters 700 Series Autosampler Remote Auto Zero Connections

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3.3.5 Waters U6K Injector Connections

The Waters 486 detector accepts the following external trigger signals from a U6K injector:

- Chart mark (inject start) signal from a contact closure signal with each injection
- Remote auto zero automatically zeroes the detector each time the U6K makes an injection

Note: *If you connect the U6K to more than one instrument, avoid connecting more than one set of cables to each set of terminal connections.*

Chart mark signal connection

To configure the detector to receive chart mark signals from the U6K (Figure 3-11), use the U6K inject start cable and make the connections indicated in the table below:

486 Mark Terminals	U6K Terminals
(+) and (-)	One set of spade lug connectors ***Note:*** *You can use either of the leads from the U6K. The black and red leads connect from the U6K to the 486 detector terminal.*

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Figure 3-11 illustrates the connections between the Waters 486 detector and the U6K for chart mark signals.

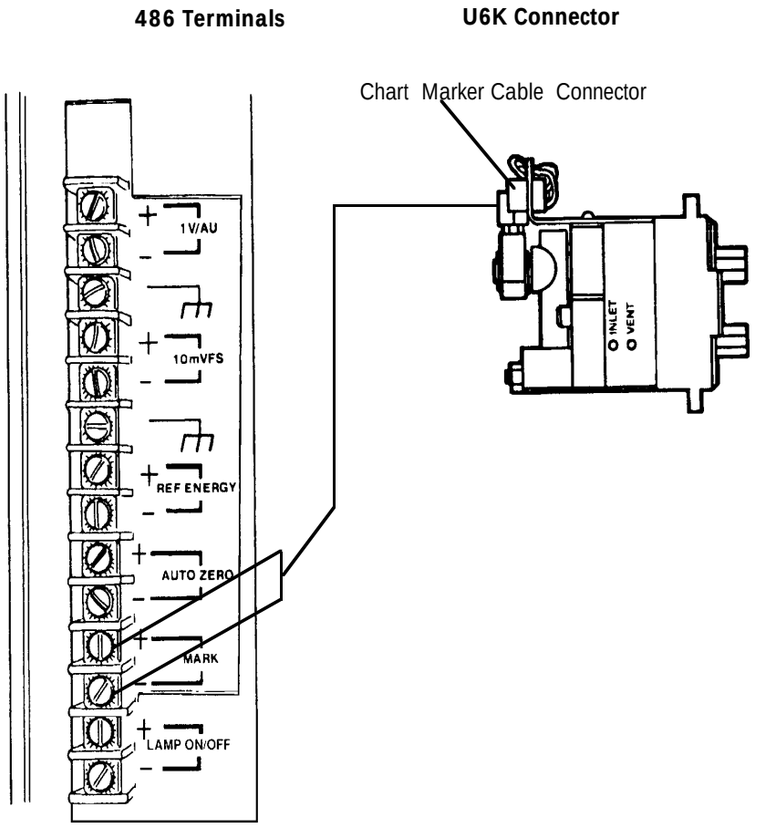


Figure 3-11 Waters U6K Chart Mark Connections

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Auto Zero signal connection

To configure the Waters 486 detector to receive auto zero signals with each U6K injection (Figure 3-12), use the U6K inject start cable to make the connections indicated in the table below:

486 Auto Zero Terminals	U6K Terminals
(+) and (-)	One set of spade lug connectors ***Note:*** *You can use either lead from the U6K. The black and red leads from the U6K connect to either 486 terminal.*

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Figure 3-12 illustrates the connections between the Waters 486 detector and the U6K for auto zero signals.

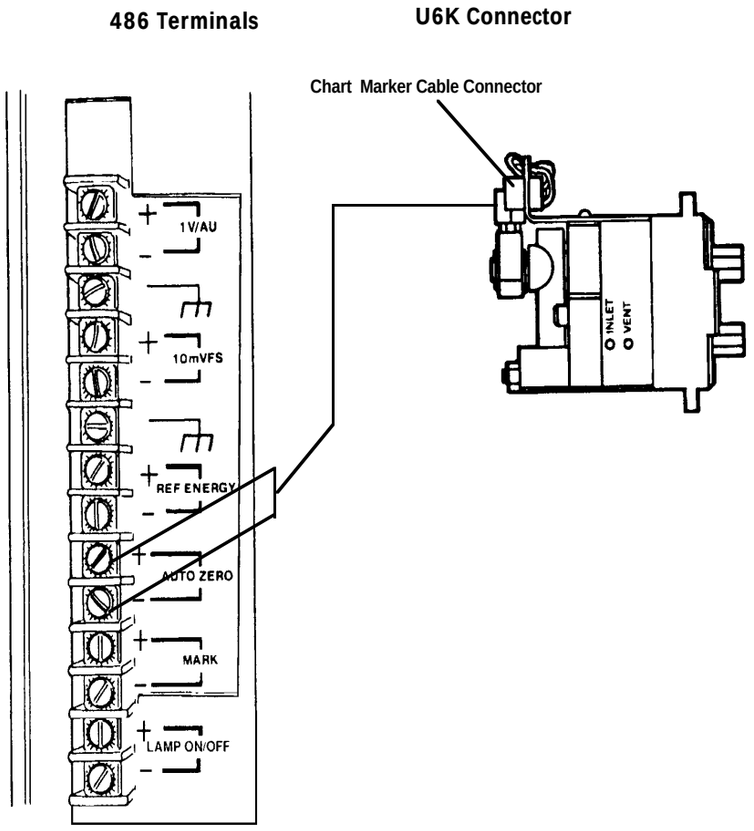


Figure 3-12 U6K Remote Auto Zero Connections

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4 Using the Waters 486 Detector

After you have installed and verified operation of the Waters 486 Detector, perform a test run. The test run allows you to verify that your detector is operating properly before running real samples.

Note: *Before you continue, be sure to perform the Instrument Verification procedures in Chapter 2, Installation and Instrument Verification, if you have not done so already.*

Using the detector as a stand-alone unit

You can use the Waters 486 Detector as a stand-alone module in conjunction with a pump, injector, recorder or integrator. To use the detector in one of the above configurations, follow the instructions provided in this chapter.

Using the detector as part of a system

You can also use the 486 Detector when it is configured with a Waters system controller, such as the Waters Millennium 2010 Chromatography Manager, Waters 600E Multisolvent Delivery System, Waters 860 Networking Computer System, or Waters 845 Chromatography Data and Control Station.

To configure the Waters 486 Detector with one of these systems, follow the instructions in the appropriate manual to set the parameters and control the 486 from the system controller. You can program the detector from the front panel, if desired, except when it is in remote mode. Refer to "Remote mode" in Section 4.1, Startup.

4.1 Startup

Powering up

To power up the Waters 486 Detector, press the On/Off switch located on the lower front right corner of the unit.

The upper LCD displays **486.** The lower LCD displays the version number of the software in the detector. The deuterium lamp illuminates after approximately one minute. Lamp illuminating is signaled by two clicks.

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Automatic calibration

The detector automatically goes through a calibration routine each time it is powered up. Refer to Section Section 1.2.2, Light Path Wavelength Calibration.

During calibration, the lower LCD displays **CAL,** indicating that the calibration routine is operating.

Delta mode

In Delta mode, the upper LCD displays **DEL,** indicating that the unit is calibrating in Delta mode.

Calibration in Delta mode takes approximately three minutes to complete. When the calibration routine is successful, the upper LCD displays an absorbance value. The lower LCD displays the last-entered wavelength value.

If calibration fails, an error code appears. Refer to Chapter 6, Error Codes and Diagnostics.

Delta mode electronically changes (inverts) the sample and reference electronics of the detector to allow samples to be run through the reference side of the flow cell.

When operating in Delta mode, flow must go through the reference side of the flow cell. If flow is not set to go through the flow cell, peaks have reversed polarity and are flat-topped at -0.1 AU (the negative absorbance unit specification limit of the detector). Be sure to clean and purge the sample side of the cell to allow proper calibration of the reference side.

Remote mode

The Waters 486 Detector operates in remote mode when it is under active control by a system controller through the IEEE-488 interface. The detector can be configured with Waters Millennium 2010 Chromatography Manager, Waters 600E Multisolvent Delivery System, Waters 860 Networking Computer System, or Waters 845 Chromatography Data and Control Station.

When the detector is in remote mode, the keyboard is locked and you cannot enter any parameters or values.

Warm-up period

For normal use, allow the detector to warm up for 30 minutes before operating.

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Powering down

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

Do not leave eluent buffers stored in the system after use. Wash the buffer out completely by flushing the lines with filtered HPLC-quality water containing at least 10 percent methanol.

Note: *If methanol and water are incompatible with your column, remove the column before flushing.*

To power down the detector, press the On/Off switch located on the front lower right corner of the unit.

Standby

If you do not want to turn off the unit, and want to preserve the lamp, you can maintain the system in standby mode by turning off only the deuterium lamp.

- To turn off the lamp, press the Lamp key.
- Pressing the Lamp key again illuminates the lamp.

The lamp may take up to 1minute to be fully operational; an LCD display indicates that the lamp is illuminated.

Programming the lamp

Select **Diagnostic 20** to access the lamp timer. You can program the lamp to turn off after a specified period of time. The timer is set in increments of 0.1 hours, from 0.1 to 99.9 hours. Refer to Chapter 6, Error Codes and Diagnostics.

If you know you will be working in remote mode, you can pre-program the lamp timer by setting the lamp before going into remote mode.

4.2 Using the Front Panel

The Waters 486 Detector front panel consists of a series of displays and keyboard indicators that facilitate adjustment of the detector.

This section describes the front panel:

- Displays
- Indicators
- Parameters

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4.2.1 Displays and Indicators

The Waters 486 Detector includes two numeric LCD displays and nine small LCD indicators. The displays and indicators are located on the upper right side of the front panel. Figure 4-1 illustrates the detector displays and indicators.

Upper display

The upper LCD display is a 4-character display that indicates current values for:

- Absorbance
- Reference energy
- Sample energy

The upper display also shows diagnostic messages generated from:

- Detector diagnostic routines
- Error conditions

Lower display

The lower LCD display is a 3-character display that shows user-selected values for the following:

- Wavelength
- Sensitivity
- Filter parameters

Figure 4-1 illustrates the Waters 486 Detector front panel keypad, display window and indicators.

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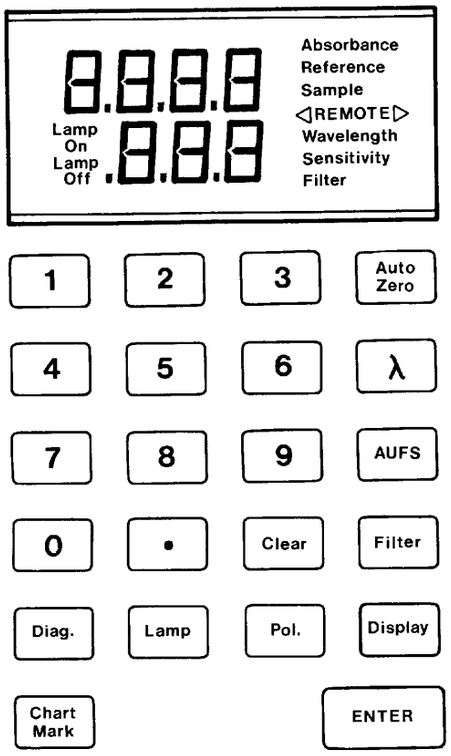


Figure 4-1 Front Panel Displays and Indicators

The display area shows diagnostic messages or numeric error codes.

To show the values in the lower display (wavelength, sensitivity, or filter) press the appropriate key. The parameter value and the corresponding LCD indicator display until you select another parameter.

Indicators

Seven small LCD indicators are located to the right of the numeric displays. One of the first six LCD indicators illuminates when you select the corresponding parameter (Section 4.2.2, 486 Detector Parameters).

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Remote indicator

The Remote LCD indicator illuminates when the detector is under active control by another system controller through the IEEE-488 interface. When the Waters 486 Detector is in remote mode, the keyboard is locked and you cannot enter the parameter values.

Lamp indicator

The Lamp-On indicator on the lower left of the numeric display illuminates when the lamp is on (Section 4.4, Displaying/Changing Parameters).

4.2.2 486 Detector Parameters

For information on accessing the Waters 486 Detector parameters, refer to Section 4.4, Displaying/Changing Parameters.

Absorbance

Indicates the amount of absorbance in the sample cell relative to the reference cell (offset corrected by auto zero) displayed in absorbance units (AU). The output is a 0 to 2 volt full scale signal that corresponds to the actual absorbance (0 to 2 AU) and is not affected by the AUFS setting. (There is some negative range on the output to allow for drift.)

Sample energy

Indicates the relative amount of light transmitted through the sample cell.

Reference energy

Indicates the relative amount of light transmitted through the reference cell. This parameter can be monitored using the terminal output strip on the back of unit (Section 3.3.2, Chart Recorder Connections).

Wavelength

Sets the wavelength at which the flow cells are illuminated. The allowable range is 190 to 600 nm.

Sensitivity

Sets sensitivity in absorbance units full scale (AUFS) depending on the entered value for AUFS. The sensitivity value affects the 10-mV output only. The allowable range is .001 to 2.0 AUFS.

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Filter (time constant)

Sets the digital noise filter time constant. The filter value can be modified to achieve the optimal signal-to-noise ratio. The allowable range is 0.1 to 5.0 seconds.

Lamp

Turns the lamp on or off to preserve the lifetime of the lamp. Refer to Section 4.4, Displaying/Changing Parameters.

Setting IEEE-488 Address

Use the front panel to set the IEEE-488 address. The front panel method for setting the IEEE-488 address is as follows:

- Select **Diagnostic 13**, press the **Diag** key, type **13,** press **Enter.** The current IEEE address displays.
- Select the new address and press **Enter.**
- Turn the unit off, then on again to store the change.

4.3 Key Descriptions

The keypad is used to enter numeric data or function parameters. However, if an IEEE cable is installed and the unit is running in remote mode, the front panel keys are inoperable. The Waters 486 Detector keypad keys and their functions are described below:

Enter

Saves the parameter settings in the detector memory.

For example, to change a parameter value, press the appropriate parameter key and enter the new value.

The LCD indicator that corresponds to the selected parameter flashes, indicating that the value has not been saved, you must press the Enter key to save the entry. The parameter value continues to flash until the detector has completed all necessary adjustments.

Auto Zero

Adjusts the zero offset of the analog output to compensate for changes in absorbance. Whenever the wavelength is changed, the detector automatically performs the Auto Zero function. Use the Auto Zero key on the front panel at any time, for example, whenever the solvent is changed.

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To activate auto zero from a remote device, refer to Section 3.3.4, Waters 700 Series Autosampler Connections, and Section 3.3.5, Waters U6K Injector Connections.

λ (Wavelength)

Changes the operating wavelength of the detector. The selected value remains in effect until changed. When the wavelength setting changes, the detector automatically performs the Auto Zero function.

To change the wavelength, press the λ key and enter the new value, then press **Enter.** Valid values are from 190 to 600 nm.

AUFS

The Absorbance Units Full Scale changes the sensitivity setting of the detector in AUFS.

To change the AUFS value, press **AUFS.** Type the new sensitivity value and press **Enter.** Valid sensitivity values are from 0.001 to 2.000 AU. This key affects the 10-mV analog signal only.

Clear

Clears the parameter from the lower display, wavelength, sensitivity, and filter.

The clear key erases incorrect parameter entries and is functional only before a value is saved.

Display

Sequences through absorbance, sample energy, and reference values in the upper display. (see Figure 4-1). Use the **Display** key to view the values in the upper display in succession.

Chart mark

The Waters 486 Detector software revision 1.0 sends a mark pulse to the 10-mV output and the 1 V/AU output. The chart is marked with a 10 percent deflection in the direction of polarity.

Starting with software revision 4.10, chart mark will automatically send a mark (only) to the 10-mV recorder output. Chart mark on the 1V output to the integrator is a user-selectable feature, that is accessible through **Diagnostic 24.**

To manually send a mark pulse, press Chart Mark. If the detector is operating under remote mode, this function is disabled. Refer to Section 3.3.3, Waters 600 Series System Connections, to set the detector for remote chart mark signals.

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Polarity

Changes the direction of the plot in the 10-mV output.

This is similar to reversing the leads to the external chart recorder. When the polarity is positive, deflection is 0 to +10 mV full scale. When polarity is negative, deflection is 0 to -10 mV full scale.

To change polarity, press the **POL** key. The display does not indicate the change.

Filter (time constant)

Adjusts the noise filter (time constant) to achieve the optimum signal-to-noise ratio without changing the sensitivity setting. Refer to "Filter constants" in Section 1.2.3, Waters Taper-Cell, for more information.

To change the filter setting value, press **Filter**. Enter the new value and press **Enter**. The Waters 486 detector accepts filter values from 0.1 to 5.0 seconds in 0.1 second increments. (A value of zero (0) turns off the filter.)

Lamp

Toggles the lamp on and off. When turned on, Lamp On displays at the lower left of the display. When turned off, Lamp Off displays. It takes roughly 3 minutes for the lamp to illuminate and stabilize.

If the detector is operating under remote control, Lamp On/Off is not controllable through the front panel or IEEE control. However, you *can* pre-program the lamp to turn off at a specified time (from the front panel) *before* you go into remote mode. You can also activate the lamp with an external signal, even if you are in remote mode.

Diag

Accesses the user diagnostic functions. Refer to Chapter 6, Error Codes and Diagnostics.

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4.4 Displaying/Changing Parameters

Use the Display key on the front panel to view and change (absorbance, reference energy, sample energy) parameter values. Refer to the front panel illustration below:

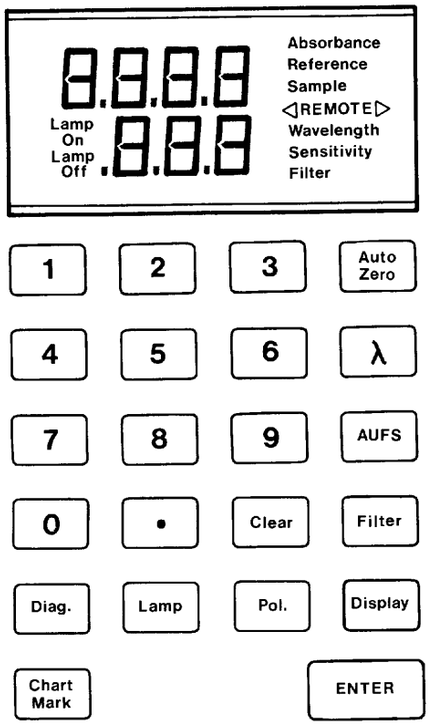


Figure 4-2 Front Panel Displays and Indicators

Changing parameter values

To change the currently displayed parameter, enter the new value. Then press the **Enter** key.

When you change the wavelength parameter, the new wavelength value flashes until the detector adjusts the optics to the new wavelength. The unit takes approximately 20 seconds to move from 190 to 600 nm.

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Cancelling a parameter setting

You can cancel and reselect a parameter setting in the lower display in one of two ways:

1. Press the **Clear** key to erase the entry.
2. Enter the correct value and press **Enter**.

- If you select a value and do not press the Enter key, the LCD indicator will flash continuously until a the value is entered. To cancel a saved entry or error message, press the appropriate parameter key. The last entry clears and the previously saved value appears.

  Note: *Example: If you enter a new value for AUFS then decide that the previous value was appropriate, to leave it unchanged, press the AUFS key to cancel the entry. The lower display returns to the previous AUFS setting.*

- If you enter an AUFS value that is out of range, the error indicator flashes. Press the **AUFS** key again to display the previous AUFS setting, then reenter the correct value.

Lamp

Use the Lamp key to toggle the lamp on and off. When the lamp is on, **Lamp On** displays in the lower left of the display window. When the lamp is turned off, **Lamp Off** displays. It takes roughly 3 minutes for the lamp to stabilize.

Lamp timer

Select **Diagnostic 20** by pressing the Diag key and typing 20 to access the lamp timer. The lamp may also be programmed to turn off after an elapsed time. For example, if a run lasts five hours, program the lamp to shut off after six hours. Refer to Chapter 6, Error Codes and Diagnostics.

The timer is set in increments of 0.1, from 0.1 to 99.9 hours.

Cancelling the lamp timer

To cancel the timer, enter zero (0).

If the detector is operating under remote control, Lamp On/Off is not controllable through the front panel or from the IEEE control; however, you can program the lamp to turn off at a specified time (from the front panel) before accessing remote mode. The lamp may also be activated using an external signal from the remote mode.

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4.5 Making a Test Run

Before you use the Waters 486 Detector for your chromatography, make a test run to be sure the results are within the standards required for your samples.

Warm-up period

Allow the detector to warm up for 30 minutes prior to operating the system.

Before you begin

Ensure that the:

- Detector is properly installed and connected to a pump, an injector, a column, and an integrator or recorder.
- Automatic start-up diagnostic tests have completed successfully, and the detector checks out properly. Chapter 2, Installation and Instrument Verification.

Once you are familiar with the keypad and have set the operational parameters, follow the test run procedures in this section to:

- Generate a *test peak* to check the microprocessor board and verify communications between the detector and the recording device connected to the detector.
- Perform a *test injection* to verify that the fluid connections are correct.

To generate a *test peak*:

Test peak procedure

1. Select **Diagnostic 14**, Test Peak.
2. Press **Enter** to start the test peak generation. The absorbance value on the front panel changes as the peak plots.

Result

If the test is successful, the peak generated should have the following characteristics:

Peak height	1.0 AU
Peak width	10 seconds
Retention time	50 seconds

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If you generate the test peak again, there should be less than 1 percent error between peaks. If these peaks are integrated, each peak should have the same area value.

To confirm that the system is plumbed correctly (from the mobile phase reservoir through the detector), perform the test injection procedure below.

Note: *Use HPLC-grade methanol for your solvent. The solvent must be thoroughly degassed before testing. Refer to* *Section 1.4.2, Solvent Degassing**.*

Test Injection Procedure

1. Without a column attached, connect approximately two feet of .009-inch I.D. tubing from the injector to the inlet port of the detector.
2. Set the pump flow rate to 1.0 mL/min. Make sure the pump flow is not pulsing.
3. Set the AUFS to 2.0, the wavelength to 254 nm, and the filter setting to 1.0.
4. Monitor the baseline until drift is negligible (approximately 10 minutes).
5. Inject 1 µL of acetone.

Result

If the system has no leaks the peak height should be equal to or larger than 75 percent of full scale. For problem assistance, contact Waters Customer Service at 1-800-252-4752.

4.6 Wavelength Scanning

Use the scan feature to determine the wavelength of maximum absorbance for a particular analyte.

Scanning is available in software version 4.10 and later. The Waters 486 Detector scan is a Stop Flow scan, which means that there cannot be any flow through the cell for the duration of the scan.

Scan data can be collected through either the 10-mV or the 1-V output from the detector.

The scan feature can be accessed by initiating the following diagnostics:

- **Diagnostic 26** - to view/edit parameters
- **Diagnostic 27** - to execute/perform scan using selected or default parameters
- **Diagnostic 26** - to view or edit the parameters that define the current scan.

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Viewing and editing parameters

These parameters include the data listed in Table 4-1.

Table 4-1 Scan Parameters

Parameter	Default	Range
Starting wavelength (in nm)	190 nm	190 to 600 nm
Ending wavelength (in nm)	600 nm	190 to 600 nm
Step size (in nm)	1 nm	1 to 5 nm
Step duration (in seconds and tenths of a second)	0.1 sec	0.1 to 5 sec

While the specified wavelength range of the detector is 190 to 600 nm, detector software allows you to scan your analyte from 180 to 700 nm. Due to increased noise in the extended wavelengths, operating scans within the chromatographic wavelengths of values outside the specified range of 190 to 600 nm (only) is strongly recommended.

To change the value of an operational parameter:

1. Press the parameter key corresponding to the appropriate field.
2. Enter a new value.
3. Press the **Enter** key.

To scroll through parameters without changing the value, press the **Enter** key.

Parameters you enter remain in effect for all scans until changed, or, until power-down. Default parameters are reinstated each time the unit is powered up.

Interpreting a scan

For easy interpretation of a scan, set the step size at the default, or at 1-nm increments, and the step duration at 4.0 seconds. Make sure the filter is set to 0.0 seconds. These parameters make it easy to follow the wavelength steps through the scan to determine the wavelength of maximum absorbance for the analyte of choice.

Note: *Be sure to dissolve the analyte in the solvent that will be used in the final chromatographic system. The wavelength of maximum absorbance can change with different solvents.*

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Performing a scan

To start the scan, use **Diagnostic 27**, (press the **Diag key** and type **27**). To abort the scan, press any key.

General parameters

Parameter settings from Table 4-2 were used in the scan. Results from using Table 4-2 settings are illustrated in Figure 4-3. Exceptions to these parameter settings are noted below each scan example.

Table 4-2 contains the parameter settings used to generate the scan examples on the following page.

Table 4-2 Scan Example Parameter Settings

Parameter	Setting
Starting wavelength	225 nm
Ending wavelength	240 nm
Step size	1 nm
Step duration	4.0 sec
Filter	0.0 sec
Wavelength of maximum absorbance	231 nm
Chart speed	10 cm/min

Figure 4-3 illustrates examples of Wavelength Scanning.

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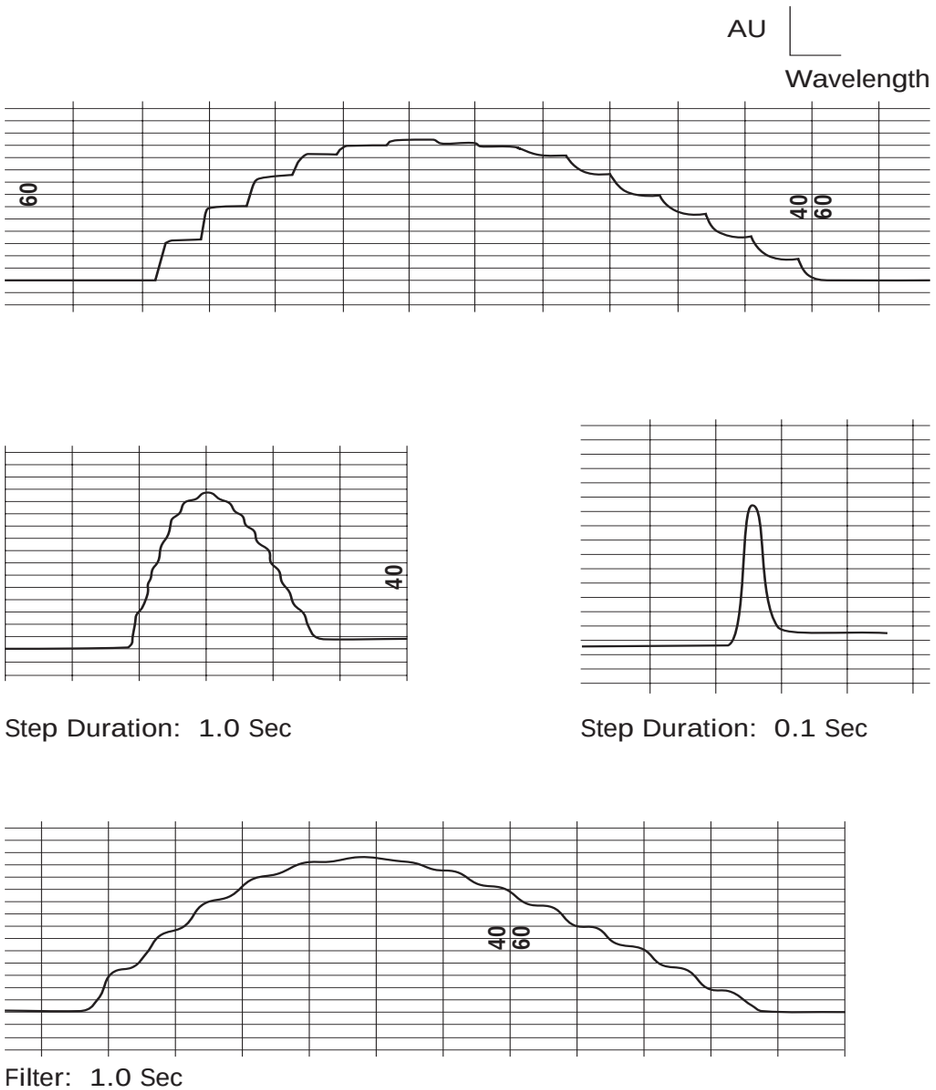


Figure 4-3 Scan Examples

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5 Maintenance Procedures

Introduction

Read the appropriate maintenance instructions before starting the procedures for any of the tasks in this chapter. If you are uncertain about how to perform the procedures, call the Waters Technical Service Department to have a trained service representative perform the procedure.

Waters Technical Service department

The Waters Chromatography Division staff of service specialists provides preventive and corrective maintenance.

If you have questions regarding repair or performance of your instrument, you can contact Waters Technical Service at (800) 252-4752, *Canadian and U.S. customers only*. Other customers, call your local Waters subsidiary or your local Waters Technical Service representative, or call Waters corporate headquarters for assistance at (508) 478-2000 (U.S.).

Spare parts

Stock the recommended spare parts to minimize downtime. Refer to Appendix B for a list of recommended spare parts. Parts not included in the list may require replacement by a trained service representative.

Safety and handling

When you perform maintenance procedures on your Waters 486 Detector, keep the following safety considerations in mind:



Caution: *To prevent the possibility of electric shock, never disconnect an electrical assembly (including the pump interface cable) while power is applied to the 486.*



Attention: *To prevent damage to the 486 electronics, wait approximately three minutes after you power off the 486 before you remove an electrical assembly.*



Caution: *When you handle eluents, change tubing, or operate the 486 in general, always observe good laboratory practices. Know the physical and chemical properties of the eluents. Refer to the Material Safety Data Sheets for the eluents in use.*



Attention: *To avoid damage due to static electricity, do not touch integrated circuit chips or other components that do not specifically require manual adjustment.*

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Caution: *To avoid the possibility of electric shock, always power off the 486 and disconnect the power cord before performing maintenance procedures.*

Removing and installing the detector cover

Some of the maintenance procedures in this chapter require that you remove and replace the detector cover.

To remove the cover:

1. Power down the unit.
2. Remove the four screws located on the lower four corners of the cover.
3. Slide the cover straight up and off the detector.

When you complete maintenance procedures, carefully replace the cover by sliding the pin in the left rear corner of the cover into the notch on the chassis and securing it with four screws.

Figure 5-1 illustrates the Waters 486 Detector with the top cover removed.

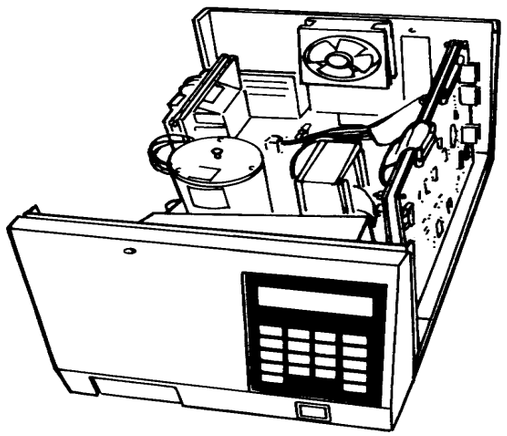


Figure 5-1 Top Inside View of the Waters 486 Detector

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5.1 Routine Maintenance

The Waters 486 Detector requires minimal routine maintenance. To achieve optimal performance perform the maintenance checks described below:

Routine Maintenance Checks

1. Replace solvent reservoir filters regularly.
2. Filter and degas solvents to prolong column life, reduce pressure fluctuations, and decrease baseline noise.

Note: *Section 1.4, Solvent Considerations, contains an overview of solvent degassing methods.*

3. Flush buffered mobile phases out of the detector with a 5 to 10 percent methanol solution each time the detector is shut down.

This process prevents:

- Plugging of the solvent lines
- Damage to the components
- Microbial growth
- Inspect instrument ventilation air filters weekly.

5.2 Cleaning Ventilation Air Filters

To keep the Waters 486 Detector running properly, inspect and clean the ventilation air filters weekly.

Procedure

To clean the ventilation filters:

1. Turn off the power and unplug the detector power cord.
2. Remove the cover.
3. Remove the filters from inside the top cover and shake it free of dust.

   If the filters are visibly dirty, replace them with new filters.
4. Replace the cover.

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5.3 Cleaning the Flow Cell

A dirty flow cell can cause baseline noise, decreased sample energy levels, calibration failure, and other problems with operation.

There are two phases in cleaning the flow cell:

- Flushing
- Removing and cleaning

If flushing is ineffective, remove and clean the flow cell. Replace flow cell components as required.

5.3.1 Flushing the Flow Cell

Flush the flow cell after each shutdown, or when dirty.



Caution: *To avoid an explosion, do not mix organics and acids.*

Procedure

To flush the flow cell:

1. Stop the pump and remove the column.
2. Attach tubing from the detector to the pump. Flush the mobile phase from the detector with a miscible solvent and water (unless mobile phase is miscible with water).
3. Flush the detector with HPLC-quality water to remove contaminants from the flow path.
4. Pump 6 M Nitric acid through the cell to clean the internal pathways.
5. Flush again with HPLC-quality water until the solvent is neutral.
6. Reattach the column to resume flow of the mobile phase. Use an intermediary solvent if using a mobile phase that is not miscible in water.

5.3.2 Removing and Cleaning the Flow Cell

If flushing the flow cell is not effective, follow the procedures to remove the flow cell and inspect for dirty or broken windows or dirty gaskets. Clean and replace parts if necessary.

This section includes procedures for:

- Removing the pre-amp module
- Disassembling and reassemble the flow cell

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- Replacing the flow cell windows
- Rebuilding the flow cell

You need the following items to remove and clean, or replace the flow cell:

- Flat-head screwdriver
- Phillips-head screwdriver
- Cotton swab
- Ethyl alcohol
- Nitrogen

Save all parts (screws, nuts) after removal unless otherwise indicated. The parts are needed to reinstall a flow cell.

Before you begin

Before removing the flow cell purge the flow cell with nitrogen as follows:

1. Turn off the detector.
2. Connect the nitrogen supply line to the sample outlet. Route the sample line to waste.
3. Purge the cell for 25 to 30 minutes at 25 to 50 psi.

5.3.3 Removing the Pre-amp Module



Caution: *To avoid the possibility of exposing your eyes to ultraviolet radiation (from the flow cell), turn off the detector while performing the following maintenance.*

Note: *When you remove the preamp, the UV lamp automatically turns off.*

To remove the pre-amp module, you must first remove the front panel.

Removing the front panel

1. Remove the detector front panel by removing the three Phillips-head screws.
2. Tilt the front panel toward you to expose the inside front cover.

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3. Disconnect the ribbon cable by pushing the ejector tabs apart (Figure 5-2). Set the front panel aside. Let the cable hang freely until it is time to reassemble.

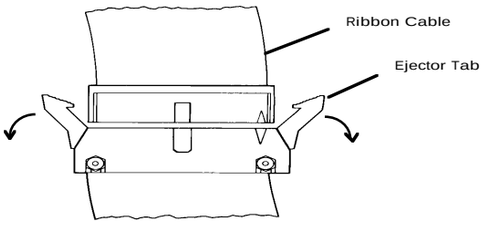


Figure 5-2 Disconnecting the Ribbon Cable

Removing the pre-amp module

4. Turn the two wing nuts counterclockwise to loosen the pre-amp module (Figure 5-3). Pull the pre-amp module away from the chassis. The ribbon cable is still connected to the microprocessor board.

Figure 5-3 illustrates the pre-amp module removal procedure.

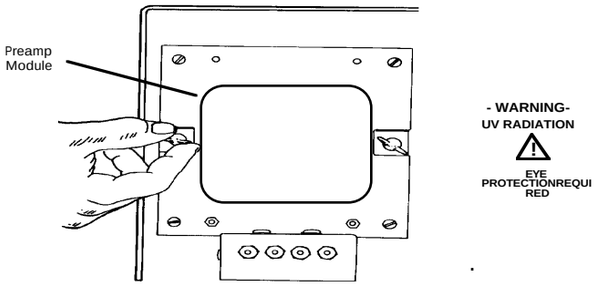


Figure 5-3 Removing the Pre-amp Module

5. Remove the ribbon cable from the clip on the back of the pre-amp module.
6. Place the pre-amp module with the photodiode facing upward on top of the detector (see Figure 5-4).

STOP ***Attention:*** *Do not touch the photodiode. Any markings on the photodiode affect the performance of the Waters 486 Detector. Adequately cover the pre-amp module to prevent dust from getting into the photodiode component.*

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Figure 5-4 illustrates the Pre-amp Module positioning.

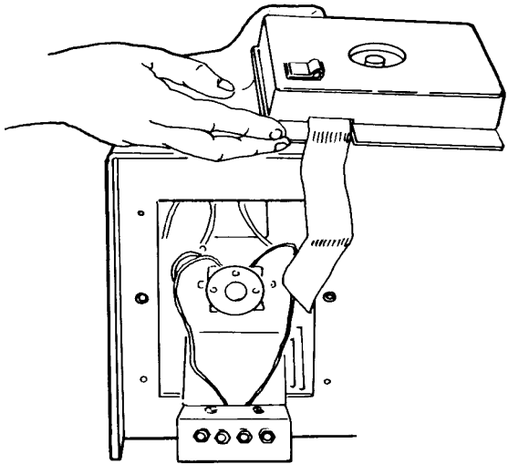


Figure 5-4 Positioning the Pre-amp Module

5.3.4 Disassembling and Reassembling the Flow Cell

Caution: *To avoid the possibility of exposing your eyes to ultraviolet radiation, turn off the unit while performing the following maintenance.*

Note: *When you remove the preamp, the UV lamp automatically turns off.*

Precautions

Take the following precautions when disassembling the flow cell:

- Do not touch the flow cell windows with your bare hands (prevents contaminating the windows).
- Do not touch the two pins on the cell; the pins are sharp.
- Do not scratch or damage the flow cell.

Figure 5-5 illustrates binder-head screw removal.

Removing the flow cell

1. Remove the two binder-head screws and star washers from the top of the fluid manifold (Figure 5-5).

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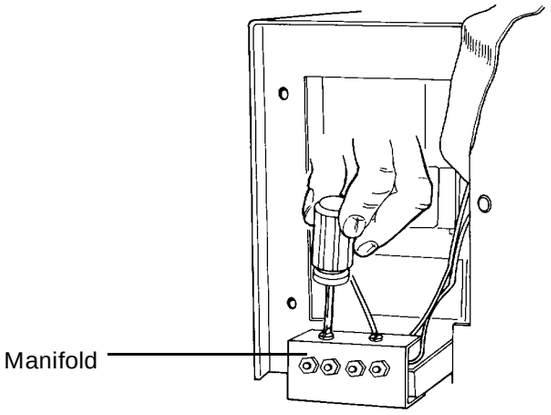


Figure 5-5 Removing Binder-head Screws on the Manifold

Figure 5-6 illustrates how to remove the binder-head screws from the flow cell.

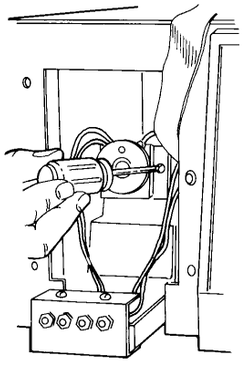


Figure 5-6 Removing Binder-head Screws from Flow Cell

2. Remove the two large binder-head screws and star washers from the front of the flow cell (Figure 5-6).

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3. Without touching the flow cell windows, grasp the flow cell and tilt it forward. Rotate or maneuver the flow cell out of the unit.
4. Push the manifold through the slot in the holding bracket. Place the flow cell unit on a flat surface.

Figure 5-7 provides an expanded view of the Analytical Flow Cell components.

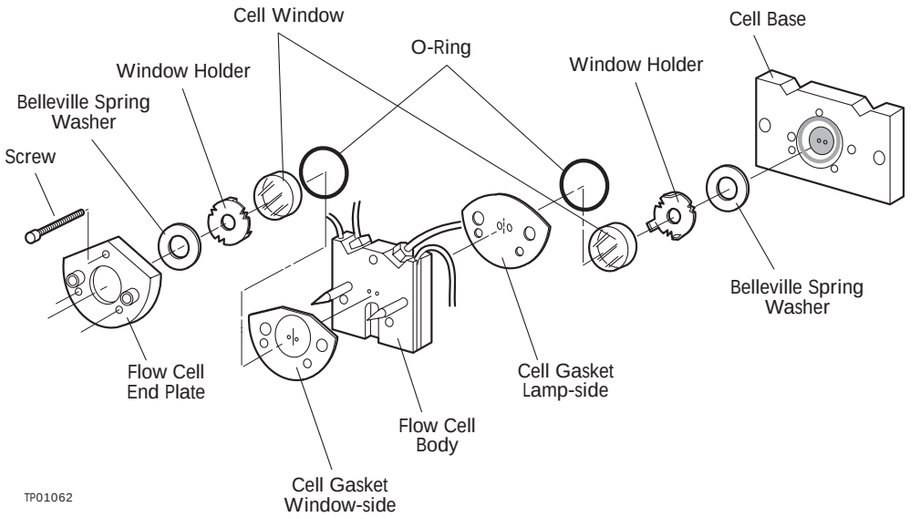


Figure 5-7 Analytical Flow Cell Components

Use the following procedure to disassemble the flow cell.

1. Using care not to touch the front pins, remove the three fillister screws from the front of the flow cell. Remove the flow cell end plate from the front of the flow cell and place the end plate on the bench front side down.
2. Remove the window-side (front) cell gasket and inspect it. Replace it if you find it to be worn or damaged.
3. Pull the flow cell body off the base.
4. Remove the lamp-side (rear) cell gasket and inspect it. Replace the gasket if you find it to be worn or damaged.
5. Inspect the windows in the flow cell end plate and cell base for cracks, scratches, or chips.
6. Clean each window with lens tissue or a cotton swab and ethyl alcohol. Replace the window if it is scratched.

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- If the windows are damaged or need replacement, refer to Section 5.3.5, Replacing a Damaged Flow Cell Window.
- If the windows are clean, refer to Section 5.3.6, Rebuilding the Flow Cell.

5.3.5 Replacing a Damaged Flow Cell Window

To replace a damaged flow cell window:

1. With a cotton swab, push the window out of the flow cell end plate or cell base.
2. Remove the window from the holder.
3. Place the spring washer, with the spring washer's concave side down, into the cavity of the end plate or base. Be careful to position spring washer concave side down or gaskets will not seal properly.
4. Place the new window in the window holder and place the entire unit into the flow cell end plate or cell base cavity.
5. Replace the O-ring.

After replacing the flow cell window, you are ready to rebuild the flow cell. Rebuild the flow cell according to the procedures in the next section.

5.3.6 Rebuilding the Flow Cell

To rebuild the flow cell:

Procedure

1. Place the lamp-side gasket onto the flow cell body, with the lamp side facing away from the flow cell body.
2. Place the flow cell body onto the cell base (Figure 5-7).
3. Place the window-side cell gasket onto the flow cell body with the window side facing away from the cell body.
4. Place the cell end plate onto the flow cell body and fit it firmly against the cell.
5. Screw in the three fillister screws. Tighten evenly.
6. Install the flow cell assembly back onto the Waters 486 Detector by feeding the manifold that holds the fluid connectors through the slot on the bracket. Reinstall the fluid manifold and tighten the two binder-head screws (reverse of Figure 5-5).
7. Install the two binder-head screws and star washers into the front of the cell, while holding the flow cell in place, (reverse of Figure 5-6).
8. Check that the gaskets are set. With the detector power off, pump solvent through the sample side and check for leaks. If the cell leaks, retighten the three small fillister screws.

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9. Reattach the ribbon cable to the clip on the back of the pre-amp module. Reinstall the pre-amp module and secure with the wing nuts.

Attention: *The ribbon cable must be secured in the clip to prevent contact with hot surfaces inside the detector.*

10. Connect the ribbon cable to the front panel PC board by inserting the cable connector into the mating connector on the PC board (Figure 5-8).

Figure 5-8 illustrates the ribbon cable positioning.

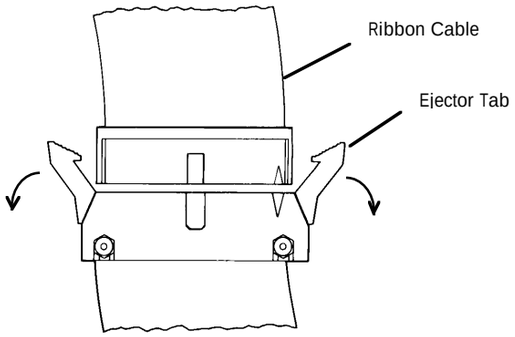


Figure 5-8 Connecting the Ribbon Cable

11. Reinstall the front panel using the three Phillips-head screws.

5.4 Replacing the Flow Cell

The Waters 486 Detector is shipped with a specific flow cell installed. Replace the flow cell when:

- The flow cell becomes damaged
- You want to use a different type of flow cell

Caution: *To avoid the possibility of exposing your eyes to ultraviolet radiation from the flow cell turn off the unit while performing the maintenance procedures described in this section.*

Note: *When you remove the preamp, the UV lamp automatically turns off.*

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Before you begin

1. Unpack and inspect the new flow cell.
2. Remove the detector front panel (Section 5.3.3, Removing the Pre-amp Module).
3. Remove the pre-amp module (see Section 5.3.3, Removing the Pre-amp Module).

Replacing the flow cell

To replace the flow cell:

1. Loosen the two binder-head screws and star washers from above the fluid manifold (Figure 5-9).
2. Remove the two large binder-head screws and star washers, while holding the flow cell in place in the detector (Figure 5-10).
3. Pull the flow cell away from the optics bench and the fluid manifold bracket.
4. Position the manifold for the new flow cell in place and tighten the two binder-head screws.

STOP ***Attention:*** *Do not touch the flow cell window. Clean dirt and fingerprints from the flow cell window with lens paper and ethanol.*

5. Secure the new flow cell to the optics bench with the two large binder-head screws.
6. Fold the ribbon cable and clip it on the back of the pre-amp module.

STOP ***Attention:*** *The ribbon cable must be secured properly in the clip to prevent contact with hot surfaces inside the detector.*

7. Replace the pre-amp module.
8. Reconnect the flat ribbon cable to the front panel PC board by inserting the cable connector into the mating connector on the PC board (Figure 5-8).
9. Replace the front panel.

Figure 5-9 illustrates how to remove the binder-head screws from the Manifold.

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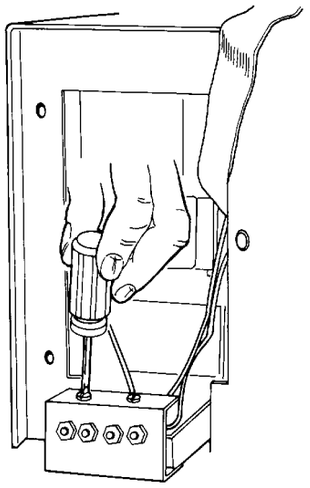


Figure 5-9 Removing Binder-head Manifold Screws

Figure 5-10 illustrates how to remove the binder-head screws from the Flow Cell.

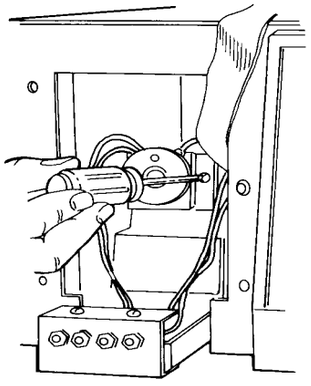


Figure 5-10 Removing Binder-head Screws from Flow Cell

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5.5 Replacing the Lamp

This section describes removing and replacing the Waters 486 Detector deuterium lamp.

The procedure include:

- Removing the old lamp
- Installing the new lamp
- Maximizing the lamp energy

Lamp characteristics

The intensity of the deuterium source lamp varies by wavelength as shown in Figure 5-11.

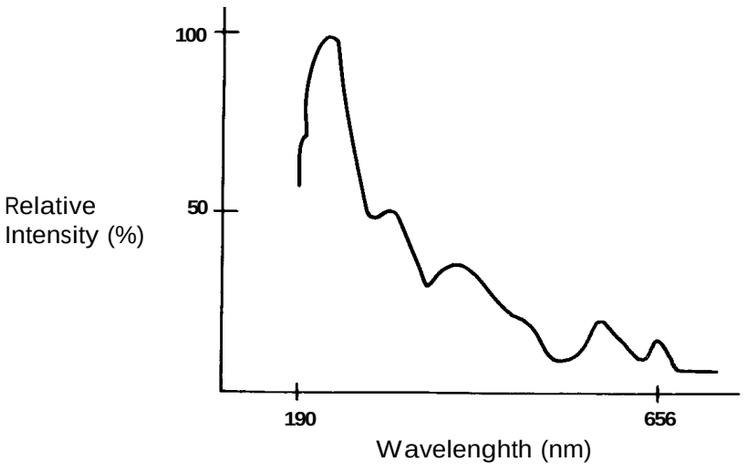


Figure 5-11 Deuterium Lamp Intensity Profile

When to replace the lamp

Performance requirements and permitted tolerances vary from application to application. If the lamp no longer provides an adequate signal-to-noise ratio for your specific application, replace it.

It is not unusual for lamps to show a 30 to 50 percent decrease in output before the noise increases by a factor of two.

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Lamp timer

The detector lamp has a life of approximately 1000 hours. One of the leads attached to the lamp includes a 1000-hour timer to indicate lamp usage.

Before you replace the lamp, check the lamp timer (see Figure 5-12).

The lamp timer is 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.

If lamp intensity is low, but the lamp has not been used for the expected number of hours, you may be able to increase the apparent lamp intensity by cleaning the flow cell, refer to Section 5.3, Cleaning the Flow Cell.

Tools required

The following tools are required to remove and replace the lamp assembly:

- Allen wrench
- Open-end wrench (3/8-inch)
- Flat-head screwdriver (small)
- Phillips-head screwdriver

Save all parts (screws, nuts, washers) after removal unless otherwise indicated. The parts are needed to install the new lamp.

5.5.1 Removing the Lamp



Caution: *To prevent eye damage from ultraviolet radiation exposure, turn off the unit and strictly adhere to the following procedures while changing the lamp.*

Note: *When you remove the protective cage, the UV lamp automatically turns off.*

Before removing the lamp

Before removing the lamp, set the wavelength to 230 nm. If the lamp is bad or fails to light and this is not possible, remove the lamp and replace as follows.



Caution: *Lamp housing becomes extremely hot during operation. To prevent burns to the skin, allow the lamp to cool for one hour before removing.*

Replacing the lamp

After turning off the unit and allowing the lamp to cool for one hour:

1. Remove the cover, as described in Section 5, Maintenance Procedures.

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2. Remove the protective cage and disconnect the lamp connector from the lamp interlock cable (Figure 5-12).

Figure 5-12 illustrates the lamp assembly connections.

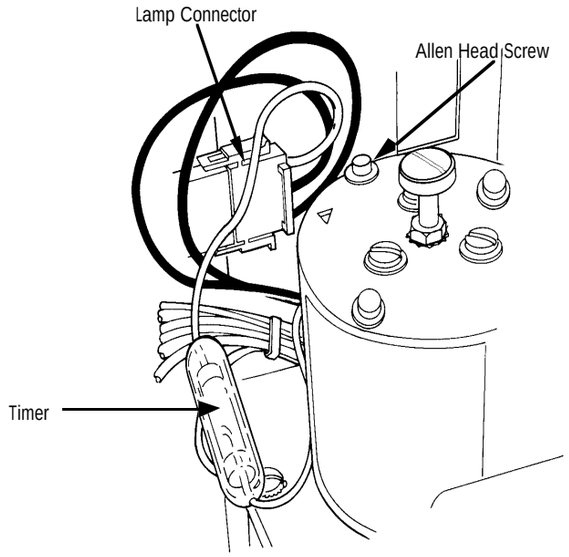


Figure 5-12 Lamp Assembly and Connections

3. From the top of the lamp assembly, remove the three Allen-head screws and flat washers (Figure 5-12).
4. Pull the lamp assembly out of the lamp housing (Figure 5-13).

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Figure 5-13 illustrates the lamp assembly removal procedure.

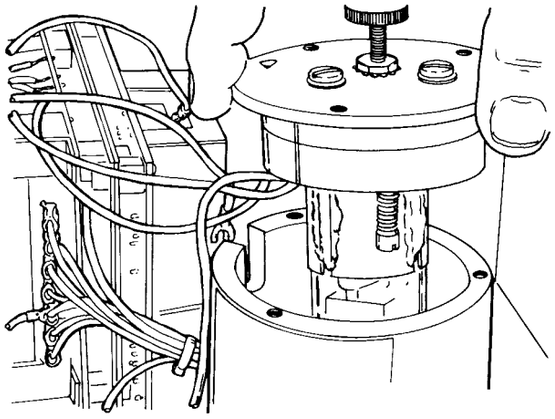


Figure 5-13 Removing the Lamp

5. From the top of the lamp assembly, remove the center adjustment screw with the star washer and the hex nut. Place the lamp assembly on a flat surface with the bulb positioned upward (Figure 5-14).

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Figure 5-14 illustrates the lamp aperture and adjustment plate.

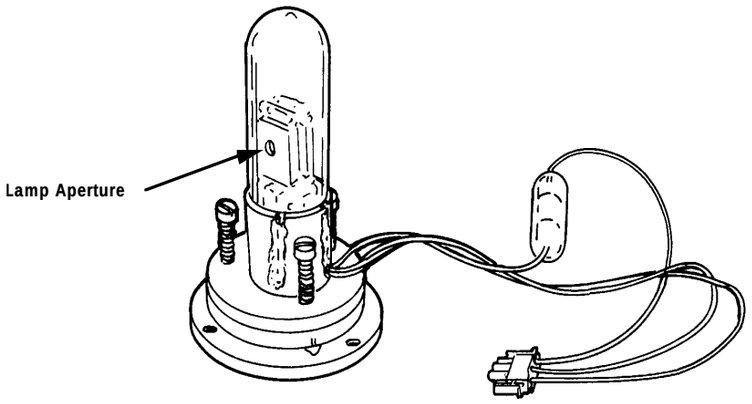


Figure 5-14 Lamp Assembly and Adjustment Plate

6. Remove the three shoulder screws with springs from the lamp. The springs are loosely coiled around the screws and do not spring off the screw when removed. The lamp adjustment plate can be separated from the deuterium lamp.

Caution: *Lamp gas is under slight pressure. Use care when disposing of the lamp to prevent shattering the glass.*

5.5.2 Installing the New Lamp

Before installing the new lamp

Unpack the lamp from its packing material. The new lamp may vary slightly from the lamp illustrated in Figure 5-14.

Attention: *Do not touch the glass bulb on the new lamp. Dirt or fingerprints on the bulb affect detector operation. If the lamp needs cleaning, gently clean the bulb with ethanol and lens tissue. Do not use any abrasive tissue or excessive pressure.*

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Procedure

To install the new lamp:

1. Position the lamp housing on a flat surface so the large cut-out on the adjustment plate faces away from you (Figure 5-15).

Figure 5-15 illustrates the lamp alignment procedure.

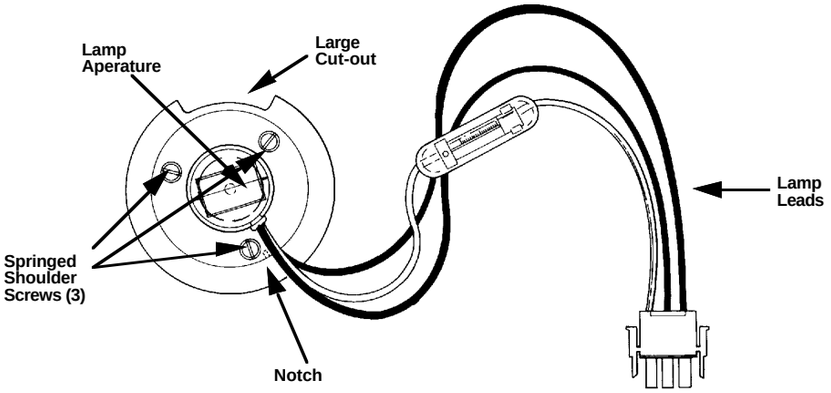


Figure 5-15 Aligning the Lamp (top view)

2. With the lamp aperture facing away from you, place the new lamp on top of the adjustment plate and secure it with the springed shoulder screws. The lamp aperture leads should line up with the notch on the lamp adjustment plate.
3. Without touching the bulb, turn the assembly so that the bulb is positioned downward.
4. Place the center adjustment screw with the hex nut and star washer in the center opening of the adjustment plate. In preparation for maximizing the lamp energy, turn the screw until the lamp assembly moves about 1/8 inch away from the adjustment plate.
5. Place the lamp assembly onto the lamp housing. Make sure the lamp leads slide freely into the housing slot.
6. Secure the lamp assembly to the housing and tighten the three Allen-head screws.
7. Plug the lamp connector into the interlock cable assembly (see Figure 5-12).
8. Replace the protective cage for access to the lamp.

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5.5.3 Adjusting the Lamp Position

After installation, position the lamp for maximum lamp energy. The detector does not have to be stabilized to perform this procedure.

To maximize lamp energy, you must:

- Access the Diagnostic Mode and monitor the reference energy.
- Make height and rotational adjustments to the lamp.
- Access Diagnostic Mode and recalibrate the detector.



Caution: *To prevent the possibility of exposing your eyes to ultraviolet radiation by wearing eye protection that filters ultraviolet light and by keeping the lamp in the housing during operation.*

Procedure

1. To adjust the position of the lamp, Plug in and power up the detector. Wait approximately one minute for the lamp to turn on.

- The display font **CAL** appears on the LCD while the Waters 486 Detector calibrates automatically.
- When calibration is complete, the operational wavelength appears.

If the lamp is too far out of adjustment, the Waters 486 Detector fails calibration.

If calibration is:	Then:
Successful,	Go to step 2
Unsuccessful,	Press any key. The detector displays the reference energy. Go to step 3 and perform the procedure to adjust the lamp energy.

2. Set the wavelength to 230 nm. Press the **DIAG** key to access Diagnostic mode. Enter **19** to monitor the reference energy. The reference energy displays on the upper LCD display.

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Height adjustment

3. Perform a lamp height adjustment to align the aperture of the lamp with the opening in the lamp housing.

To perform the height adjustment, watch the reference energy display on the front panel. At the same time, turn the center adjustment screw on the adjustment plate clockwise or counterclockwise until the highest reference energy displays.

Wait until you see a stable value to determine if you have reached peak energy.

Rotational adjustment

4. Perform a lamp rotational adjustment to allow the brightest portion of the arc of light to project through the opening in the lamp housing.



Caution: *The lamp assembly gets very hot and could burn you. Exercise caution when performing the height and rotation adjustments.*

To perform a rotational adjustment, loosen the Allen-head screws and move the lamp assembly (within the slots) clockwise or counterclockwise. Watch the reference energy display until the highest peak energy number displays, then tighten the screws.

5. Repeat the height adjustment to fine-tune the unit. When the reference energy peaks, tighten the hex nut on the center adjustment screw to prevent the center adjustment screw from moving.
6. Press the **Enter** key to exit Diagnostic 19.

If:	Then:
You skipped step 2 earlier because the unit did not successfully calibrate,	Turn off the unit and restart. Go to step 2 and repeat lamp adjustment procedure at 230 nm.
The unit now successfully calibrates,	Go to step 7.
Calibration fails,	Follow instructions in steps 3 to 6 to adjust the lamp energy. Refer to Chapter 6, Error Codes and Diagnostics, for a description of calibration error codes.

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7. Recalibrate the detector by pressing the **DIAG** key and entering **4**. Calibration takes approximately two and one-half minutes to complete.

If calibration is:	LCD displays:	Then:
Successful,	Precalibration settings.	Go to step 8.
Unsuccessful,	FAIL (upper LCD) Calibration error code 4 ##, where ## is a number from 00 to 99(lower LCD).	Adjust the lamp energy again as described in steps 3 through 6. Refer to Chapter 6, Error Codes and Diagnostics, for a description of the calibration error codes.

8. When the calibration procedure is successful, reinstall the cover by sliding the pin in the left rear corner of the cover into the notch on the chassis and securing it with four screws.

5.6 Replacing Fuses

This section contains the procedure for replacing the:

- Rear panel fuse
- Lamp power supply board fuses



Caution: *To avoid the possibility of electric 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

A faulty fuse usually has a smoked glass area or broken filament. If no break is visible, you can remove the fuse and test with an Ohm meter.

Check the rear panel fuse if the detector does not powerup.

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Check the lamp power supply fuses when:

- The fan starts but the front panel does not illuminate. Verify that the plug connections on the lamp power supply board are inserted tightly before checking the fuses.
- The detector displays zero energy or if it fails calibration.

5.6.1 Replacing the Rear Panel Fuse

The rear panel fuse is the main A/C power fuse. Refer to Chapter 2, Installation and Instrument Verification for information on replacing the rear panel fuse.

Caution: *To avoid the possibility of electric shock, power down unit and unplug the power cord before replacing fuses.*

5.6.2 Replacing Lamp Power Supply Fuses

The lamp power supply board requires seven fuses:

- Four low-voltage fuses
- Three high-voltage fuses

Figure 5-16 shows the fuse locations.

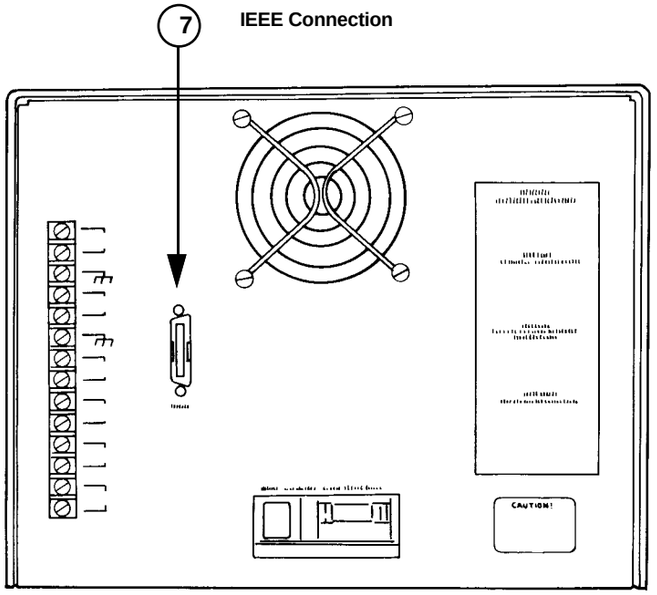


Figure 5-16 Lamp Power Supply Board Fuses

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Caution: *To avoid the possibility of electric shock, turn off the unit and unplug the power cord before replacing the fuses.*

Replacing low and high voltage fuses

1. Turn off the detector and disconnect the power cord.
2. Remove the cover and the protective cage.

   Note: *The unit will automatically turn off when you remove the protective cage.*

3. Remove the two binder-head screws on the base of the lamp power supply bracket. Tilt the lamp power supply away from the unit as shown in Figure 5-17.

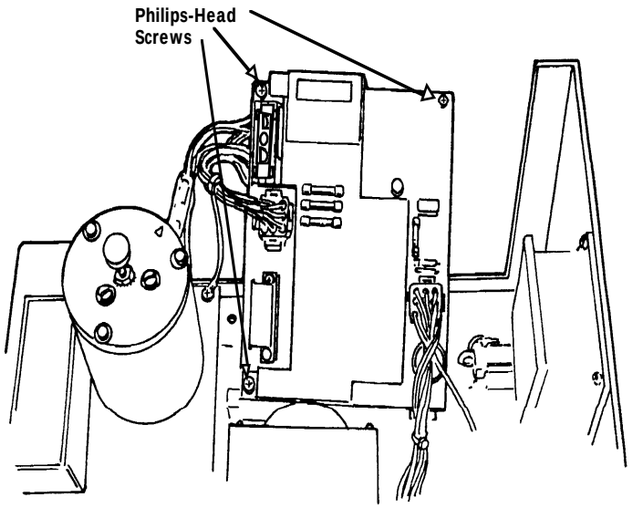


Figure 5-17 Low and High Voltage Fuse Locations

4. Visually inspect the filament on all low-voltage fuses to determine which has failed.
5. Remove and replace the faulty fuse(s).
6. Reinstall the protective cage and the detector cover.
7. Connect the power cord and turn the unit on.

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6 Error Codes and Diagnostics

The Waters 486 Tunable Absorbance Detector uses error-code-producing self-test diagnostic routines and user diagnostics to troubleshoot system problems.

- **Error Codes** - Describe powerup and calibration error codes and recommended actions for correcting the errors.
- **Diagnostics** - Describe user diagnostics for troubleshooting and configuring the detector.

6.1 Error Codes

The Waters 486 Detector performs a self-test diagnostic routine each time the unit is turned on or calibrated. The error codes are generated when automatic powerup or the calibration diagnostics fail.

Diagnostic error messages and corresponding corrective actions are listed in tables provided in this chapter.

When the suggested corrective action fails to resolve the problem, refer to Chapter 7, Troubleshooting for additional information.

6.1.1 Powerup Error Codes

Powerup diagnostics are executed automatically when the is turned on. These diagnostics verify the proper operation of the detector electronics. Some powerup diagnostics can be used to troubleshoot the Waters 486 Detector, these are described in greater detail in Section 6.2, User-Selected Diagnostics.

Powerup diagnostic failures

When powerup diagnostics detect a failure, the upper LCD on the front panel displays the word FAIL and the lower LCD displays the specific error code number.

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Powerup diagnostic error codes and recommended corrective actions are provided in the table below:

Error Code	Probable Cause	Corrective Action
FAIL 1	RAM not preserved.	Reprogram the parameter settings. Use Detector to check battery. Call Waters Technical Service for battery replacement.
FAIL 2	One or more DIP switches on the CPU board is/are closed (ON).	Turn off DIP switches.
FAIL 4	Scan parameters entered are out of range.	Press any key to continue.
FAIL 100	CPU RAM test failed.	Call Waters Technical Service.
FAIL 102	EPROM checksum test failed.	Call Waters Technical Service.

6.1.2 Calibration Error Codes

The Waters 486 Detector automatically calibrates when the unit is turned on. Calibration may also be initiated manually, using Diagnostic 4. Refer to Section 6.2, User-Selected Diagnostics.

Wavelength Calibration, describes the calibration routine. Refer to Section 1.2.2, Light Path Wavelength Calibration.

Calibration diagnostic failures

When calibration fails, calibration diagnostic error codes are displayed.

The upper LCD on the front panel displays the word **FAIL** and the lower LCD displays the specific error code number.

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Calibration diagnostic error codes and suggested corrective actions are provided in the table below:

Error Code	Probable Cause	Corrective Action (in sequence)
FAIL 402 FAIL 403 FAIL 404 FAIL 405 FAIL 406 FAIL 407	Insufficient reference energy found by the software	1. Clean cell and recalibrate. Refer to Section 5.3, Cleaning the Flow Cell. 2. Check fuses on lamp power supply board; replace if necessary. Refer to Section 5.6, Replacing Fuses. 3. Align the lamp. Refer to Section 5.5.3, Adjusting the Lamp Position. 4. Replace lamp and recalibrate. Refer to Section 5.5, Replacing the Lamp. 5. If unit still fails to calibrate, call Waters Technical Service.
FAIL 400 FAIL 401 FAIL 408 FAIL 409 FAIL 410	Wavelength tuner not functioning properly	6. Recalibrate. If unsuccessful, call Waters Technical Service.

6.2 User-Selected Diagnostics

In addition to the automatic self-diagnostic routine, user-selectable diagnostics are also available for use in:

- Troubleshooting the detector
- Configuring the detector
- Verifying the detector electronics and optics

Refer to Section 2.4, Instrument Verification

Some of the automatic self-diagnostics are also user-selectable diagnostics. Refer to Section 6.1, Error Codes for recommended actions on failed diagnostics.

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User-selected diagnostics are activated by accessing diagnostic mode and entering specific key sequences on the keypad.

Procedure

To perform user-selectable diagnostics:

1. Press the **Diag** key.

   The detector displays **ddd** on the lower display, indicating diagnostic mode.

2. To access a particular diagnostic, enter the corresponding number code of the diagnostic and press **Enter**. Refer to the diagnostic codes in Table 6-1.

   Once you press the **Enter** key, subsequent processing depends on the code entered.

   As in normal operation, you may use the **Clear** key to remove the entry and start over.

3. After the diagnostic code and response is displayed, you may have to press any key to return to normal operation. In some cases, you may return to normal operation automatically.

Diagnostic overview

Table 6-1 lists diagnostic codes alphabetically to help locate diagnostics by name. For a detailed description of each diagnostic, refer to the Diagnostics Description section following Table 6-1.

Table 6-1 Diagnostic Quick Reference

Name	Number
Absorbance, fix input	5
Automatic auto zero, disable	23
Automatic auto zero, enable	22
Auto zero, cancel	9
Auto zero, display offset	8
Calibrate wavelength	4
Disable 1V chart mark	25

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Table 6-1 Diagnostic Quick Reference *(Continued)*

Name	Number
Enable 1V chart mark	24
EPROM checksum test	3
Filter, hamming	17
Front panel display test	10
IEEE-488 address	13
Integrator, fix output	6
Integrator, set offset	21
Keypad test	11
Perform scan	27
RAM test	1
Recorder, fix output	7
Reference energy	19
Sample and reference, invert	16
Sample and reference, normal	15
Software version number	12
Solicit scan parameters	26
Test peak	14

Table 6-2 details the user-selectable diagnostics. The diagnostics are arranged numerically by code number.

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Table 6-2 User-Selectable Diagnostics

Diag Code	Description
1	**Non-destructive RAM Test (automatic on powerup):** Tests the battery-backed static RAM on the CPU board. The front panel blanks during this test. When the test is complete, the front panel indicates PASS or FAIL. If the test fails, the detector cannot be used. Press any key to exit.
3	**EPROM Checksum Test (automatic on powerup):** Verifies the checksum in the EPROM on the CPU board. When the test is complete, the front panel indicates PASS or FAIL. Press any key to exit.
4	**Calibrate Wavelength (automatic on powerup):** Calibrates the wavelength. The keypad is locked during the diagnostic. If calibration fails, the word FAIL appears in the upper display. Press any key, the unit displays the reference energy on the upper display. If calibration is successful, the unit returns to normal operation.
5	**Fix Absorbance Input:** Electronically sets a fixed absorbance value. Values you can select are: .000, .001, .010, .100, 1.00, 2.00 AU. The value 1 AU is equal to 1V. Verify the absorbance value by connecting a recorder or volt meter to the 1-V output on the detector rear panel. When you enter this diagnostic, the first absorbance value displays. Press **Enter** to advance through additional absorbance values. Each value takes effect as it is displayed. Press **Enter** after the last selection to exit.

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Table 6-2 User-Selectable Diagnostics *(Continued)*

Diag Code	Description
6	**Fix Integrator Output:** Sets a fixed voltage for the integrator output. Values you can select are: 0.0, 0.5, 1.0, 2.0 V and OFF. When you enter this diagnostic, the first allowable voltage value is displayed. Press **Enter** to advance through additional voltage values. Each value takes effect automatically as it is displayed. Press **Enter** after the last selection to exit.
7	**Fix Recorder Output:** Sets a fixed voltage for the recorder output. Selectable values are minus ten, minus five, minus one, zero, 1.5, 10-mV. When you enter this diagnostic, the first allowable voltage value is displayed. Press **Enter** to advance through entries. Each value takes effect automatically as it is displayed. Press **Enter** after the last selection to exit.
8	**Display Auto Zero Offset:** Displays the total auto zero correction in AU in the upper display window. Press any key to exit.
9	**Cancel Auto Zero:** Removes the effect of auto zero and displays the true absorbance (in AU).
10	**Front Panel Display Test:** Tests the front panel indicators and character displays. Each LCD annunciator flashes alternately, then each numeric display shows the digits 0 through 9, with the decimal point and the zero. The detector exits this diagnostic automatically.

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Table 6-2 User-Selectable Diagnostics *(Continued)*

Diag Code	Description
11	**Keypad Test:** Tests each key on the keypad. Press each key once. As you press each key, the column/row designation displays on the upper display (see Figure 6-1). For example, when you press the **1** key, **A1** appears on the display. Columns are labeled A, B, C, and D; rows are labeled 1, 2, 3, 4, 5, 6. The detector exits this diagnostic automatically after all the keys have been pressed at least once.
12	**Display software version number (automatic on powerup):** Displays the software version number on the upper display. Press any key to continue.
13	**Display IEEE-488 Address:** Displays the IEEE-488 bus address. If the address is 0, IEEE operation is disabled. Enter the new address in the range 1 to 29, then press **Enter**. The unit must be powered off and on again for the new IEEE bus address to take effect. If your unit is not equipped for IEEE operation, the address must be set to zero.
14	**Generate Test Peak:** Generates a Gaussian test peak. Default settings are 1.0 AU, with a standard deviation of 10 seconds, and a retention time of 50 seconds. This diagnostic is useful for checking the operation of chart recorders and integrators, or for examining the effects of different filter settings on peak shape.
15	**Normal Sample and Reference:** Restores the detector to its normal state after it has been inverted by Diagnostic 16.

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Table 6-2 User-Selectable Diagnostics *(Continued)*

Diag Code	Description
16	**Invert Sample and Reference (Delta mode):** Electronically adjusts the detector to allow you to run samples through the reference side of the flow cell. When operating in Delta mode, the display shows **DEL** and **CAL** during the powerup calibration routine.
17	**Select Hamming Filter:** With the Hamming filter selected, the maximum allowable time constant is 5.0 seconds.
18	**Select RC Filter:** Selects first-order RC filter. With the RC filter, the maximum allowable time constant is 99.9 seconds.
19	**Monitor Reference Energy:** Continuously monitors the reference energy. (The reference energy value displayed normally on the front panel is updated only every 10 seconds.) This diagnostic is useful when maximizing lamp energy. Reference energy is also included on the recorder output while this diagnostic is in effect. Press any key to exit.
20	**Lamp Elapsed Timer:** Used to program an elapsed time for the lamp to shut off. The timer is set in increments from 0.1 to 99.9 hours.
21	**Set Integrator Offset:** Offsets the integrator output voltage by 0, 10, 20, 30, 40, 50, 100, or 150 mV. When you enter this diagnostic, the first allowable voltage value is displayed. This increases the baseline above zero to allow for detection of negative peaks and drift. This is a voltage offset, not a pen offset. Press any key except **Enter** to advance through the voltage values. When the correct value displays, press **Enter** for normal operation. This value remains in effect between powerups.

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Table 6-2 User-Selectable Diagnostics *(Continued)*

Diag Code	Description
22	**Enable Automatic Auto Zero:** Sets the unit to auto zero with each wavelength change.
23	**Disable Automatic Auto Zero:** Disables automatic auto zero with each wavelength change.

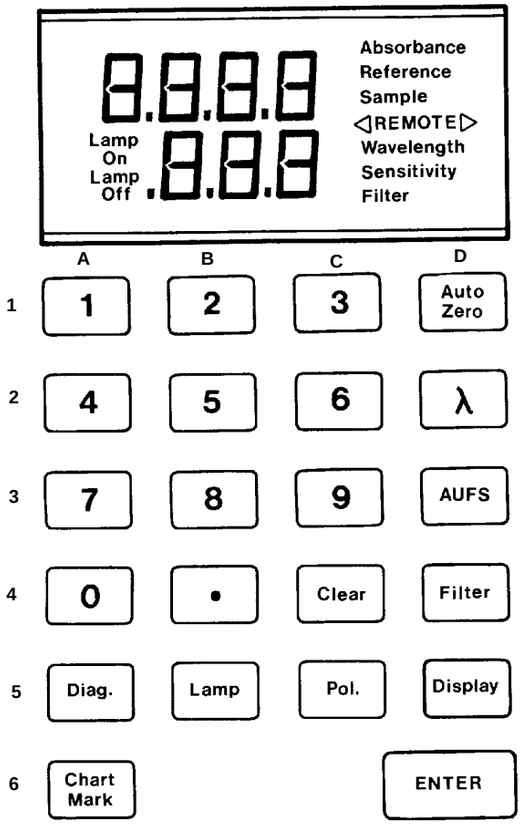


Figure 6-1 Keypad Test

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The diagnostic functions listed in Table 6-3 are available with version 4.06 software (or later).

Table 6-3 Diagnostics for Version 4.06 or Later

Diag Code	Description
24	**Enable Chart Mark on 1V (integrator) Output:** Enables the chart mark function to apply to the integrator (1 V/AU) output as well as the recorder (10 mV full scale) output. A deflection of 10% of full scale (.2V) appears on the output. Once this function is enabled, it remains enabled between powerups. To disable the function, use the Diagnostic 25.
25	**Disable Chart Mark on 1V (integrator) Output:** Cancels the chart mark signal to the integrator and recorder.
26	**Solicit Scan Parameters:** View and/or change the parameters which define the current scan, including starting wavelength, ending wavelength, step size, and step duration. Each parameter is displayed in the order listed above. Step size is in nanometers and Step duration is in seconds and tenths of a second. You may enter new values for one or more of these parameters, or press **Enter** to toggle through to the next parameter. Scan parameters remain in effect for all scans until altered or until powerdown. The default scan parameters, listed in Table 4-2, appear upon powerup.
27	**Perform Scan:** Performs a scan in accordance with the current scan parameters. To abort a scan, press any key.

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7 Troubleshooting

This chapter contains procedures for troubleshooting the Waters 486 Tunable Absorbance Detector and provides a checklist of probable causes, and recommended corrective actions.

Keep in mind that the source of apparent detector problems may lie within the chromatography or hardware of your unit.

This chapter is structured as follows:

Section	Describes
Section 7.1, General Troubleshooting	A general approach to isolating problems
Section 7.2, Chromatography Troubleshooting	Problems and corrective actions associated with chromatography systems, such as a contaminated column.
Section 7.3, Hardware Troubleshooting	Problems and corrective actions associated with the Waters 486 Detector, such as the lamp not lighting.

Most detector problems are relatively easy to correct. If you are unable to correct a problem or a failed condition, contact your Waters Technical Service Specialist for information.

When you call Waters Technical Service

To expedite your request for service, have the following information available when you call Waters Technical Service.

1. Problem symptom(s)

1. Operating wavelength
2. Sensitivity setting (.001 to 2.00)
3. Flow rate
4. Filter setting

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5. Type of column
6. Operating pressure
7. Solvent(s)
8. Diagnostic error code
9. System configuration

Note: *The Waters 486 Detector may be configured with a 600E, 860, or a non-Waters product.*

Diagnostics

The Waters 486 Detector performs user-selected diagnostics to help you troubleshoot basic system problems. Refer to Chapter 6, Error Codes and Diagnostics, for diagnostic descriptions and instructions on how to use them.

Diagnostic error codes

Diagnostic error codes and suggested corrective actions are described in Chapter 6, Error Codes and Diagnostics.

Power surges

Power surges, line spikes, and transient energy sources can adversely affect detector operations. Be sure that the electrical supply used for the detector is properly grounded and free from any of these conditions.

7.1 General Troubleshooting

This section describes general troubleshooting procedures using trees and corresponding Chromatography Troubleshooting Tables to isolate possible causes

Troubleshooting procedures

This section provides a logical approach to troubleshooting different symptoms.

Step 1: Take a step back and look at the system. Determine if something obvious is causing the problem.

Example: If the unit does not power up when you turn it on, verify that the unit is plugged in. This may seem like an oversimplified example, but always eliminate simple problem causes first.

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Step 2: Compare current system operation with system operation before the problem started.

Example: If your system usually runs at X psi with a certain method, is the system pressure currently in the same range, or drastically higher (possibly caused by a plug), or lower (possibly caused by a leak)? Are pressure fluctuations in the same pressure range as during normal operation?

Note: *This step points out the importance of keeping track of system parameters and the results of your chromatography during normal operation. Troubleshooting the system is easy if you know the working system conditions.*

When your system is installed, get in the habit of keeping a record of system conditions under normal operations.

Step 3: Isolate the parameter that varies from normal operation. The parameters to observe are:

- Baseline noise
- Peak retention time
- Peak resolution
- Qualitative/quantitative chromatography results
- System pressure

Evaluate the parameters in the order given above to rule out simple problem causes.

Step 4: Use the Troubleshooting Trees that correspond to parameters in step 3 to narrow the possible cause. Refer to Chapter 7.2, Chromatography Troubleshooting.

Step 5: Use the appropriate troubleshooting tables to determine a corrective action for the possible cause that you isolated in step 4.

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7.2 Chromatography Troubleshooting

Before using the chromatography troubleshooting tables in this section, read the general troubleshooting hints in the previous section. Follow the procedural steps recommended in that section to isolate the cause of the chromatography problem.



Caution: *To avoid harmful accidents, be sure to observe proper laboratory practices when handling solvents or performing maintenance.*

7.2.1 Troubleshooting Trees and Tables

The troubleshooting trees and tables in this section help to isolate possible causes of problems related to:

- High baseline noise
- Erratic or incorrect retention times
- Poor peak resolution
- Incorrect qualitative/quantitative results
- High system pressure

If your system is exhibiting symptoms addressed in a troubleshooting tree, refer to the following chromatography troubleshooting table for additional help.

If your system is exhibiting symptoms *not* addressed in a troubleshooting tree, refer to the troubleshooting table in Chapter 7.3, Hardware Troubleshooting.

High baseline noise

If baseline noise is high, determine if it is a short or long term variation. You can eliminate many causes of baseline abnormality by identifying the rate at which the baseline is changing.

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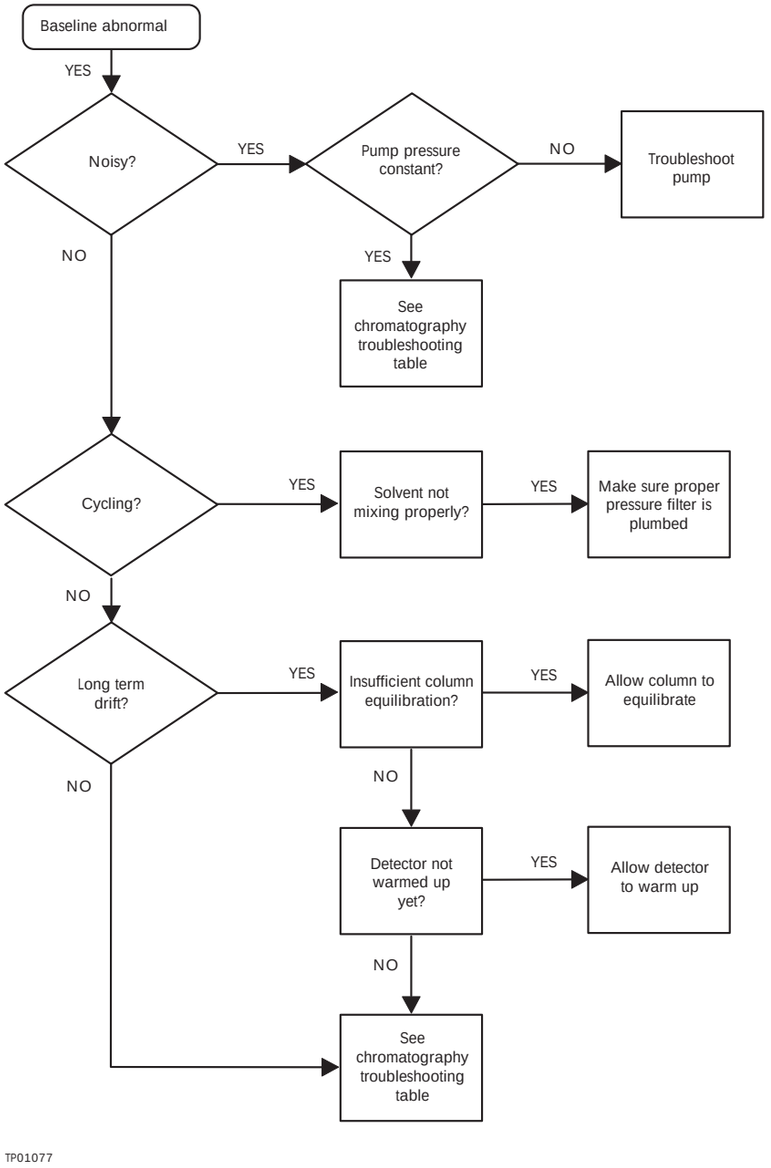
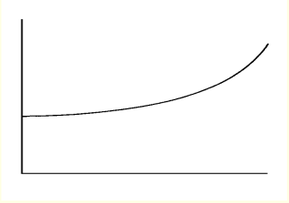
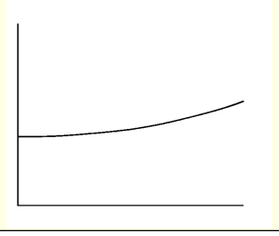


Figure 7-1 Abnormal Baseline Troubleshooting Flowchart

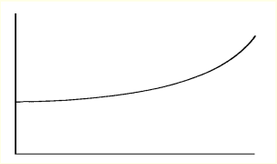
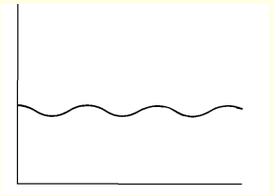
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Table 7-1 Baseline Abnormality Troubleshooting

Symptom	Possible Cause	Corrective Action
Baseline drift, rapid 	Column not equilibrated	Equilibrate column.
	Detector not allowed to warm-up	Allow detector to warm up until baseline is stable. Time varies based on wavelength and sensitivity.
	Solvent contaminated or not 4752 grade	Use fresh solvent.
	Solvent not properly degassed (rapid or slow drift)	Degas solvent.
	Flow fluctuations (rapid or slow drift)	Fix pump problems, replace pump seals, check valves.
	Incorrect wavelength for solvent	Use correct wavelength or solvent (make sure solvent does not absorb UV light at the wavelength used).
Baseline drift, slow 	Solvent contaminated	Use fresh solvent.
	Decreased UV lamp energy	Check lamp energy using the detector diagnostics. Refer to Table .

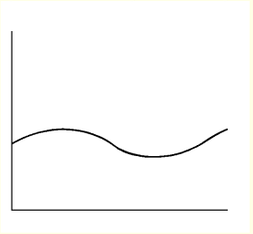
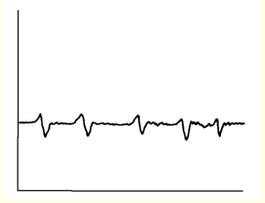
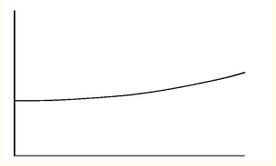
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Table 7-1 Baseline Abnormality Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline drift, slow (continued) 	Ambient temperature fluctuations	Stabilize operating environment temperature enough to allow full equilibration.
	Flow cell leaking (internal, cross-port)	Check flow cell, tighten connections.
	Dirty flow cell	Clean flow cell.
Baseline noise cycling **Short term (30 sec to 1 min)** 	Pump pulsing	Add pulse dampener.
	Inadequate solvent blending in pump	Connect high flow pulse dampener. Use gradient mixer.
	Flow fluctuating	Stabilize flow.
	Solvent not mixed (short-term or long-term cycling)	Stir solvent.
	AC power source (short -term or long-term cycling)	Disconnect other instruments on the power line, try using a different wall outlet, have line voltage checked, or use a power conditioner.

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Table 7-1 Baseline Abnormality Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
	Radio frequency noise (short-term or long-term cycling)	Eliminate interference.
Long term (approximately 1 hour) 	Ambient temperature fluctuations	Stabilize ambient temperature.
	Integrator or recorder faulty	Check integrator or recorder for excessive baseline noise.
Baseline noise, random 	Air in detector	Purge detector to remove air.
	Solvents not properly degassed or sparged	Degas/sparge solvents. Refer to Section 1.4.2, Solvent Degassing, for more information. Add 12 inches of 0.009-inch tubing to the detector outlet to apply some backpressure.
	Flow erratic, pump not primed	Prime the pump.
		Check for air in the pump, failing seals.
	Solvents contaminated	Use fresh solvent.
	Column contaminated	Clean/replace column.

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Table 7-1 Baseline Abnormality Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Baseline noise, random (continued)	Dirty flow cell	Clean flow cell. Refer to Section 5.3, Cleaning the Flow Cell.
	Analog output cable not properly connected to the detector and data system, recorder, or integrator	Properly connect cable.
	System improperly grounded	Plug into different electrical circuit. Use power conditioner.
	Recorder voltage incorrect	Set recorder to correct voltage.
	Radio frequency noise	Eliminate interference.
	Deuterium lamp has deteriorated significantly	Replace lamp.

7.2.2 Erratic or Incorrect Retention Times

Retention time changes

When troubleshooting retention time problems, determine whether the retention times:

- May vary from one run to another
- Are constant from run to run, but are outside the allowable range for the assay

When troubleshooting a retention time problem, check the system pressure and watch for the following:

- **Pressure fluctuations** - Are they short term (with each pump cycle) or long-term (over the course of several minutes)?
- **An absolute pressure change** - If the pressure is not fluctuating, is it higher or lower than the normal operating pressure?

Determine whether retention time problems occur:

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- Suddenly at the end of a series of test runs. This may indicate that the air is dissolving in the mobile phase, that the mobile phase is degrading, or that the column is contaminated.
- Early in a series of runs and tend to become constant or within range after 3 to 4 minutes. This may indicate that the column was not equilibrated, or that the solvent is not properly degassed and sparged.

Figure 7-2 and Figure 7-3 (troubleshooting flowcharts) can be used to assist you in determining the cause of erratic retention time problems.

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Pressure fluctuations and changes

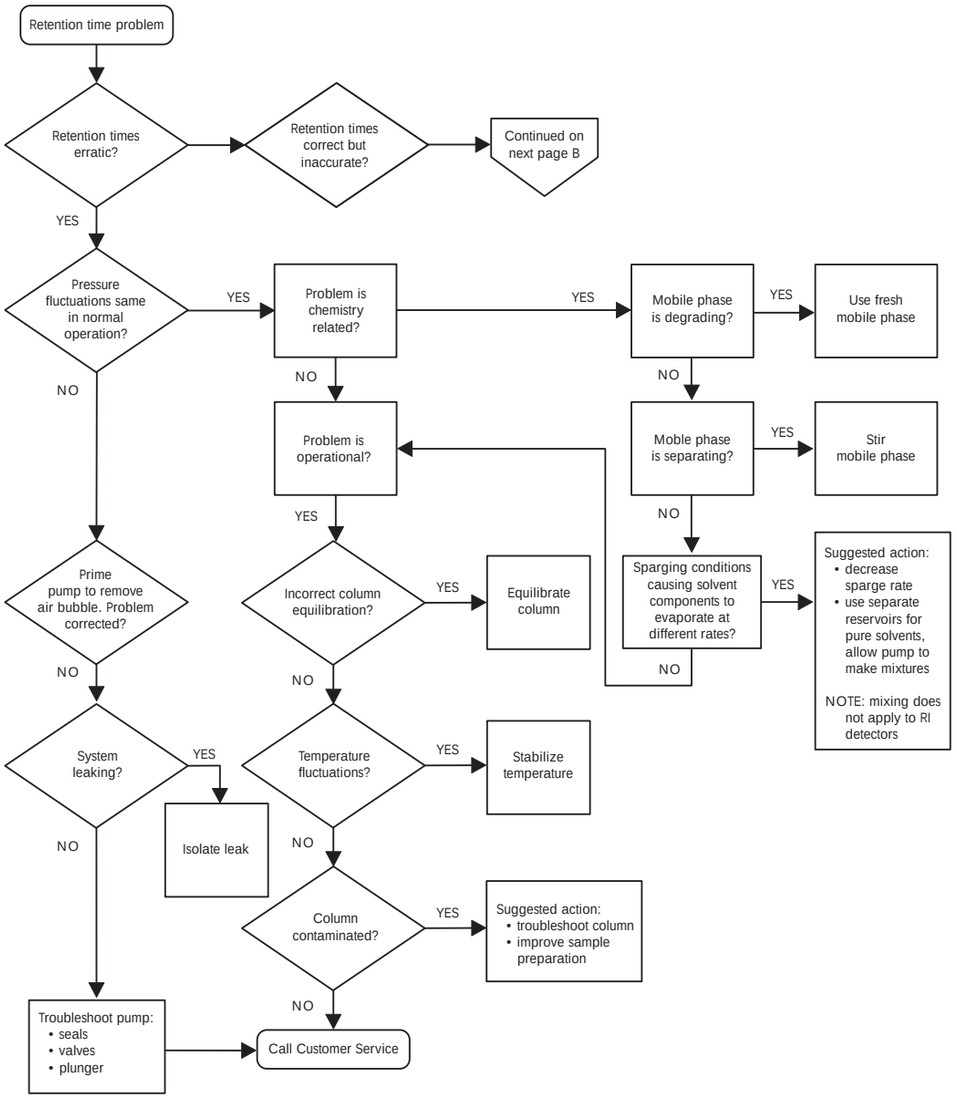


Figure 7-2 Erratic Retention Time Troubleshooting, Part 1

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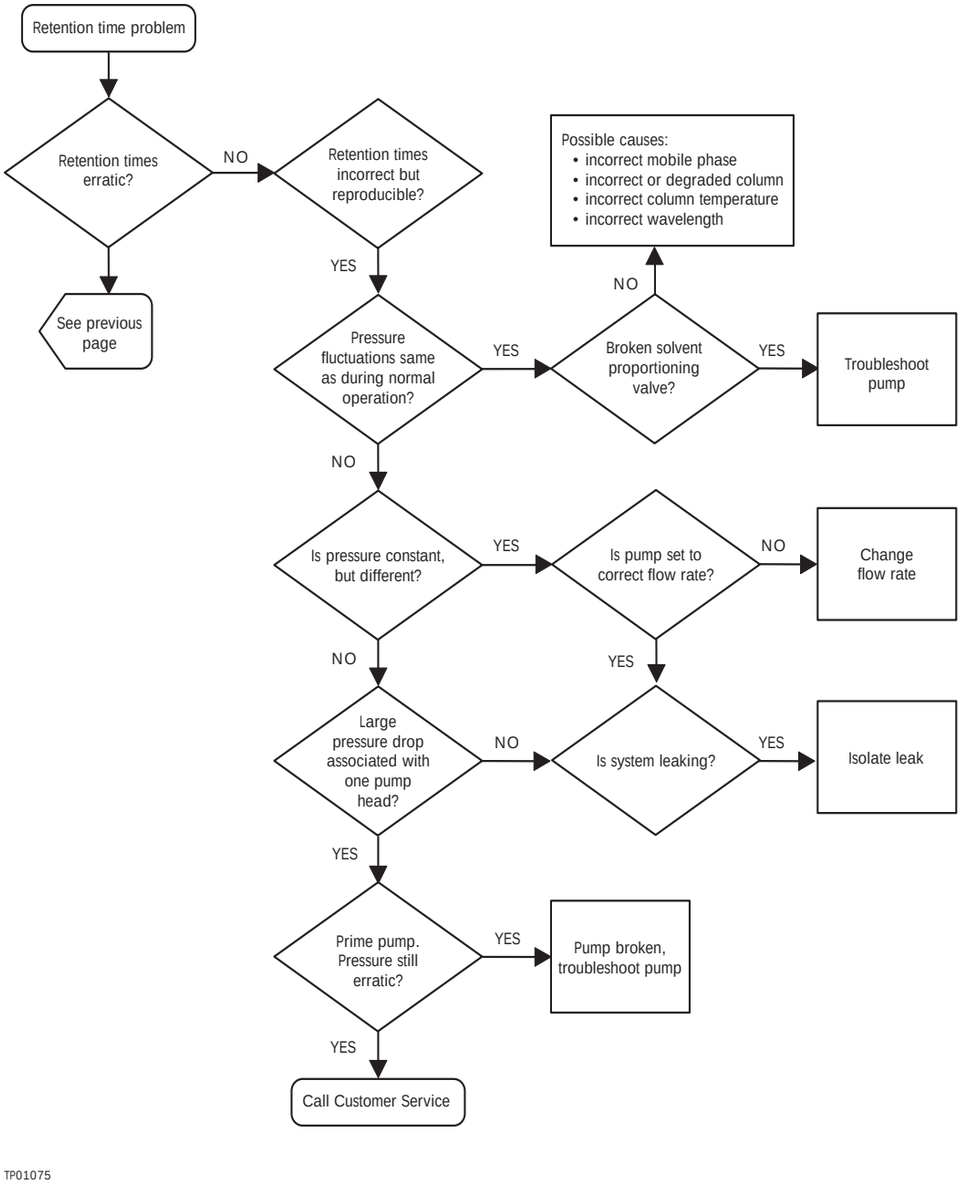


Figure 7-3 Erratic Retention Time Troubleshooting, Part 2

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Table 7-2 Retention Time Troubleshooting

Symptom	Possible Cause	Corrective Action
Erratic retention times	Air bubble in pump head	Degas all solvents, prime pump. Check sparge rate.
	Malfunctioning pump check valves	Clean/replace pump, check valves.
	Leaking pump seals	Replace pump seals.
	Separation chemistry	Check mobile phase and column.
	Clogged solvent filters	Replace filters.
Increased retention times	Incorrect flow rate	Change flow rate.
	Incorrect solvent composition	Change solvent composition.
	Column heater module not turned on	Turn on column heater module.
	Incorrect mobile phase	Use correct mobile phase.
	Column contaminated	Clean/replace column.
	Incorrect column	Use correct column.
Doubled retention times	Air bubble in pump head	Prime pump to remove bubble.
	Malfunctioning pump	Clean/replace pump, check valves.
	Broken pump plunger	Replace the plunger.
Reduced retention times	Incorrect flow rate	Change flow rate.

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Table 7-2 Retention Time Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Reduced retention times (continued)	Incorrect solvent composition	Change composition.
	High column temperature	Reduce column temperature.
	Incorrect mobile phase	Use correct mobile phase.
	Column contaminated	Clean/replace column.
	Incorrect column	Use correct column.
Reproducibility errors	Solvent not properly degassed/sparged	Degas/sparge solvent. Refer to Section 1.4.4, Solvent Degassing Methods.
	Incorrect chemistry/integration	Check chemistry/integration.
	Auto-injector problem	Troubleshoot auto-injector.

7.2.3 Poor Peak Resolution

Before addressing problems with peak resolution, be certain that peaks elute at the correct retention time. Common causes of poor peak resolution, may appear to be retention time problems.

If the peak the retention time is correct, determine if poor resolution has occurred:

- Throughout the chromatogram
- At a single peak pair

In a gradient method, if resolution of early peaks is poor, but improves later in the chromatogram, pre-column band broadening, such as auto-injector or guard column failure may be at fault. If peak resolution is poor throughout the chromatogram, post-column band-broadening or loss of column efficiency may implied.

If one peak in the chromatogram is badly-shaped, the peak component may be interacting with the column, in a way that is different from the interaction of other components. To troubleshoot this resolution problem, you need an understanding of the separation chemistry.

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Table 7-4 and Table 7-5 (flowcharts) can be used to troubleshoot the cause of peak resolution problems.

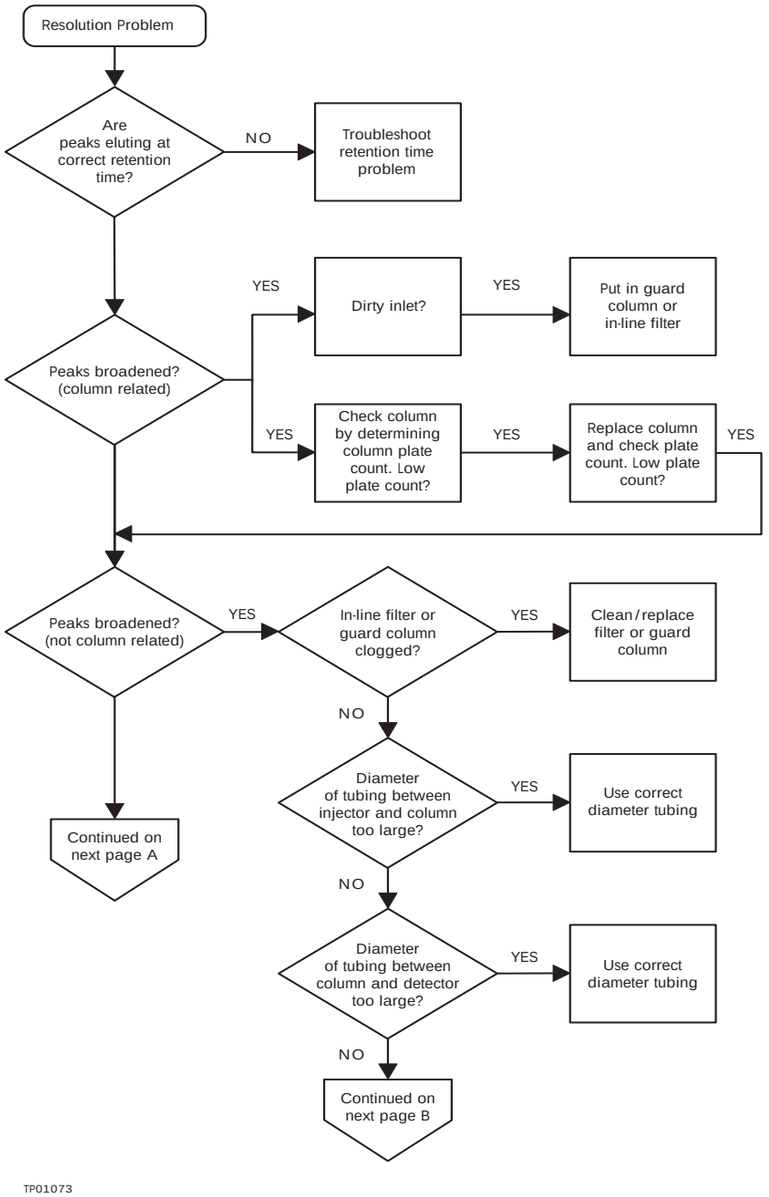


Figure 7-4 Peak Resolution Troubleshooting, Part 1

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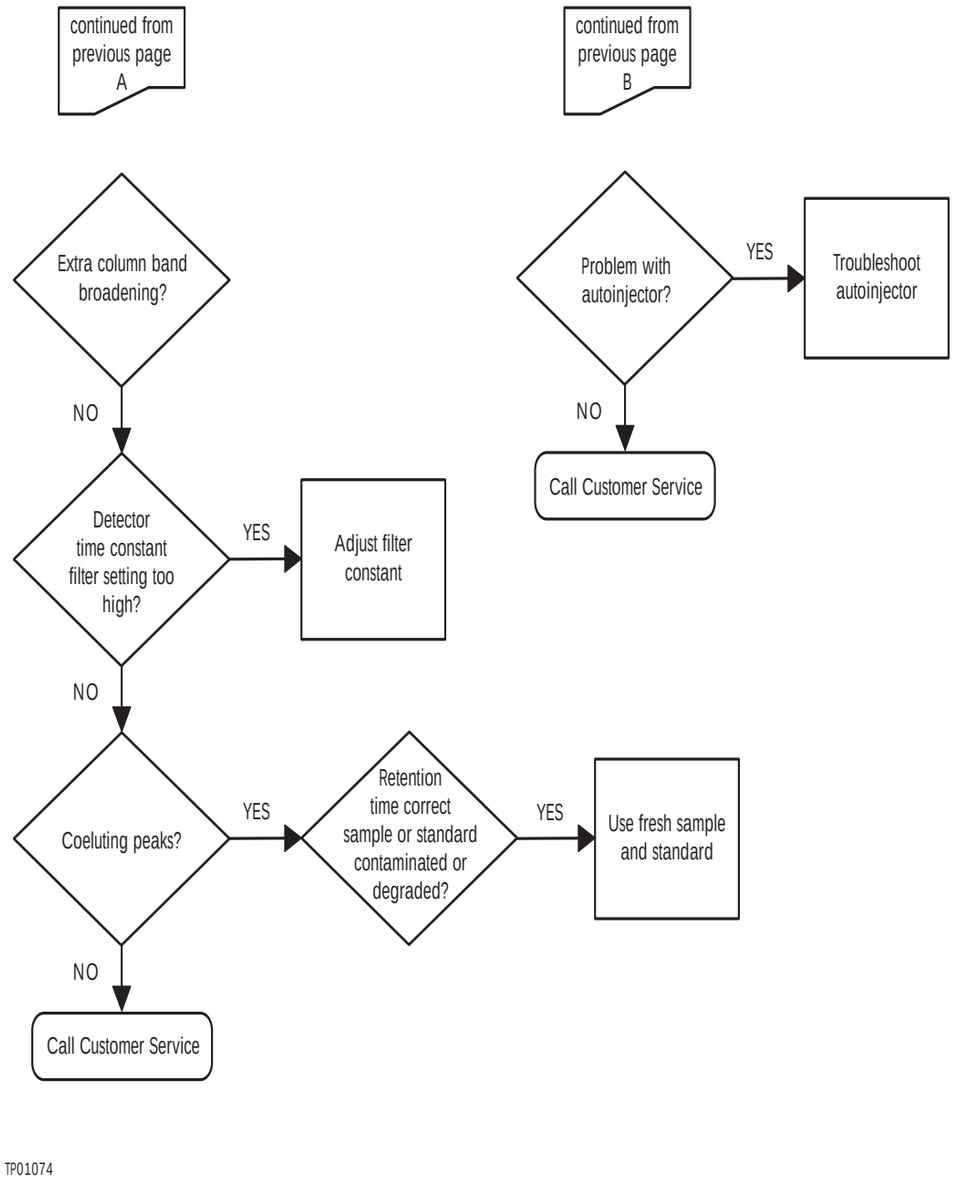
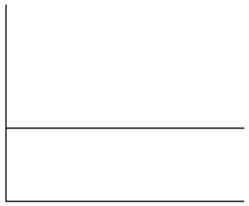
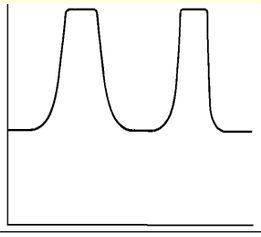


Figure 7-5 Peak Resolution Troubleshooting, Part 2

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Table 7-3 Peak Resolution Troubleshooting

Symptom	Possible Cause	Corrective Action
Straight baseline, no peaks 	No pump flow	Set pump flow rate.
	Lamp not on	Use detector diagnostics to check reference/sample energy. Zero energy indicates that the lamp is not on. Refer to Section 6.2, User-Selected Diagnostics.
		Power unit down then up to light lamp.
		Replace lamp.
	Detector not zeroed	Zero detector baseline.
	Improper connection between the detector unit and the recorder.	Check cabling between unit and recorder.
	Incorrect wavelength	Check wavelength setting.
	Leak in solvent path	Check fittings.
Flat-topped peaks 	Detector not zeroed	Zero detector baseline.
	Incorrect recorder input voltage. Sample concentration or injection value too high.	Adjust recorder input voltage, or adjust detector output cable to correct position. Decrease sample concentration or injection volume.

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7.2.4 Incorrect Qualitative/Quantitative Results

If a peak is incorrectly identified by a data system or integrator, make sure that the retention time is correct.

If retention times are correct and peak resolution is good, the cause of qualitative and quantitative errors is not likely to be chromatographic. It is more likely due to sample preparation or manipulation of the data (integration).

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Figure 7-6 (troubleshooting flowchart) can be used to assist you when your quantitative results are incorrect.

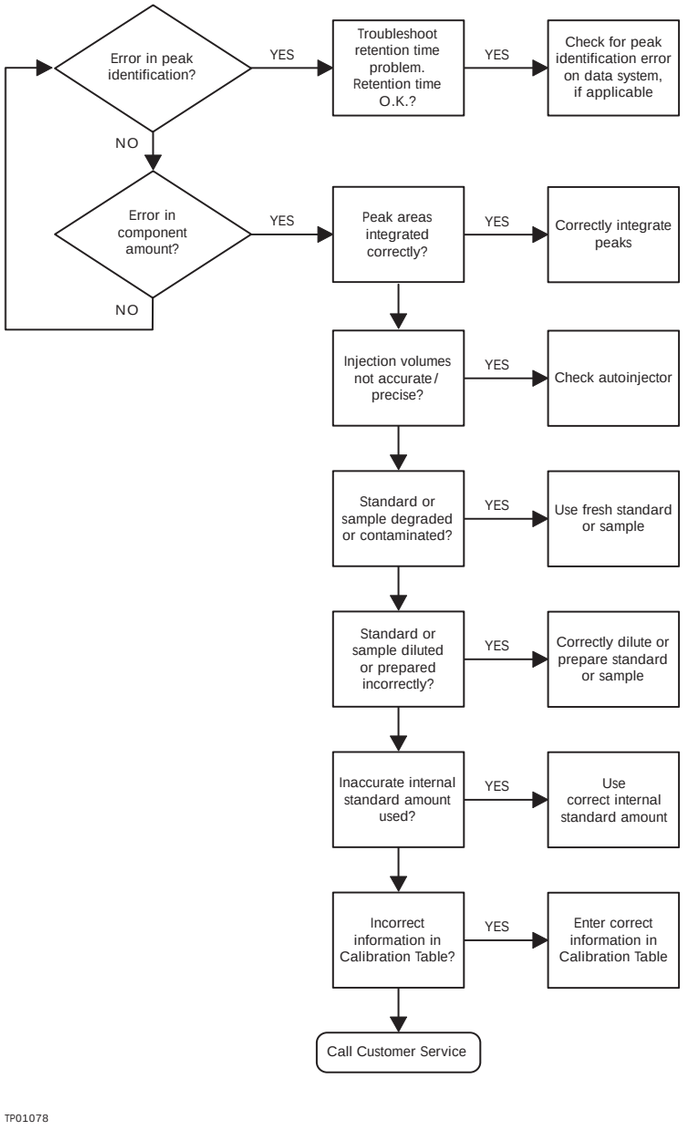


Figure 7-6 Incorrect Results Troubleshooting

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Table 7-4 Incorrect Results Troubleshooting

Symptom	Possible Cause	Corrective Action
Sensitivity loss	Leak in auto-injector.	Troubleshoot the auto-injector.
	Degraded, contaminated, or improperly prepared sample.	Use fresh sample.
	Column contaminated.	Clean/replace column.
	Loss of column efficiency.	Clean/replace column.
	Peaks wider than expected.	Troubleshoot auto-injector.
	Change in mobile phase composition.	Correct mobile phase pH or ionic composition.
	Leak in flow cell.	Tighten the flow cell seal.
	Incorrect flow rate.	Change flow rate.
Sample energy decreased, reference energy not decreased	Contaminated mobile phase.	Use fresh mobile phase.
	Dirty flow cell.	Remove column, and flush system with stronger solvent to remove particulate or filmy matter from flow cell windows. Clean flow cell.

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Table 7-5 High System Pressure Troubleshooting

Symptom	Possible Cause	Corrective Action
Flow restriction causing high pressure in the detector	Dirty flow cell	Flush with water.
		Remove column, and flush system with stronger solvent to remove particulate or filmy matter from flow cell windows.
		Reverse flow path by pumping mobile phase at 1 ml/min into the outlet port of the flow cell and flush with water or mobile phase. Disassemble and clean flow cell. Refer to Chapter 5, Maintenance Procedures.
	Dirty flow cell tubing	Reverse flow path by pumping mobile phase into the outlet port of the flow cell and flush with water or mobile phase.

Figure 7-7 (troubleshooting flowchart) can be used to assist you in determining the source of the problem when your system pressure is too high.

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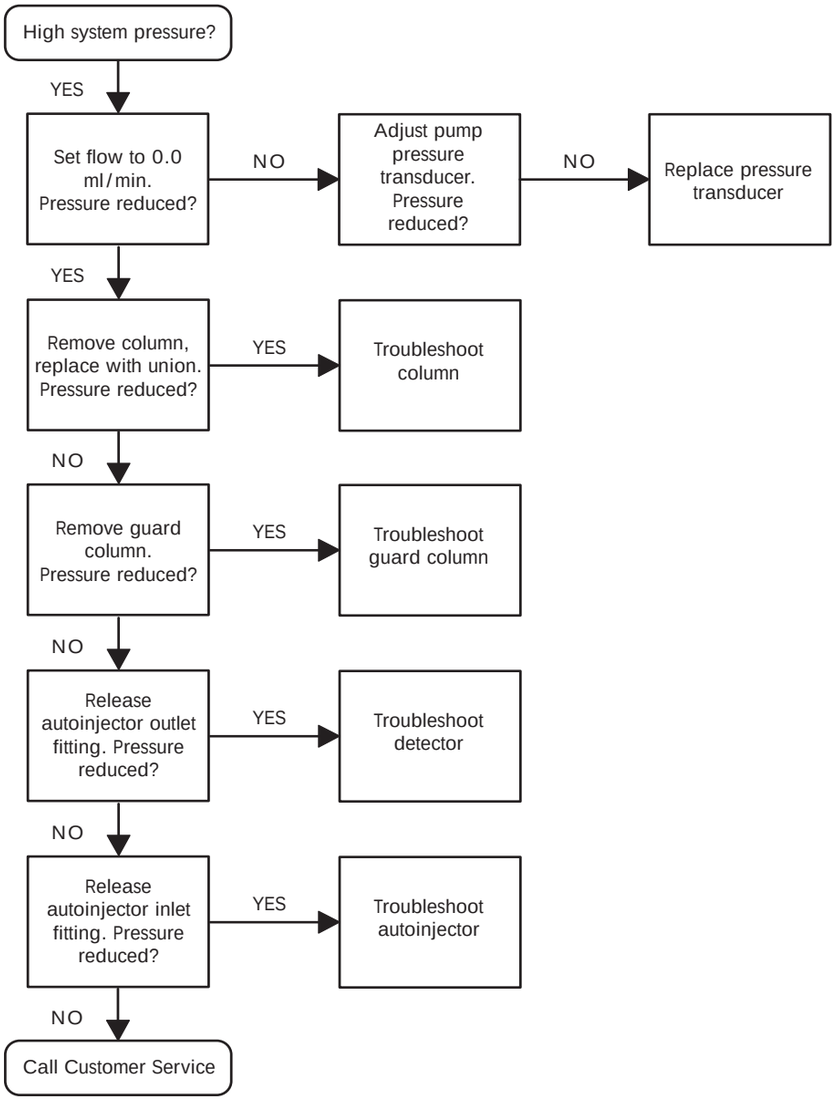


Figure 7-7 High System Pressure Troubleshooting

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7.3 Hardware Troubleshooting

This section contains general system troubleshooting for the Waters 486 Detector.

Table 7-6 General System Troubleshooting

Symptom	Possible Cause	Corrective Action
Detector inoperative	Fuse blown.	Check that the fan and the LCD displays are operational; if neither, replace AC rear panel fuse.
	No power at outlet.	Check outlet by connecting another electrical unit known to be in working order and see if it operates.
Front panel LCD fails to illuminate	Broken electrical connection. Fuse blown. Bad LCD control board.	Check electrical connections. Replace any faulty fuses on the lamp or power supply board. Call Waters Technical Service.
Front panel indicators do not light	Fuse blown Front panel defective.	Check and replace faulty fuses. Perform Diagnostic 10 to verify, then call Waters Technical Service.
Front panel displays odd characters	Faulty EPROMs. Bad LCD control board.	Perform Diagnostic 3 to verify. Call Waters Technical Service.
IEEE problems	Incorrect IEEE address. Bad IEEE cable (external).	Perform Diagnostic 13 to display IEEE address, then set correct address. Replace IEEE cable.
Keypad not functioning	Keypad defective. Bad EPROM. Bad microboard.	Run Diagnostic 11 to verify, if defective call Waters Technical Service. Run Diagnostic 3 to check EPROM. Call Waters Technical Service.

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Table 7-6 General System Troubleshooting *(Continued)*

Symptom	Possible Cause	Corrective Action
Deuterium source lamp does not light	Faulty fuse. Lamp not plugged in. Bad lamp power supply board. Lamp switch off.	Replace lamp power supply board fuse. Refer to Section 5.6.2, Replacing Lamp Power Supply Fuses. Plug in the lamp connector. Call Waters Technical Service. Press **Lamp** key.
Unit overheating	Dirty ventilation filters. Faulty fan.	Clean ventilation air filters. Refer to Section 5.2, Cleaning Ventilation Air Filters. Call Waters Technical Service.
No sample or reference energy	Lamp burned out. Lamp switch off.	Replace lamp. Press **Lamp** key.

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Appendix A Specifications

Appendix A provides several tables that list individual operating specifications for the Waters 486 Tunable Absorbance Detector.

Table A-1 Operational Specifications

Condition	Specification
Wavelength range (single, fixed)	190 to 600 nm
Wavelength accuracy	± 2 nm
Wavelength repeatability	± 0.25 nm
Noise	±1.0 x 10^{-5} AU, (1 second filter setting, 210 to 280 nm) dry cell
Drift	1.0 x 10^{-4} AU/° C (after warm-up) 1.0 x 10^{-4} AU/hour (after warm-up)
Sensitivity setting range	0.001 to 2.0 AUFS (under software control)
Filter setting range	0.1 to 5.0 seconds (under software control)
Bandwidth	8 nm
Optical Component Specifications	
Lamp source	Deuterium arc lamp
Flow cell	Dual beam Taper-Cell® design
Path length	10 mm (standard, analytical)
Cell volume	8 µL (standard, analytical)
Pressure limit	1000 psi

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Table A-1 Operational Specifications *(Continued)*

Condition	Specification
Materials	316 stainless steel, Dynasil®, Suprasil II®, Teflon®
Environmental Specifications	
Operating temperature	15 to 32.2 °C
Operating humidity	20 to 80%, non-condensing
Shipping and storage temperature	(minus) -40° to 65° C
Shipping and storage humidity	20 to 80%, non-condensing
Dimensions	
Height	9.2 inches (24 cm)
Length	21.5 inches (54.5 cm)
Width	11.2 inches (28.5 cm)
Weight	38 pounds (17.5 kg)
Electrical Specifications	
Line frequency	50 Hz, 47 to 53 Hz 60 Hz, 57 to 63 Hz
Fuse ratings	100/120 V ac: 1.5A, SLO-BLO, 1 1/4-inch long F 1.25A/250V, FAST BLO, 20 mm long 220/240 V ac: 1.5 A, SLO-BLO, 1 1/4-inch long F 1.25A/250V, FAST BLO, 20 mm long
Power consumption	100 VA (nominal)

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Table A-1 Operational Specifications *(Continued)*

Condition	Specification
DC power supply	±5V dc at 2.2 A (power limited at 11W) ±15V dc at 300 mA
Lamp power supply	309 mA regulated constant current (fused at 3/8 A) 3.0V ac filament heater (unregulated) (fused at 6 1/4 A) 500V dc Arc Strike (nominal)

Table A-2 Instrument Operating Voltages

Voltage Setting (V ac)	Operating Range (V ac)
100	85 to 110
120	102 to 132
220	187 to 242
240	204 to 264

Table A-3 Optical Specifications

Monochromator	Seya-Namioka Configuration
Grating	Cancave holographic
Optical Bandwidth	8 nm
Lamp Power	30 W

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Table A-4 Optional Flow Cell Specifications

	Volume (µl)	**Path Length** (mm)	**Sample Path Diameter** (Inches)		**Reference Path Diameter** (Inches)	
			In	**Out**	**In**	**Out**
Analytical Cell	8	10 (mm)	0.009	0.009	0.009	0.009
Semi-Prep Cell	2.5	3 mm	0.040	0.040	0.009	0.009
Microbore Cell	2.5	3 mm	0.005	0.005	0.009	0.009
Non-metallic Cell	8	10 mm	0.010	0.010	0.020	0.020
Mass Spec Cell	8	10 mm	0.009	0.009	0.009	0.009

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Appendix B Spare Parts

Appendix B provides table listings of spare parts for the Waters 486 Tunable Absorbance Detector.

Table B-1 Spare Parts

Item	Part Number
Fuses	
MDC, 1.5A primary fuse 100/120 V ac SLO-BLO, 1.25-inch long	99542
F 1.25A primary fuse, 100/120 V ac, FAST BLO, 20 mm long	163-12
0.75A primary fuse 220/240 V ac SLO-BLO 1.25-inch long	99543
F.63A primary fuse 220/240 V ac, FAST BLO, 20 mm long	163-09
6.25A lamp heater fuse	40214
3/8 lamp power supply fuse	32148
3A pigtail fuse	30425
3/8A pigtail fuse	80404
Optical Parts/Assemblies	
Deuterium source lamp assembly, 486	80678
Analytical cell kit	80733
Non-metallic cell kit	80735
Semi-prep cell kit	80734
Microbore cell kit	80736

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Table B-1 Spare Parts *(Continued)*

Item	Part Number
Second order fitter kit (to enhance operation over 360 nm)	80747
Solvent clar. kit, w/vacuum pump 110 V, 60 Hz	85113
Solvent clar. kit, w/V ac pump 220 V, 50 Hz	85122
Solvent clar. kit, w/o vacuum pump	85124
General Parts	
Instrument ventilation air filter (1)	80369
A/C voltage power cord (120 V)	97194
A/C voltage power cord (240 V)	49760
Nuts and ferrules (kit of 5 each)	25604
Stainless steel tubing 0.009-inch x 120 inches	26973
Stainless steel tubing 0.040-inch x 120 inches	26805
Recorder cable 4 ft. (1)	48918
Waters start-up tool kit	96146
Tubing cutter for 1/16-inch stainless steel tubing	22384
Tubing cutter, spare blades (package of 3)	22385
486 Second order filter kit	80747
IEEE Cable Lengths	
3.3 feet	87198
6 feet	87141
13 feet	87191

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Table B-1 Spare Parts *(Continued)*

Item	Part Number
Analytical Flow Cell Components	
Screw, 4-40 x 16 fillisters, ss	21988
Flow cell end plate	80271
Belleville spring washer	46430
Window kit (1 window, 1 holder)	97091
O-ring	46376
Cell gasket, window side	80335
Flow cell body	80251
Cell gasket, lamp side	80336
Cell base	80304
Analytical flow cell assembly	80381

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Appendix C Warranty/Serial Number Information

This appendix includes information on:

- Limited Product Warranty
- Shipments, Damages, Claims, and Returns

C.1 Limited Product Warranty

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

The Warranty is as follows:

Waters warrants that all products sold by them 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.

The foregoing warranty is exclusive and in lieu of all other express and implied warranties, including but not limited to fitness for any other purpose(s). In no event is Waters liable for consequential, economic or incidental damages of any nature. Waters reserves the right not to honor this warranty if the products are abused by the customer. The Warranty is not deemed to have failed of its essential purpose so long as Waters is able and willing to repair or replace any nonconforming part or product.

Warranty service

Warranty service is performed at no charge and at Waters's 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.

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Non-conforming 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 notified 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, 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 which 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

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.

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The warranty period for the Waters 486 Tunable Absorbance Detector is as follows:

Assemblies and Serviceable Parts	Warranty
Microprocessor Board Lamp Power Supply Board Low Voltage Power Supply Board Pre-Amplifier Board Stainless Steel Analytical Cell Body Keyboard LCD Display Assembly	1 year

Normal Wear and Maintenance Items	Warranty
Cables	90 days
Grating Drive Belt	

General Warranty Policies	Warranty
Replacement parts	30 days
Service repair and maintenance workmanship	

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C.2 Shipments, Damages, Claims, Returns

Shipments

As all shipments are made 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 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 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 us, please contact our Technical Service Department or your nearest Waters subsidiary or representative for a return merchandise authorization (RMA) number and forwarding address.

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