Waters

Division of MILLIPORE

WATERS
410 REFRACTOMETER

Operator's Manual

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Address Comments
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U.S.A. ONLY

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This equipment generates and uses radio frequency energy. If not installed and used strictly in accordance with the *Waters 410 Refractometer Operator's Manual,* it may cause harmful interference to radio Communications. This equipment has been tested and found to comply with the limits for a Class A computing device pursuant to Subpart J of Part 15 of FCC rules. These rules are designed to provide reasonable protection against such interference when operated in a commercial environment.

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HOW TO USE THIS BOOK

Purpose The *Waters 410 Refractometer Operator's Manual* desenbes the features and use of the Waters 410 Refractometer. It also includes installation and maintenance procedures.

Audience The *Waters 410 Refractometer Operator's Manual* is intended for anyone using, maintaining, and troubleshooting the 410.

Structure of this document The 410 Operator's Manual is divided into chapters. Each chapter is marked with a tab, providing easy access to all information.

Chapter 1 Introduction and Theory of Operation	Includes a product description and describes principles of differential refractometry and 410 operation.
Chapter 2 Installation	Contains installation procedures.
Chapter 3 Connecting Components	Includes procedures for connecting other components of your chromatography systern to the 410.
Chapter 4 Solvent Preparation	Discusses the importance of filtering and degassing solvents for effective operation.
Chapter 5 Using the 410	Provides instructions for powering up and down, using the front panel, and selecting operating parameters such as sensitivity.
Chapter 6 Maintenance	Contains maintenance and replacement procedures.
Chapter 7 Troubleshooting	Includes tables of symptoms, possible causes, and corrective actions for 410 operational problems.

Additional information **Appendix A** contains system specifications.

Appendix B provides warranty and service information, and includes shipping and ordering information.

Appendix C includes recommended spare parts.

Related documents Operator's manuals for specific instruments provide information necessary to operate units connected to the 410.

Special print type This manual uses these print types to make text easier to understand:

ALL CAPS indicates keys to press, for example, press the SENS key.

Italics introduces new or important words. It is also used for emphasis.

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INTRODUCTION/THEORY OF OPERATION

This chapter contains:

- Description of the 410 refractometer
- Principles of refractometry
- Principles of operation

Refer to Appendix A for 410 refractometer specifications.

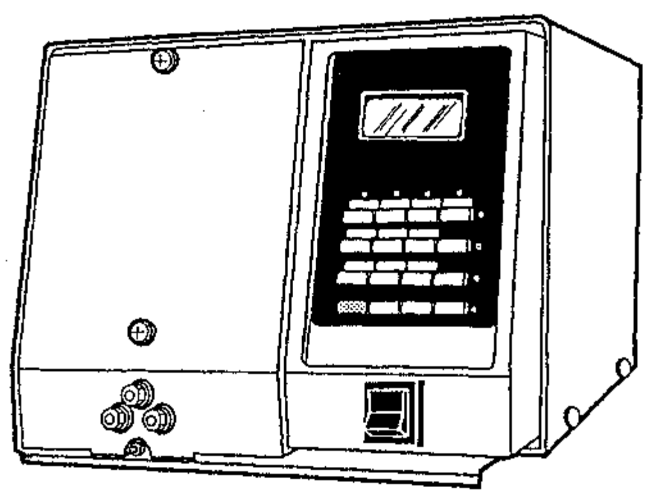


Figure 1-1 Waters 410 Differential Refractometer

1.1 WATERS 410 REFRACTOMETER DESCRIPTION

Overview This section describes the Waters 410 Differential Refractometer (the 410) and its features.

The 410 is a differential refractive index detector designed for high performance liquid chromatography applications. It can operate as a stand-alone unit with an integrator or chart recorder, or with a system controller or data system.

Range and sensitivity The 410 functions with solvents with refractive indices between 1.00 and 1.75. The measurement range of the instrument is $5x10^{-8}$ to $5x10^{-3}$ refractive index units (RIU) full scale.

Features Features of the 410 refractometer include:

- Patented countercurrent heat exchanger and temperature-controlled cell for stable operation under varying conditions
- Auto zero and auto purge for automated operation
- Built-in pressure relief to protect flow cell
- Auto diagnostics
- Two optional external column heater controls
- Battery backup to retain parameter settings when the detector is powered down or during power interruptions
- Long-life pulsed LED light source

1.2 410 REFRACTOMETER THEORY OF OPERATION

The 410 refractometer uses optica! refraction to monitor the concentrations of sample components in your eluent. This section describes:

- Optical refraction
- Differential refractometry
- Common problems in refractometry

1.2.1 Optical Refraction

When a beam of light passes from one medium into another, it changes its speed. If the light enters at an angle that is not perpendicular to the surface, it bends, or refracts.

Refractive index The ability of a medium to refract light is its *refractive index* (RI). RI is the ratio of the velocity of light in a vacuüm to the velocity of light in the medium. It is a physical property of the medium, with a dimensionless integer value represented by the letter n.

This section discusses:

- Factors which affect RI
- Measuring refraction
- Using changes in RI for sample detection

1.2.1.1 Factors Which **Affect RI**

The refractive index for a medium is solely dependent upon the speed of light in the medium. The speed of light in a medium is constant for a given wavelength of light at a specified temperature and pressure.

Effect of wavelength **on** RI The refractive index of a medium has a specific value that changes with the wavelength of the light beam. Since the 410 refractometer uses monochromatic light, the effect of different wavelengths of light is not discussed in this manual.

The refractive index of a medium is often listed with a subscript indicating the wavelength of the light at which it is measured. All refractive indices referred to in this manual are at 930 nm, which is the wavelength of the pulsed LED light source in the 410 refractometer.

Effect of density on RI

The factors that affect the density of the medium also affect its RL The most important of these are:

- Composition
- Temperature
- Pressure

At a fixed wavelength, the relationship between the density of a medium and its RI is generally, but not necessarily, linear.

Figure 1-2 illustrates the effect of density on RI for two solutions. The refractive index of a sucrose solution changes iinearly with concentration, but a methanol solution exhibits a nonlinear region between concentrations of 45 to 55 percent.

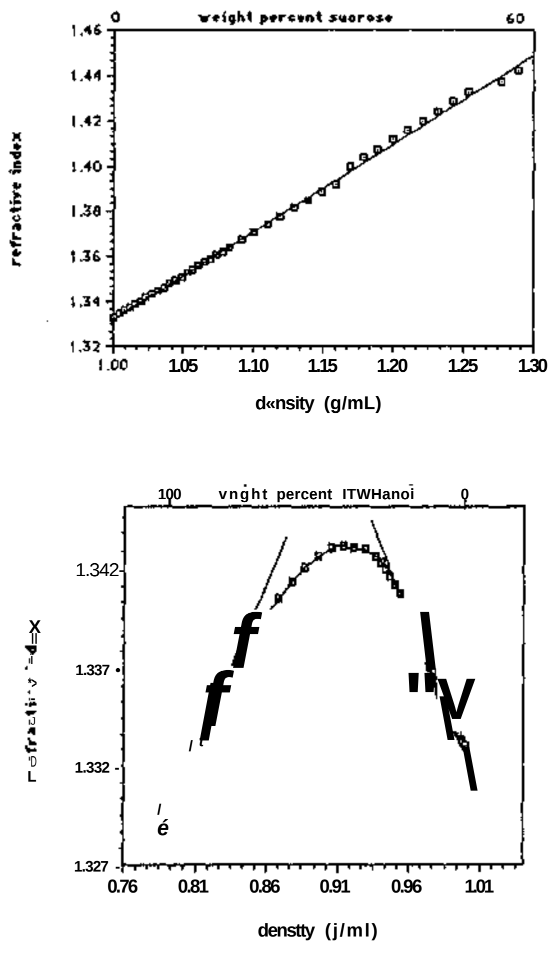


Figure 1-2 Effect of Density on RI

1.2.1.2 Measuring Refraction

The degree a beam of light refracts when it enters a medium depends on two factors:

- The angle at which it enters the new medium (the *angle of incidence)*
- The refractive index of the new medium

The angle of the light beam through the new medium is its *angle of refraction.*

Figure 1-3 illustrates the relationship between angle of incidence, angle of refraction, and refractive index.

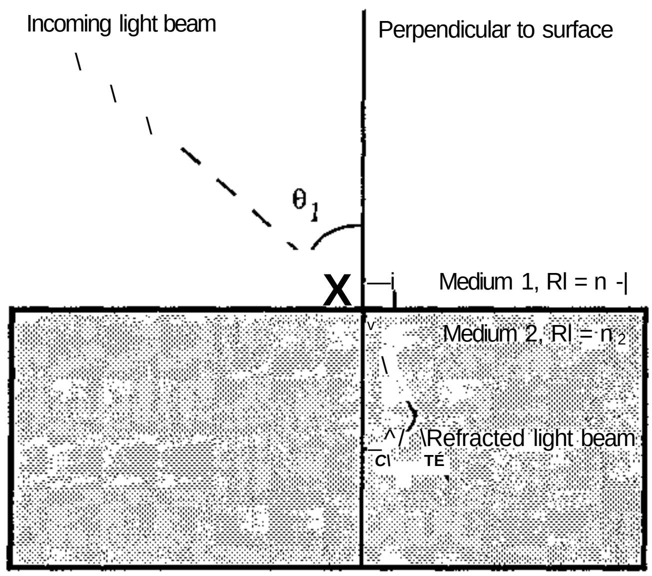


Figure 1-3 Refraction of Light

The relationship between the refractive indices of the two media and the angles of incidence and refraction is described by Snell's Law:

$$n_1 \sin \theta_1 = n_2 \sin \theta_2$$

θ_1 = Angle of incidence	θ_2 = Angle of refraction
n_1 = RI of medium 1	n_2 = RI of medium 2

You can use Snell's Law to calculate the RI of a solvent/sample solution from the angle of incidence, the RI of the solvent, and the angle of refraction.

1.2.1.3 Using Changes in RI for Sample Detection

As the separated components of a sample pass through the flow cell:

1. The composition of the solvent/sample solution changes.
2. The RI of the solution changes.
3. The light beam passing through the solution is refracted.

The refractometer detects the position of the deflected light beam, creating a signal that differs from the baseline signal.

Figure 1-4 shows the deflection of the image on the photodiode when the flow cell contains sample, refracting the light beam.

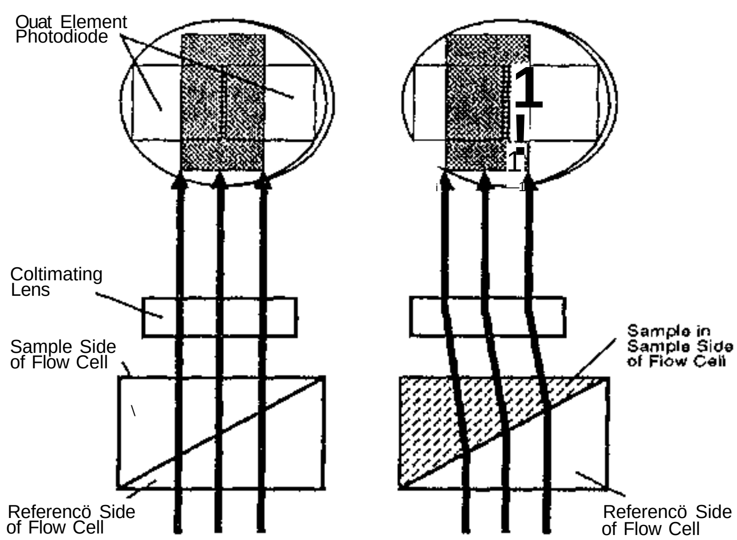


Figure 1-4 Presence of Sample Changes the Photodiode Signal

Detecting sample with the refracted light beam By keeping wavelength, temperature, and pressure constant, the refractometer measures only changing sample concentration. A solution with a high concentration of a solute refracts a beam of light more sharply than a dilute solution. Therefore, high concentrations of sample yield large peaks.

1.2.2 Differential Refractometry

The 410 differential refractometer measures extremely small changes in refractive index to detect the presence of sample. This section covers the principles of differential refractometry and the production of a chromatographic peak from a change in sample concentration.

Overview The function of a differential refractometer is to measure the small differences in RI between a reference solution and a sample solution. The difference in RI is referred to as An. An is measured in RI units (RIU).

The 410 measures An values as small as 5 x 10"^{8} RIU by detecting the difference in the amount of light falling upon the elements of a dual element photodiode.

External angle of deflection The amount of light falling upon the elements of the photodiode is determined by the angle of the light beam. The final angle of the refracted beam is the *external angle of deflection* (ϕ). The external angle of deflection determines the shift (Ax) of the image cast on the photodiode by the light beam.

Figure 1-5 illustrates the external angle of deflection and its dependence upon the difference in RI.

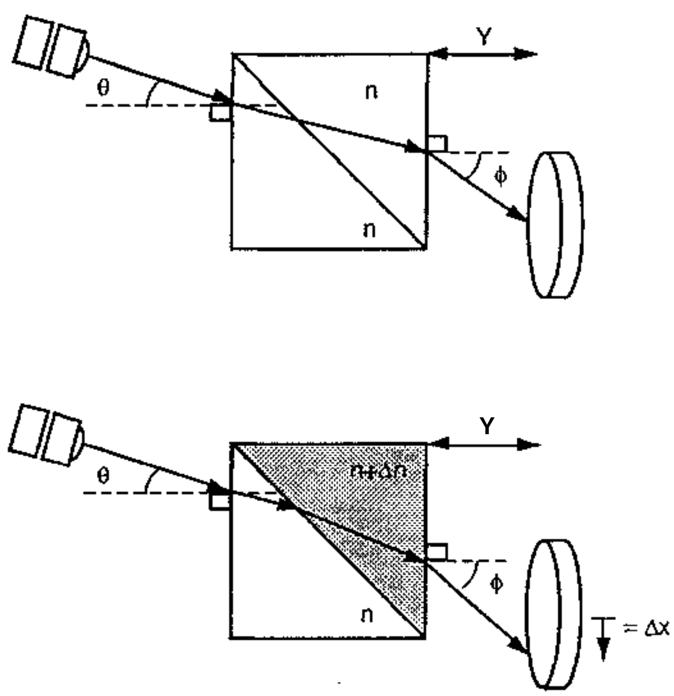


Figure 1-5 Refraction Changes <j>

Effect of refraction on ϕ

As the beam of light moves along the light path to the photodiode, it encounters and is refracted by the air in the opties bench, the fused quartz walls of the flow cell, the solution in the sample side of the flow cell, and the solvent in the reference side of the flow cell.

Figure 1-6 shows the path of the light beam to the photodiode.

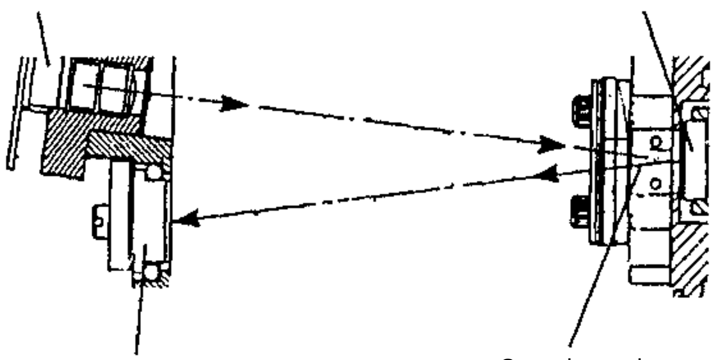


Figure 1-6 Light Beam Path to the Photodiode

Effect of change in ϕ

Of these refractors, only the solution in the sample side of the flow cell changes. As a result, the signal from the photodiode does not change until the light beam is deflected from its zero position by a change in the RI of the sample solution.

Relationship of ϕ and An

For the very small An and Aϕ encountered in differential refractometry, the relationship between the external angle of deflection and the RI of the solution is summarized:

An = ϕ / tan 9

Where: An = ' Difference in RI between the solvent and the solvent/sample solution

ϕ = External angle of deflection in radians

9 = Angle of incidence

Deflection at the photodiode

The change in ϕ determines the displacement (Ax) of the image cast by the light beam on the photodiode. Because the 410 refractometer uses a dual pass opties bench, the light beam passes through the flow cell twice before reaching the photodiode, doublinl the image shift.

The relationship between the image shift at the 410 photodiode and the change in RI of the solution is summarized:

$$Ax = 2Y \tan 9 \ An$$

Where:	Ax	=	Deflection at the photodiode
	Y	=	Distance from flow cell to photodiode
	An	=	Difference in RI between solvent and sample solution

The angle of incidence (0) and the distance to the photodiode (Y) are fixed in the refractometer, so the equation becomes:

$$Ax = CAn$$

Where:	C	=	A constant representing the fixed values

By detecting how far the image shifts (Ax), the refractometer measures the difference in RI between the solvent/sampie solution and the solvent alone (An).

Changes in output voltage

The changing signal from the elements of the dual element photodiode results in a change in the output voltage from the 410 refractometer. The integrator or chart recorder registers changes in output voltage as peaks on your chromatogram.

1.2.3 Common RI Detection Problems

Changes in solution density caused by something other than sample concentration are the most common source of problems in RI detection. There are two causes for changes in solution density:

- Environmentally induced density changes
- Inhomogeneities in the solution

Environmentally induced density changes

Environmentally induced density changes include changes in the temperature or pressure of the solution. Even small changes in ambient temperature can cause drift. Backpressure pulses from a dripping waste tube can cause short-term cycling.

Refer to Chapter 7, Troubleshooting, for more information.

Inhomogeneities in solution

The differential refractometer measures the difference in refraction between a pure reference solvent and a homogeneous sample solution. If the sample solution is not homogeneous, the light passing through the sample may be absorbed, scattered, or refracted unpredictably. This can result in shifts in retention time and broad, tailing peaks. Most common inhomogeneity problems are due to improper solvent preparation.

See Chapter 4, Solvent Preparation, for proper solvent preparation procedures.

1.3 410 PRINCIPLES OF OPERATION

To effectively use the Waters 410 Differential Refractometer, you must be familiar with its design and principles of operation.

This section describes:

- Waters 410 Fluidics
- Waters 410 Opties
- Waters 410 Electronics

1.3.1 Waters **410** Fluidics

The fluid path of the 410 includes the following components:

- Countercurrent heat exchanger
- Flow cell, with sample and reference sides
- Solenoid valve
- Pressure relief valve
- IN port
- OUT port
- PRG OUT port

Countercurrent heat exchanger The 410 refractometer uses a patented countercurrent heat exchanger to minimize temperature fluctuations in the sample stream. in the countercurrent heat exchanger, the sample and reference inlet and outlet lines run adjacent to each other. All four lines are copper-coated to facilitate heat exchange.

Flow cell The flow cell consists of two fused quartz hollow prisms. Each has an inlet and outlet. One of the prisms is the sample side of the flow cell, through which a constant flow of fluid passes during analysis.

The other prism is the reference side of the flow cell. It fills with clean solvent when you purge the 410 during equilibration. When you switch from purge to normal operation, the solenoid valve opens and the pressure relief valve doses. The flow of solvent through the reference prism stops.

Solenoid valve During normal operation, the solenoid valve remains open. Fluid that has passed through the sample side of the flow cell passes out through the solenoid valve to the OUT port.

When you purge the 410, the solenoid valve doses. Fluid that has passed through the sample side of the flow cell cannot flow through the closed solenoid valve, so it flows through the reference side of the flow cell.

Pressure relief valve During norma! operation, the pressure relief valve is closed. It is pressure actuated, opening when pressure gets too high. This protects the flow cell, which has a maximum pressure rating of 100 psi.

During purging, fluid that has passed through the sample and reference sides of the flow cell passes out through the pressure relief valve to the PRG OUT port.

Figure 1-7 shows the 410 refractometer fluidics and illustrates the path of the solvent and sample as they pass through the 410.

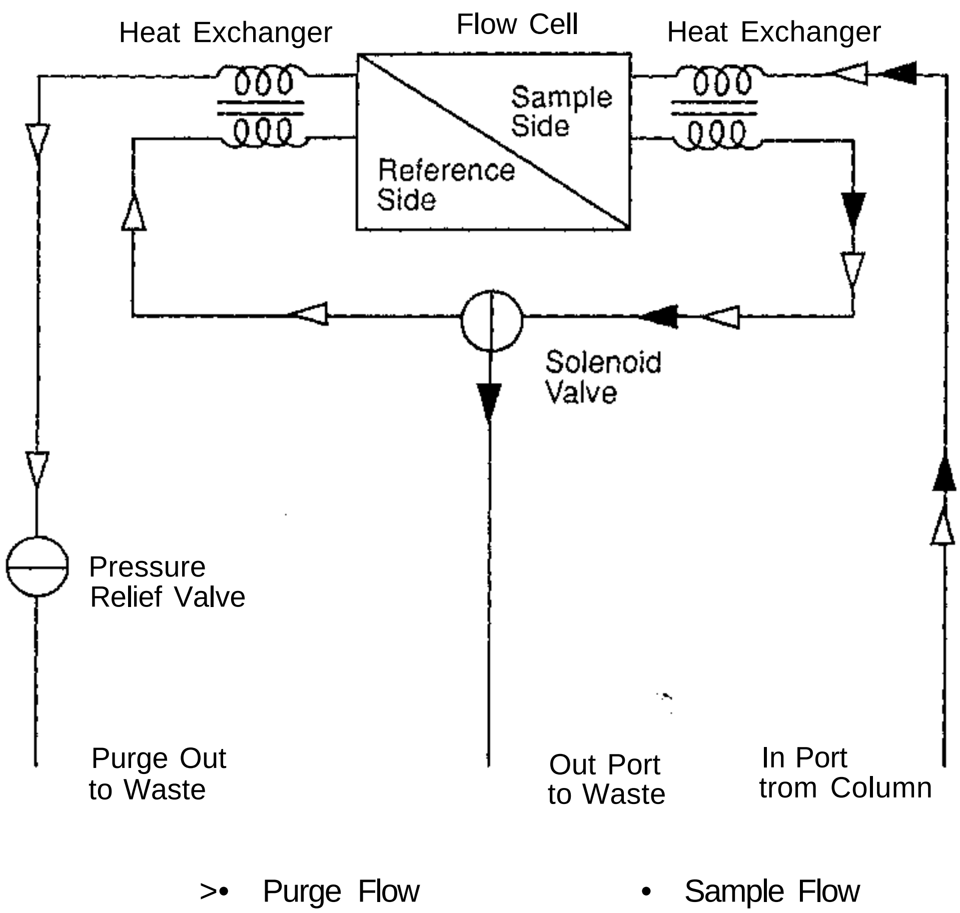


Figure 1-7 Waters 410 Fluid Path

Fluid Path Line	Inside Diameter
Sample In	.009 inch
Sample Out	.040 inch
Reference In	.020 inch
Reference Out	.040 inch

Fluid path during analysis

During analysis, solvent and sample follow this route:

1. Fluid goes in through the IN port.
2. It stabilizes its temperature as it passes through the countercurrent heat exchanger.
3. It flows through the sample side of the flow cell.
4. It returns through the countercurrent heat exchanger.
5. It passes through the solenoid valve to the OUT port.

Fluid path during purge

When you purge the 410 fluid path, solvent follows this route:

1. Fluid enters through the IN port.
2. It passes through the Sample In tube of the countercurrent heat exchanger.
3. It.flows through the sample side of the flow cell.
4. It returns through the Sample Out tube of the countercurrent heat exchanger to the closed solenoid valve.
5. It passes through the Reference In tube of the countercurrent heat exchanger.
6. It flows through the reference side of the flow cell.
7. It returns through the Reference Out tube of the countercurrent heat exchanger.
8. It goes out through the relief valve to the PRG OUT port.

1.3.2 Waters 410 Opties

The 410 refractometer opties bench ineludes the foliowing components:

- LED source lamp
- Aperture
- Focusing lens
- Flow cell, with sample and reference sides
- Mirror
- Collimating lens
- Dual element photodiode

Figure 1-8 shows the 410 opties and illustrates the path of the light beam as it passes through the components in the opties assembly.

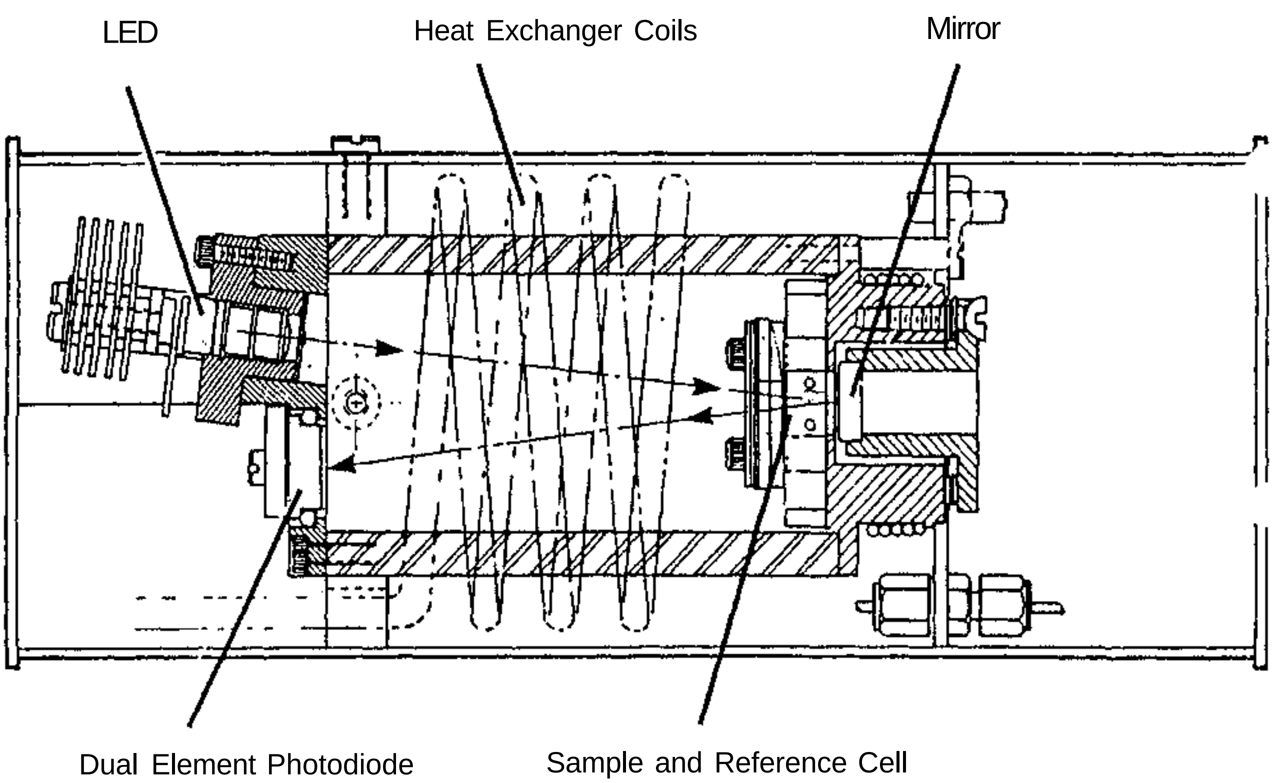


Figure 1-8 410 Opties Assembly Light Path

Opties assembly light path

Light travels the following route:

1. The light from the LED is focused by the focusing lens through the aperture and collimating lens to form a beam.
2. The light beam passes through the sample and reference sides of the flow cell to the mirror.
3. The light beam reflects back through the flow cell and the collimating lens to the dual element photodiode.

The change in response from the elements of the photodiode results in a deflection from the baseline on the chromatogram.

1.3.3 Waters 410 Electronics

The 410 refractometer contains both analog and digital components, and includes hardware such as the front panel keyboard, printed circuit (PC) boards and their interconnections. The following PC boards are included in the 410 electronics:

Analog board Handles the interface of analog input signals from the opties to the microprocessor for further signal conditioning. Provides analog output signals. Drives the LED, Auto Zero, and signal compensation electronics.

CPU board Receives inputs from the Analog board. Stores and executes parameters programmed by the user. Provides communication between the 410 and external devices.

Front panel board Controls the keypad, indicators, and display.

Rear panel board Handles the IEEE interface, terminal strips, and external column heaters.

2

INSTALLATION

This chapter describes the procedures for:

- Selecting the site
- Unpacking and inspecting the instrument
- Selecting voltage and instailing the fuse
- Making fluid connections

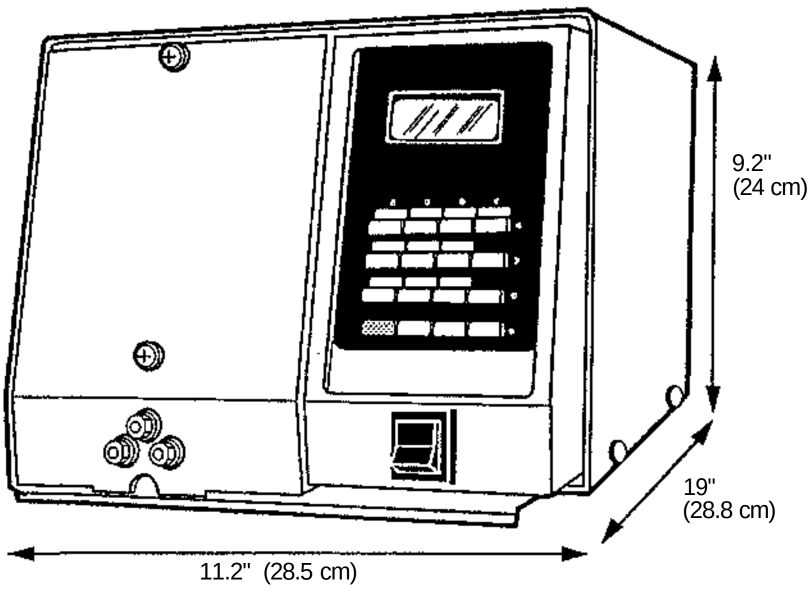


Figure 2-1 Dimensions of the Waters 410

2.1 SITE SELECTION AND POWER REQUIREMENTS

Site selection requirements Install the 410 refractometer in an area where:

- Temperature is maintained at 15 to 32 °C. Avoid exposure to direct sunlight and air conditioning vents.
- Relative humidity is 20 to 80 percent, non-condensing.
- Surface is level to allow proper function of the drip tray, which directs solvent leaks to the front of the unit.
- Bench space is:

Width	15 inches (38 cm)
Depth	24 inches (61 cm)
Height	11 inches (28 cm)

Power requirements The 410 refractometer requires:

- Line voltage of 100 to 120 VAC or 220 to 240 VAC
- A grounded alternating current (AC) power source
- Minimal power transients and fluctuations

The power consumption is 100 watts.

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

2.2 UNPACKINGANDINSPECTION

The Waters 410 refractometer shipping carton includes:

- *Read Me First* sheet
- Waters 410 Differential Refractometer
- Startup Kit
- *Waters 410 Differential Refractometer Operator's Manual*

Unpack carefully Find the *Read Me First* sheet. As you unpack, check the contents of the shipping carton against the packing list on the *Read Me First* sheet.

Save the shipping carton for future transport or shipment.

Inspect all items Inspect all items for damage. Immediately report any damage to both the shipping carrier and to the Waters Chromatography Division Order Entry Department.

The toll-free number at Waters is 1-800-252-4752 in the U.S.A. Refer to the subsidiary list at the end of this manual for international phone numbers.

If any items are damaged, use the shipping container for subsequent claim purposes. See Appendix C, Warranty/Service Information, for information on returning a damaged shipment.

Record the startup date Record the serial number (found on both the bottom of the front panel and on the rear panel) and startup date in the spaces provided in Appendix C, Warranty/Service Information.

2.3 VOLTAGE SELECTION/ FUSE INSTALLATION

You can configure the 410 to work at either of two voltage ranges.

This section describes procedures for selecting the voltage setting and installing the correct fuse.

Table 2-1 Voltage and Fuse Requirements

Nominal Voltage	Voltage Range	Fuse Required
110/120 Vac, 50/60 Hz	90-132	1 A (time delay)
220/240 Vac, 50/60 Hz	198-264	1/2 A (time delay)

***WARNING:** Avoid electrical shock. Before performing these procedures, make sure the refractometer is turned off and unplugged.*

2.3.1 Voltage Selection

Set the LINE and HTR voltage selectors to the correct voltage for your workplace. The voltage selectors are located below the fan, and to the left of the AC receptacle on the rear panel (Figure 2-2).

Procedure Insert a screwdriver into the slot and push it to the desired voltage setting.

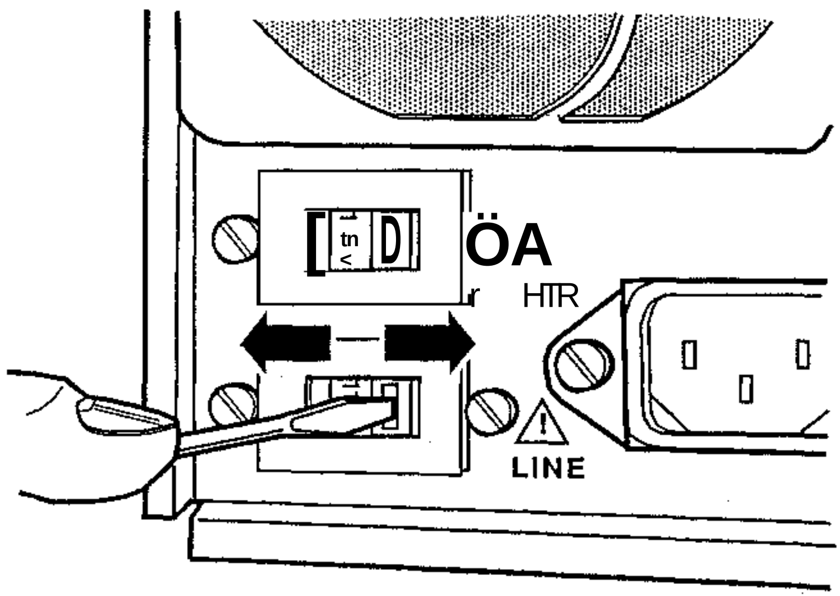


Figure 2-2 Voltage Selection

2.3.2 Fuse Installation

The fuses are located to the right of the AC receptacle on the rear panel (Figure 2-3). The 410 line fuse (F1) is in the lower, gray fuse holder. The optional upper fuse holder is for a secondary fuse.

Procedure To install a fuse:

1. Use a screwdriver to turn the gray fuse holder counterclockwise and pull it out, as shown in Figure 2-3.
2. Install a properly rated fuse (see Table 2-1).
3. Replace the fuse holder.

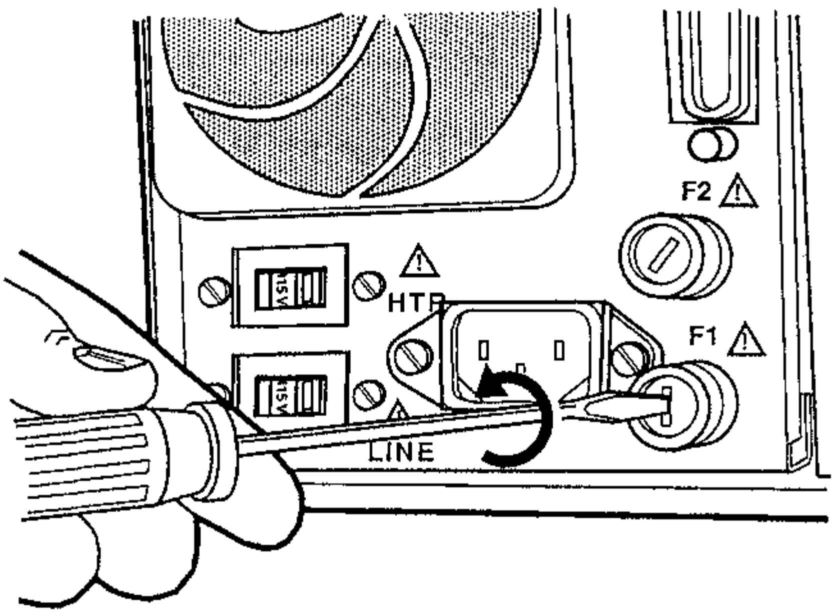


Figure 2-3 Fuse Installation

2.4 MAKING FLUID CONNECTIONS

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

This section describes the procedures for connecting the 410 refractometer to:

- Column or another detector
- Waste
- Drip tray

The fluid connections for the 410 refractometer are located on the left side of the front panel (Figure 2-4).

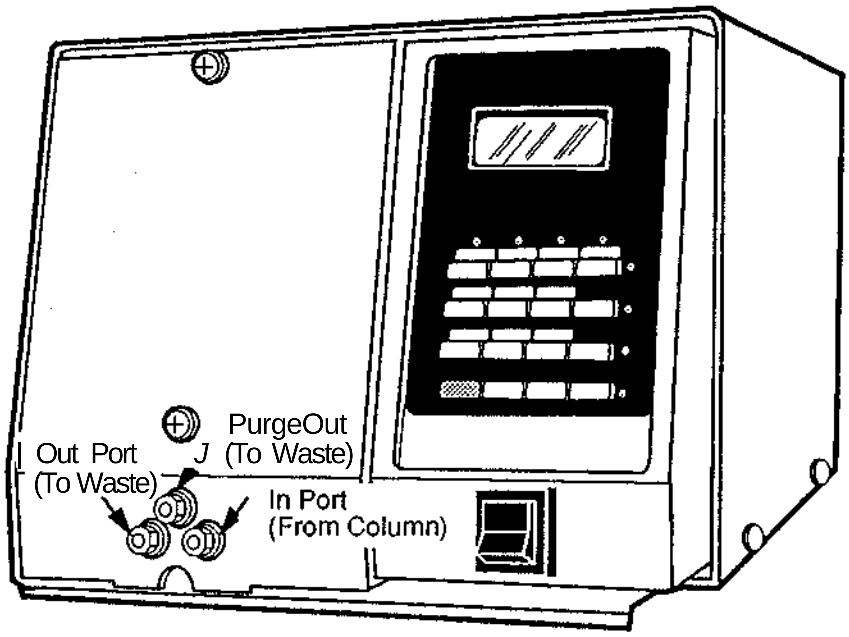


Figure 2-4 Fluid Connections

2.4.1 Connecting a Column or Second Detector

Supplies When connecting a column or second detector to the 410 refractometer, you need the following supplies from the Startup Kit:

- 2 compression fittings and ferrules
- 1/16-inch stainless steel tubing, .009-inch ID

Tools You also need these tools:

- 5/16-inch open-end wrench
- Waters 1/16-inch stainless steel tubing cutter or other method for cutting tubing

Procedure To connect a column or other detector:

1. Measure the minimum length of tubing needed to connect the column or other detector outlet to the IN port.
2. Cut a length 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.
   - 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 connection from the column outlet tubing to the 410 inlet on the front panel by sliding the compression fitting over the .009-inch tubing followed by the ferrule, large end over the tubing first (Figure 2-5).

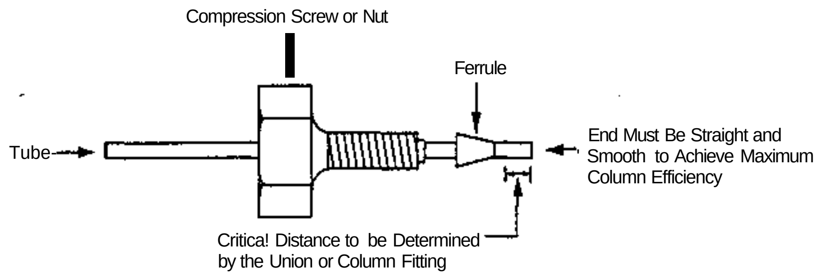


Figure 2-5 Ferrule and Compression Screw Assembly

4. Bottom the tubing in the fitting seat to determine the distance between the end of the tubing and the ferrule.

5. Seat the ferrule by tightening the compression screw 3/4 turn past finger tight with the 5/16-inch open-end wrench.

6. Repeat steps 3, 4 and 5 to connect the tubing to the column.

2.4.2 Connecting to Waste

The 410 flow cell is very sensitive to backpressure. Use only .040-inch ID tubing for waste. Use 18 to 24 inches (45 to 60 cm) of waste tubing.

If you have more than one detector in line, the 410 refractometer must be the last.

Supplies When connecting the 410 to waste, you need the following supplies from the startup kit:

- 1 compression fitting and ferrule
- 1/16-inch stainless steel tubing, .040-inch ID

Tools You also need these tools:

- 5/16-inch open-end wrench
- Waters 1/16-inch stainless steel tubing cutter or other method for cutting tubing

Procedure To connect the 410 to waste:

1. Cut the minimum length of tubing needed as in step 2, above (see Section 2.4.1).
2. Assemble the connection from the 410 OUT port on the front panel by sliding the compression fitting over the .040-inch tubing followed by the ferrule.
3. Bottom the tubing in the fitting seat to define the distance between the end of the tubing and the ferrule.
4. Seat the ferrule by tightening the compression screw 3/4 turn past finger tight with the 5/16-inch open-end wrench.
5. Put the waste container lower than or at the same level as the 410.
6. Position the end of the tubing to rest against the side of the container, or immerse the end of the waste line in the waste container, to prevent dripping.

\ *CA UTION: The maximum pressure for the flow cell is 100 psi. The flow cell could be damaged if this pressure is exceeded.*

2.4.3 Drip Tray Connection

The detector contains a drip tray underneath the flow cell behind the front panel to direct solvent leaks from the cell or fluid connections to the front of the unit.

Connecting the drip tray is usually unnecessary. If you connect it, be sure to position the waste container below the drip tray outlet.

Supplies When connecting the drip tray to waste, you need the Teflon® tubing from the Startup Kit.

Tools You also need a razor blade or other method for cutting the Teflon tubing.

Procedure To connect the drip tray:

1. Cut as much of the Teflon tubing as you need.
2. Connect the Teflon tubing supplied in the Startup Kit to the white plastic fitting below the three bulkhead fittings.
3. Put the other end of the Teflon tubing in the waste container.

3

CONNECTING COMPONENTS TO THE 410

This chapter contains procedures for connecting components to the 410 refractometer. These procedures include:

- Terminal strip connections, including:
  - Waters 740 Data Module
  - Waters 745/745B/746 Integrators
  - Chart recorder
  - Waters SAT/IN™ Interface Module for the Waters 845 and 860 Data Systems
  - Waters SIM™ Interface Module for the Waters 810, 820, 840, 845, and 860 Data Systems
  - Waters WISP™ 700 Series Autosampler
  - Waters U6K Injector
  - Waters Watchdog/Event Box Interface for the Waters 810, 815, 820, and 825 Data Systems
  - Waters 590 Programmable Solvent Delivery Module
- Optional IEEE-488 interface connections, including:
  - Waters 840/845/860 Data Systems
  - Waters LAC/E Module
  - Waters PowerLine™System Controller (used with the Waters 600E, Waters 650E, Waters 625 LC, Waters ACTION Analyzer, or Waters Prep 4000)
- Optional extemal column heater connections

3.1 ELECTRICAL CONNECTION DESCRIPTION

The 410 can be connected to components in three ways:

- **Terminal strip** for attaching input and output connections (using two or three-wire cables) between components. The terminal strip includes two types of connections:
  - Analog outputs
  - Event inputs
- **IEEE-488 interface** for communication with a data system (such as the Waters 840 or 845/860 via a LAC/E™ module) or with a PowerLine or other system controller (as part of the 600E, 650E, 625 LC, ACTION Analyzer, or Prep 4000 system).
- 5- **or 9-pin DIN connectors** for control of up to two external column heaters.

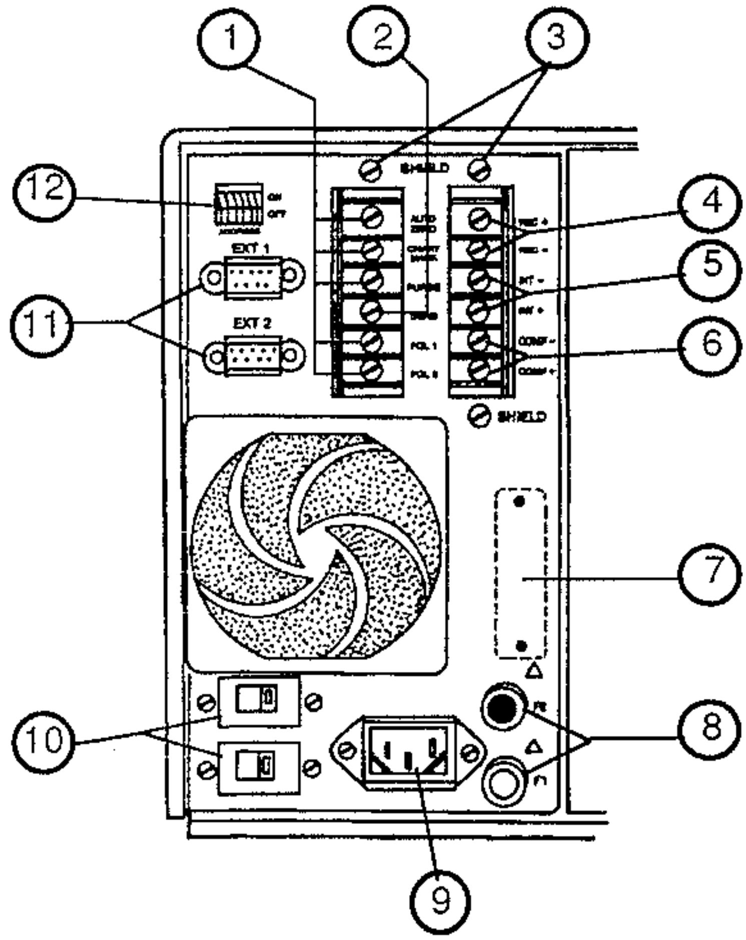


Figure 3-1 Waters 410 Back Panel

Table 3-1 describes the function of the 410 connections.

Table 3-1 410 Electrical Connections

Items (Figure 3-1)	Connection	Function
1	AUTO ZERO CHART MARK PURGE POL 1 POL 2	Accept TTL-level (0 to +5 V) or contact closure signals from an external instrument.
2	DGND	Digital ground for all event input signals.
3	SHIELD	Chassis ground terminal, internally connected to the 410 housing. Connect the shield from the analog signal cable to this ground (always at the 410 terminal strip).
4	REC (Recorder out)	Sends a 0 to ±10 mV (full scale) signal to a chart recorder.
5	INT (Integrator out)	Sends a 0 to 10 V (full scale) signal to an integrator or computer.
6	COMP (Compressed out)	Sends a compressed 4 mV maximum output signal to a chart recorder/integrator.
7	IEEE-488 connector	Connector for optional IEEE-488 interface cable between the 410 refractometer and the data collector or system controller.
8	F1 F2	Line fuse. Optional second fuse.
9		Power cord connection.
10	LINE, HTR	Voltage selection switches.
11	EXT1 EXT2	9-pin DIN connectors for optional external column heaters.
12	ADDRESS	DIP switches for setting IEEE-488 addresses.

Table 3-2 summarizes 410 connections:

Table 3-2 Summary of Component Connections to 410

Electrical Connection on 410	Used to connect
Terminal Strip	
Analog output screw terminals	• 740 Data Module • 745/745B/746 Integrators • Chart recorder • SIM(to810, 820, 840,845, or 860) • SAT/IN Module (to 845, or 860, or to LAC/E) • Watchdog board interface (to 810, 815, 820, and 825) • Computer (via an A/D interface)
Event input screw terminals	• System Controller (used with the 600E, 625 LC, ACTION Analyzer, 650E, and Prep 4000) • WISP 700 Series Autosampler • U6K manual injector
IEEE-488 Connector	
IEEE-488	• 840 • LAC/E Module (to 845/860) • PowerLine System Controller
External Column Heater	
Two 9-pin DIN connectors	• Optional external column heaters

3.2 CONNECTING COMPONENTS TO THE TERMINAL STRIP

This section describes Waters 410 connections to:

- Waters 740 Data Module
- Waters 745/745B/746 Integrator
- Chart recorder
- Waters SAT/IN Satellite Interface
- Waters SIM System Interface Module
- Waters WISP 700 Series Autoinjector
- Waters U6K Injector
- Waters Watchdog Board
- Waters 590 Solvent Delivery System

WARNING: To avoid electric shock, power down the 410 before connecting components. Power down the system controller or data collector before powering down the 410.

When powering up, turn on the 410 before turning on external devices connected through IEEE-488.

3.2.1 Waters 740 Data Module

INT analog output signal To send an INT analog output signal (0 to 10 V) from the 410 to the Waters 740 Data Module (Figure 3-2):

Connect signal cable from INT on the 410:	**To the 740 rear panel:**
Red (INT +)	9-pin ANALOG INPUT DIN receptacle **(pins 1** and 2)
White/blue (INT -)	

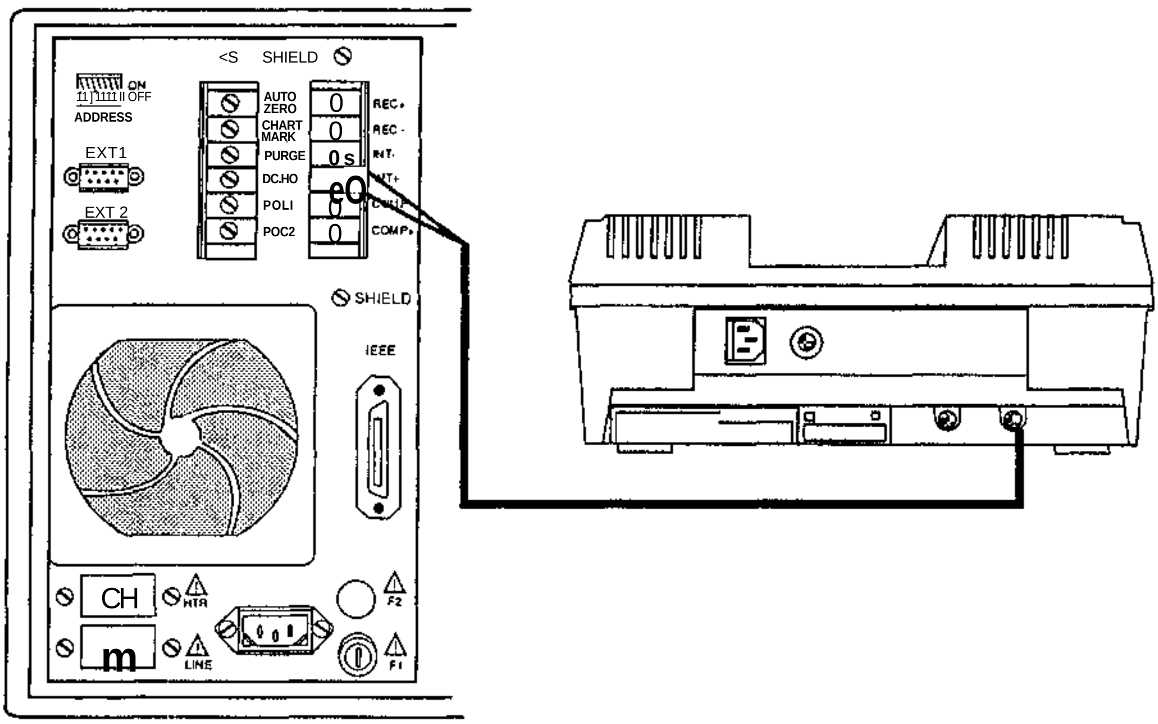


Figure 3-2 Waters 740 Connection

3.2.2 Waters 745/745B/746 Integrator

INT analog output signal To send an INT analog output signal (0 to 10 V) from the 410 to the Waters 745/745B/746 Integrator (Figure 3-3):

Connect signal cable from INT on the 410:	To the 745/745B/746 rear panel:
Red (INT+)	CHA connector (+)
Black (INT-)	CHA connector (-)
White is not used; tape it back to prevent shorting.	

For best results,. if using the Waters 745/745B/746 with a chart recorder, use two separate channels for plotting and integration. Otherwise, changes in chart recorder attenuation may affect the integration of the peaks.

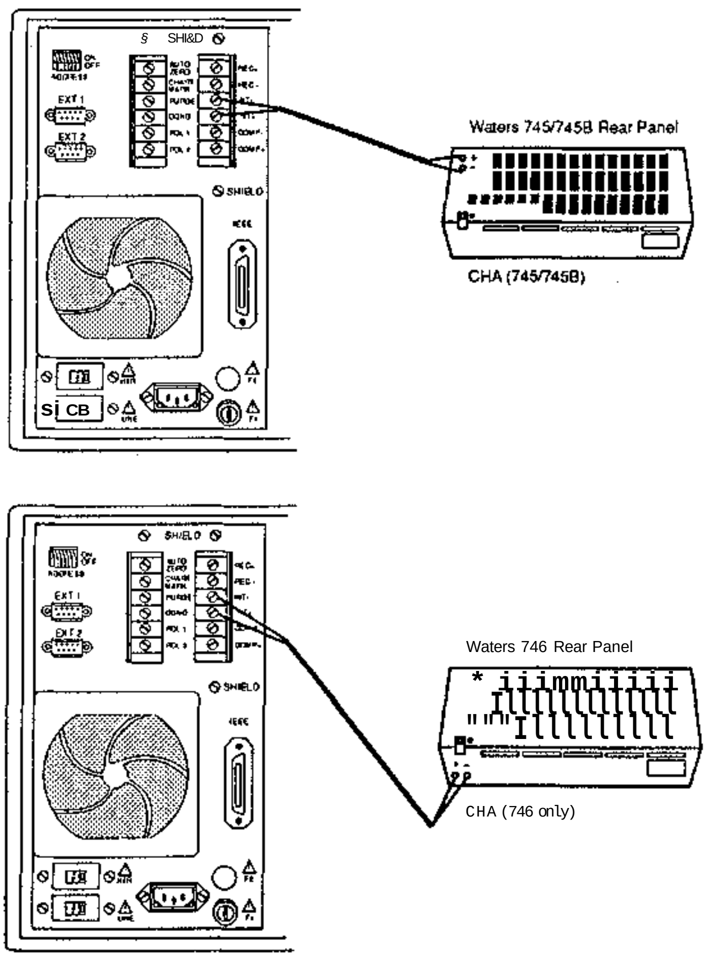


Figure 3-3 Waters 745/745B/746 Connections

3.2.3 Chart Recorder Connections

10 mV analog output signal To send **a** 10 **mV analog signal from the 410 to a chart** recorder (Figure 3-4):

Connect signal cable from REC on the 410:	**To the Chart Recorder rear panel:**
Red (REC+)	Pen **1 (+)**
Black (REC -)	Pen **1 (-)**
White is not used; tape it back to prevent shorting.	

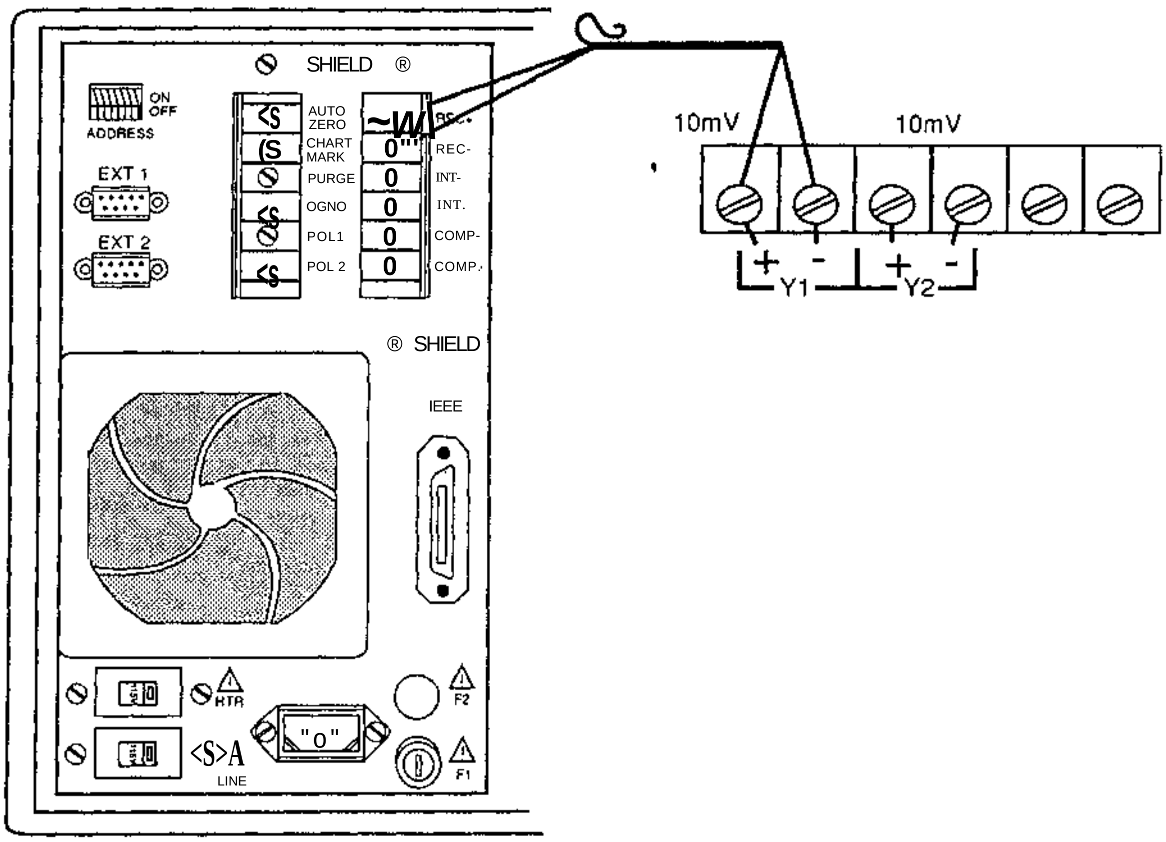


Figure 3-4 Chart Recorder Connections

Performing chart **mark** with **the** chart **recorder** To set the 410 to automatically send a chart mark pulse to the chart recorder at the start of each run, connect the extemal device (System Controller, autosampler, or manual injector) to the 410 CHART MARK screw terminal as described in the appropriate component connection sections of this chapter.

3.2.4 Waters SAT/IN for the 845 and 860

INT analog output signal To send an INT analog output signal (0 to 10 V) trom the 410 to a 2-channel SAT/IN (Satellite Interface) module (Figure 3-5):

Connect signal cabie from INT on the 410:	To the SAT/IN front panel:
Red (INT +)	CHANNEL 1 or
Black (INT-)	CHANNEL2
White is not used; tape it back to prevent shorting.	

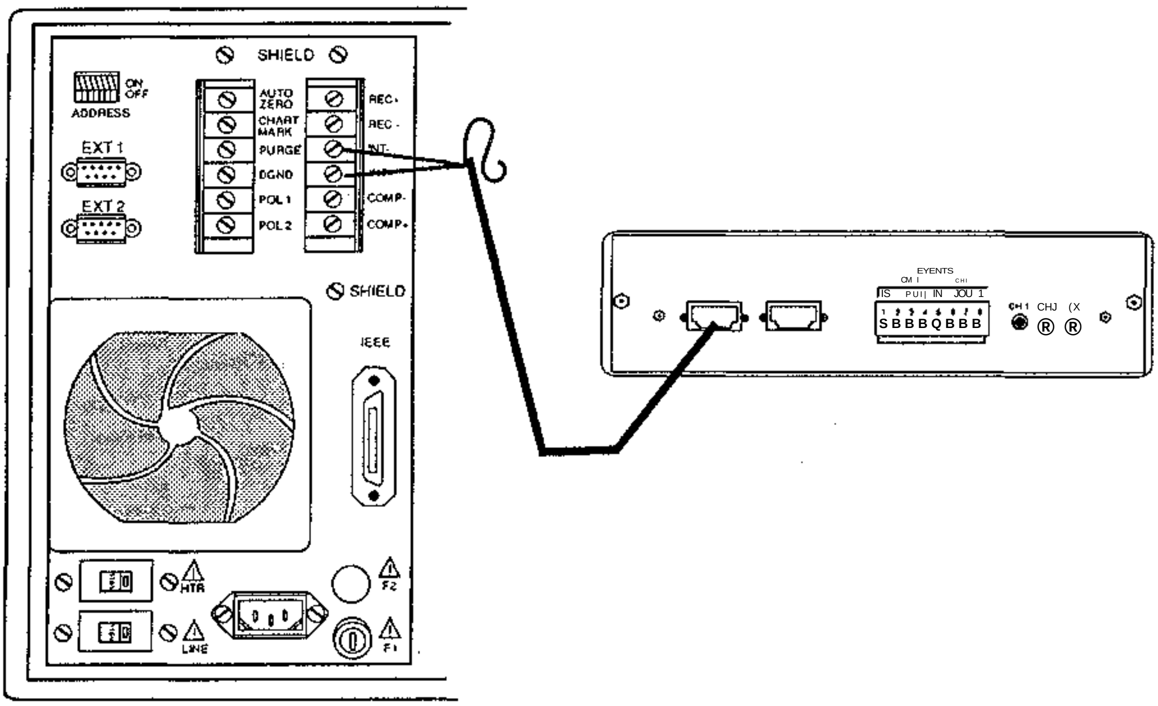


Figure 3-5 Waters SAT/IN Front Panel

3.2.5 Waters SIM forthe 810, 820,840,845, and 860

INT analog output signal To send an INT analog output signal (0 to 10 **V)** from the 410 to a System Interface Module (SIM) (Figure 3-6):

Connect signal cable from INT on the 410:	To the SIM rear panel:
Red (INT+)	CHAN1 or2
Black (INT-)	COM(-)
White (SHIELD)	Not connected

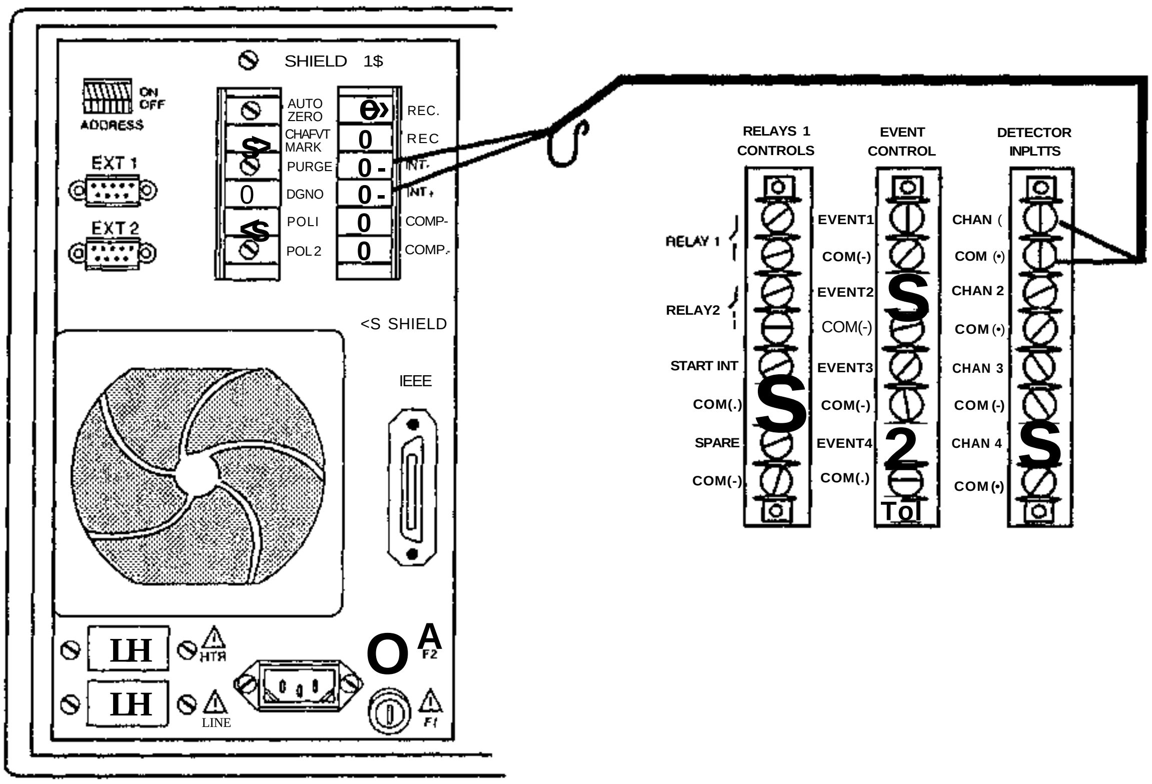


Figure 3-6 SIM Rear Panel

3.2.6 Waters WISP 700 Series Connections

The Waters 410 accepts the following external injection trigger signals from a non-IEEE WISP 700 Series autosampler:

- Chart mark (inject start) signal from a contact closure signal with each injection.
- Remote auto zero signal to auto zero the 410 each time the WISP makes an injection.

Each time the 410 receives a signal, it performs the specific chart mark or auto zero function.

Chart Mark To connect the 410 to the WISP to generate a chart mark signal (Figure3-7):

Connect signal cable from CHART MARK on the 410:	To the WISP rear panel:
Red (CHART MARK)	CHART MARK
Black (DGND)	terminals
White (SHIELD)	

If you connect the WISP to more than one detector, avoid connecting more than one set ofcables to the same WISP rear panel terminal connection.

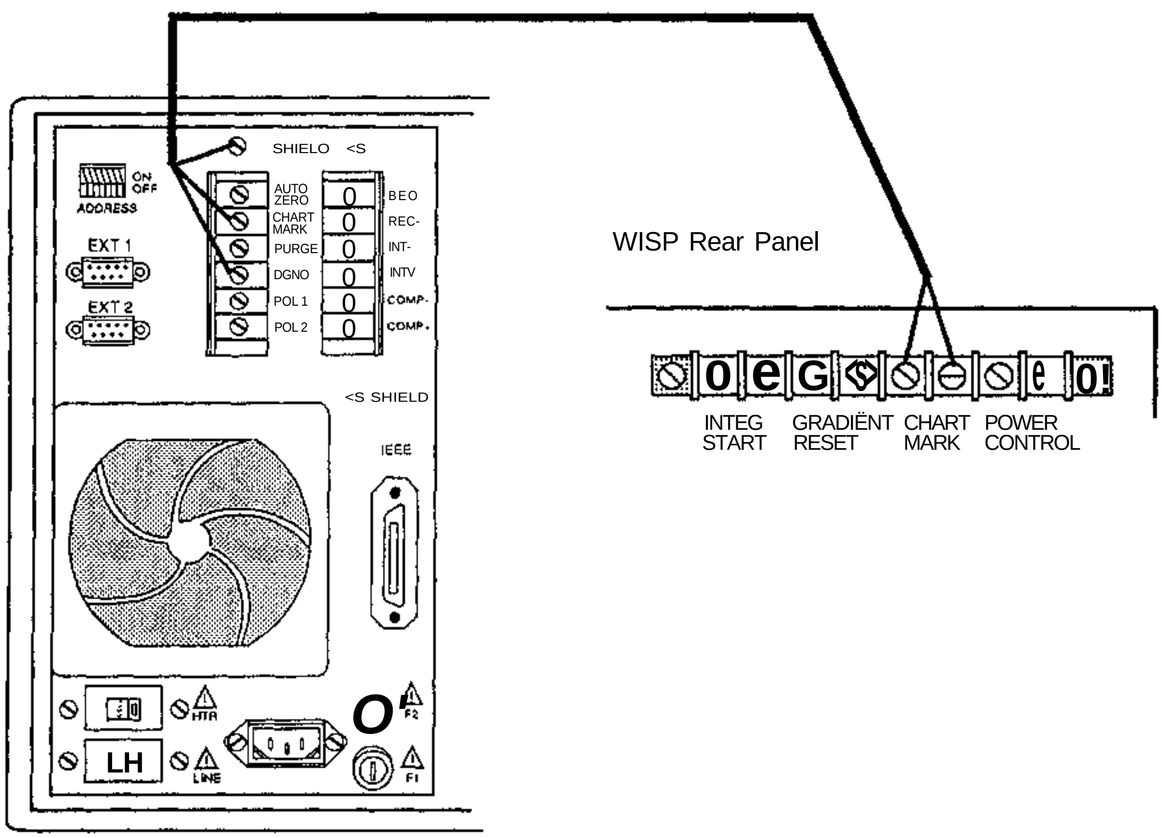


Figure 3-7 Waters WISP ***Chart Mark Connection***

Remote auto zero To connect the 410 to a WISP to perform remote auto zero (Figure3-8):

Connect signal cable from AUTO ZERO on the 410:	**To the WISP rear panel:**
Red (AUTO ZERO)	INTEG START or
Black (DGND)	INJECT START
White (SHIELD)	terminals

If you connect the WISP to more than one detector, avoid connecting more than one set ofcables to the same WISP rearpanel terminal connection.

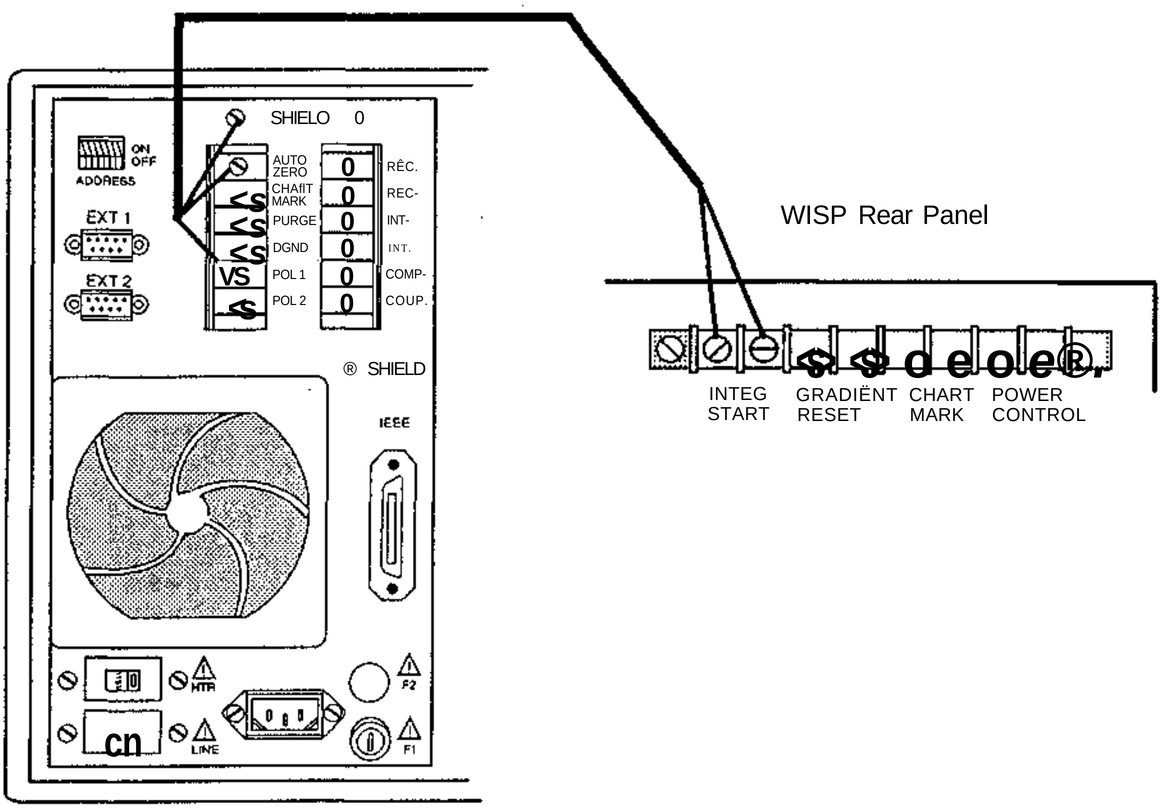


Figure 3-8 Waters WISP Remote ***Auto Zero Connection***

3.2.7 Waters U6K Injector Connections

The 410 accepts the following external injection trigger signals from a U6K injector:

- Chart mark (inject start) signal from a contact closure signal with each injection
- Remote auto zero signal to automatically adjust the zero offset of the 410 each time the U6K makes an injection

Each time the 410 receives a signal, it performs the specific chart mark or auto zero function.

If you connect the U6K to more than one detector, avoid connecting more than one set of cables to each set of terminal connections.

Auto zero and chart mark

To connect the 410 to a U6K to generate an autozero or chart mark signal (Figure 3-9):

Connect signa! **cable from AUTO ZERO or CHART MARK on** the **410:**	**To the back of the U6K:**
Red (AUTO ZERO or CHART MARK)	6-pin connector (The first and second U6K cables are functionally identical)
Black (DGND)	
White (SHIELD)	Not connected

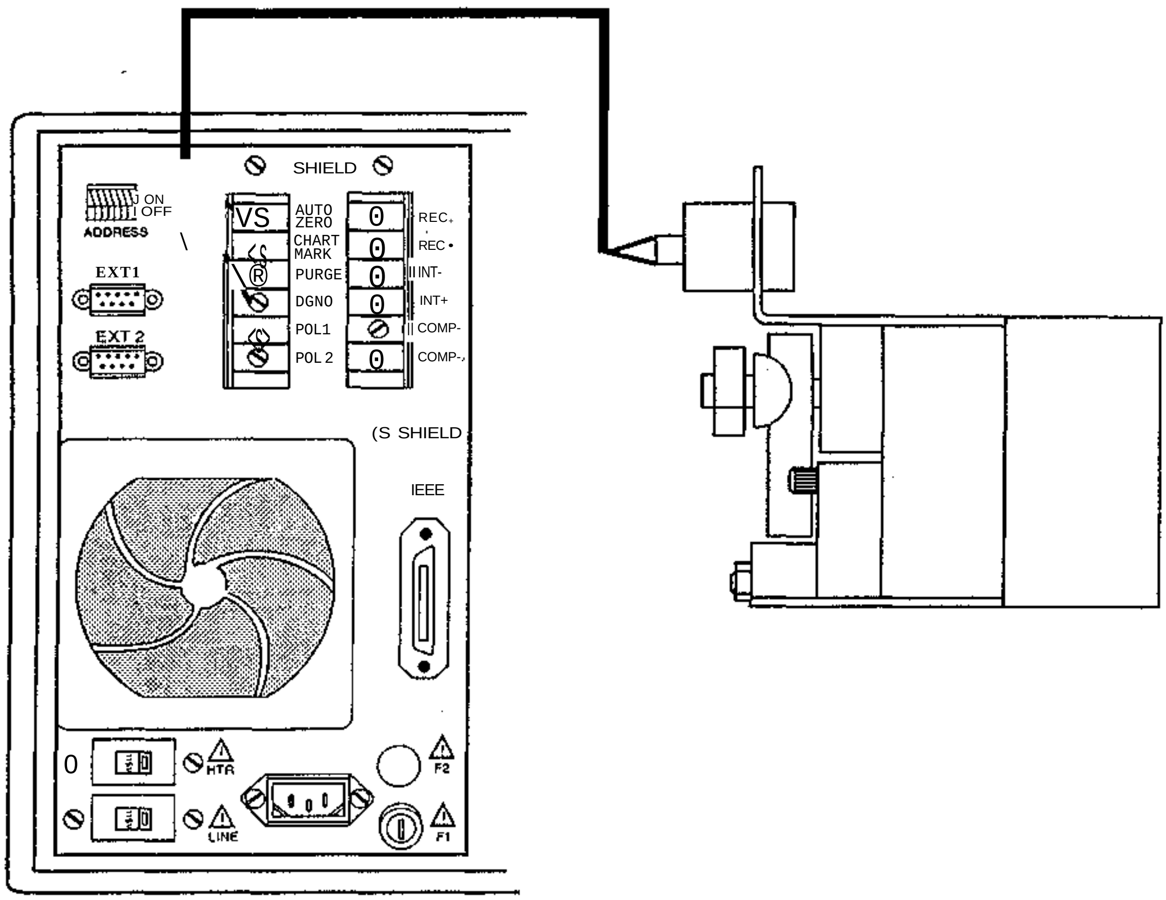


Figure 3-9 Waters U6K Auto Zero or Chart Mark Connection

3.2.8 Waters Watchdog Interface tor the 810, 815, 820, and 825

Watchdog board Figure 3-10 illustrates an 810/815/820/825 rear panel with a four-channel watchdog board (WD24) installed, as recommended, in slot 3.

If desired, your watchdog board may be installed in a different slot.

If you have a WD22 watchdog board, one A/D converter with two channels is available. If you have a WD24 watchdog board, two A/D converters with four channels are available.

An Event Box may be attached to the watchdog board.

INT analog output signal To send an INT analog output signal (0 to 10 V) from the 410 to the watchdog board (Figure 3-10):

Connect the signal cable **from INT** on **the 410:**	**To the watchdog board rear panel:**
Red (INT+)	CH1 or2onWD22
Black (INT -)	or
White (SHIELD)	CH1,2,3or 4onWD24

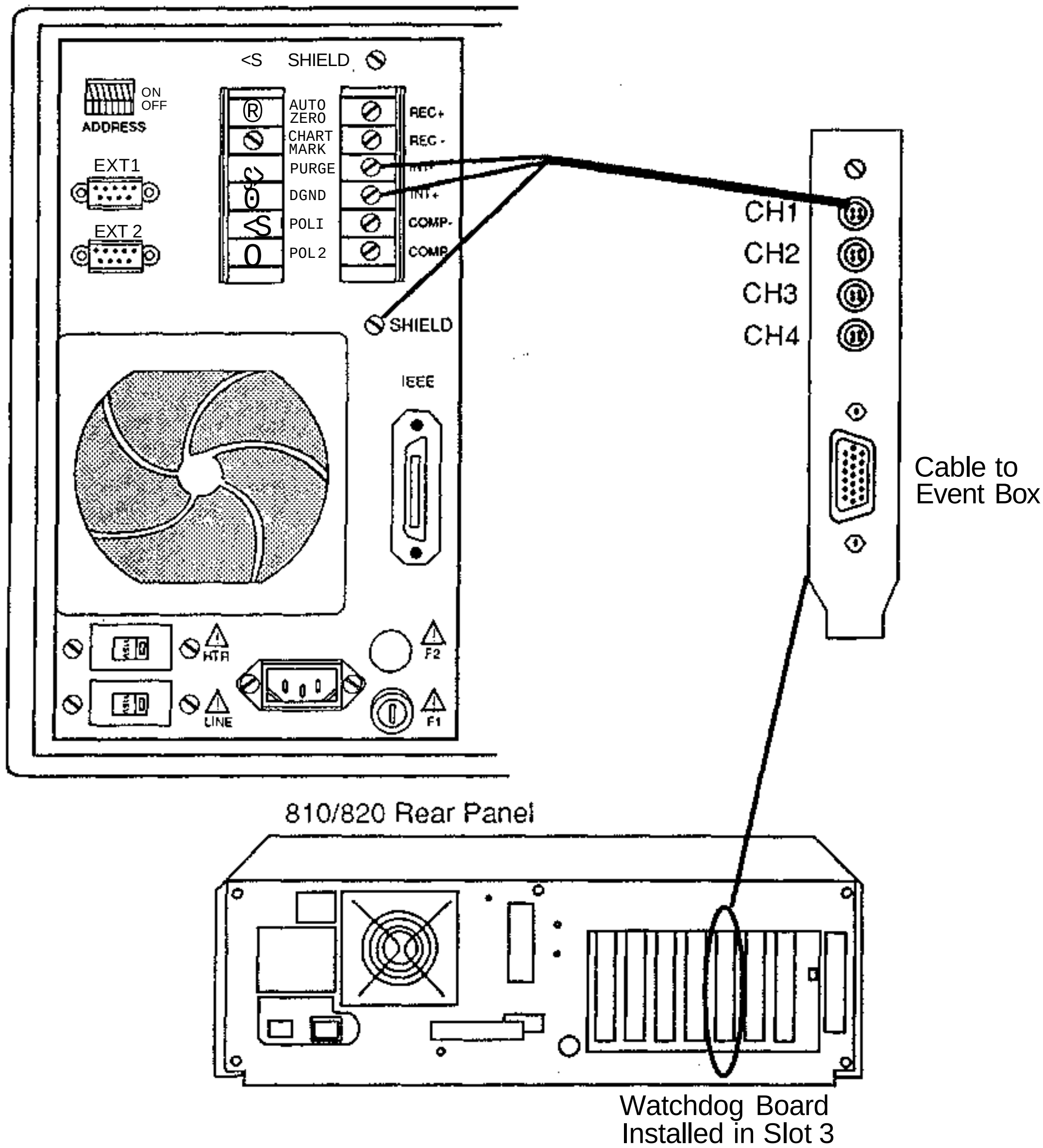


Figure 3-10 *Waters* ***810/815/820/825 Rear Panel Connections***

3.2.9 Waters 590 Connections

The 410 accepts the foilowing external injection trigger signals from a 590 solvent delivery system:

- Chart mark (inject start) signal from a contact closure signal with each injection
- Remote auto zero signal to automatically adjust the zero offset of the 410 each time the 590 makes an injection

Each time a signal is received by the 410, the 410 performs the specific chart mark or auto zero function.

If you connect the 590 to more than one detector, avoid connecting more than one set of cables to each set of terminal connections.

Auto zero or chart mark

To connect the 410 to a 590 to generate an auto zero or chart mark signal, you go through an Event I/O Box (Figure 3-11):

Connect signal cable from AUTO ZERO or CHART MARK on the 410:	To the Event I/O Box output terminal (1 to 7, not terminal 8):
Red (AUTO ZERO or CHART MARK)	Red (+12 V terminal)
Black (DGND)	Black (other terminal)
White (SHIELD)	Not connected

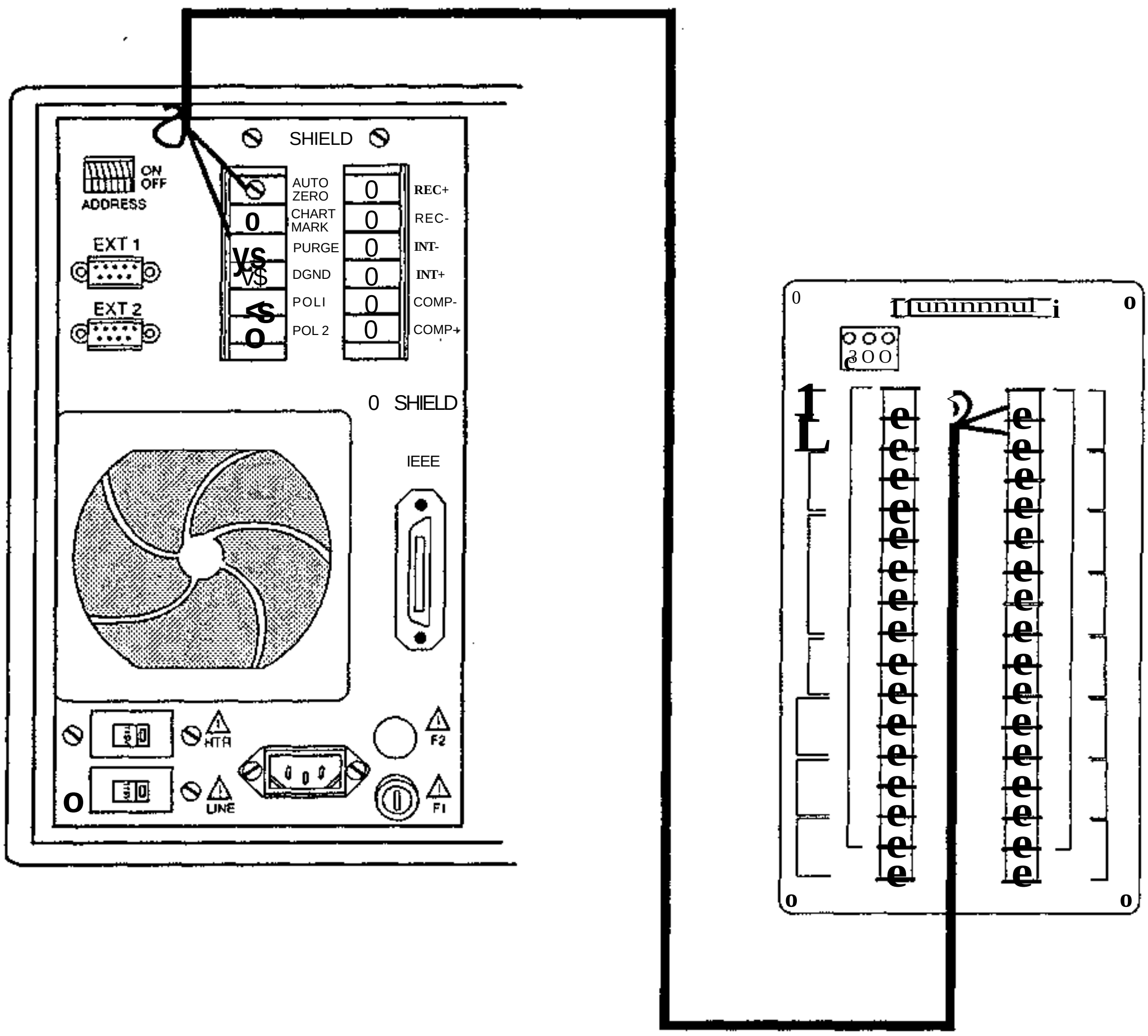


Figure 3-11 Waters 590 Connection

3.3 CONNECTING COMPONENTS TO THE IEEE-488 CONNECTOR

The optional IEEE-488 Communications interface on the 410 rear panel allows you to connect the 410 Refractometer to:

- Data systems such as the Waters 840 Chromatography Workstation, Waters 845 Chromatography Data and Control Station, and Waters 860 Networking Computer System
- System controllers, such as the Waters PowerLine System Controller

3.3.1 IEEE-488 with a Data System

To use the 410 with an IEEE data system:

1. Make the IEEE-488 connection.
2. Connect a trigger wire between the manual injector (or autosampler) and the 410 AUTO ZERO or CHART MARK.

Data system connection overview Depending upon the data system in use, the 410 refractometer can be connected to the data system as shown in Table 3-3:

Table 3-3 Overview **of Connections to Data Systems**

Data System	If using with	Then
840	410 directly	Connect the IEEE^488 cable between the 410 and the 840 rear panel (Figure 3-12).
840	SIM	Connect the 410 analog output to the SIM rear panel (see Section 3.2.5).
845/860	LAC/E	Connect the IEEE-488 cable between the 410 rear panel and the LAC/E IEEE-488 option board on the LAC/E rear panel (Figure 3-13).
845/860	SAT/IN	Connect the 410 analog output (see Section 3.2.4).
845/860	SIM	Connect the 410 analog output (see Section 3.2.5).

3.3.1.1 Data System Connection

Refer to Figures 3-12 and 3-13 for IEEE-488 data system connections to the 410.

840 IEEE connection The 410 can be connected directly to the 840 by IEEE-488 (Figure 3-12). See Section 3.2.5 for the 410 connection to a SIM.

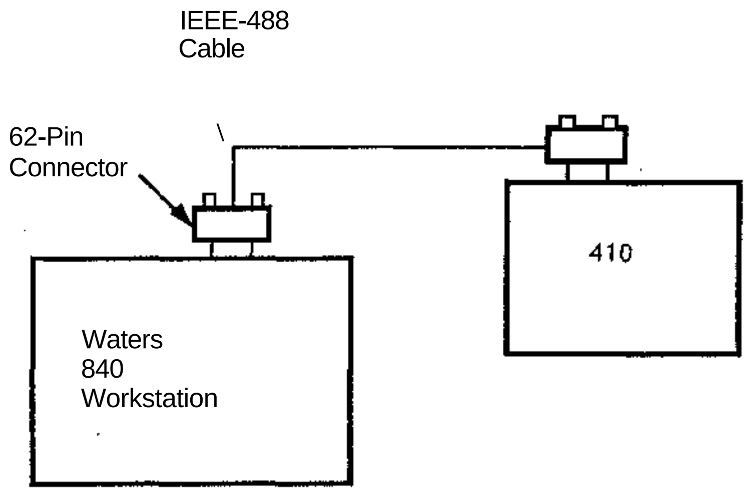


Figure 3-12 410 Connected Directly to an 840 via IEEE-488

860/845 IEEE connection The 410 is connected^o an 860 or 845 system through a LAC/E module (Figure 3-13).

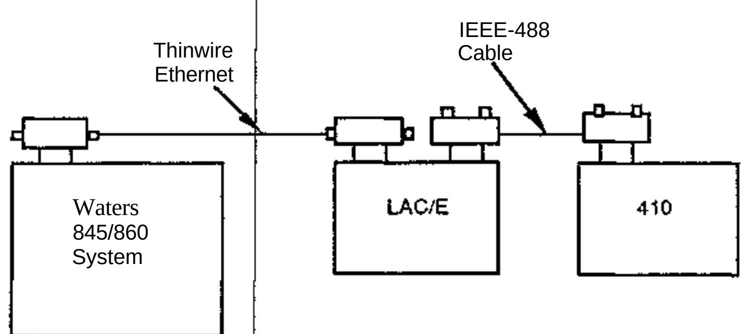


Figure 3-13 Conhection to an 845/860 by a LAC/E Moldule via IEEE-488

3.3.1.2 Injector Connections

When using the 410 with an IEEE data system, the data system must receive an inject start signal to initiate the data collection and time-based programs.

Depending upon the injector used, the inject start signai comes from:

Injector	Source of Inject Start Signal
U6K	CHART MARK terminal
WISP 712	CHART MARK terminal
WISP715	IEEE-488 interface (see Figure 3-14) *In this configuration, the 845/860 multi-method must be programmed to START BY LAC/E (refer to the Waters 845/860 Operators Manual for details).*

If multiple devices in your system require this connection, connect cables between the inject start terminal on the injector and each device.

U6K connection If using the U6K:

Connect signal cable from terminal strip on the 410:	To **the U6K:**
Red (CHART MARK)	One set of spade lug chart mark cables on U6K (the U6K inciudes two pairs of cables; both are **functionally** identical)
Black (DGND)	
White (SHIELD)	

WISP 712 connection If using a WISP 712 autosampler:

Connect signal cable from terminal strip on the 410:	To the WISP rear panel:
Red (CHART MARK)	INTEG START terminals
Black (DGND)	or
White (SHIELD)	CHART MARK terminals

3.3.2 IEEE-488 with a Waters PowerLine System Controller

To controi the 410 refractometer with a PowerLine System Controller, connect the 410 with the IEEE-488 interface cable as illustrated in Figure3-14.

The Waters System Controller is used with the foilowing fluid handling units:

- Waters 600E Multisolvent Delivery System
- Waters 650E Advanced Protein Purification System
- Waters 625LC System
- Waters ACTION Analyzer System
- Waters Delta Prep 4000 System
- Waters Prep LC 4000 System

Each fluid handling unit is configured with either an:

- Integrated manual injector (built in as part of the drawer or shelf unit)
- Externally connected manuai injector or autosampler

The WISP can also be connected directly to the PowerLine Controller over IEEE.

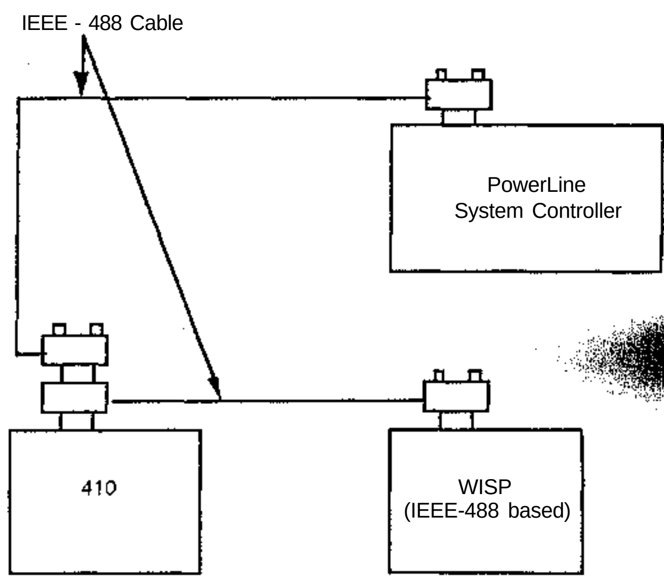


Figure 3-14 Connection to a PowerLine System Controller via IEEE-488

3.4 SETTING IEEE-488 ADDRESSES

The 410 needs an IEEE-488 address to be recognized by the IEEE-488 controller (840, LAC/E module, Powerüne System Controller).

Set the data system or controller IEEE-488 address with the five DIP switches located in the upper left-hand corner of the back panel.

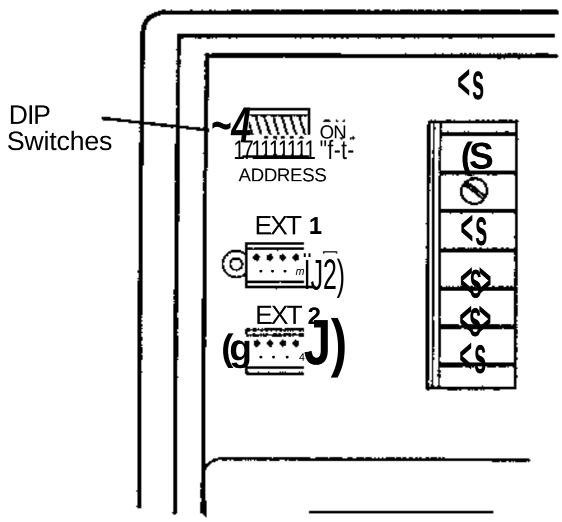


Figure 3-15 IEEE-488 DIP Switches

Setting an address The five switches can each be set to either OFF or ON, yielding 32 possible address combinations from 0 (OFF, OFF, OFF, OFF, OFF) through 31 (ON, ON, ON, ON, ON). Address 0, 1, 30, and 31 are reserved. The 28 remaining addresses are available to the 410 for connection to a data system or controller. Table 3-4 lists available IEEE-488 addresses.

Once selected, turn the 410 off and on to reconfigure the system for the new IEEE-488 address.

IEEE-488 address requirements

IEEE-488 addresses must be unique. Some components require that the IEEE-488 address for the 410 be higher than those for other devices. Consult your system operator's manual for more information on IEEE-488 Communications.

Table 3-4 IEEE-488 Address Switch Settings

IEEE Address	Switch Settings					IEEE Address	Switch Settings				
	1	2	3	4	5		1	2	3	4	5
0 and 1	are reserved for internal use					16	OFF	OFF	OFF	OFF	ON
2	OFF	ON	OFF	OFF	OFF	17	ON	OFF	OFF	OFF	ON
3	ON	ON	OFF	OFF	OFF	18	OFF	ON	OFF	OFF	ON
4	OFF	OFF	ON	OFF	OFF	19	ON	ON	OFF	OFF	ON
5	ON	OFF	ON	OFF	OFF	20	OFF	OFF	ON	OFF	ON
6	OFF	ON	ON	OFF	OFF	21	ON	OFF	ON	OFF	ON
7	ON	ON	ON	OFF	OFF	22	OFF	ON	ON	OFF	ON
8	OFF	OFF	OFF	ON	OFF	23	ON	ON	ON	OFF	ON
9	ON	OFF	OFF	ON	OFF	24	OFF	OFF	OFF	ON	ON
10	OFF	ON	OFF	ON	OFF	25	ON	OFF	OFF	ON	ON
11	ON	ON	OFF	ON	OFF	26	OFF	ON	OFF	ON	ON
12	OFF	OFF	ON	ON	OFF	27	ON	ON	OFF	ON	ON
13	ON	OFF	ON	ON	OFF	28	OFF	OFF	ON	ON	ON
14	OFF	ON	ON	ON	OFF	29	ON	OFF	ON	ON	ON
15	ON	ON	ON	ON	OFF	30 and 31	are reserved for internal use				

3.5 CONNECTING EXTERNAL COLUMN HEATERS

The 410 controls up to two optional extemal column heaters through the EXT 1 and EXT 2 ports to the left of the terminal strip. The ports are Standard 9-pin DIN connectors.

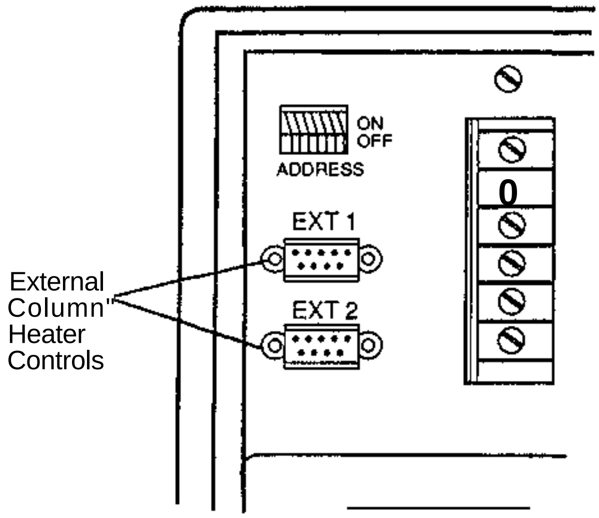


Figure 3-16 Column Heater Ports

4

SOLVENT PREPARATION

Changes in the composition or temperature of a solvent can cause baseline changes which may appear as drift, cycling, noise, or an erratic baseline. Proper solvent selection and preparation are critical in differential refractometry. This chapter presents information on:

- Common Solvent Problems
- Selecting a Solvent
- Solvent Degassing

STOP *WARNING: Observe good laboratory practices when handling solvents. Refer to the Material Safety Data Sheets shipped with solvents for handling information.*

4.1 COMMON SOLVENT PROBLEMS

The 410 refractometer measures changes in the concentration of the solution flowing through the sample side of the flow cell (see Section 1.2, Theory of Operation, for more information on measuring concentration).

However, factors other than the presence of dissolved sample molecules can affect the solution's refractive index. Common problems include:

- Changes in temperature
- Changes in pressure
- Contaminants
- Separation of mixed solvents
- Outgassing of dissolved gases

4.2 SELECTING A SOLVENT

Choosing the correct solvent for your analysis is important. An ideal solvent:

- Has good soiubility characteristics for your application
- Has a significantly different RI than the sample components
- Gives satisfactory baseline noise performance
- Provides optimum optical sensitivity characteristics

Solvent quality Use spectral-grade or HPLC-grade solvent. Pure solvents provide:

- Reproducible results
- Operation with minimal instrument maintenance
- Minimal optical interference

A dirty or impure solvent can cause:

- Baseline noise and drift
- Plugged columns
- Blockages in the fluid path

Refractive indices of common solvents Table 4-1 lists the refractive indices for some common chromatographic solvents. Use this table to verify that the solvent you intend to use for your analysis has a significantly different RI than the sample components.

Table 4-1 Refractive Indices of Common Solvents

Solvent	RI	**Solvent**	RI
Fluoroalkanes	1.25	Tetrahydrofuran (THF)	1.408
Hexafluoroisopropanol (HFIP)	1.2752	Amyl alcohol	1.410
Methanol	1.329	Diisobutylene	1.411
Water	1.33	n-Decane	1.412
Acetonitrile	1.344	Amyl chloride	1.413
Ethyl ether	1.353	Dioxane	1.422
n-Pentane	1.358	Ethyl bromide	1.424
Acetone	1.359	Methylene chloride	1.424
Ethanol	1.361	Cyclohexane	1.427
Methyl acetate	1.362	Ethylene glycol	1.427
Isopropyl ether	1.368	N,N-Dimethyl Formamide (DMF)	1.428
Ethyl acetate	1.370	N,N-Dimethyl Acetamide (DMAC)	1.438
1-Pentene	1.371	Ethyl sulfide	1.442
Acetic acid	1.372	Chloroform	1.443
Isopropyl chloride	1.378	Ethyiene dichloride	1.445
Isopropanol	1.38	Carbon tetrachloride	1.466
n-Propano!	1.38	Dimethyl sulfoxide (DMSO)	1.477
Methylethylketone	1.381	Toluene	1.496
Diethyl amine	1.387	Xylene	-1.50
n-Propyl chloride	1.389	Benzene	1.501
Methylisobutylketone	1.394	Pyridine	1.510
Nitromethane	1.394	Chlorobenzene	1.525
1-Nitropropane	1.400	o-Chiorophenol	1.547
Isooctane	1.404	Aniline	1.586
Cyclopentane	1.406	Carbon disulfide	1.626

4.3 SOLVENT DEGASSING

Using degassed solvents is the most important step in solvent preparation. Degassing provides:

- Stable baselines and enhanced sensitivity
- Reproducible retention times
- Stable pump operation

This section presents information on the solubility of gases, solvent degassing methods, and solvent degassing considerations.

4.3.1 Gas Solubility

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

- The chemical affinity of the gas for the liquid
- The temperature of the liquid
- The pressure applied to the liquid

Changes in the composition, temperature, or pressure of the mobile phase can all lead to outgassing.

Effects of intermolecular forces Nonpolar gases (N_2, O_2, CO_2, He) are more soluble in nonpolar solvents than in polar solvents. Generally, a gas is most soluble in a solvent with intermolecular attractive forces similar to those in the gas ("like dissolves like").

Effects of temperafure Temperature affects the solubility of gases. If the heat of solution is exothermic, the solubility of the gas decreases when you heat the solvent. If the heat of solution is endothermic, the solubility increases when you heat the solvent. For example, the solubility of He in H_2O decreases with an increase in temperature, but the solubility of He in benzene increases with an increase in temperature.

Effects of partial pressure The mass of gas dissolved in a given volume of solvent is proportional to the partial pressure of the gas in the vapor phase of the solvent. If you decrease the partial pressure of the gas, the amount of that gas in solution also decreases.

4.3.2 Solvent Degassing Methods

This section describes the solvent degassing techniques that wili help you to attain a stable baseline. Degassing your solvent also improves reproducibility and pump performance.

There are three common methods used to degas solvents:

- Sparging with helium
- Reducing pressure by vacuüm
- Sonication

These methods may be used individually or in combinations. Vacuüm sonication followed by sparging is the most effective technique for most solvents.

Sparging Sparging removes gases from solution by saturating the solvent with a less soluble gas, usually helium. Well-sparged solvent improves pump performance. Helium sparging brings the solvent to a state of equilibrium, which may be maintained by slow sparging or by keeping a blanket of helium over the solvent. Blanketing inhibits reabsorption of atmospheric gases.

Sparging may change the composition of mixed solvents.

Vacuüm A vacuüm 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.

Vacuüm degassing may change the composition of mixed solvents.

Sonication Sonication with high energy sound waves drives energy into the solvent and causes the submicron-sized "bubbles" of gas to aggregate. As the gas bubbles aggregate, they become large enough to float out of the solvent and dissipate. Sonication alone degasses 4 liters of solvent in approximately 22 minutes.

4.3.3 Solvent Degassing Considerations

Select the most efficiënt degassing operation tor your application. To remove dissolved gas quickly, consider the following:

Sonication plus vacuüm Sonication plus vacuüm degasses a liter of solvent very quickly. This technique is less likely to change the composition of mixed solvents because the mixed solvents are not held under vacuüm as long (less than a minute is usually sufficient).

WARMING: *Do not apply vacuüm to the brown gallon bottles in which solvent is shipped. There is a high risk of implosion under these conditions. Use a thick-walled container designed for vacuüm applications.*

Sparging Helium sparging gives stable baselines and better sensitivity in a detector, and prevents reabsorption of atmospheric gases. Use this method to retard oxidation when you are using THF or other peroxide-forming solvents.

Vacuüm Vacuüm alone is too slow to be an acceptable means of degassing solvent. However, a 0.45 \im membrane filter with 300 mm of vacuüm can filter and degas 4 liters of solvent in approximately 8 minutes.

4.3.4 Guidelines

Use HPLC-grade solvents to ensure the best possible results. Filter through 0.45 |im membrane filters before use.

Preparation checklist

The foliowing soivent preparation guidelines help to ensure stable baselines and good resolution:

- Filter solvents with a 0.45 |j.m filter.
- Degas and/or sparge the soivent.
- Stir the soivent.
- Put the soivent reservoir in a piace free from drafts and shock.

Water

when using water, use a high quality source such as a Milli-Q® water purification system. If the water system does not provide filtered water, filter it through a 0.45 p.m membrane filter before use.

Buffers

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

THF

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

STOP *WARN1NG: THF contaminants (peroxides) are potentially explosive if concentrated or taken to dryness.*

5

USING THE 410 REFRACTOMETER

Using the 410 refractometer as a stand-alone unit

The 410 may be used as a stand-alone module in conjunction with *a* pump, injector, column, and a recorder or integrator. To use the 410 in this way, follow the instructions given in this chapter.

Using **the 410 refractometer as part of a system**

The 410 may also be configured and controlled by a Waters system controller, such as the Waters 600E Multisolvent Delivery System, Waters 860 Networking Computer System, or Waters 845 Chromatography Data and Control Station. If the 410 is set up in this way, follow the instructions given in the appropriate manual to set parameters and control the 410 from the system controller. The 410 can still be programmed from the front panel, if desired, except when in remote mode (see Section 5.4, Powering Down).

Read Chapter 4, Solvent Preparation, before using the 410.

This chapter covers:

- Using the Front Panel
- Selecting Parameters
- Routine Startup
- Powering Down

5.1 USING THE FRONT PANEL

The 410 front panel consists of a display, a series of LED parameter indicators, and a keypad.

This section describes the 410 front panel:

- Display
- Indicators
- Keypad

5.1.1 Display and Indicators

The front panel of the Waters 410 includes a numeric LED display and eight small circular LED parameter indicators. Figure 5-1 shows the display and parameter indicators of the Waters 410 refractometer.

Display The 410 shows parameter values and commands with a four character LED display. To display the value of a parameter, press the appropriate parameter key. The parameter displays and its corresponding indicator illuminates until you select another parameter.

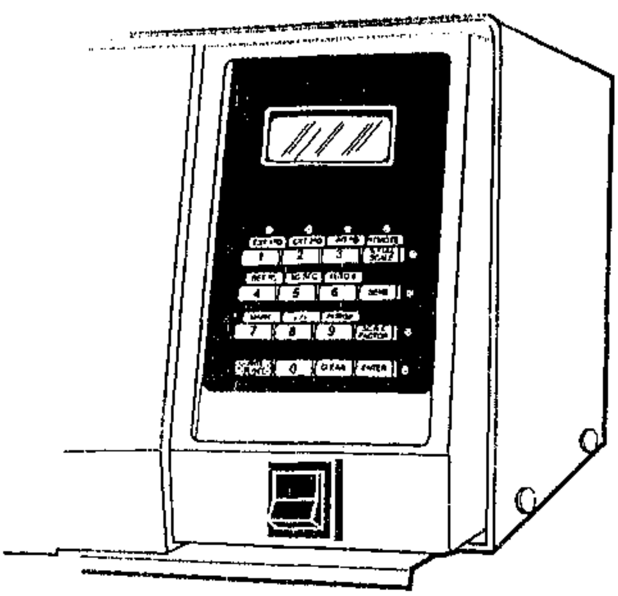


Figure 5-1 Display and Indicators

Parameter Indicators Eight round parameter indicators are located above and to the right of the numeric keypad. The indicators illuminate when you select the corresponding parameter.

Table 5-1 Indicator Functions

Parameter Indicator	Is lighted when:
EXT1°C EXT2°C	The display shows the current settings for the external column heaters, and when you are changing the settings for the external column heaters.
INT°C	The display shows the temperature of the internal oven, and when you are changing it.
%FULL SCALE	The display shows the current %full scale recorder output.
SENS	The display shows the current sensitivity setting, and when you are changing the sensitivity.
SCALE FACTOR	The display shows the current scale factor setting, and when you are changing the scale factor.
REMOTE	The 410 is underthe control of a remote controller.
2ND FUNC	The 2ND FUNC indicator is to the right of the ENTER key. This indicator illuminates when you press the blue 2ND FUNC key. It stays illuminated for five seconds, waiting for you to press the key whose second function you want to access.

5.1.2 The Keypad

The keypad is located on the right front panel of the 410. Use the keys to enter numeric data or function parameters. If the unit is running in remote mode with the keyboard locked, the front panel keys are inoperable.

Some keys scroll through several specific values. To scroll through the values, hold down the key until the desired value appears. Then release the key and press ENTER.

Numeric keypad The numeric keypad includes the number keys 0 to 9 and the decimal point key. Use these keys to enter numeric values.

Primary function and secondary function

Each key is labeled with its *primary function.* When you press a key, the function named on the key is performed. For example, when you press the SENS key, the 410 prompts you to enter a sensitivity value.

Most keys also have a *secondary function.* Secondary functions are listed above the key. To use the secondary functions, press the 2ND FUNC key, then the key below the function. For example, press 2ND FUNC then the 9 key (the key below PURGE) to set the 410 to purge mode.

This section describes:

- Primary functions
- Secondary functions
- Using the keypad

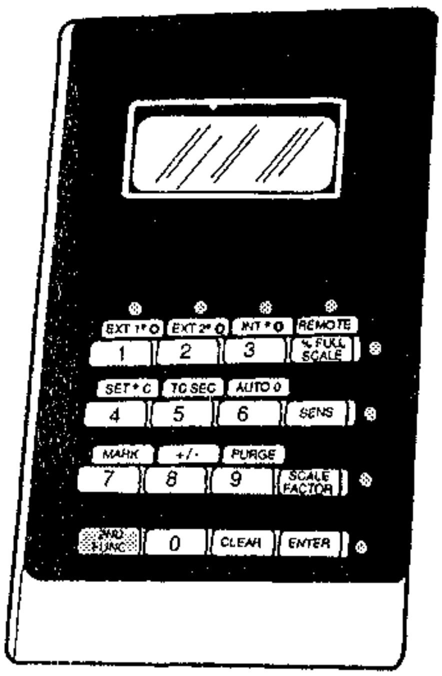


Figure 5-2 The Waters 410 Keypad

5.1.2.1 Primary Functions

This section describes the primary functions of the keys on the 410 keypad. Change a parameter by pressing the key, entering the new parameter, then pressing ENTER.

Numeric buttons — Used to enter values for parameters. After entering a numeric value, press ENTER.

%FULL SCALE — Displays the REC output of the detector (in millivolts) as a percent of full scale. When the display reads 0001, the output is 1 percent of full scale, or 0.1 mV. A value of 0100 means that the output is 100 percent of full scale, or 10.0 mV.

SENS — Selects sensitivity. Pressing the key scrolls through the allowable values between 1 (least sensitive) and 1024 (most sensitive). The default value is 4.

SCALE FACTOR — Selects the scale factor. Allowable values range from 1 to 100. The default value is 20.

Scale factor affects the magnitude of the peaks on the chart recorder (10 mV output) only. A high scale factor setting results in a larger plot, which may cause larger peaks to go offscale. Scale factor does not affect integrator output.

Section 5.2.3, Scale Factor, discusses the effects of different scale factor settings and selecting scale factor.

ENTER — Saves parameter settings in the memory of the 410.

CLEAR — The CLEAR key erases unsaved parameter entries.

2ND FUNC — Accesses second functions. The 2ND FUNC LED indicator is located to the right of the ENTER key. Pressing the blue 2ND FUNC key activates second functions. You have 5 seconds to press the key whose second function you want to access.

Second functions are discussed in the next section, 5.1.2.2, Second Function Keys.

5.1.2.2 Second Function Keys

Each of the numeric buttons on the 410 keypad (except zero) has a second function to use when you change settings. Access second functions by pressing the blue 2ND FUNC key, then the key below the name of the function you want to access.

When you press 2ND FUNC, the indicator to the right of the ENTER key lights up. 2ND FUNC stays activated for 5 seconds, waiting for you to press the key whose second function you want to access.

EXT1 °C & EXT2 °C Display the temperature of a selected external column heater, in degrees Celsius.

To change the temperature of a column heater, press 2ND FUNC followed by EXT1 °C or EXT2 °C. The temperature of the column heater appears in the display, and the corresponding indicator lights up. Press 2ND FUNC, SET °C, and enter the new temperature. Then press ENTER.

The default value of 245.7 appears when no column heater is connected, or if a connected column heater is turned off.

INT°C Displays the temperature of the internal oven. This is the value that flashes on startup. Press CLEAR to stop it from flashing.

To change the temperature, press 2ND FUNC followed by INT °C. The temperature of the internal oven appears in the display, and the ccrresponding indicator lights up. Press 2ND FUNC, SET °C, and enter the new temperature. Then press ENTER.

It takes several hours for the opties bench to stabilize at the new temperature. Do not make a run until the temperature has stabilized; the changing temperature causes baseline drift.

SET °C Sets the temperature of a column heater or the internal oven. Allowable values range from 30 to 50 °C for the internal oven and ambient to 150 °C for the column heaters.

To turn off a heater, press 2ND FUNC followed by EXT1 °C, EXT2 °C, or INT °C (for either column heater or the internal oven). The temperature of the column heater or oven appears in the display, and the corresponding indicator lights up. Press 2ND FUNC, SET °C, CLEAR, then press ENTER.

TC SEC Adjusts the time constant of the noise filter to achieve the optimum signal-to-noise ratio.

Hold the key down to scroll through the values of 0.2, 1, 3, and 10. Press ENTER when you get to the value you want.

Section 5.2.5, Time Constant, discusses the effects of different time constant settings, calculating time constants for special applications, and the relationship between time constant and response time.

AUTO ZERO Adjusts the zero offset of the analog output to compensate for changes in baseline position. Use AUTO ZERO at any time, for example, before beginning a new run.

To activate AUTO ZERO from a remote device, see Sections 3.2.6 and 3.2.7, Waters WISP Remote Auto Zero and Waters U6K Remote Auto Zero Connections.

Shorter TC SEC settings decrease the length of time it takes for the 410 to auto zero. Higher TC SEC settings increase auto zero time.

MARK Sends a chart mark signal to the recorder or data module. The chart mark is aiways a 10 percent deflection in the positive direction, regardless of chart polarity.

To activate MARK from a remote device, see Sections 3.2.6 and 3.2.7, Waters WISP Remote Chart Mark and Waters U6K Remote Chart Mark Connections.

+/- Changes the chart polarity. Pressing the key once shows the current setting. Keeping the key pressed altemates through "POS" and "neg". When the display shows the polarity you want, press ENTER.

PURGE Purges the reference and sample sides of the 410 fluid path with fresh solvent. Purging requires pressing the PURGE button twice, once to start and once to finish the purge. During the purge, the display shows the letters "PgE".

Purge the fluid path whenever you change solvents or experience an unexpected loss in sensitivity due to excess noise or drift.

5.1.2.3 Using the Keypad

Checking values To use the keypad to view the value for a parameter, press the key for the parameter whose value you want to see. To view a second function value, press 2ND FUNC first. The value stays on the display until you select another parameter.

Changing SENS or SCALE FACTOR To change a value for SENS or SCALE FACTOR:

1. Press the key for the parameter whose value you want to change.
2. Enter a new value by scrolling (SENS only) or from the numeric buttons.
3. Press ENTER to save the new value. If you enter an unacceptable value, the 410 beeps and returns to the previous value.

Changing TC SEC To change a value for TC SEC:

1. Press the 2ND FUNC key.
2. Press the TC SEC key (5).
3. Enter a new value by scrolling (keep the key pressed) or from the numeric buttons.
4. Press ENTER to save the new value. If you enter an unacceptable value, the 410 beeps and returns to the previous value.

Changing EXT1 °C, EXT2°C,orINT°C To change a value for EXT1 °C, EXT2 °C, or INT °C:

1. Press the 2ND FUNC key.
2. Press the key for the unit whose temperature you want to change.
3. Press the 2ND FUNC key.
4. Press the SET °C key (4).
5. Enter a new temperature from the numeric buttons. Pressing CLEAR turns the internal oven or column heater off.
6. Press ENTER to save the new temperature. If you enter an invalid temperature, the 410 beeps and returns to the previous value.

Changing polarity To change output polarity:

1. Press the 2ND FUNC key.
2. Press the +/- key (8) to access the +/- function.
3. Press the +/- key again to reverse the polarity.
4. Press ENTER to save the new value.

AUTO ZERO, MARK, and PURGE To use AUTO ZERO, MARK, or PURGE, press 2ND FUNC followed by the key for the function you want to access.

To purge the 410 refractometer, press 2ND FUNC followed by PURGE (9) and ENTER. The letters "PgE" appear on the display.

To stop purging, press 2ND FUNC followed by PURGE and ENTER again. The display returns to the function it displayed before the purge began.

5.2 SELECTING PARAMETERS

You can adjust noise leve!, peak height, peak direction, and temperature of the internal oven and column heaters to optimize detector performance.

This section describes the effects of the following parameters:

- Sensitivity
- Scale factor
- Time constant
- Temperature
- Polarity

5.2.1 Sensitivity

Sensitivity affects both the integrator and the recorder signal. The higher the SENS setting, the larger the response for a given RI change, and the higher the baseline noise. For easiest operation, choose the lowest sensitivity that yields satisfactory peaks.

Effects of settings Higher sensitivity settings:

- Increase resulting peak area
- increase baseline noise
- Increase sensitivity to environmentai fluctuations

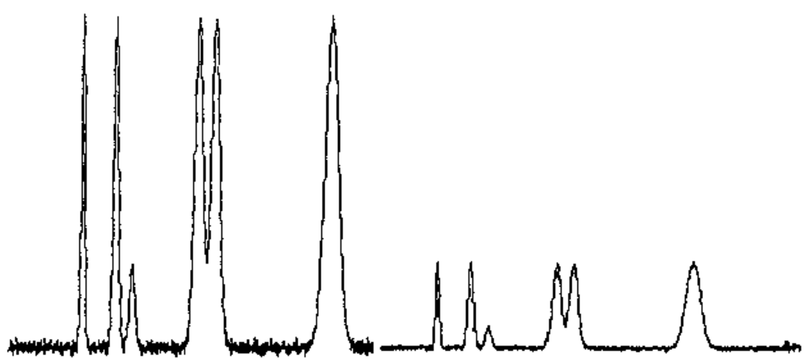


Figure 5-3 Effects of Sensitivity Settings

5.2.2 Sending Output to an Integrator

Voltage output as a function of sensitivity

The output of the 410 refractometer increases with increasing sensitivity. The following equation displays the relationship between SENS setting (S), change in refractive index (Δn), and output voltage (V):

$$V = S \times \frac{\Delta n}{5 \times 10^{-3}}$$

Maximum integrator output

The maximum change in refractive index (Δn) that the 410 can **optically** measure is 5×10^{-3} RIU). The integrator output is limited to ±10 V. For sensitivities of 1, 2, 4,or 8, the 5×10^{-3} RIU optical limit is less than the 10 V output limit.

Table 5-2 illustrates the relationships between sensitivity setting, maximum voltage output at each sensitivity setting, and RIU/V for the integrator output.

Table 5-2 Integrator output

Sensitivity	Maximum Output (V)	RIU/V
1	1	5.00×10^{-3}
2	2	2.50×10^{-3}
4	4	1.25×10^{-3}
8	8	6.25×10^{-4}
16	10	3.12×10^{-4}
32	10	1.56×10^{-4}
64	10	7.81×10^{-5}
128	10	3.91×10^{-5}
256	10	1.95×10^{-5}
512	10	9.77×10^{-6}
1024	10	4.88×10^{-6}

! ***CAUTION:*** *Many data systems are limited to 1 or 2 V input voltages.*

Example

At a sensitivity of 1, an integrator output signal of 1 volt corresponds to the maximum change in RI measurable by the 410 (5×10^{-3} RIU). The minimum detection limit of the 410 (5×10^{-8} RIU) at a sensitivity of 1 corresponds to a 10 μV integrator output signal.

Selecting SENS for your application The dynamic range of the 410 is the range of difference in RI over which it provides meaningful voltage output. Dynamic range decreases as you increase sensitivity. Use the lowest SENS setting that gives you acceptable results.

The optical limits are 5×10^{-3} to 5×10^{-8} RIU. The voltage limits are 0.02 to 10.02 volts.

Figure 5-4 shows the dynamic range and integrator output for each SENS setting.

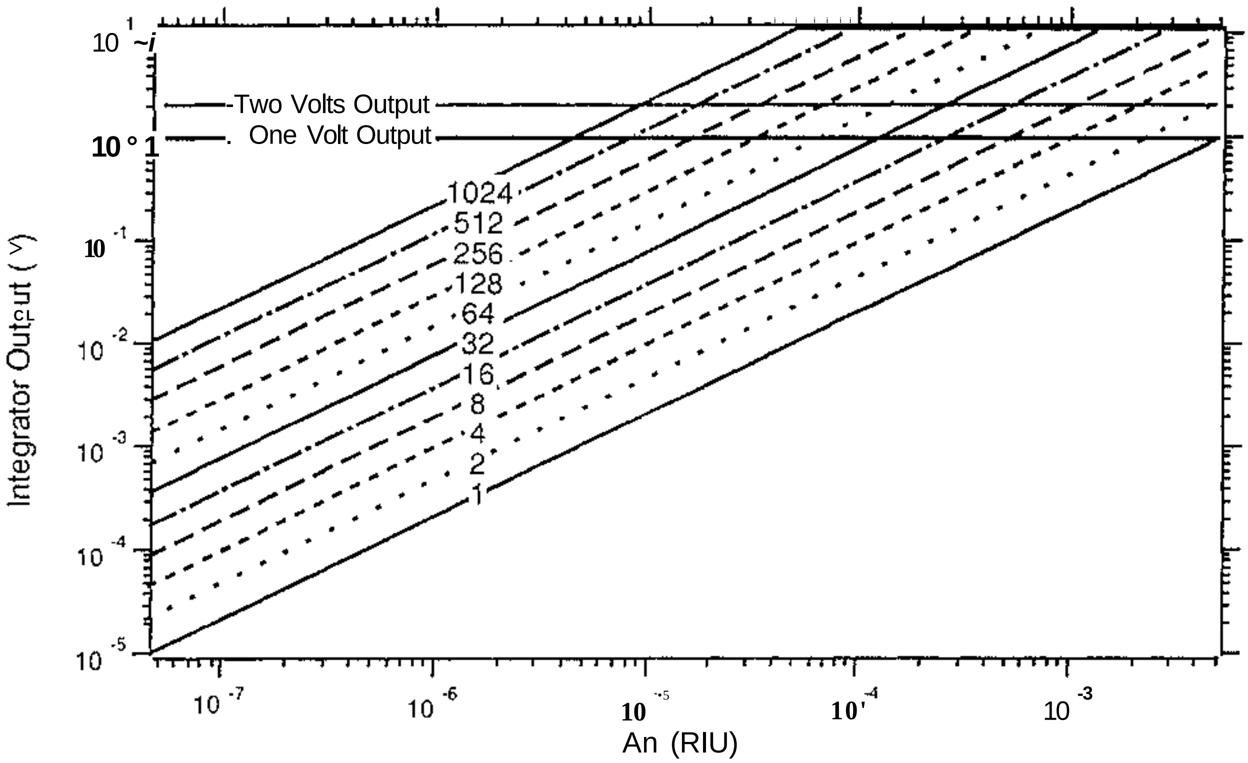


Figure 5-4 Determining An from Integrator Output and SENS

Examples At a SENS setting of 4, a change in refractive index (An) of 1.25 x 10-3 results in an integrator output of 1 volt.

For a data system with a maximum input voltage of 2 V, a 410 with a SENS setting of 512 has an effective dynamic range of 1.95x10-5 RIU.

Selecting sensitivity for 18 or 20-bit data systerns

A data system or integrator with an 18 or 20-bit A/D (analog to digital) converter has a greater dynamic range than the 410. For example, an 18-bit A/D converter with a 1 V input range can resolve 2"^{18} Volt or 3.8 |iV. A 20-bit A/D converter with a 2 V input range can resolve 2-19 Volt or 1.9 p.V.

The smallest An that the 410 refractometer can measure is 5 x 10"^{8} RIU. At a sensitivity of 1, this generates a signal of 10 (xV. Because the 18-bit, 1 V data system has 2.6 times the resolution of the 410, the data system can track the smallest An that the 410 can measure. A 20-bit, 2V data system has 5.2 times the resolution of the 410.

When operating the 410 refractometer with an 18 or 20-bit data system or integrator, set the sensitivity to 1 if the full scale input is 1 volt, and to 2 if the full scale input is 2 volts.

5.2.3 Scale Factor

At high sensitivities, some peaks may be too large for the chart recorder. Use scale factor to reduce the plot. Scale factor affects only the recorder (10 mV) output.

Effects of settings

A high scale factor setting resuits in a larger plot, which may cause some peaks to go offscale. It does not affect peak integration. A small scale factor setting reduces the size of the plot, so small peaks may not be well-defined, but integration remains unaffected.

Scale factor settings:

- Reduce large peaks to fit the chart recorder scale, but reduce smaller peaks as well.
- Have no effect on peak resolution, only amplitude.

Calculating scale factor

To caiculate an appropriate scale factor setting, use the following equation:

$$SF = \frac{10{,}000}{\%FS}$$

Where: %FS = The value displayed when the 410 reads the largest peak.

5.2.4 Sending Output to a Chart Recorder

The chart recorder output of the 410 refractometer has a zero offset of 20 mV.

REC output and INT output The maximum voltage output to a chart recorder is always 10 mV, regardless of the sensitivity or scale factor setting.

REC output and scale factor When sending output through the 10 mV REC terminals, you can adjust your plot with the scale factor function of the 410.

The following equation shows recorder output in millivolts as a function of difference in refractive index (An), sensitivity setting (S), and scale factor (SF):

$$mV = \frac{An}{5 \times 10^{-4}} \times S \times SF$$

Table 5-3 Recorder Output in RIL) Full Scale

Sensitivity	RIUFS (SF = 1)
1	5.00x10-3
2	2.50 x IO-3
4	1.25x 10-3
8	6.25 x 10-"
16	3.12 x 10-»
32	1.56 x 10-4
64	7.81 x 10-5
128	3.91 x 10-5
256	1.95x10-5
512	9.77 x 10-6
1024	4.88 x 10-6

Examples At a sensitivity of 1 and scale factor of 100, a full scale deflection (% full scale reads 100, or 10 mV) yields a ARIU of:

5x 10-3 RIUx 100%:=5x 10-3 RIUFS

At the same settings, a deflection of 1% (.1 mV) means the corresponding ARIU is one hundred times smaller, or:

5x 10-3 RIUx 1% = 5x 10-s RIUFS

At a sensitivity of 1 and scale factor of 20, a 10 mV deflection (10 mV) corresponds to a change in RIU of:

5x10-3 RIU x 0.2 = 5 x 10-4 RIUFS

If the 410 is set to the most sensitive scale (sensitivity = 1024, scale factor = 100), a 1% full scale reading (0.1 mV deflection) corresponds to a ARIU of:

4.88 x 10-6 R|U x 1% = 4.88 x 10-8 RIUFS.

5.2.5 Time Constant

The time constant adjusts the response time of the noise filter to achieve optimum signal-to-noise ratio by reducing short-time noise.

Effects of lower settings

Lower time constant settings:

- Produce narrower peaks with minimum peak distortion and time delay
- Result in greater baseline noise
- Reduce the amount of time it takes to auto zero

Effects of higher settings

Higher time constant settings:

- Decrease baseline noise
- Shorten and broaden peaks
- Increase the amount of time it takes to auto zero

Default setting

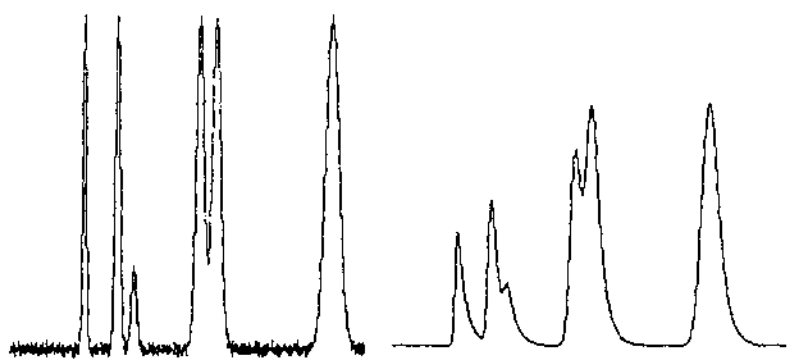
The default time constant of 1.0 second is satisfactory for most applications.

Calcuiating time constant

To calculate an appropriate time constant:

$$TC = 0.2 \times PW$$

Where: TC = time constant setting
PW = peak width in seconds at half height of the narrowest peak



Low Time Constant High Time Constant

Figure 5-5 Effects of Time Constant Settings

5.2.6 Temperature

The 410 refractometer permits temperature ranges of 30 to 50 °C for the internal oven, and ambient to 150 °C for up to two external column heaters. The general operating temperature for the internal oven should be about 5 °C above the ambient temperature for room temperature applications. This guards against drift caused by variations in the ambient temperature.

High temperature operation Set the internal oven temperature 3 to 5 °C below column temperature for high temperature applications, depending upon the length of tubing between the column heater and the 410 refractometer.

Effects of higher temperatures Higher temperature settings:

- Reduce the viscosity of the mobile phase
- Increase the solubility of the sample
- Increase mass transfer rates, improving column efficiency
- Decrease retention times
- Make the system less susceptible to fluctuations in ambient temperature
- Let dissolved gases come out of poorly degassed cooler solvents, resulting in bubbles

Default temperature The default temperature of 30 °C is satisfactory for most room-temperature applications.

5.2.7 Polarity

The polarity button reverses the direction of peaks. Samples detected with the 410 refractometer can yield positive or negative peaks, depending on whether their RI is greater than or less than that of the mobile phase.

Effect of reversing polarity Reversing polarity has no effect on peak integration or resolution. It may aid proper integration.

Default setting The default polarity setting is positive.

5.3 ROUTINE STARTUP

Powering up To power up the 410, press the ON/OFF switch located on the lower front right corner of the unit. The indicators each light once while the 410 performs internal tests. The display flashes the internal oven temperature setting. Press CLEAR to stop the flashing and display the current internal oven temperature.

Turn on peripherals after powering up the 410.

Remote mode The 410 detector is in remote mode when it is under active control by a system controller through the IEEE-488 interface. The 410 can be configured with units such as the Waters 600E Multisolvent Delivery System, Waters 860 Networking Computer System, or Waters 845 Chromatography Data and Control Station. When the 410 is in remote mode, the controller can lock the keypad and you cannot enter any parameters or values.

Warm-up period For normal use, allow the detector to warm up for 24 hours before operating.

Changing Solvents When you change solvents:

STOP *WARNING: öbserve good laboratory practices when handling solvents. Refer to the Material Safety Data Sheets shipped with solvents for handling Information.*

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1. Make sure the PRG OUT line goes to waste.
2. Replace the column with a union.
3. Set the pump flow rate to 5 mL/min.
4. Press 2ND FUNC, PURGE.
5. Let the 410 purge for 5 minutes.
6. Press 2ND FUNC, PURGE again to stop purging.

Follow the same procedure to purge the 410 with 10 percent methanol/water or helium before storing it.

5.4 POWERING DOWN

Storage Do not turn the 410 off unless you are storing it.

If you are not storing the 410, set the flow rate to 0.1 mL/min and keep pumping. This minimizes the amount of time the 410 will need for re-equilibration when you use it again.

Purging the 410 Huid path Do not ieave buffers in the system after use. Flush out the lines with a suitable solvent (for example 100 percent methanol), or with helium.

If your storage solvent is incompatible with your column, remove the column before flushing.

Turning off peripherals Turn off peripheral devices before powering down the 410.

Powering down To power down the 410, press the black ON/OFF switch located on the lower front right corner of the unit.

6

MAINTENANCE PROCEDURES

Introduction The 410 refractometer requires minimal routine maintenance. Perform these procedures to help keep the 410 refractometer running smoothly:

- Clean the fluid path.
- Replace the fuse.
- Clean the fan filter.

STOP *WARNING: To avoid electrical shock, power down the 410 refractometer before cleaning the fan filter or changing a fuse.*

Waters Service Department The Waters Chromatography Division service specialists provide preventive and corrective maintenance assistance.

In the USA, contact the Waters Chromatography Division Service Department at 1-800-252-4752 or your local service representative if you have questions regarding repair or performance.

Outside of the USA, contact the nearest Waters Chromatography Division subsidiary. There is a list of subsidiaries at the back of this manual.

Spare parts Stock the recommended spare parts to optimize uptime. Refer to Appendix B for a list of recommended spare parts. Parts not inciuded in this list may require replacement by a Waters service representative. Contact Waters Service for assistance.

6.1 CLEANINGTHEFLUIDPATH

A dirty **fluid** path can cause baseline noise, inaccurate sample refraction, and other problems with operation.

Contaminated tubing Contaminated tubing anywhere along the flowpath can cause erratic drift or a saw-tooth baseline. If you suspect that the tubing has been contaminated, follow this procedure. Read through it carefully first, and pay strict attention to the warning.

Supplies To clean the fluid path, you need:

- A wrench suitable for removing and replacing the column.
- A solvent miscible in both the mobile phase and water. (Methanol is commonly used.)
- Milli-Q® or equivalent HPLC-grade water.
- A strong cleaning solvent suitable for your system. (6 N Nitric acid is commonly used.)
- A separate waste container for acid waste.
- A means for measuring the pH of acid effluent, if you use an acid as your cleaning solvent.

WARNING: *Strictly adhere to this procedure. Préparé a separate waste container for the acid.*

Be careful to keep organic waste from mixing with acid waste.

Procedure To clean the fluid path:

1. Stop the pump and replace the column with a union.
2. Replace the mobile phase with an intermediary solvent miscible in both the current solvent and water.
3. Put the 410 refractometer into Purge mode.
4. Restart the pump. Set the flow rate to 5 mL/min to flush the mobile phase from the 410 refractometer.

5. Switch the pump to HPLC-grade water. Flush the 410 with water to remove contaminants from the flow path.

6. Switch the pump to the cleaning solvent. Flush the 410 for 6 to 10 minutes. Use a clean waste container when pumping cleaning solvent. Do not mix acidic and organic waste.

Ifyou use 6 N nitric acid, do so with care. When working at high sensitivities, it may require extensive flushing with water to remove all traces of the nitric acid.

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7. Switch the pump back to HPLC-grade water. Flush until the pH of the waste effluent is neutral (a pH value of 6.0 to 7.0).

8. Switch the pump back to the water-miscible intermediate solvent. Flush for 10 minutes.

9. Switch the pump back to the mobile phase. Flush for 5 minutes.

10. Take the 410 out of Purge mode and stop the pump.

11. Reattach the column and re-equilibrate the 410.

6.2 REPLACING THE FUSE

This section describes replacing the fuse. You need a Standard screwdriver for this procedure.

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

Check the fuse if the detector does not power up.

For information on detecting the cause of a blown fuse, refer to Section 7.3, Hardware Troubleshooting.

Procedure

STOP *WARNING: To avoid electric shock, power down and unplug the refractometer before checking the fuse. For continued protection against fire hazard, replace the fuse only with another of the same type and rating.*

1. Turn the gray fuse holder (F1) with the screwdriver and pull it out.
2. Replace the fuse.
3. Put the fuse holder back in the rear panel.

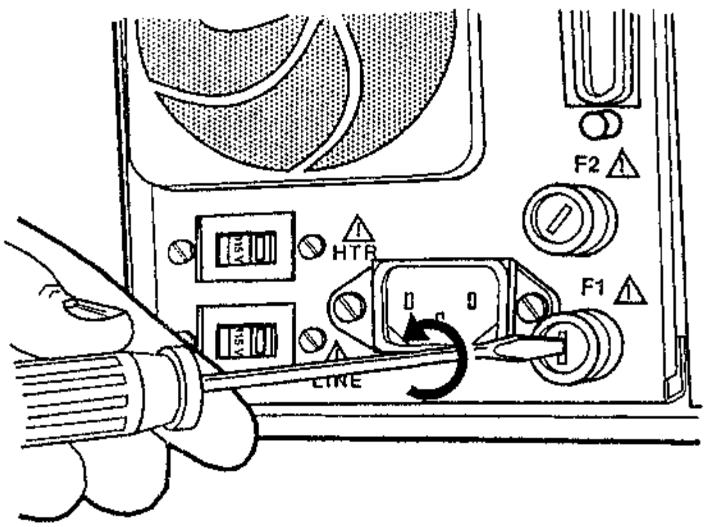


Figure 6-1 Replacing the Fuse

The 410 refractometer uses the following fuses:

Table 6-1 Fuse Requirements

Nominal Voltage	Voltage Range	Fuse Requlred
100/120, 50/60 Hz	90-132	1.5 A time delay
220/240, 50/60 Hz	198-264	0.75 Atime delay

6.3 CLEANING THE FAN FILTER

To keep the 410 refractometer running properly, inspect and clean the fan filter monthly.

Procedure To clean the fan filter:

1. Snap the filter cover off from the back of the refractometer.
2. Shake the filter free of dust. If the filter is visibly dirty, replace it with a new filter.
3. Replace the filter cover.

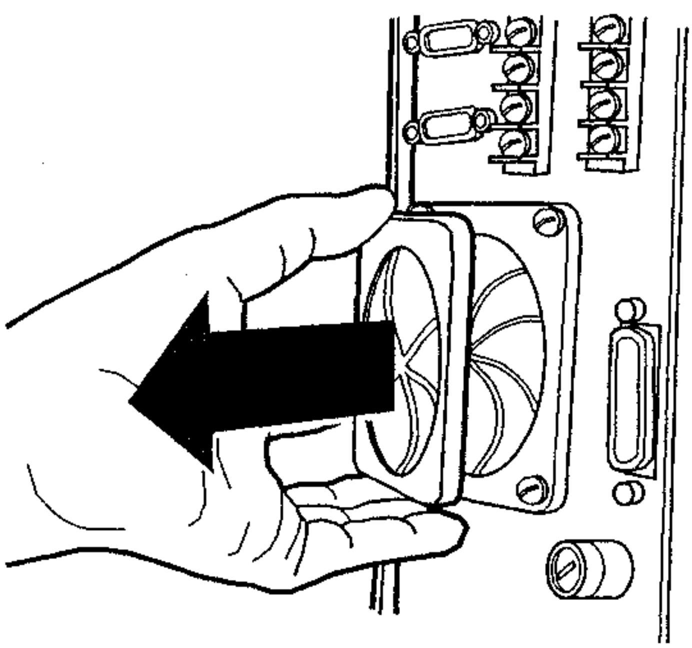


Figure 6-2 Cleaning the Fan Filter

7

TROUBLESHOOTING

This chapter provides information for troubleshooting the 410 refractometer. It includes a checklist of possible causes, and recommended corrective action for each problem.

This chapter is structured as follows:

Section	Title	Describes
7.1	**General** Troubleshooting Hints	Isolating the problem
7.2	**Chromatography** Troubleshooting Tables	Problems with the chromatography system, such as a contaminated column, and corrective action.
7.3	Hardware Troubleshooting Table	Problems with the 410 refractometer, and corrective action.

Most probiems are easily corrected. If you cannot correct a condition, contact your Waters Service Specialist or Waters Chromatography Division Service at 1-800-252-4752.

When you call Waters Service To expedite your request for service, have the following information avaiiable when you call:

1. Symptom
2. Sensitivity setting
3. Scale factor
4. Flow rate
5. TC setting
6. Type of column
7. Operating pressure
8. Solvent(s)
9. Diagnostic results
10. System configuration (for example with a 600E, an 860, a non-Waters product)
11. Serial number

Diagnostics The 410 includes diagnostics helpful in troubleshooting. Refer to Section 7.3.1, 410 Diagnostics, for a description of each diagnostic and instructions for performing diagnostics.

Power surges Power surges, line spikes, and transient energy sources can adversely affect operation. Be sure **that** the electrical supply used for the 410 is properly grounded and free from any of these conditions.

7.1 GENERAL TROUBLESHOOTING HINTS

This section lists the basic steps for troubleshooting different symptoms.

Step 1 Take a step back and look at the system. Is something obvious causing the problem?

Example: If the unit does not power up when you turn it on, check that the unit is plugged in to a live outlet. This may seem like an oversimplified example, but always eliminate the obvious causes first.

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

Example: If your system usually runs at 50 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?

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 instalied, get in the habit of keeping a record of system conditions during normal operation.

Step 3 Isolate the parameter that varies from normal operation:

- Baseline (drift, noise, or cycling)
- Peak retention time
- Peak resolution
- Qualitative/quantitative chromatography results
- System pressure

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

Step 4 Use the Troubleshooting Tables to find suggested corrective action.

7.2 CHROMATOGRAPHY TROUBLESHOOTING

This section contains Chromatography Troubleshooting Tables that describe symptoms and provide suggested corrective actions.

Before using the Chromatography Troubleshooting Tables in this section, read the General Troubleshooting Hints in the previous section and follow the steps to isolate the cause of the chromatography symptom.

WARNING: Always observe good laboratory practices when handling solvents and performing maintenance.

7.2.1 Troubleshooting Tables

The Troubleshooting Tables in this section help to isolate the possible causes of problems related to:

- Abnormal baseline (drift, noise, or cycling)
- Erratic or incorrect retention times
- Poor peak resolution
- Incorrect qualitative/quantitative results

If your system is exhibiting symptoms *not* addressed in a Chromatography Troubleshooting Table, referto the 410 Hardware Troubleshooting Table in Section 7.3. If you need further help, call Waters Customer Service at 1-800-252-4752.

7.2.1.1 Abnormal Baseline

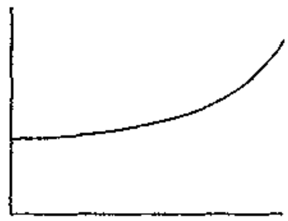
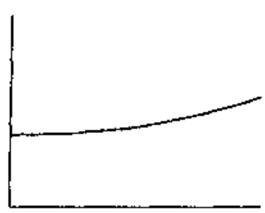
Drift The most common difficulty with the 410 refractometer is baseline drift. Drift may be flow-related or result from changing ambient conditions, especially temperature. Determine if drift is flow-related by shutting down the pump.

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

Cycling If the baseline is cycling, determine the period of the cycling and if it is related to the flow rate or fluctuations in ambient temperature.

Table 7-1 Baseline Troubleshooting

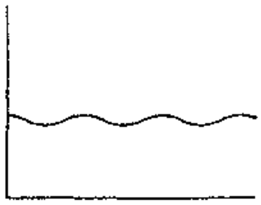
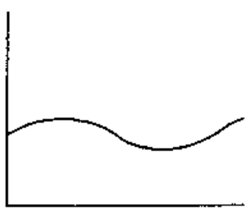
Waters Customer Service 1-800-252-4752

	Possible Cause	Corrective Action
Baseline drift, rapid	Column not equiübrated	Equilibrate column.
	Detector not allowed to warm up	Allow detector to warm up until baseline is stable. Warm-up time varies based on sensitivity.
	Solvent contaminated or not HPLC grade	Use fresh solvent.
	Solvent not properly degassed (rapid or slow drift)	Degas the solvent. Capthe solvent reservoir.
	Tubing contaminated	Clean the tubing with the procedure in Section 6.1, Cleaning the Fluid Path.
	Fiow fluctuations (rapid or slow drift)	Fix pump problems, replace pump seals, check valves.
Baseline drift, slow	Solvent contaminated	Use fresh, degassed solvent.
	Ambient temperature fluctuations	Stabilize operating environment temperature enough to allow full equilibration. Keep the system away from air conditioning vents, chance breezes, and direct sunlight.
	Dirty flow cell	Clean flow cell (Section 6.1, Cleaning the Fluid Path).
Baseline **drift,** descending	Leaky flow cell	Call Waters Service.

Contlnued

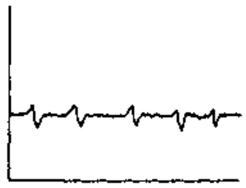
Table 7-1 (Cont.) Baseline Troubleshooting

Waters Customer Service 1-8OO-252-4752

Symptom	**Possible Cause**	**Corrective Action**
Baseline noise cycling	Pump pulsing	Add pulse dampener.
Short **term (30 sec-1 min)** 	Inadequate solvent blending in pump	Connect high flow pulse dampener. Use gradiënt mixer.
	Flow fluctuating	Stabilize flow.
	Solvent not mixed (short or long term cycling)	Stir the solvent.
	Faulty check valve	Ciean/replace/rebuild pump check valves.
	AC power source (short or long term cycling)	Disconnect other instruments on the power line, try a different wall outlet, have line voltage checked, use power conditioner.
	Radio frequency noise (short or long term cycling)	Eliminate interference.
Long term (approximately 1 hour) 	Ambient temperature fluctuations	Stabilize ambient temperature.
	Integrator or recorder faulty	Check integrator or recorder for excessive baseline noise.
	Faulty check valve	Clean/replace/rebuild pump check valves.

Continued

Table 7-1 (Cont.) Baseline Troubleshooting

Symptom	Possible Cause	Corrective Action
Baseline noise, random 	Air in flow cell	Purge fluid path to remove air.
	Solvents not properly degassed or sparged	Degas/sparge solvents (see Section 4.3, Solvent Degassing, for more information).
	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.
	Dirty flow cell	Clean fluid path (Section 6.1, Cleaningthe Fluid Path).
	Anaiog output cable not properly connected between 410 and data system or recorder	Properly connect cable.
	System improperly grounded	Plug into different outlet on different electrical circuit.
		Use power conditioner.
	Recorder voltage incorrect	Set recorder to correct voltage.
	Radio frequency noise	Eliminate interference.

7.2.1.2 Erratic or Incorrect Retention Times

Retention time changes

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

- Change from run to run
- Are constant from run to run, but are outside the aliowable range for the assay

Examining pressure fluctuations and changes

When troubleshooting a retention time problem, check system pressure first. Look for:

- **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?

It is also important to note if the retention time changes:

- Appear suddenly at the end of a series of runs. This may indicate that air is dissolving in the mobile phase, that the mobile phase is degrading, or that the column is contaminated.
- Occur 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.

Table 7-2 Retention Time Troubleshooting

Symptom	Possible Caüse	Corrective Action
Erratic retention times	Air bubble in pump head	Degas all solvents, prime pump (Section 4.3.2, Solvent Degassing Methods).
	Malfunctioning pump check yalves	Clean/replace/rebuild 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	Verify flow rate.
	Incorrect solvent composition	Change solvent composition.
	Column heater module not on	Turn column heater module on.
	Column not equilibrated	Equilibrate column.
	Incorrect column or guard column	Use correct column or guard column.
Doubled retention times	Air bubble in pump head	Prime pump to remove bubble.
	Malfunctioning pump check valve(s)	Clean/replace/rebuild pump check valve(s).
	Broken pump plunger	Replace the plunger.
Reduced retention times	Incorrect flow rate	Verify flow rate.
	Incorrect solvent composition	Change composition.
	High column temperature	Reduce column temperature.
	Incorrect column pretreatment	See column manual.
	Column contaminated	Clean/replace column.
	Incorrect column or guard column	Use correct column or guard column.
Reproducibility errors	Solvent not properly degassed/sparged	Degas/sparge solvent (Section 4.3.2).
	Incorrect chemistry/integration	Check chemistry/integration.
	Column not equilibrated	Equilibrate column
	Injector problem	Troubleshoot injector.

7.2.1.3 Poor Peak Resolution

Before addressing problems with peak resolution, be certain that peaks elute at the correct retention time. The most common causes of poor peak resolution can also appear as retention time problems.

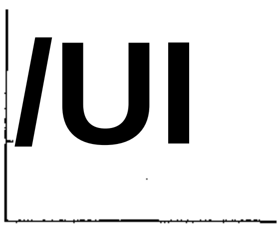
If peak retention time is correct, determine if poor resolution occurs:

- Throughout the chromatogram
- At a single peak pair

If efficiency of early peaks is poor, this may imply extra-column band broadening, such as autoinjector or guard column failure. If peak efficiency is poor throughout the chromatogram, it may imply post-column band-broadening or loss of column efficiency.

If only one peak in the chromatogram is badly-shaped, it may imply that this peak interacts with the column with a different chemical mechanism. To troubleshoot this resolution problem, you need an understanding of the separation chemistry.

Table 7-3 Resolution Troubleshooting

Symptom	Possible Cause	Corrective Action
Straight baseline, no peaks 	No pumpflow	Set pump flow rate.
	LED nol on	Use Detector Diagnostics to check reference/sample energy (Section 7.3.1, Diagnostics). Zero energy indicates LED not on.
		Power unit down then up to light lamp.
		Call Waters Service.
	Detector not zeroed	Zero detector baseline.
	Improper connection between 410 unit and recorder	Check cabling between unit and recorder.
	Solvent and sample have similar refractive indices	Select another solvent.
	Sensitivity too low	Select higher sensitivity.
	No sample injected	Check injector.
	Leak in solvent path	Check fittings and drip tray.
Flat-topped peaks 	Detector not zeroed	Zero detector baseline.
	Incorrect recorder input voltage	Adjust recorder input voltage, or adjust detector output cable to correct position.
	Sensitivity too high	Select a lower sensitivity.
	Scale factor too high (recorder only)	Select a lower scale factor.
	Sample concentration or injection volume exceeds voltage output of detector	Decrease sample concentration or injection volume.

7.2.1.4 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 resoiution 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).

Table 7-4 Incorrect Results Troubleshooting

Symptom	Possible Cause	Corrective Action
Decreased peak height	Leak in injector	Troubleshoot injector.
	Degraded, contaminated or improperly prepared sample	Use fresh sample.
	Column contaminated	Clean/replace column.
	Loss of column efficiency	Clean/replace column.
	Change in mobile phase composition	Correct mobile phase pH or ionic composition.
	Incorrect flow rate	Change flow rate.
	Dirty flowcell	Clean the fluid path (Section 6.1, Cleaningthe Fluid Path).
Increased noise	Electronic noise	Use the shortest lengths of cabling possible. Make sure cables are shielded.
	Dirty flow cell	Clean the fluid path (Section 6.1, Cleaning the Fluid Path).
	Air bubble in flow path	Replace column with union and purge flow path at 10 mL/min.
	Mobile phase not degassed	Degas/sparge mobile phase.
	Contaminated mobile phase	Use fresh mobile phase.

7.3 410 HARDWARE TROUBLESHOOTING

This section contains information about user diagnostics and hardware troubleshooting for the 410 refractometer.

7.3.1 410 Refractometer Diagnostics

This section describes user diagnostics for troubleshooting the 410 refractometer. Use the diagnostics to determine:

- LED current strength
- Total voltage transmitted from the photodiode
- Voltage transmitted from the sample side of the photodiode

This information may be helpful when you call Waters Customer Service at 1-800-252-4752.

Using **diagnostics** To use the 410 Diagnostics:

1. Press the 2ND FUNC key, then CLEAR, CLEAR, ENTER. The display goes dark.

2. To determine the LED current strength, press the 2ND FUNC key, then 3, and press ENTER. The vaiue displayed should be between 70 and 150.

3. To determine the total voltage transmitted from the photodiode, press the 2ND FUNC key, then 4, and press ENTER. The value displayed should be between 3.9 and 4.1.

4. To determine the voltage transmitted from the sample side of the flow cell, press the 2ND FUNC key, then 5, and press ENTER. The value displayed should be half of the total transmitted voltage, or between 1.95 and 2.05.

5. To exit Diagnostic Mode, press the 2ND FUNC key, then CLEAR, and ENTER. Each red indicator lights up for an instant, starting next to the ENTER button and ending above the 1 key.

6. After exiting Diagnostic Mode, you must reset your desired operating parameters. It is particularly important to reset the internal oven temperature, which may take a long time to equilibrate.

7.3.2 Hardware Troubleshooting

This section describes symptoms, causes, and corrective actions reiated to the 410 refractometer hardware. Use this table when you know the problem is in the detector.

Table 7-5 410 Hardware Troubleshooting

Symptom	Possible Cause	Corrective Action
Detector inoperative	No power at outlet	Check outlet by connecting another electrical unit known to be in working order and see if it operates.
	Fuse blown	Check that the fan and display are operational; if neither, replace fuse (see Section 6.2).
Display fails to illuminate	Weak electrical connections	Check connections.
	Fuse blown	Check and replace faulty fuse.
	Faulty Analog board	Call Waters Service.
Indicators do not light	Fuse blown	Check and replace faulty fuse.
	Faulty Display board	Call Waters Service.
Display shows odd characters or FAIL	Fauity Control board	Call Waters Service.
	Faulty Analog board	
IEEE trouble	Incorrect IEEE-488 address	Set correct address (Section 3.4, Setting IEEE-488 Addresses).
	IEEE-488 cable not connected	Connect IEEE-488 cable (Section 3.4).
	Bad IEEE-488 cable (external)	Replace IEEE-488 cable.

Continued

Table 7-5 (Cont.) 410 Hardware Troubleshooting

Symptom	Possible Cause	Corrective Action
Keypad not functioning	Keypad defective	Call Waters Service.
LED source lamp does not light	Faulty fuse	Call Waters Service.
	LED burned out	
Unit overheating (410 beeping)	Dirty fan filter	Clean fan filter (Section 6.3, Cleaning the Fan Filter).
	Faulty fan	Call Waters Service.
	Ambient temperature is 5 °C above set oven temperature	Remove externai heat sources from around 410. Check column heater settings.
Internal oven overheating	Relay stuck	Call Waters Service.
	Faulty Analog board	
Internal oven does not heat	Thermal switch needs to be reset	Press white button on back left side of 410 to reset.
No signal from LED	Air bubble in flow cell	Purge the 410.
	LED burned out	Call Waters Service.

APPENDIX A

SPECIFICATIONS

APPENDIX A Specifications

Operational Specifications

Conditions	Specifications
RI Range	1.00 to 1.75 RIU
Measurement Range	5×10^{-3} RIU maximum FS (SENS= 1, SF=1) 5 x 10-a RIU minimum FS (SENS=1024, SF=100)
Flow Rate	0.3 -10 mL/min
Noise	2.0×10^{-8} RIU, (TC = 1, SF = 20, SENS = 32, THF at 24 °C with restrictor coil)
Drift	2.5×10^{-7} RIU/hr (static with THF)
Sensitivity Settings	1, 2, 4, 8, 16, 32, 64, 128, 256, 512, 1024
Time Constant Filter Settings	0.2, 1, 3, 10 seconds
Recorder Output	0 to 10 mV
Temperature Control	Internal oven 30 to 50 °C External column heaters Ambient to 150 °C

Integrator output

Sensitivity setting	Output (V)
1	1
2	2
4	4
8	8
16 to 1024	10

Optical Component Specifications

Light Source	930 nm pulsed Light Emitting Diode (LED)
Flow Cell	Fused quartz
Cell Volume	10 µL
Pressure Limit	100 psi
Fluid Path Materials	316 stainless steel, Dynasii®, Suprasil II®, Teflon®, Kalrez®, quartz

SPARE PARTS / ACCESSORIES

APPENDIX B

APPENDIX B Spare Parts/Accessories

RECOMMENDED SPARE PARTS

DESCRIPTION	PART NUMBER
General Parts	
Fan filter	097610
Pressure relief valve	070377
Solenoid valve	070376
Ferrule, 1/16-inch, Teflon	070215
Nuts and ferrules (kit of 5 each)	025604
Stainless steel tubing 0.009-inch ID x 10 feet	026973
Stainless steel tubing 0.040-inch ID x 10 feet	026805
Recorder cable 4 ft (1)	048918
Waters Startup Tool Kit	096146
Tubing cutterfor 1/16 inch stainless steel tubing	022384
Tubing cutter, spare blades, 3/pkg	022385
Fuses (time delay)	
1 A main fuse (110 V, 50/60 Hz)	022615
1/2 A main fuse (220 V, 50/60 Hz)	022628
3/4 A internal heater fuse (110 V units)	072778
3/8 A internal heater fuse (220 V units)	032148
IEEE-488 Cable Lengths	
3.3 feet	087198
6 feet	087141
13 feet	087191
26 feet	087192
52 feet	087193
Solvent Preparation Accessories	
Solvent Clarification Kit Contains: 1 pump, 110V, 60 Hz 1 liter flask 300 mLfunnel damp tubulated base 100 Durapore™ filters	085113
Mobile Phase Stabilization Accessory	037302
0.45 µm membrane filters Fluoropore™ organic filters Triton™ free aqueous filters	 085118 085147

APPENDIX C

WARRANTY/
SERVICE INFORMATION

APPENDIX C Warranty/Service Information

Waters Service Department
1-800-252-HPLC

Serial Number________________Startup Date.__________

410 LIMITED PRODUCT WARRANTY

Millipore Corporation, including its Waters Chromatography Division (Waters), provides this limited warranty (the Warranty) to protect customers from non-conformity 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 quaiity 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, economie 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 will not be deemed to have failed of its essential purpose so long as Waters is able and willing to repair or replace any non-conforming part or product.

Warranty Service Warranty service is performeel at no charge and at Waters option in one of three ways:

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

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 retumed. 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 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 failed to perform maintenance, calibration checks, or to observe good operating procedures.

Repair or replacement is not made:

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

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

The warranty period for the 410 refractometer is as follows:

1 year warranty

- Analog Board
- Control (CPU) Board
- Front panel Board
- Rear panel Board
- Fan
- Photodiode assembly
- Heater
- Resistance thermometers
- Relays
- Switches
- AC Transformer

90 day warranty

- Cables
- Solenoid
- Pressure relief valve

30 day warranty

- Replacement parts
- Service workmanship

Expendable items (Must be replaced by Customer Service)

- Flow cell assembly
- Source light (LED)
- Countercurrent heat exchanger

Expendable items (Customer replaceable)

- Tubing/Fittings
- Fuses
- Fan filter

410 ORDERING INFORMATION

Where to place orders

Write: Millipore Corporation,
Waters Chromatography Division,
34 Maple St.
Milford, MA 01757
Attn: Order Processing Department

Cal!:* 1-800-252-4752 - Customer Sales Department

Telex: 174166 or 174128
Fax: 1-508-872-1990

International Consult the listing of Waters Sales/Service offices at the end of this manual.

"Confirming orders mailed after a telephone order has been placed must be clearly marked "CONFIRMING" to avoid duplication.

How to place orders Delays or errors in processing orders are frequently caused by incorrect or incomplete information. To minimize delays and errors in processing your orders, please list part numbers in ascending numerical order, and provide all of the following information:

1. Catalog numbers and descriptions.
2. Quantity desired.
3. Complete purchase order number - orders cannot be processed without it. Requisition numbers are insufficient.
4. Complete Ship To address and marking if applicable.
5. Complete Bill To address if other than Ship To.
6. Required delivery date.
7. Method of transportation desired.
8. Name and telephone number of person to contact if clarification is required.

Pricing Prices listed are FOB Milford, MA, unless otherwise agreed. Prices and product information contained in any catalog or price list are current at the time of printing. In a continuing effort to provide the finest products available, Waters reserves the right to change specifications, models, or prices without notice and without liability for such changes. Where price **changes** occur, prices prevailing at time of receipt of your order apply.

Terms of payment Waters' terms are net 30 days from invoice date with approved credit. If your credit has not been previously established with Waters, our terms are payment in advance or COD.

SHIPMENTS-DAMAGES-CLAIMS-RETURNS

Shipments As all shipments are made FOB Milford, MA, we suggest you authorize insurance 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, 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 authorization number.

Claims After a damage inspection report has been secured, Waters will cooperate 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 Customer Service Department or your nearest Waters subsidiary/representative for a return authorization number and forwarding instructions.

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W, X, Y, Z

Waters

Division of MILLIPORE

U.S. Sales Offices

Ne*1/ Ëngland Waters Chromalography Division
Millipore Corporalion/Milford, MA
Tel. 508-478-2000/FAX 508-872-1990

Chicago Watefs Chfomalography Division
Wood Dole, Il/Tel. 708-766-6060
FAX 708-766-6385

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Morrislown, NJ/Tel. 201-285-1404
FAX 201-285-0694

North Carolina Waters Chromalography Division
Cary, NC/Tel.919-469-2501 /FAX 919-481-3118
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Korea Young-In Scientific Co. Ltd./Seoul
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Mexico Millipore S.A. de C.V./Waters
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